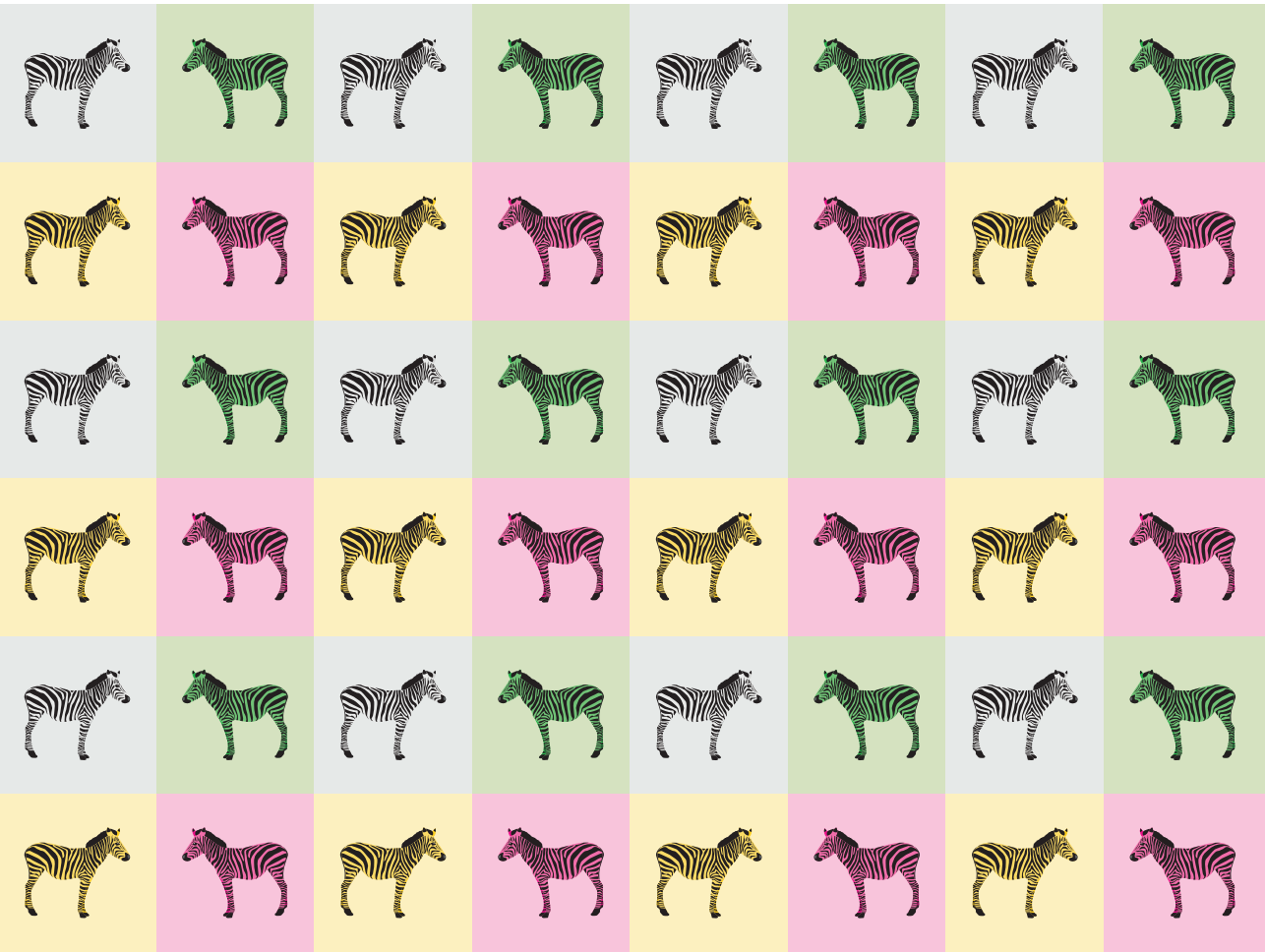
^{177}Lu-octreotate in Neuroendocrine Tumors: Treatment Effects

Esther I. van Vliet



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The zebra is the international symbol for neuroendocrine tumors.

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Voor papa

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Chapter 1

General Introduction

Somatostatin is a 14-amino acid peptide that inhibits the secretion of a wide range of hormones and acts by binding to somatostatin receptors (ssts), which are, amongst others, expressed on most neuroendocrine tumors (NETs).[1] The biological stability of native somatostatin (SS-14) is poor due to rapid enzymatic degradation within the range of minutes after intravenous administration. The somatostatin analog octreotide, that contains eight amino acids, is more resistant to this enzymatic degradation due to various modifications including the introduction of D-amino acids and shortening of the molecule to the bioactive core sequence. Ssts are a family of five G-protein coupled receptors, sst_1–sst_5 (somatostatin subtype 1-5), which have been cloned in the early 1990s.[2] The majority of NETs abundantly express ssts, and these tumors can be visualized in patients using the radiolabeled somatostatin analog [^{111}Indium-DTPA^0]octreotide (OctreoScan®; Covidien, Petten, the Netherlands). NETs mainly express sst_2.[3]

EPIDEMIOLOGY OF NETS

NET is a rare disease with an incidence of 2–5 per 100,000 inhabitants.[4-6] In the Netherlands, the incidence of NETs (not including functioning or nonfunctioning pancreatic NETs) was 1.8 per 100,000 inhabitants for men and 1.9 per 100,000 inhabitants for women in the period of 1989–1996.[7] The highest incidence of NETs occurs in the 7th decade of life.[6-7] There is a rise over time in incidence of NETs.[5-6, 8] Although this may partly reflect an increased use of imaging and improved techniques in diagnostic modalities, a real increase in incidence seems likely. Also increased awareness of NETs by clinicians may explain part of the rise in incidence.

In an American epidemiological study, comprising 13,715 patients with NETs, 66.9% of all NETs occurred in the gastrointestinal tract, and 24.5% in the tracheobronchopulmonary tract.[5] In the subanalysis for the most recent time period (i.e. 1992–1999), most NETs were located in the small bowel (41.8% of gastrointestinal NETs), followed by the rectum (27.4% of gastrointestinal NETs). Most NETs in the small bowel were located in the ileum (47.3% of small bowel NETs). Because this study used the, by that time common, term 'carcinoid' for their search, no patients with a functioning or nonfunctioning pancreatic NET were included.

Pancreatic NETs account for approximately 1.3–2% of all pancreatic cancers in incidence[9-10], whereas they represent almost 10% of pancreatic cancers in prevalence analyses[9], due to their slow-growing nature. Contrary to the previous observation, small bowel NETs are the most common small bowel cancer (incidence of 37.4% in a large series of 67,843 patients with small bowel neoplasms, compared with 36.9% of patients with an adenocarcinoma of the small bowel).[11] Nonfunctioning pancreatic NETs are more common than functioning pancreatic NETs, and occurred in 90.8% of 1,483 patients with a pancreatic NET in an American population-based study.[12] Of note, benign insulinomas were not included in this study.

DIAGNOSIS

Clinical Aspects

The presenting clinical aspects of NET patients can be divided into specific hormone-related symptoms and non-specific tumor-related symptoms. NETs may also be found by coincidence, during diagnostic procedures for other conditions. The specific hormone-related symptoms occur in so-called functioning NETs. Functioning NETs are defined by the presence of a clinical syndrome caused by hormonal hypersecretion, such as hypoglycemia in insulinoma, peptic ulcer disease in gastrinoma, and the Verner-Morrison syndrome (see table 1) in VIPoma. Non-specific tumor-related symptoms are for example pain, weight loss, or anorexia. NETs can either be sporadic, or occur in the setting of a genetic syndrome, e.g. the Multiple Endocrine Neoplasia Type-1 (MEN-1) syndrome (reviewed in[15]), von Hippel-Lindau disease[16], and neurofibromatosis type 1.[17] The various clinical classifications of NET patients are presented in table 1.

Because functioning NETs give specific symptoms early in the disease course, they are mostly diagnosed much earlier than nonfunctioning NETs, which only give symptoms due to mass effects (e.g. pain, jaundice). The carcinoid syndrome, which consists of secretory diarrhoea, flushing, wheezing, and right-sided valvular heart disease, is caused by serotonin production. Serotonin production also may lead to mesenteric fibrosis, which in turn can cause small bowel ischemia. Because serotonin is inactivated in the liver, the carcinoid syndrome occurs only in case of hepatic metastases, or in case of retroperitoneal tumor depositions which have their drainage through the caval vein instead of the portal vein, or in NETs which originate in the testis/ovary, which also have (part of) their drainage through the caval vein.

Diagnostic Procedures

Diagnostic procedures for NETs encompass laboratory tests, imaging procedures, and pathological diagnosis, amongst others.

Laboratory tests used in monitoring of therapy and follow-up in NETs include the measurement of various tumor markers (chromogranin A (CgA) and neuron-specific enolase (NSE)), specific hormones in case of functioning NETs, and more general blood tests, such as liver function tests in the case of hepatic metastases.

CgA is a glycoprotein, which is present in the secretory granules of neuroendocrine cells. It has a sensitivity of 53–85% and a specificity of 84–98% in the detection of NETs (reviewed in[18]). It is important to realize that CgA levels can also be elevated due to proton pump inhibitor use, and in patients with chronic kidney failure or chronic gastritis, amongst others.[18-19] CgA can also be used for prognostic purposes in NET patients. In a study, evaluating prognostic factors for survival in midgut NET patients, patients with plasma CgA levels >5,000 µg/l had a significantly shorter median overall survival (OS) than patients with plasma CgA levels <5,000 µg/l (33 versus 57 months, $p<0.001$).[20] NSE, which is present

Table 1. Clinical symptoms of neuroendocrine tumors (Partly adapted from[13-14]).

Tumor	Clinical symptoms
General	General tumor-related symptoms, e.g. pain, weight loss, anorexia
Specific	
Bronchial NET	Cough, hemoptysis, postobstructive pneumonia
	Cushing's syndrome based on ectopic adrenocorticotropic hormone (ACTH) production
	Rarely carcinoid syndrome
	Incidental funding on conventional radiography or CT scan
Gastric NET	Hematemesis, hypergastrinemia
	Atypical carcinoid syndrome (mainly flushing (presumably histamine-mediated))
	Incidental finding on upper gastrointestinal endoscopy
Pancreatic NET	
Nonfunctioning	Mass-related effects, e.g. abdominal pain, jaundice, anorexia, nausea, vomiting, back pain, weight loss
	Incidental finding on abdominal ultrasonography/CT scan/MRI scan
Functioning	
Gastrinoma	Zollinger-Ellison syndrome; peptic ulcer; diarrhoea
Insulinoma	Fasting hypoglycemia; Whipple triad (low blood glucose level, symptoms of hypoglycemia at the time of the low glucose level, symptom relief with treatment of hypoglycemia)
VIPoma	Verner-Morrison syndrome (watery diarrhoea, hypokalemia, achlorhydria)
Glucagonoma	Diarrhoea, cachexia, diabetes mellitus, necrolytic migratory erythema
Somatostatinoma	Gall stones, diabetes, steatorrhea, achlorhydria
Duodenal NET	Pain, jaundice, nausea, vomiting, gastrointestinal bleeding, duodenal obstruction
	Rare: Zollinger-Ellison syndrome
	Incidental finding on upper gastrointestinal endoscopy
Ileo-Jejunal NET	Small bowel obstruction, gastrointestinal bleeding, abdominal pain, mesenteric ischemia (caused by mesenteric fibrosis)
	Carcinoid syndrome (i.e. secretory diarrhoea/flushing/wheezing/right-sided valvular heart disease, based on serotonin production) in case of metastatic disease (liver metastases)
Appendiceal NET	Incidental finding during appendectomy
Colon NET	Incidental finding on colonoscopy
Rectum NET	Rectal bleeding, constipation, pain
	Incidental finding on endoscopy
Unknown Primary	Incidental finding of metastases (often liver metastases) on imaging (abdominal ultrasonography/CT scan/MRI scan)

in the cytoplasmic compartment of cells, is also a potential NET serum biomarker.[21] It has a sensitivity of 32.9% and a specificity of 100% in NET patients.[21] It has to be said that the number of patients who were considered tumor-free, and hence were used for specificity calculations, was low (i.e. 21 patients), partly reducing the validity of the specificity calculations. The number of patients with disease, used for the sensitivity calculations, was 106 patients. Elevated NSE levels are associated with poor tumor differentiation[22], and therefore NSE measurement will have a greater role in G3 tumors than in G1-G2 tumors (see Grading and Staging for grade assignment). An advantage of CgA and NSE measurement, is that they can be used in both functioning and nonfunctioning NETs. For the diagnosis of functioning NETs, specific hormones can be assessed as tumor marker, e.g. insulin for insulinoma, gastrin for gastrinoma, vasoactive intestinal peptide for VIPoma, and glucagon for glucagonoma. Measurement of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in 24-hour urine collection can also be used for the diagnosis of NETs. However, this can only be done in patients with hepatic metastatic midgut NETs, or patients with retroperitoneal tumor deposits, or NETs with a primary tumor in the testis/ovary, since in these patients hepatic breakdown of serotonin is absent, as explained above. For the analysis of 5-HIAA, patients preferably need to be on a diet free of tryptophan/serotonin-rich foods (bananas, avocados, plums, eggplant, tomatoes, pineapples, and walnuts) to avoid false elevations in urinary 5-HIAA. One study reported the sensitivity of 5HIAA for various NETs (also including non-midgut NETs) to be 35.1%, whereas the specificity was 100%.[21]

Imaging of NETs can be divided into anatomical and functional imaging. Anatomical imaging of NETs can be performed with conventional radiography, ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and angiography.[23-26] The advantage of these imaging techniques is that they give information on anatomical localization of the tumor, tumor size, and tumor boundaries with surrounding tissue. A limitation of these techniques is that they usually only scan a part of the body. Another disadvantage is that lymph nodes which are considered non-pathological on a CT scan (i.e. lymph nodes <10 mm), are not by definition tumor-free. Furthermore, to improve the detection rate of neuroendocrine liver metastases on a CT scan, a so-called three-phasic CT examination is required.[26] This involves examination before (nonenhanced, native) and during intravenous contrast enhancement in the arterial phase and in the venous phase. Functional imaging, like somatostatin receptor scintigraphy using [^{111}Indium-DTPA^{0}]octreotide (SRS)[27] and positron emission tomography-computed tomography (PET-CT) with for example ^{68}Ga-DOTA-Tyr^{3}-octreotide[28] or ^{68}Ga-DOTA-Tyr^{3}-octreotate[29] can screen the total body and provide information about the presence of stts on the tumor. Other functional imaging techniques for the detection of NETs, not based on sst receptor targeting, include PET imaging with 6-^{18}F-fluoro-L-DOPA[30-31] or ^{11}C-5-hydroxytryptophan.[31] ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET imaging, reflecting glucose metabolism, may be of value in NETs with a high proliferation index. Binderup et al. compared [^{111}Indium-DTPA^{0}]octreotide scintigraphy (SRS), ^{18}F-FDG PET

scan, and ^{123}I-metaiodobenzylguanidine (MIBG), in 96 NET patients.[32] Although the overall sensitivity of SRS was higher than that of the ^{18}F-FDG PET scan (sensitivity of 89% versus 58%), ^{18}F-FDG PET scan showed a higher sensitivity than SRS in NETs with a Ki67 index above 15% (sensitivity of 92% versus 69%, respectively). Furthermore, in patients with a negative SRS (this occurred in 11/96 patients), ^{18}F-FDG PET scan was positive in 7 patients (of note, the Ki67 index was very variable in these 7 patients and ranged between 2 and 95%). The authors concluded that SRS should be the method of choice, and that ^{18}F-FDG PET scan provides complementary diagnostic information, and can be used in patients with a high Ki67 index, or with a negative SRS. The same group showed that a positive ^{18}F-FDG PET scan in NET patients was associated with significantly lower OS and progression-free survival (PFS) compared with a negative ^{18}F-FDG PET scan.[33]

Endoscopy of the gastrointestinal tract, e.g. upper gastrointestinal endoscopy or colonoscopy, may also be used in the diagnosis of NETs. These endoscopies can be performed in the search for a primary tumor in the setting of metastastic disease. With the increased use of endoscopy of the gastrointestinal tract for other reasons, e.g. pyrosis, hematemesis, or rectal bleeding, NETs more often are found as an incidental finding during these endoscopies. For the diagnosis of pancreatic NETs, often endoscopic ultrasonography is used to assess tumor localization, tumor relation with surrounding structures, and the presence of pathological lymph nodes.[34]

The definitive diagnosis of a NET is pathology-based. Therefore, a biopsy of the primary tumor/metastasis should always be performed, when feasible. Fine-needle aspiration or core biopsy of a liver metastasis, or fine-needle aspiration of a pancreatic tumor during endoscopic ultrasonography, is often performed for this purpose. Next to hematoxylin and eosin (HE) staining, immunostaining for chromogranin A and synaptophysin, and the assessment of the mitotic index using a mitotic count, and the Ki67 proliferative index (see Staging and Grading) should be performed.[35-37] Immunohistochemical staining for sst_2 is optional.[35, 37] Furthermore, immunostaining for insulin, gastrin, glucagon, or vasoactive intestinal peptide may help to find the primary tumor (in case of carcinoma of unknown primary), and may provide verification of hormonal production in functioning NETs[36]; however, it is not diagnostic for a functional NET syndrome.

STAGING AND GRADING

Traditionally, NETs were subdivided into foregut (lung, stomach, pancreas, biliary system, and duodenum), midgut (jejunum, ileum, appendix, coecum, and right colon), and hindgut (left colon and rectum) tumors.[38] NETs were formerly either called (bronchial) carcinoids or islet-cell tumors. In 2000, the WHO classification abandoned the term 'carcinoid', and introduced the general terms 'neuroendocrine tumor' and 'neuroendocrine carcinoma'.[39] However, in the medical literature the term carcinoid is still being used afterwards.

Nowadays, the staging and grading of NETs is done according to the WHO 2010 classification[40], incorporating a TNM (tumor, node, metastasis) staging system, and a grading system, based on mitotic count or proliferative activity (Ki67). The differences between the WHO 1980, WHO 2000, and WHO 2010 classifications are presented in table 2. The TNM staging of NETs of the gastroenteropancreatic system is site-specific. The grading system, incorporated in the WHO 2010 classification, was initially proposed by the European Neuroendocrine Tumor Society (ENETS)[41-42], and divides tumors according to mitotic count or Ki67, into G1 (mitotic count <2 per 10 high-power fields (HPF) and/or Ki67: 0–2%), G2 (mitotic count 2–20 per 10 HPF and/or Ki67 >2–20%), and G3 (mitotic count >20 per 10 HPF and/or Ki67 >20%) tumors. All G3 tumors are by definition neuroendocrine carcinomas (NECs). This grading system has been validated in patients with pancreatic NETs.[43-44] Also in midgut NETs, Ki67 is an important prognostic factor for survival.[45-46] The range of Ki67 of 3–20% for G2 tumors may be too broad, and it is suggested that a cut-off value of 5 instead of 2% may be more accurate in stratifying patients with pancreatic NETs into different prognostic groups.[43, 47]

Table 2.

WHO 1980	WHO 2000	WHO 2010
I Carcinoid	1. Well-differentiated endocrine tumor (WDET)	1. NET G1 (carcinoid)
	2. Well-differentiated endocrine carcinoma (WDEC)	2. NET G2
	3. Poorly differentiated endocrine carcinoma/ small cell carcinoma (PDEC)	3. NEC (large cell or small cell type)
II Mucocarcinoid III Mixed forms carcinoid-adenocarcinoma	4. Mixed exocrine-endocrine carcinoma (MEEC)	4. Mixed adenoneuroendocrine carcinoma (MANEC)
IV Pseudotumor lesions	5. Tumor-like lesions (TLL)	5. Hyperplastic and preneoplastic lesions

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In 2010, the American Joint Committee on Cancer (AJCC) manual (seventh edition) introduced a TNM staging classification for pancreatic NETs[48], which is derived from the staging system for exocrine pancreatic adenocarcinomas. This staging system is incorporated in the WHO 2010 classification. Another broadly used staging system for pancreatic NETs is the ENETS staging classification.[41] The AJCC staging classification differs from the ENETS classification in several ways. First, it recommends tumor grade to be recorded, but does not include specific guidelines for tumor grade assignment. Second, the T2–T4 definitions are different for both classification systems, and consequently also the stages I–III. In a study by Strosberg et al., including 425 patients with functioning and nonfunctioning pancreatic NETs, AJCC

staging, as well as ENETS staging, was strongly correlated with OS.[49] By contrast, in a European study, including 1,072 patients with functioning and nonfunctioning pancreatic NETs, better prediction of survival was achieved with the ENETS staging system than with the AJCC staging system.[44] The differences in staging systems make comparisons between studies using the different systems difficult.

TREATMENT

Surgery

The only potential to cure patients with NETs is surgery. However, surgery is often not possible due to widespread disease.

Even in patients with liver metastases, surgery may play a role. Kleine et al. described 41 patients with a pancreatic NET who all had pancreatic surgery with curative intent.[50] Thirteen patients had extended surgery (partial liver resection/portal vein resection/ partial gastric resection/liver transplantation), next to their pancreatic surgery. Patients who underwent extended resection had similar disease-specific survival compared to patients with pancreatic resection alone. Surgical complications were more common in the extended resection patient group; however, postoperative mortality rate and length of hospital stay were equal for both groups. Furthermore, patients who had a liver resection had similar disease-specific survival compared to patients without liver metastases. These data show that extended surgery for pancreatic NETs is feasible in highly selected patients. Norton et al. described 13 carcinoid patients and 5 gastrinoma patients, who all had surgery for liver metastases, in some patients combined with resection of their primary tumors.[51] There were no operative deaths. The 5-year survival rate was 82%. These data show that surgery of hepatic metastases of NETs is feasible, and, although this was a retrospective, nonrandomized study, the encouraging 5-year survival rate suggests that major surgery may extend survival. The same group reported results of extended (hepatic) surgery in 20 patients with pancreatic or duodenal NETs.[52] There was no operative mortality. Six patients (30%) had postoperative complications, such as abscess and pancreatic fistula. The 5-year survival rate was 80%.

Surgery can also be performed for symptom control. In a study by Sarmiento et al., hepatic resection was associated with a partial or complete response with respect to hormonal symptoms in 104/108 gastroenteropancreatic NET patients (96%).[53] The median time to symptom recurrence was 46 months.

Resection of the primary tumor is associated with increased OS in patients with functioning and nonfunctioning pancreatic NETs.[54-55] However, because these data are all based on retrospective analyses, selection bias may play a role. It is likely that more patients in a better clinical condition (in itself leading to better survival) are present in the operated patient group.

Chemotherapy

The role of chemotherapy in patients with well differentiated NETs of non-pancreatic origin is limited. In patients with pancreatic NETs, various chemotherapy regimens have been applied.

Streptozocin in combination with doxorubicin resulted in an objective tumor response in 69% of 36 patients with a pancreatic NET.[56] Median time to tumor progression was 20 months, and median survival was 2.2 years. Side effects consisted of nausea, vomiting, alopecia, leukopenia, heart failure, and chronic renal insufficiency.

Another study showed the objective response rate after treatment with fluorouracil, doxorubicin, and streptozocin to be 39% in 84 patients with a pancreatic NET.[57] The median response duration was 9 months. The 2-year PFS rate was 41%, and the 2-year OS rate was 74%. Grade 3 or 4 toxicity occurred in 19/84 patients (23%), and included nausea, vomiting, myelosuppression, fatigue, and alopecia.

Moertel et al. reported on the results of combination therapy of cisplatin and etoposide.[58] An objective response occurred in 2/27 patients (7%) with well differentiated NETs. In patients who were classified as having anaplastic neuroendocrine carcinomas, an objective response occurred in 12/18 patients (67%). Toxicity was a major problem; hematologic toxicity was universal, and two thirds of patients had renal toxicity.

In a more recent study, including NET patients (with foregut, midgut, or pancreatic origin) with poorly differentiated NETs or with a rapidly progressing clinical course, 18/33 patients (55%) had a radiological and/or biochemical response.[59] The median duration of the response was 9 months. Neutropenia grade 3 or 4 occurred in 23/36 patients (64%), and nephrotoxicity grade 1 or 2 occurred in 19/36 patients (53%).

Strosberg et al. reported on the treatment effects of the chemotherapeutic agents capecitabine and temozolomide in patients with metastatic pancreatic NETs.[60] A partial response occurred in an encouraging rate of 21/30 patients (70%). Median PFS was 18 months. The 2-year survival rate was 92%. A grade 3 or 4 adverse event occurred in 4/30 patients (13%).

Hentic et al. published their results on the combination of 5-fluorouracil, leucovorin, and irinotecan (FOLFIRI), in 19 patients with neuroendocrine carcinomas grade 3 according to the WHO 2010 classification in whom treatment with cisplatin and etoposide was discontinued due to progression or severe neurotoxicity.[61] An objective response occurred in 6/19 patients (32%). Median PFS was 4 months, and median OS was 18 months. Six out of 19 patients (32%) had a grade 3 or 4 toxicity (3 neutropenia, 3 diarrhoea).

The combination of temozolomide and thalidomide was studied in 29 patients with metastatic carcinoid, pheochromocytoma, or pancreatic NETs.[62] An objective response occurred in 7/28 assessable patients (25%) (45% in pancreatic NET patients, and 7% in carcinoid tumor patients). The median duration of response was 14 months. The 2-year

survival rate was 61%. Grade 3 or 4 lymphopenia occurred in 20/29 patients (69%), and was associated with an opportunistic infection in 3 patients.

The combination of temozolomide and bevacizumab, a monoclocal antibody targeting vascular endothelial growth factor, has been investigated in a phase II study in 19 patients with a carcinoid tumor and in 15 patients with a pancreatic NET.[63] The studied combination showed antitumor activity in pancreatic NETs. Partial responses occurred in 5/15 patients (33%) with a pancreatic NET, and in 0/19 patients (0%) with a carcinoid tumor. Also, survival rates were different for both tumors. The median PFS was 14 months for patients with a pancreatic NET, and 7 months for carcinoid patients. The median OS was 42 months for patients with a pancreatic NET, and 19 months for carcinoid patients. Toxicities were mainly hematological, with grade 3 or 4 lymphopenia in 53% of patients, and grade 3 or 4 thrombocytopenia in 18% of patients.

Although some chemotherapy regimens show encouraging results with regard to objective tumor responses, its use is hampered by the mostly short duration of response, and significant toxicity.

Medical Therapy (Somatostatin Analogs and Interferon Alfa)

Treatment with somatostatin analogs such as octreotide and lanreotide can reduce hormonal overproduction and may result in symptomatic relief in most patients with metastasized disease.[64-66] Furthermore, Rinke et al. demonstrated that treatment with the long-acting somatostatin analog octreotide LAR (Sandostatin LAR; Novartis, Basel, Switzerland) significantly lengthens time to tumor progression when compared with placebo in patients with functionally active and inactive metastatic midgut NETs.[67]

Treatment with recombinant interferon alfa in patients with NETs resulted mainly in biochemical responses.[68] The combination of somatostatin analogs and interferon alfa was suggested for enhancing anti-tumor activity in some retrospective studies.[69-70] However, in a prospective, randomized clinical trial, no significant difference in OS was found in patients with midgut NETs treated with the combination of octreotide and interferon alfa compared with patients treated with octreotide alone.[71] Also, the time to tumor progression in patients with various NETs treated with the combination of lanreotide and interferon alfa, compared with patients treated with lanreotide or interferon alfa alone, was not significantly different.[68] Side effects of treatment with interferon alfa can be severe, and include flu-like symptoms, chronic fatigue syndrome, mental depression, the development of anti-nuclear, thyroid antibodies, and thyroid dysfunction.[72]

The percentage of objective tumor responses after treatment with somatostatin analogs or interferon alfa is low. In a prospective study by Rinke et al., a partial response was observed in one of 42 patients (2%) with midgut NETs treated with octreotide LAR.[67] No complete responses were observed. In a prospective study by Faiss et al., a partial response was observed in one of 25 patients (4%) treated with lanreotide, one of 27 patients (4%) treated

with interferon alfa, and two of 28 patients (7%) treated with the combination of lanreotide and interferon alfa.[68] No complete responses occurred in this study.

Radiotherapy

External beam radiotherapy has a limited place in the treatment of NETs. Radiotherapy can be performed in case of brain metastases, spinal cord compression due to bone metastases, or painful bone metastases. Furthermore, radiotherapy may be of value in localized bronchial NETs.[73] Another application of radiotherapy may be its use in combination with chemotherapy (i.e. chemoradiation), which has been performed in a few patients with locally advanced pancreatic NETs.[74]

Molecular Targeted Agents

Recently, the results of large phase III trials on new targeted therapies for the treatment of NETs have been presented. Treatment with sunitinib (Sutent; Pfizer Inc, New York, NY), a tyrosine kinase inhibitor, resulted in a longer median progression-free survival (PFS) than placebo (11.4 versus 5.5 months) in patients with pancreatic NETs.[75] Also, treatment with everolimus (Afinitor; Novartis Pharmaceuticals, Basel, Switzerland), an inhibitor of mammalian target of rapamycin (mTOR), resulted in a longer median PFS than placebo (11.0 versus 4.6 months) in patients with pancreatic NETs.[76] Of note, sunitinib and everolimus are now both registered therapies for the treatment of patients with pancreatic NETs in the Netherlands. Also in patients with advanced NETs, with radiological documented disease progression within the past 12 months, and a history of secretory symptoms (diarrhoea or flushing), treatment with everolimus and octreotide LAR 30 mg every 28 days resulted in a longer median PFS than placebo and octreotide LAR 30 mg every 28 days (16.4 versus 11.3 months).[77] Moreover, everolimus and octreotide LAR resulted in greater reductions in serum CgA and urinary 5–HIAA levels compared with placebo and octreotide LAR.[77]

However, objective tumor responses with these new targeted therapies are rare and were reported in 8/86 patients (9%) with pancreatic NETs treated with sunitinib[75], in 10/207 patients (5%) with pancreatic NETs treated with everolimus[76], and in 5/216 patients (2%) with advanced NETs treated with everolimus.[77]

Liver-Directed Therapies

In case of predominant liver disease, liver-directed therapies, such as embolization, chemo-embolization, radioembolization, radiofrequency ablation (RFA), or liver transplantation may be an option to reduce the tumor mass. These therapies may also be used if there are otherwise uncontrolled hormonal symptoms due to hormone-producing liver metastases.

The rationale of performing embolization, chemobolization, and radioembolization of liver metastases is based upon the fact that liver metastases derive their blood supply almost

entirely from the hepatic artery, as opposed to the normal liver parenchyma, which mainly depends on the portal vein.

Gupta et al. reported their experience of hepatic arterial (chemo)embolization in 81 NET patients.[78] Fifty patients were treated with bland hepatic arterial embolization; 31 patients with hepatic arterial chemoembolization (mostly containing microencapsulated cisplatin). Response rates were reported for 69 patients who had baseline and follow-up imaging available. A partial response occurred in 46/69 patients (67%), a minor response in 6/69 patients (9%), and stable disease in 11/69 patients (16%). These numbers are very encouraging. However, it is not reported why the patients who were excluded did not have baseline and follow-up imaging available. In the most extreme situation, it could be that they all died while on-study. The median PFS was 19 months, and the median OS was 31 months. Serious complications, such as hepatorenal syndrome and sepsis, occurred in 11% of the procedures.

Recently, the results of a prospective, randomized trial, comparing hepatic arterial embolization and hepatic arterial chemoembolization (containing doxorubicin) in patients with midgut NETs, were published.[79] There were no significant differences in the 2-year PFS rate (38% for hepatic arterial chemoembolization versus 44% for hepatic arterial embolization, p=0.90), which was the primary end point of the study. Grade 3 toxicity occurred in 3 patients in the hepatic arterial chemoembolization group (2 neutropenia, 1 acute liver failure), and in 2 patients in the hepatic arterial embolization group (2 acute liver failure). The incidence of total adverse events was similar in both groups (p=0.30). Although this was a small study (including 26 patients), it is the only study to date comparing embolization with chemoembolization in a prospective, randomized manner. Based on these data, there seems to be no additive antitumoral effect of chemotherapy combined with embolization.

Radioembolization of neuroendocrine liver metastases is being performed with ^{90}Yttrium (^{90}Y) microspheres, consisting of embolic microparticles of glass or resin impregnated with the isotope ^{90}Y. ^{90}Y microspheres are being delivered directly in the hepatic artery through a catheter. The results of 12 studies on ^{90}Y radioembolization in patients with neuroendocrine liver metastases are reviewed in[80]. The rates of an objective response ranged between 13% and 100% (median 63%). The postembolization syndrome, comprising of nausea, vomiting, fever, and abdominal pain, was common. Other complications included cirrhosis, hepatic failure, portal vein thrombosis, jaundice, radiation gastritis, and duodenal ulceration. Grade 3 or higher toxicities occurred in 0–13% of patients.

RFA is based on the cytotoxic effects of non-physiologic temperature that is locally administered with probes placed in the liver, and can be performed either percutaneously or intraoperatively. Indications for RFA are <5 liver lesions and a tumor size <5 cm.[81] RFA is often associated with relief of symptoms (reviewed in[81] and in[82]). RFA can be used in adjunct to hepatic resections, or in the case of liver metastases at locations in the liver not

amenable to surgery. Recurrences of liver metastases are a major problem after RFA and new liver lesions can occur in up to 63% of patients.[83] Other local ablative techniques for neuroendocrine liver metastases include alcohol ablation, or intraoperative cryotherapy.[82]
Because liver metastases of NETs often exhibit a slow-growing character, orthotopic liver transplantation may be an option for patients with liver-only disease. Patients can be considered for orthotopic liver transplantation when they have hormonal symptoms refractory to surgical or medical therapy, uncontrolled complaints due to tumor load (hepatomegaly), or when curation can be achieved with orthotopic liver transplantation. However, the scarcity of donor organs, and the high recurrence rate after orthotopic liver transplantation, makes this approach not standard in neuroendocrine liver metastases.
In a large, multicenter, French study, the results of 85 NET patients undergoing liver transplantation were reported.[84] The primary tumor was located in the pancreas/duodenum in 40 patients, in the digestive tract in 26 patients, in the bronchial tract in 5 patients, and in 14 patients the primary tumor was undetermined at the time of liver transplantation. Twelve patients (14%) died during the postoperative period (interval 2–157 days). OS at 5 years was 47%. Disease-free survival at 5 years was 20%. Prognostic factors associated with worse survival at multivariate analysis were upper abdominal exenteration (performed concomitantly with liver transplantation), a primary tumor in pancreas/duodenum, and hepatomegaly.
Gedaly et al.[85] published the results of liver transplantation in 150 patients with NETs (including 6 children), derived from an American database. Fifty-one patients had a carcinoid tumor, 6 patients an insulinoma, 3 patients a glucagonoma, 11 patients a gastrinoma, and 9 patients a VIPoma; 70 patients had an unspecified NET. Overall 5-year survival rate for patients undergoing isolated liver transplantation was 49%. Disease-free survival rate at 5 years was 32%. Data on surgical complications were not available from the database used.
The ENETS Guidelines for patients with liver metastases stated the following minimal requirements for consideration of liver transplantation: mortality <10%, absence of extrahepatic disease as determined by PET/CT (with ^{68}Ga-radiolabeled somatostatin analogs, or with ^{18}F-FDG), primary tumor removed prior to transplantation, and well-differentiated NET (NET G1, G2).[86]

Peptide Receptor Radionuclide Therapy

Peptide Receptor Radionuclide Therapy (PRRT) with radiolabeled somatostatin analogs is a promising novel treatment modality in patients with NETs, and is reviewed in **Chapter 2.**
In summary, PRRT is and has been performed with various compounds, such as [^{111}Indium-DTPA^{0}]octreotide (^{111}In-octreotide), [^{90}Y-DOTA^{0},Tyr^{3}]octreotide (^{90}Y-DOTATOC), and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-octreotate or ^{177}Lu-DOTATATE). Although treatment with ^{111}In-octreotide often resulted in symptom relief in patients with metastasized NETs, objective tumor responses were rare.[87-88] Complete and partial responses obtained after treatment

with ^{90}Y-DOTATOC are in the same range as after treatment with ^{177}Lu-octreotate (i.e. 10–30%).[89-93] Nephrotoxicity is the major side effect after treatment with ^{90}Y-DOTATOC[94-96], and severe permanent kidney toxicity may occur in up to 9% of patients treated with ^{90}Y-DOTATOC.[96] Severe late side effects after treatment with ^{177}Lu-octreotate, such as renal insufficiency and myelodysplastic syndrome, occur in less than 1% of patients.[93] The median time to progression is 29 months for ^{90}Y-DOTATOC[92] and 40 months for ^{177}Lu-octreotate.[93]

MEASURING TREATMENT EFFECT

Tumor Response

The tumor response assessment of NETs after treatment can be done through various criteria. The ones most commonly used are the Response Evaluation Criteria in Solid Tumors (RECIST) criteria[97] and the Southwest Oncology Group (SWOG) solid tumor response criteria.[98] The differences and specifications for these two response criteria are outlined in **Chapter 4**. In 2009, the revised RECIST criteria (version 1.1)[99] were published, incorporating, amongst others, criteria for the assessment of pathological lymph nodes, and reducing the maximum number of lesions to be measured from 10 lesions with the RECIST criteria to 5 lesions with the revised RECIST criteria. Tumor response after treatment can also be assessed through functional imaging. For this purpose, response criteria for functional imaging with ^{18}F-FDG PET scan after treatment have been developed in the so-called PET Response Criteria in Solid Tumors (PERCIST).[100] For NETs, tumor response through functional imaging may be performed with SRS; PET-CT with ^{68}Ga-DOTA-Tyr^3-octreotide, ^{68}Ga-DOTA-Tyr^3-octreotate, or 6-^{18}F-fluoro-L-DOPA.

Tumor Markers

CgA and NSE can be used in the response assessment after treatment. Elevated baseline CgA and NSE levels were associated with shorter PFS and OS compared with patients without elevated baseline levels in patients with pancreatic NETs treated with everolimus.[101] Furthermore, patients with an early CgA or NSE response (defined as a 30% or greater reduction from baseline or normalization at wk 4) had a longer PFS than patients without an early biomarker response.[101] In another study, an 80% or more reduction in CgA level following cytoreductive surgery for neuroendocrine hepatic metastases, was associated with symptom relief and stabilization of disease.[102] Also measurements of 5–HIAA in 24-hour urine collection can be used for evaluation of treatment response. However, this can only be done in patients with hepatic metastatic midgut NETs, or patients with retroperitoneal tumor deposits, or NETs with a primary tumor in the testis/ovary, since in these patients hypersecretion of serotonin is present.

Survival

Table 3 lists the different endpoints which can be used for clinical studies evaluating cancer patients. For NETs, PFS as endpoint has advantages over OS for various reasons.[103] First, patients with NETs often have a long survival after progression, due to the often slow-growing nature of NETs. Second, the use of OS as endpoint may be complicated by different treatment regimes after progression, or by a cross-over from placebo to the active medicine as defined in study protocols. Lastly, OS tends to require a larger sample size than PFS, because the time to reach an event is mostly longer. Disease-free survival is often used in surgical studies evaluating (curative) resections of NETs.

Table 3. Various endpoints used in clinical studies evaluating cancer treatments.

End Point	Definition
Progression-free Survival (PFS)	Time from first treatment until progression (radiological or clinical) or death from any cause
Overall Survival (OS)	Time from first treatment until death from any cause
Overall Survival (OS) From Diagnosis	Time from diagnosis until death from any cause
Disease-specific Survival	Time from first treatment until tumor-related death
Time to Progression (TTP)	Time from first treatment until progression (radiological or clinical) or death from any cause in patients who had a Complete Response/ Partial Response/Minor Response/Stable Disease as treatment outcome
Disease-free Survival	Time from (curative) intervention until relapse
…Year Survival Rate (e.g. 5-Year Survival Rate)	Percentage of patients who are alive … years after diagnosis/start treatment

Quality of Life

Quality of life is an important outcome of treatment, specifically in NET patients, because the majority of these patients have a good quality of life before treatment start.[104-105] However, hormone-related symptoms, such as diarrhoea and/or flushing in patients with serotonin-producing NETs, or symptoms of hypergastrinemia in patients with gastrinomas, can place a heavy burden on the patient's life. Reducing these symptoms can significantly improve the patient's quality of life. The most widely used questionnaire in cancer clinical trials to assess quality of life is the European Organization of Research and Therapy in Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30).[106] Specific quality of life questionnaires for NET patients have been developed.[107]

AIMS AND OUTLINE OF THIS THESIS

The aims of the studies presented in this thesis are to:

1. Evaluate tumor response after treatment with ^{177}Lu-octreotate regarding:
   a. differential response of bone versus soft-tissue lesions
   b. the application of different response criteria (i.e. the Response Evaluation Criteria in Solid Tumors (RECIST), the Southwest Oncology Group (SWOG) solid tumor response criteria, and their modified variants (mRECIST and mSWOG))
2. Evaluate the neoadjuvant application of ^{177}Lu-octreotate in patients with initially irresectable nonfuntioning pancreatic NETs
3. Evaluate the possible etiology of hypocalcemia after treatment with ^{177}Lu-octreotate
4. Evaluate the treatment effects of ^{177}Lu-octreotate in patients with metastasized insulinoma

Chapter 2 gives an overview on the current literature on peptide receptor radionuclide therapy in patients with gastroenteropancreatic neuroendocrine tumors. In **Chapter 3** the response of bone lesions is compared with the response of soft-tissue lesions after treatment with ^{177}Lu-octreotate, by measuring bone and soft tissue lesions on CT scans performed before and at different time points after treatment with ^{177}Lu-octreotate. In **Chapter 4** the application of different response criteria (i.e. the RECIST, SWOG, mRECIST, and mSWOG criteria) are being compared in a large number of patients with gastroenteropancreatic and thoracic NETs treated with ^{177}Lu-octreotate. The outcomes will be correlated with progression-free survival and overall survival. In **Chapter 5** the neoadjuvant use of ^{177}Lu-octreotate in a large group of patients with nonfunctioning pancreatic NETs is described. We aim to assess if successful surgery after ^{177}Lu-octreotate is associated with increased survival. **Chapter 6** describes a prospective study aiming to evaluate the potential mechanisms of the occurrence of hypocalcemia after treatment with ^{177}Lu-octreotate. In the same chapter, a second group of patients is described which is retrospectively analyzed, to assess the occurrence of hypocalcemia in a larger group. **Chapter 7** describes the anti-proliferative and the clinical effects of treatment with ^{177}Lu-octreotate in patients with metastasized insulinoma with severe complaints of hypoglycemia. **Chapter 8** and **9** provide a summary of the presented data in this thesis and a general discussion.

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Chapter 2

Treatment of Gastroenteropancreatic Neuroendocrine Tumors with Peptide Receptor Radionuclide Therapy

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ABSTRACT

The primary treatment of gastroenteropancreatic neuroendocrine tumors (GEPNETs) is surgery with curative intent or debulking of the tumor mass. In case of metastatic disease, cytoreductive options are limited. A relatively new therapeutic modality, peptide receptor radionuclide therapy (PRRT) with radiolabeled somatostatin analogs, is currently available in a number of mostly European centers. Complete and partial responses obtained after treatment with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide are in the same range as after treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (i.e. 10–30%). However, significant nephrotoxicity has been observed after treatment with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide. Options to improve PRRT may include combinations of radioactive labeled somatostatin analogs, intra-arterial administration, and the use of radiosensitizing drugs combined with PRRT. Other therapeutic applications of PRRT may include additional therapy cycles in patients with progressive disease after benefit from initial therapy, PRRT in adjuvant or neoadjuvant setting, or PRRT combined with new targeted therapies, such as sunitinib or everolimus. Randomized clinical trials comparing PRRT with other treatment modalities, or comparing various radioactive labeled somatostatin analogs should be undertaken to determine the best treatment options and treatment sequelae for patients with GEPNETs.

Key Words: gastroenteropancreatic neuroendocrine tumors; peptide receptor radionuclide therapy; somatostatin analogs; radiolabeled somatostatin analogs

INTRODUCTION

Gastroenteropancreatic neuroendocrine tumors (GEPNETs), which comprise functioning and non-functioning endocrine pancreatic tumors and carcinoids, are usually slow-growing and are often metastasized at diagnosis. Treatment with somatostatin analogs such as octreotide and lanreotide can reduce hormonal overproduction and may result in symptomatic relief in most patients with metastasized disease.[1-3] Furthermore, Rinke et al.[4] demonstrated that treatment with the longacting somatostatin analog octreotide LAR (Sandostatin LAR; Novartis, Basel, Switzerland) significantly lengthens time to tumor progression when compared with placebo in patients with functionally active and inactive metastatic midgut neuroendocrine tumors (NETs).

The majority of GEPNETs abundantly express somatostatin receptors (ssts), and these tumors can be visualized in patients using the radiolabeled somatostatin analog [^{111}Indium-DTPA^{0}]octreotide (^{111}In-octreotide; OctreoScan; Mallinckrodt, Petten, The Netherlands), or using newer radiotracers used in PET, e.g. [^{68}Ga-DOTA-Tyr^{3}]octreotide[5] or [^{68}Ga-DOTA-Tyr^{3}] octreotate.[6] A logical next step to this tumor visualization in vivo was to also try to treat these patients with radiolabeled somatostatin analogs.

CHELATORS AND PEPTIDES

Radiolabeled somatostatin analogs that are used both for diagnostic and therapeutic purposes consist of 3 parts: a cyclic octapeptide, a chelator and a radionuclide. The chelators commonly used are DTPA (diethylene triamine penta-acetic acid) and DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetic acid). The most used combinations of peptide-chelator complexes are [DOTA^{0},Tyr^{3}]octreotide (DOTATOC) and [DOTA^{0},Tyr^{3}] octreotate (DOTATATE). Other complexes include DTPA-octreotide and [DOTA^{0}-1-Nal^{3}] octreotide (DOTANOC). Figure 1 shows the chemical structures of DTPA-octreotide, DOTATOC, DOTATATE and DOTANOC. Changing the peptide or chelator can considerably affect the binding affinities for the five different ssts (sst_1–sst_5), as has been shown by Reubi et al.[7] The affinity profiles of various somatostatin analogs for the different ssts are shown in table 1. From this table it is also clear that the addition of a radiometal (i.e. yttrium or gallium) also affects the binding properties.

THERAPY STUDIES

Therapy Studies with ^{111}In-Octreotide

Because at that time somatostatin analogs labeled with beta-emitting radionuclides were not available for clinical use, early studies in the 1990s used high activities of the Auger electron emitting ^{111}In-octreotide for peptide receptor radionuclide therapy (PRRT). These treatments often resulted in symptom relief in patients with metastasized NETs, but

objective tumor responses were rare[8-9] (table 2). Toxicity was mainly hematological, most frequently thrombocytopenia, but also the development of myelodysplastic syndrome (MDS) or leukemia was observed. It is not surprising that CT-assessed tumor regression was observed only in rare cases, because ^{111}In-coupled peptides have a small particle range and therefore a short tissue penetration. Therefore, ^{111}In-coupled peptides are not ideal for PRRT.

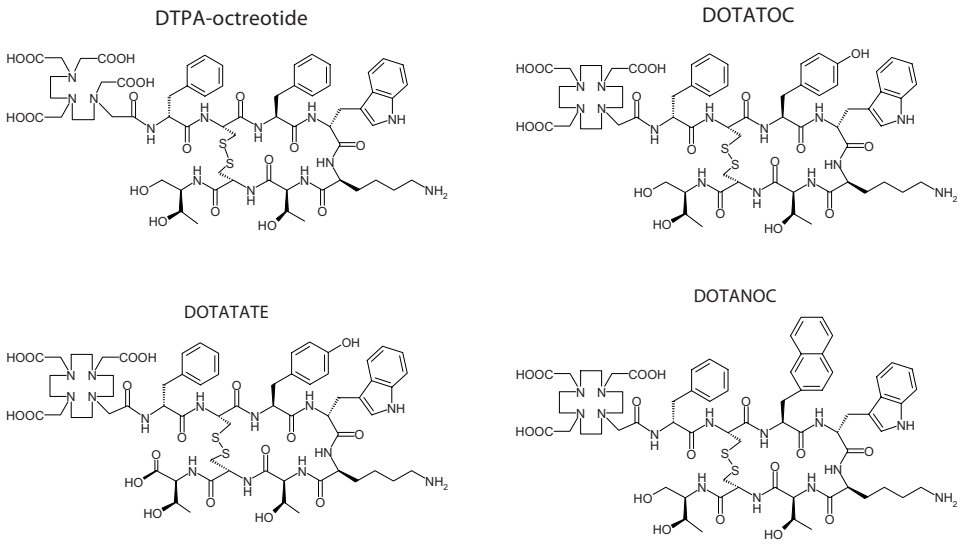

Figure 1. Structures of DTPA-octreotide, DOTATOC, DOTATATE and DOTANOC.

Therapy Studies with ^{90}Y-DOTATOC

The next generation of analogs used in PRRT consisted of a modified somatostatin analog, [Tyr^3]octreotide, with a higher affinity for the sst_2, and a different chelator, DOTA instead of DTPA, which allows stable binding of the β-emitting radionuclide ^{90}Yttrium (^{90}Y). ^{90}Y has a maximum energy of 2.27 MeV. Its maximal tissue penetration is 12 mm and its half-life is 2.7 days. ^{90}Y-DOTATOC (OctreoTher) was used in several phase I and phase II PRRT trials in various countries (table 2).

Chinol et al.[10] described dose-finding studies with ^{90}Y-DOTATOC. They observed no major acute reactions when administering doses of up to 5.6 GBq (150 mCi) per cycle. None of the patients developed acute or delayed kidney failure, although follow-up was short. Complete response (CR) and partial response (PR) were reported by this group in 28% of 87 patients with NETs.[11] A subsequent study by the same group[12] was a phase I study in 40 patients with somatostatin receptor positive tumors, including 21 patients with GEPNETs. Cumulative total treatment doses ranged from 5.9 to 11.1 GBq (160–300 mCi), given in two

treatment cycles. Six of 21 (29%) patients had tumor regression (table 2). Median duration of the response was 9 months.

Table 1. Affinity profiles (IC50, half maximal inhibitory concentration) for human sst_1–sst_5 receptors of a series of somatostatin analogs.

Peptide	sst_1	sst_2	sst_3	sst_4	sst_5
Somatostatin-28	5.2 (0.3)	2.7 (0.3)	7.7 (0.9)	5.6 (0.4)	4.0 (0.3)
Octreotide	>10,000	2.0 (0.7)	187 (55)	>1,000	22 (6)
DTPA-octreotide	>10,000	12 (2)	376 (84)	>1,000	299 (50)
In-DTPA-octreotide	>10,000	22 (3.6)	182 (13)	>1,000	237 (52)
In-DTPA-[Tyr^3]octreotate	>10,000	1.3 (0.2)	>10,000	433 (16)	>1,000
DOTA-[Tyr^3]octreotide	>10,000	14 (2.6)	880 (324)	>1,000	393 (84)
DOTA-[Tyr^3]octreotate	>10,000	1.5 (0.4)	>1,000	453 (176)	547 (160)
DOTA-lanreotide	>10,000	26 (3.4)	771 (229)	>10,000	73 (12)
Y-DOTA-[Tyr^3]octreotide	>10,000	11 (1.7)	389 (135)	>10,000	114 (29)
Y-DOTA-[Tyr^3]octreotate	>10,000	1.6 (0.4)	>1,000	523 (239)	187 (50)
Y-DOTA-lanreotide	>10,000	23 (5)	290 (105)	>10,000	16 (3.4)
Y-DOTA-vapreotide	>10,000	12 (2)	102 (25)	778 (225)	20 (2.3)
Ga-DOTA-[Tyr^3]octreotide	>10,000	2.5 (0.5)	613 (140)	>1,000	73 (21)
Ga-DOTA-[Tyr^3]octreotate	>10,000	0.2 (0.04)	>1,000	300 (140)	377 (18)

All values are IC50 (SEM) in nanometer. (Adapted from Reubi et al.[7]). No data are available for Lu-loaded somatostatin analogs.

Table 2. Tumor responses in GEPNET patients treated with different radiolabeled somatostatin analogs.

Ligand	Tumor Response						
	CR+PR %	Patient, n [ref.]	CR (%)	PR (%)	MR (%)	SD (%)	PD (%)
^{111}In-octreotide	0%	26[8]	0	0	2 (8)	15 (58)	9 (35)
^{111}In-octreotide	8%	26[9]	0	2 (8)	NA	21 (81)	3 (12)
^{90}Y-DOTATOC	29%	21[12]	0	6 (29)	NA	11 (52)	4 (19)
^{90}Y-DOTATOC	24%	74[17-18]	2 (3)	16 (22)	NA	49 (66)	7 (9)
^{90}Y-DOTATOC	9%	58[13a]	0	5 (9)	7 (12)	29 (50)	14 (24)
^{90}Y-DOTATOC	4%	90[14b]	0	4 (4)	NA	63 (70)	15 (17)[c]
^{90}Y-DOTATOC	23%	53[19]	2 (4)	10 (19)	NA	34 (64)	7 (13)[d]
^{177}Lu-DOTATATE	29%	310[23]	5 (2)	86 (28)	51 (16)	107 (35)	61 (20)

[a] Three patients had a tumor response of unknown.
[b] Eight patients had a tumor response of unknown.
[c] In contrast to the authors, we included another 4 patients to the PD group who died during the study.
[d] This study describes a subgroup of Danish GEPNET patients treated in Basel, Switzerland. In contrast to the authors, we included another patient to the PD group who died without CT follow-up.
GEPNET: gastroenteropancreatic neuroendocrine tumor; CR: complete response; PR: partial response; MR: minor response; SD: stable disease; PD: progressive disease; NA: not applicable.

In a multicenter trial[13] with ^{90}Y-DOTATOC, 58 GEPNET patients received escalating doses up to 14.8 GBq (400 mCi)/m^2 in 4 cycles or up to 9.3 GBq (250 mCi)/m^2 in a single dose, without reaching the maximum tolerated single dose. The cumulative radiation dose to the kidneys was limited to 27 Gy. Amino acids were given concomitantly with ^{90}Y-DOTATOC for renal protection. Three patients had dose-limiting toxicity: 1 had liver toxicity, 1 thrombocytopenia grade 4 (<25 × 10^9/l), and 1 MDS. Five out of 58 (9%) patients had PR, and 7 (12%) had a minor response (MR; 25–50% tumor volume reduction) (table 2). Median time to progression was 29 months.

Recently, the results of another multicenter trial were published.[14] Ninety patients with carcinoid tumors received a fixed dose of 3 × 4.4 GBq (3 × 120 mCi) ^{90}Y-DOTATOC. Four out of 90 patients had PR (table 2). Three patients had reversible grade 3 to 4 renal failure despite the coadministration of amino acids. Median progression-free survival (PFS) was 16 months and median overall survival (OS) was 27 months.

Very recently, the treatment effects of ^{90}Y-DOTATOC in a large group of patients with various NETs, treated in Basel, Switzerland, were published.[15] Results in a smaller number of patients were also reported earlier.[16-19] Patients were treated with 3.7 GBq (100 mCi)/m^2 ^{90}Y-DOTATOC. If patients had a clinical or biochemical response, or morphologic disease control (morphologic response or stable disease (SD)) after the first treatment cycle, additional cycles were given. Of 1,109 patients, 378 (34%) had a morphologic response. However, this was not RECIST or SWOG based. Fifty-eight (5%) patients had SD. Median survival from diagnosis was 95 months. Data on median PFS were not given. Morphologic, biochemical and clinical response, and high tumor uptake on somatostatin receptor scintigraphy were associated with longer median survival. Since morphologic, biochemical and/or clinical response were the criteria for treatment with additional cycles of ^{90}Y-DOTATOC, a dose-effect relation on longer survival cannot be ruled out. MDS and acute myeloid leukemia occurred in 1 patient each. One hundred and two (9%) patients had severe permanent renal toxicity [grade 4 (GFR 15–29 ml/min/1.73 m^2): 67 patients, and grade 5 (GFR <15 ml/min/1.73 m^2 or dialysis): 35 patients], although amino acids had been given for renal protection.

Therapy Studies with ^{177}Lu-DOTATATE

^{177}Lutetium (^{177}Lu) is a medium energy β-emitter, with a maximum energy of 0.5 MeV and a maximal tissue penetration of 2 mm. Its half-life is 6.7 days. ^{177}Lu also emits low-energy γ-rays at 208 and 113 keV with 10 and 6% abundance, respectively, allowing scintigraphy and subsequent dosimetry using the same therapeutic compound. The somatostatin analog [DTPA^0,Tyr^3]octreotate differs from [DTPA^0,Tyr^3]octreotide only in that the C-terminal threoninol is replaced with threonine. Reubi et al.[7] showed that octreotate had a higher affinity for sst_2 than octreotide (table 1). Also, in a study in patients, the uptake of radioactivity, expressed as percentage of the injected dose, was comparable for ^{177}Lu-DOTATATE to ^{111}In-octreotide for kidneys, spleen and liver, but was three- to four-fold

higher for ^{177}Lu-DOTATATE in four out of five tumors.[20] Also, Esser et al.[21] showed that the mean residence time of ^{177}Lu-DOTATATE was longer than that of ^{177}Lu-DOTATOC in tumors of GEPNET patients, who had both analogs injected sequentially, with a mean ratio of 2.1 in favor of ^{177}Lu-DOTATATE. Figure 2 shows the uptake of both radiopharmaceuticals in a patient. Comparing ^{177}Lu-DOTATATE with ^{177}Lu-DOTATOC, the mean residence time ratio for the kidneys was 1.4. Taking this into account, it was concluded that the mean administered dose to tumors would still be advantageous by a factor of 1.5, assuming that a fixed maximum kidney dose is reached when using ^{177}Lu-DOTATATE. Forrer et al.[22] demonstrated no difference in tumor uptake of ^{111}In-DOTATATE compared with ^{111}In-DOTATOC, whereas ^{111}In-DOTATOC showed a higher tumor-to-kidney absorbed dose ratio. In this study, a low amount of peptide (10 µg of peptide) was used. In contrast, Esser et al.[21] used 200 µg of peptide, which corresponds more to the clinical therapeutic setting. Furthermore, in contrast to Esser et al.[21], Forrer et al.[22] did not give concomitant amino acids, which affects the biodistribution of the compound.

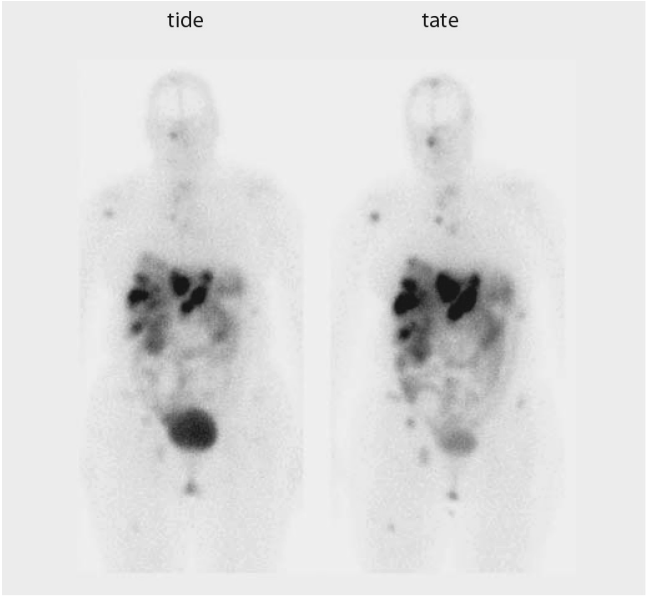

Figure 2. A typical example of a longer mean residence time of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate than that of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotide in the tumor of a patient with a GEPNET (mean residence time ratio of 2.3 in favor of octreotate for this example). Adapted from Esser et al.[21]

tide: [^{177}Lu-DOTA^{0},Tyr^{3}]octreotide; tate: [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

The treatment effects of ^{177}Lu-DOTATATE therapy were described in a large group of GEPNET patients.[23] Patients were treated up to an intended cumulative activity of 22.2–29.6 GBq (600–800 mCi). Serious delayed toxicities were observed in 9 out of 504 patients. There were 2 cases of renal insufficiency, both of which were probably unrelated to treatment with ^{177}Lu-DOTATATE. There were 3 patients with serious liver toxicity, 2 of which were probably treatment related. Lastly, MDS occurred in 4 patients, and was potentially treatment related in 3. In 6 patients with highly hormonally active NETs, a hormone-related crisis occurred after administration due to massive release of bioactive substances.[24] All patients recovered after adequate care. Tumor size was evaluated in 310 GEPNET patients. CR was found in 5 (2%) patients, PR in 86 (28%) and MR in 51 (16%) patients (table 2). Prognostic factors for predicting tumor remission (CR, PR, or MR) as treatment outcome were high uptake on the Octreoscan and Karnofsky Performance Score >70. Median time to progression was 40 months from start of treatment. Median OS was 46 months and median disease-related survival was >48 months. Figure 3 shows an example of a patient with an insulinoma with liver metastases treated with 29.6 GBq (800 mCi) ^{177}Lu-DOTATATE with a PR after therapy. Also, quality of life (QoL) improved after treatment with ^{177}Lu-DOTATATE.[25] Fifty GEPNET patients filled out the EORTC Quality of Life Questionnaire C30 before therapy and at follow-up visit 6 weeks after the last cycle. Global health status/QoL scale improved significantly after therapy with ^{177}Lu-DOTATATE. The patients reported a significant improvement in symptom scores for fatigue, insomnia, and pain. Improvement of QoL domains was most frequently observed in patients with proven tumor regression.

Other Radiolabeled Somatostatin Analogs

Cwikla et al.[26] described the results of treatment with ^{90}Y-DOTATATE in 60 GEPNET patients who received a mean cumulative dose of 11.2 GBq (300 mCi). PR was reported in 13 out of 57 (23%) patients. Three patients who died during therapy due to extensive, progressive cancer were not included in the analysis, resulting in seemingly better therapy results. Median PFS was 17 months and median OS was 22 months. After 24 months, 7 out of 23 evaluable patients had WHO grade 2 renal toxicity.

Lanreotide, another somatostatin analog, can be labeled with ^{111}In for diagnostic purposes and with ^{90}Y for therapeutic use. It has been advocated because of its better binding to sst_3/sst_4 than ^{111}In-octreotide[27], but this claim can be questioned.[7] Although this compound has been used to treat patients with GEPNETs, it shows poorer affinity than radiolabeled DOTATOC/DOTATATE for sst_2 (table 1), the receptor subtype that is predominantly overexpressed in GEPNETs.[28]

DOTANOC, in which the third amino acid of octreotide (phenylalanine) is replaced by (1-naphthyl)-alanine, has a good affinity for sst_2 but also for sst_3 and sst_5 as opposed to DOTATOC and DOTATATE.[29] This may be important for imaging and treatment of tumors with less pronounced expression of sst_2 and more pronounced expression of sst_3 and sst_5.

^{68}Ga-DOTANOC has been used for imaging purposes[30]; however, ^{177}Lu-DOTANOC showed higher mean absorbed whole-body dose than ^{177}Lu-DOTATATE[31], whereas tumor uptake was the same for the two compounds. Therefore, DOTANOC is currently not considered suitable for PRRT.

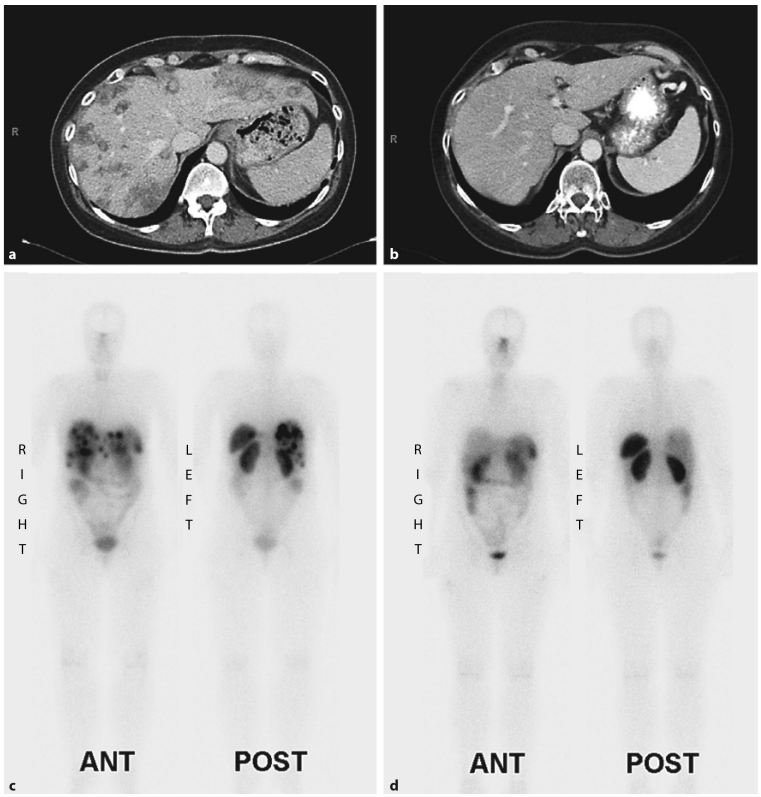

Figure 3. PR in a patient with an insulinoma with liver metastases treated with 29.6 GBq (800 mCi) ^{177}Lu-DOTATATE. **(A)** CT scan showing multiple liver metastases of an insulinoma before treatment with ^{177}Lu-DOTATATE. **(B)** CT scan 6 weeks after treatment with ^{177}Lu-DOTATATE, showing regression of liver metastases, consistent with a PR. **(C)** Post-therapy scan of the same patient after the first treatment with ^{177}Lu-DOTATATE, showing intense uptake in multiple liver metastases. **(D)** Post-therapy scan after the fourth (and last) treatment with ^{177}Lu-DOTATATE, showing reduced uptake in liver metastases.
ANT: anterior; POST: posterior.

Additional PRRT

Forrer et al.[32] reported their results of one additional cycle of 7.4 GBq (200 mCi) ^{177}Lu-DOTATOC in patients with disease progression after an initial benefit from ^{90}Y-DOTATOC treatment. ^{177}Lu-DOTATOC was chosen for additional treatment rather than ^{90}Y-DOTATOC because

further treatment with ^{90}Y-DOTATOC might cause renal failure. Two out of 27 (7%) patients had PR and 5 (19%) had MR. Mean time to progression was 8 months. At the time of analysis, the treatment seemed safe, but time of follow-up was rather short. However, the relatively low administered dose of ^{177}Lu-DOTATOC and the short time of follow-up make it hard to draw any firm conclusions from this study.

Van Essen et al.[33] reported on the effects of retreatment with two cycles of 7.4 GBq (200 mCi) ^{177}Lu-DOTATATE in 33 GEPNET patients with CT-assessed tumor progression before the start of retreatment. Twenty-eight of these had had a radiological response (at least MR) after the regular treatment with usually 4 cycles of ^{177}Lu-DOTATATE, and 5 had experienced a significant clinical improvement. In 8 (24%) patients, renewed tumor size reduction was observed, and 8 (24%) had SD at follow-up. Median time to progression was 17 months. No major side effects were observed during a median follow-up of 16 months. It was concluded that this salvage therapy is safe and can be effective in selected patients.

OPTIONS TO IMPROVE PRRT

Combination of Compounds

From experiments in rats[34], it became clear that ^{90}Y-labeled somatostatin analogs may be more effective for larger tumors, and ^{177}Lu-labeled somatostatin analogs may be more effective for smaller tumors, whilst their combination may be the most effective. The tumor in this rat model is rapidly growing, which may cause a more heterogeneous receptor distribution because of tumor necrosis associated with rapid tumor growth. In contrast, NETs in man in general have a homogeneous receptor distribution and grow slowly, hence making it difficult to extrapolate these findings directly to NETs in man. A preliminary report[35] on a study in patients stated that those patients treated with the combination of ^{90}Y-DOTATATE and ^{177}Lu-DOTATATE had a longer median time of survival than the patients treated with ^{90}Y-DOTATATE only. However, this was not a randomized trial comparing the two compounds, and group sizes were small (only 16 patients in each group), so the results may be influenced by an inclusion bias.

Seregni et al.[36] described a study protocol using a dual treatment with ^{90}Y-DOTATATE and ^{177}Lu-DOTATATE. Patients with NETs were treated with an intended dose of 5.55 GBq (150 mCi) ^{177}Lu-DOTATATE alternating with 2.59 GBq (70 mCi) ^{90}Y-DOTATATE for a total of four administrations. Tumor response was PR in 8 out of 13 (62%) GEPNET patients. However, 2 patients who had a deterioration of their health condition during treatment were excluded from analysis, resulting in a more favourable treatment outcome. Again, this was not a randomized trial. Furthermore, long-term follow-up data are not available yet.

Intra-Arterial Administration

Several groups have investigated the feasibility of locoregional, i.e. intra-arterial, administration of radiolabeled somatostatin analogs. Kratochwil et al.[37] found that the mean standardized uptake value of liver metastases and primary tumors in GEPNET patients was higher after intra-arterial infusion of ^{68}Ga-DOTATOC than after intravenous administration. A preliminary report[38] stated that the administration of ^{111}In-octreotide via the hepatic artery compared with locoregional administration resulted in a two-fold higher uptake in liver metastases in rats with sst_2-positive tumors, and in a more than three-fold higher uptake in liver metastases in patients with NETs. Limouris et al.[39] described the treatment results of intra-arterial administration of ^{111}In-octreotide in 16 patients with GEPNETs with liver metastases only. These patients received a mean cumulative dose of 58 GBq (1,570 mCi) ^{111}In-octreotide. The mean number of treatments per patient was 11. Tumor response was CR in 1 (6%) patient and PR in 8 (50%) patients. Median survival time for the patients with a CR, PR or SD as tumor response was 32 months. Mild hematological toxicity was observed in this study. Very recently, preliminary data have been presented on the therapeutic application of intra-arterial administration of the α-emitter ^{213}Bi-DOTATOC in 10 patients with NETs.[40] Long-term responses and toxicity are not available yet. The intra-arterial administration of PRRT looks promising for GEPNET patients with a predominant tumor load in the liver.

Radiosensitizing Drugs and PRRT

Using radiosensitizing chemotherapeutical agents [e.g. 5-fluorouracil (5-FU) or capecitabine] may be another way to improve PRRT. 5-FU was used in many of the numerous trials investigating the effects of (fractionated) external beam radiotherapy with chemotherapy. More recent trials used capecitabine, a prodrug of 5-FU, which has the advantage of oral administration. The enzyme thymidine phosphorylase (TP) is needed to convert the inactive form (capecitabine) into its active form (5-FU). Many tumors have a higher amount of TP and this results in a higher concentration of the active form in such tumors than in normal tissues. In addition, irradiation can induce an upregulation of TP.[41] With the combination of radiotherapy and capecitabine, an increased efficacy in terms of tumor growth control was reported if compared with radiotherapy as single treatment modality for a variety of tumors.[42] Also, ^{90}Y-labeled antibody radioimmunotherapy in combination with 5-FU as radiosensitizer was feasible and safe.[43] Therefore, after proving the safety of the combined therapy[44], we started a randomized multicenter trial comparing treatment with ^{177}Lu-DOTATATE with and without capecitabine (Xeloda; Roche, Basel, Switzerland) in patients with GEPNETs.

Recently, the results of a study using ^{177}Lu-DOTATATE in combination with capecitabine in 33 GEPNET patients[45] were published. The cumulative intended dose was 31.2 GBq (840 mCi), with 14 days of 1,650 mg/m^2 capecitabine per day per treatment cycle. Capecitabine had to be discontinued in 3 patients due to transient angina. Tumor response was PR in 8 out of 33

(24%) patients and SD in 23 out of 33 (70%) patients according to the revised RECIST version 1.1 guideline. However, the percentage of 70% SD may be too optimistic, since the authors defined an increase of >30% in target lesions as PD, but in the revised RECIST version 1.1 guideline PD is defined as an increase of >20% in target lesions.[46] This could mean that responses now classified as SD should actually be classified as PD. Furthermore, it should be emphasized that this was not a randomized trial comparing treatment with ^{177}Lu-DOTATATE with and without capecitabine.

LIMITATIONS OF PRRT

The kidneys are the dose-limiting organ for PRRT, especially when ^{90}Y is used. Valkema et al.[47] estimated a median decline in creatinine clearance of 7.3% per year in patients treated with ^{90}Y-DOTATOC and of 3.8% per year in patients treated with ^{177}Lu-DOTATATE. All patients received concomitant amino acids for kidney protection. The cumulative renal dose was higher in patients treated with ^{90}Y-DOTATOC than in patients treated with ^{177}Lu-DOTATATE (26.9 vs. 19.8 Gy). Age, hypertension and diabetes were probable contributing factors to a decline of creatinine clearance after PRRT. Another study[48] confirmed hypertension, diabetes, age, and in addition renal morphological abnormalities, to be risk factors for creatinine clearance loss after treatment with ^{90}Y-DOTATOC. These authors suggested that patients with these risk factors should not receive a renal bio-effective dose higher than 28 Gy, whereas patients without risk factors could receive a renal bio-effective dose up to 40 Gy. In a recently published study by Imhof et al.[15], evaluating 1,109 patients treated with ^{90}Y-DOTATOC, 9% of patients developed severe permanent renal toxicity after treatment with ^{90}Y-DOTATOC. The authors stated that 1 liter of 0.9% NaCl, containing 20 g of lysine and 21 g of arginine, had been given for renal protection. However, it is not clear if all study patients had received this combination. In earlier studies from the same group[16-18], it is stated that patients had received Hartmann-Hepa 8% amino acid solution, mostly 2 liters, containing approximately 10 g of lysine and 16 g of arginine. One has to assume that these patients were also analyzed by Imhof et al. Since it is known that higher amounts of lysine lead to a greater reduction in renal uptake of radioactivity[49], it would be of interest to know whether the patients who had received the Hartmann-Hepa solution had a higher incidence of severe renal toxicity than the patients who had received the solution containing 20 g of lysine and 21 g of arginine. Similarly, other authors have reported on renal toxicity after treatment with ^{90}Y-DOTATOC. Bodei et al.[48] described WHO grade 1–3 creatinine toxicity in 9 out of 23 patients who were selected for dosimetric studies out of a group of 211 patients. Four of these 9 patients had not received any amino acids, and almost all patients had risk factors for kidney disease as described above. In a multicenter trial[13], end-stage renal disease occurred in 2 out of 58 (3%) patients. All patients received amino acids (2 liters, containing 11 g of lysine and 16 g of arginine). In another multicenter trial[14], transient grade 3/4 renal

toxicity was described in 3 out of 90 (3%) patients. Again, all patients received amino acids (2 liters, containing 28 g of lysine and 28 g of arginine). However, follow-up time was short in this study.

Amino acid solutions used for renal protection during PRRT should contain sufficient lysine. However, it is important to know that high amounts of lysine are associated with hyperkalemia, which occurred at amounts of 75 g of lysine in a study by Rolleman et al.[49] Gelofusine, a succinylated gelatine, reduced the uptake of radiolabeled octreotide as effectively as did lysine in an animal study[50], whereas it caused no side effects in humans.[51] Furthermore, the addition of gelofusine to lysine decreased the renal uptake further in animal studies.[52-53] Other modalities for renal protection during PRRT include albumin fragments, amifostine (a radioprotective drug), angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers (for a review, see Rolleman et al.[54]). Lastly, fractionation of treatment cycles (i.e. giving the same cumulative dose in a higher number of cycles, thus reducing the radiation dose per cycle) may result in a reduced effective dose on kidneys and hence may lower the nephrotoxicity.[55]

The bone marrow is the dose-limiting organ for ^{177}Lu-DOTATATE. The cumulative excreted activity in the urine was higher for ^{177}Lu-DOTATOC than for ^{177}Lu-DOTATATE[21], resulting in a higher amount of ^{177}Lu-DOTATATE in the 'remainder of the body', thereby leading to higher absorbed doses of ^{177}Lu-DOTATATE to the bone marrow. MDS occurred in 4 out of 310 (1%) patients treated with ^{177}Lu-DOTATATE[23], and was potentially treatment related in 3 patients.

FUTURE DIRECTIVES

New applications of PRRT may include the neoadjuvant use of PRRT for pancreatic NETs. A few case reports have described the neoadjuvant use of PRRT in patients with pancreatic NETs who could be operated on successfully after PRRT.[56-57] Since surgery is the only curative option for patients with GEPNETs, this neoadjuvant treatment is very promising.

PRRT may also be used in an adjuvant setting after surgery of GEPNETs, preventing tumor development after spread due to manipulation of the tumor during surgery or preventing further growth of already present micrometastases. In an animal study, therapy with ^{177}Lu-DOTATATE prevented or significantly reduced the growth of tumor deposits in the liver after injection of tumor cells via the portal vein mimicking perioperative tumor spill.[58] So far, we have treated 3 patients with ^{177}Lu-DOTATATE in an adjuvant setting. To detect a difference in survival and/or tumor recurrence rate in patients treated with and without adjuvant PRRT, a large, multicenter trial with years of follow-up should be performed.

Recently, the results of new targeted therapies for the treatment of GEPNETs have been presented. Treatment with sunitinib (Sutent; Pfizer Inc., New York, N.Y., USA), a tyrosine kinase inhibitor, resulted in a longer median PFS than placebo (11.4 vs. 5.5 months) in patients with pancreatic NETs.[59] Also, treatment with everolimus (Afinitor; Novartis Pharmaceuticals,

Basel, Switzerland), an inhibitor of mammalian target of rapamycin (mTOR), resulted in a longer median PFS than placebo (11.0 vs. 4.6 months) in patients with pancreatic NETs.[60] The combination of PRRT with sunitinib or everolimus, or the sequential use of PRRT with one of these compounds may be of interest in the treatment of patients with pancreatic NETs.

Lastly, chelated pansomatostatin agonists[61-62] and chelated somatostatin antagonists[63] are currently under investigation. Chelation of these compounds enables their radiolabeling with ^{111}In, ^{90}Y or ^{177}Lu, therewith making them candidates for tumor targeting.

CONCLUSIONS

PRRT is a promising new treatment modality for inoperable or metastasized GEPNET patients. CRs and PRs obtained after treatment with ^{90}Y-DOTATOC are in the same range as after treatment with ^{177}Lu-DOTATATE. However, the significant nephrotoxicity observed after treatment with ^{90}Y-DOTATOC may hamper the use of this compound. Options to improve PRRT may include combinations of radioactive labeled somatostatin analogs, intra-arterial administration, and the use of radiosensitizing drugs combined with PRRT. Other therapeutic applications of PRRT may include additional therapy cycles in patients with progressive disease after benefit from the initial therapy, PRRT in adjuvant or neoadjuvant setting, or PRRT combined with new targeted therapies. Randomized clinical trials comparing PRRT with other treatment modalities, or comparing various radioactive labeled somatostatin analogs should be undertaken to determine the best treatment options for patients with GEPNETs.

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Chapter 3

Tumor Response Assessment to Treatment with [^{177}Lu-DOTA^{0},Tyr^{3}] Octreotate in Patients with Gastroenteropancreatic and Bronchial Neuroendocrine Tumors: Differential Response of Bone Versus Soft-Tissue Lesions

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ABSTRACT

Purpose

We have noted that bone lesions on CT respond differently from soft-tissue lesions to treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-octreotate). We therefore compared the response of bone lesions with that of soft-tissue lesions to treatment with ^{177}Lu-octreotate in patients with gastroenteropancreatic and bronchial neuroendocrine tumors (NETs).

Methods

Forty-two patients with well-differentiated NETs who had bone metastases that were positive on [^{111}In-DTPA^{0}]octreotide somatostatin receptor scintigraphy (SRS) before treatment, and who had soft-tissue lesions, were studied. All patients had had a minimum of 1 follow-up CT scan. Lesions were scored on CT and bone lesions also on SRS before and after treatment. Tumor markers (chromogranin A and 5-hydroxyindoleacetic acid) before and after treatment were compared.

Results

Because bone lesions were not visible on CT before treatment in 11 of 42 patients (26%), bone and soft-tissue lesions were evaluated in 31 patients. Whereas bone lesions increased in size, soft-tissue lesions decreased in size. The percentage change in bone and soft-tissue lesions was significantly different at all time points up to 12 mo of follow-up ($p<0.001$). The intensity or number of bone lesions on SRS decreased after treatment in 19 of 23 patients (83%) in whom SRS after treatment was available. The tumor markers also decreased significantly after treatment. In 1 patient, bone lesions became visible on CT after treatment, mimicking progressive disease with "new" bone lesions, although there was an overall treatment response.

Conclusion

In patients with NETs, the apparent increase in size of bone lesions or the appearance of new bone lesions on CT after treatment with ^{177}Lu-octreotate should be interpreted cautiously, as this finding may be therapy-related rather than indicative of tumor progression.

Key Words: neuroendocrine tumor; peptide receptor radionuclide therapy; [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate; bone metastases; treatment response

INTRODUCTION

Gastroenteropancreatic and bronchial neuroendocrine tumors (NETs) are rare neoplasms that usually grow slowly and have a relatively indolent course. These tumors were formerly called either (bronchial) carcinoids or islet-cell tumors. Today, new classifications, such as the World Health Organization[1] and TNM[2-3] classifications, are being used for the staging and grading of NETs. The primary treatment is surgery with curative intent or debulking of the tumor mass. In cases of metastatic disease, cytoreductive options are limited. A relatively new therapy, peptide receptor radionuclide therapy with radiolabeled somatostatin analogs, is currently available in several centers (reviewed in[4]).

The prevalence of bone metastases in NETs is 7%–22%.[5-7] Bone metastases are associated with poor clinical outcome[6-10] and can have multiple sequelae, including bone pain, pathologic fractures, nerve root compression, spinal cord compression, and hypercalcemia.[11-12] The detection of bone metastases may change the clinical management in NET patients; chemotherapy or localized radiation may be indicated, instead of liver-directed therapy.[5-6]

Bone metastases in NETs can be visualized with several imaging modalities, including conventional radiography, CT, MRI, somatostatin receptor scintigraphy (SRS), bone scintigraphy, and PET/CT with, for example, ^{68}Ga-DOTA-Tyr^3-octreotide or 6-^{18}F-fluoro-L-DOPA.[5, 13-16] MRI has the highest sensitivity for the detection of bone metastases in NETs (nearly 100%[17]); however, a limitation of MRI is that usually only a part of the skeleton is scanned. Therefore, it is advocated that SRS be used as a total-body screening method. SRS has an acceptable sensitivity of around 80%.[18] MRI can be used to evaluate the possibility of pathologic fractures or spinal cord compression in areas of intense uptake on SRS.

In our clinical practice, we have noted that on CT, the response of bone metastases to treatment with [^{177}Lu-DOTA^0,Tyr^3]octreotate (^{177}Lu-octreotate) tends to differ from that of, for example, liver metastases. We therefore compared the radiologic response on CT of bone lesions in NETs with that of soft-tissue lesions after treatment with ^{177}Lu-octreotate. We also evaluated the imaging characteristics of bone and soft-tissue lesions in NETs on CT performed before and after treatment with ^{177}Lu-octreotate.

MATERIALS AND METHODS

Patients

From the patients with NETs who had been treated with ^{177}Lu-octreotate according to protocol in our institution between January 2000 and January 2010, we retrospectively selected those with bone metastases that were positive on [^{111}In-DTPA^0]octreotide scintigraphy (SRS) before treatment, with soft-tissue lesions on CT, with digitally available CT, with a minimum of 1 follow-up CT scan, and with lesions at baseline that met the Response Evaluation Criteria in Solid Tumors (RECIST) criteria[19] for a measurable lesion (i.e., longest diameter on CT ≥10 mm). Exclusion criteria included radiotherapy, chemotherapy,

hepatic artery embolization or chemoembolization 3 mo or less before the treatment with ^{177}Lu-octreotate, or the presence of a second primary tumor. This study was part of the ongoing prospective study on NET patients treated with ^{177}Lu-octreotate at the Department of Nuclear Medicine of Erasmus University Medical Center Rotterdam, which was approved by the local medical ethical committee. All patients gave written informed consent to participate in the study.

Treatment

[DOTA^0,Tyr^3]octreotate was obtained from Mallinckrodt. $^{177}LuCl_3$ was obtained from the Nuclear Research and Consultancy Group and was distributed by IDB-Holland. ^{177}Lu-octreotate was locally prepared as described previously.[20]

Granisetron (Kytril; Roche), 3 mg, was injected intravenously 30 min before the start of the ^{177}Lu-octreotate infusion. To reduce the radiation dose to the kidneys, an infusion of amino acids (2.5% arginine and 2.5% lysine, 1 L) was started 30 min before administration of the radiopharmaceutical and lasted 4 h. The radiopharmaceutical was coadministered using a second pump system. Cycle doses were 7.4 GBq, injected over 30 min. The interval between treatments was 6–10 wk. Patients were treated with up to a cumulative intended dose of 22.2–29.6 GBq. If dosimetric calculations indicated that the radiation dose to the kidneys would exceed 23 Gy with a dose of 29.6 GBq, the cumulative dose was reduced to 22.2–27.8 GBq. Routine testing of hematology and liver and kidney function was performed before each therapy and at follow up visits.

Comparison of Bone and Soft-Tissue Lesions

Bone and soft-tissue lesions were scored on CT (Somatom, Sensation 64; Siemens Healthcare) at baseline; 6 wk after treatment; 3, 6, and 12 mo after treatment; and when progressive disease (PD) occurred. Soft-tissue lesions were categorized as liver lesions or as other lesions.

A maximum of 3 bone lesions, 5 liver lesions, and 3 other lesions was chosen. If patients had received previous radiotherapy, only nonirradiated lesions were chosen for measurements. If a liver lesion had been treated with radiofrequency ablation, it was not assessed. If the liver was enlarged and liver lesions could not be measured separately, the size of the total liver was measured instead. This decision was made by an experienced radiologist.

Lesions were measured according to RECIST[19] and the Southwest Oncology Group criteria.[21] For RECIST, the sum of the longest diameters of lesions was calculated. For the Southwest Oncology Group criteria, the sum of the products of the perpendicular diameters of lesions was calculated.

SRS and Laboratory Values

The intensity and number of bone lesions on SRS before and after treatment were compared visually. Various tumor markers and potential tumor-volume-related determinants in serum at baseline were compared with the values at the time point of best response, which was defined as the time point of the best response achieved in soft-tissue lesions according to RECIST.

Aspect of Lesions

In addition to lesion size measurement, various aspects of lesions were scored. For all lesions, the visual appearance of a boundary with surrounding tissue was scored and the Hounsfield units (HUs) were determined by placing a region of interest as large as possible. For bone lesions, cortical destruction was also assessed. Liver metastases (which were assessed in the venous phase of contrast enhancement) were also scored according to homogeneity or heterogeneity of lesions, aspect of heterogeneity if applicable, and density of lesions when compared with normal liver parenchyma (hypodense, hyperdense, or isodense).

Best-Response Categories

Best-response category was defined as the best response according to RECIST achieved in soft-tissue lesions after treatment. Best response had to be confirmed on a subsequent CT scan. If a patient had only 1 follow-up scan (and thus no confirmatory scan), the best response was unknown.

Statistics

Independent *t* tests, paired *t* tests, Wilcoxon signed-rank tests, McNemar tests, Mann–Whitney *U* tests, and χ^2 tests (or, if applicable, Fisher exact tests) were used. To compare the response of bone and soft-tissue lesions, paired *t* tests were used and a repeated-measurement analysis was performed. In this analysis, different regression lines were fitted for bone and soft-tissue lesions. The dependency between measurements of the same tumor was fitted using an unstructured covariance matrix. The SPSS (version 15.0; IBM) and SAS (version 9.2; SAS Institute Inc.) packages were used. Two-sided P values are reported. P values of less than 0.05 were considered to be significant.

RESULTS

Seventy-five patients had bone metastases and soft-tissue lesions. In 23 patients, a baseline CT scan was not digitally available (which was necessary for HU measurement). Four patients had no follow-up CT scan. Six patients did not meet other inclusion criteria. Thus, 42 patients were evaluated. All patients had well-differentiated (G1 or G2) tumors.[2-3] None of the patients were pretreated with ^{90}Y-coupled somatostatin analogs. Bone metastases

were not visible on CT before treatment in 11 of 42 patients (26%). In 2 of these patients, bone metastases occurred on CT after treatment. In 1 patient, this was probably due to PD, because new bone lesions were also seen on post-therapy scintigraphy. In the other patient, PD was unlikely, since SRS after treatment showed a reduced number of bone lesions, and CT showed a reduction of liver metastases, consistent with a partial response (PR). Baseline characteristics of the 42 patients are presented in Table 1. Patients with a primary tumor in the small bowel had visible bone lesions on CT before treatment less often than patients with a bronchial NET.

Bone and soft-tissue lesions were evaluated in 31 patients: liver lesions in 25 patients, the total liver in 3 patients, mediastinal lymph nodes in 2 patients, and a pancreatic tumor in 1 patient. The results below apply to this group of 31 patients.

Comparison of Bone and Soft-Tissue Lesions

Figure 1 compares the response of bone and soft-tissue lesions on CT after treatment with ^{177}Lu-octreotate according to RECIST. Whereas, on average, bone lesions increased in size, soft-tissue lesions regressed. The percentage change in bone and soft-tissue lesions was significantly different at all time points up to 12 mo of follow-up ($P<0.001$). These outcomes were the same when the Southwest Oncology Group criteria were used, when separate analyses were done according to categorized treatment outcome, and when repeated-measurement analysis was performed.

Figure 2 shows the best response (percentage change) on CT of soft-tissue lesions and the corresponding bone lesions at the same time point in the same patients. The best response did not always match the best-response outcome category, since the confirmation criterion was not always met. There was a clear difference in response between bone and soft-tissue lesions. An example of a patient whose bone metastases apparently progressed on CT (i.e., "new" bone lesions appeared) after treatment is shown in Figure 3 (this patient had no measurable bone metastases on CT before treatment and does not belong to the abovementioned group of 31 patients).

SRS and Laboratory Values

The intensity or number of bone lesions on SRS decreased after treatment in 19 of 23 patients (83%) in whom SRS after treatment was available. This decrease was observed in 11 of 13 patients (85%) with a PR and in 8 of 10 patients (80%) with stable disease (SD) as the best response. In the remaining 8 patients, SRS was not available for the following reasons: death in 5 patients, PD in 1 patient, and loss to follow-up in 2 patients.

Table 2 shows various tumor markers and potential tumor-volume-related determinants in serum before treatment and at the time point of best response in patients with elevated values at baseline. Median chromogranin A and mean 5-hydroxyindoleacetic acid (5-HIAA) levels decreased significantly after treatment.

Table 1. Baseline characteristics of patients with and without bone lesions visible on CT before treatment with ^{177}Lu-octreotate.

Characteristic	Bone lesions visible on CT	Bone lesions not visible on CT	*P*
No. of patients	31	11	
No. of male patients	19	7	1.00
Age (y)			0.85
Mean	61	62	
Range	43–77	51–79	
Time from diagnosis to treatment (mo)			0.91
Median	16	43	
Range	4–354	3–313	
Time from development of bone metastases to treatment (mo)			0.51
Median	4	3	
Range	1–44	1–14	
Location of primary tumor (*n*)			
Lung	11	0	0.01*
Small intestine	5	7	
Colon/rectum	2	0	
Other	4	0	
Unknown	9	4	
Previous therapy (*n*)	25	8	0.68
Octreotide	20	8	0.72
Surgery	16	7	0.73
Radiotherapy	4	2	0.64
Chemotherapy	3	1	1.00
Embolization/chemoembolization	3	1	1.00
Liver radiofrequency ablation	1	0	1.00
Total administered dose (GBq)			0.50
Median	29.7	29.9	
Range	22.0–30.2	14.7–30.3	
5-HIAA elevated (*n*)	18	9	0.28

*Significant difference (Fisher exact test using Monte Carlo method).
Elevated 5-HIAA is ≥50 µmol/L in 24-h urine collection.
GBq: gigabecquerel; 5-HIAA: 5-hydroxyindoleacetic acid.

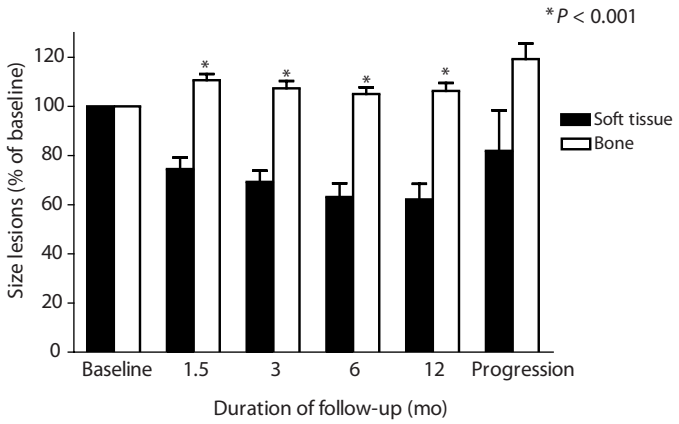

Figure 1. Size of bone lesions and soft-tissue lesions on CT as percentage of baseline at various time points (mean ± SEM). *P*<0.001 for difference between mean bone and soft-tissue lesion size (paired *t* test).

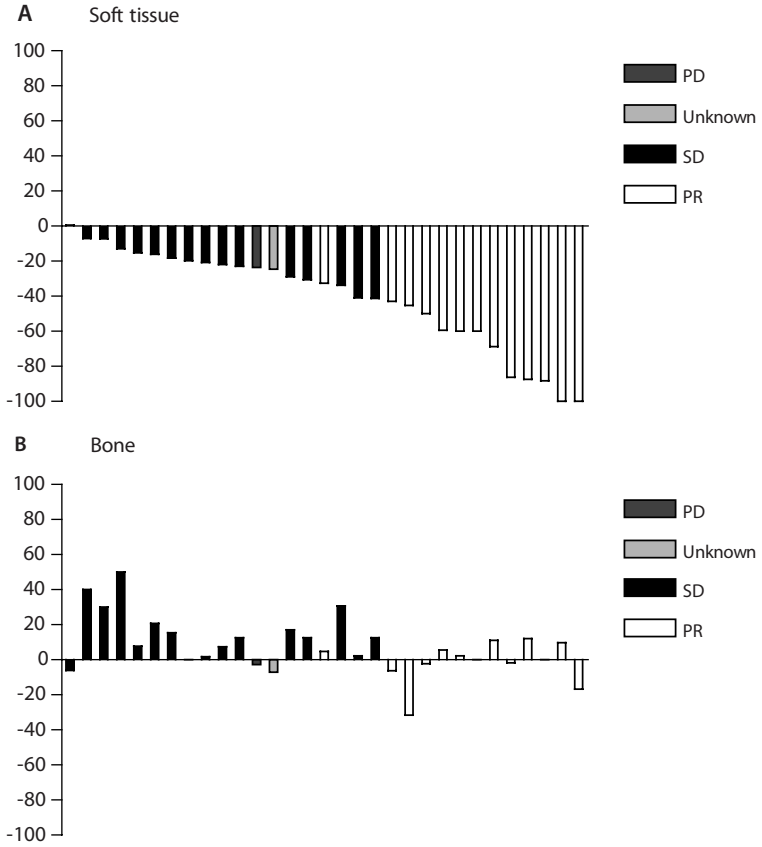

Figure 2. (**A**) Best CT response (percentage change) of soft-tissue lesions. (**B**) Corresponding bone lesions at same time point in same patients (*n*=31). Bars indicate best-response outcome categories based on assessment of soft-tissue lesions according to RECIST.

PD: progressive disease; PR: partial response; SD: stable disease.

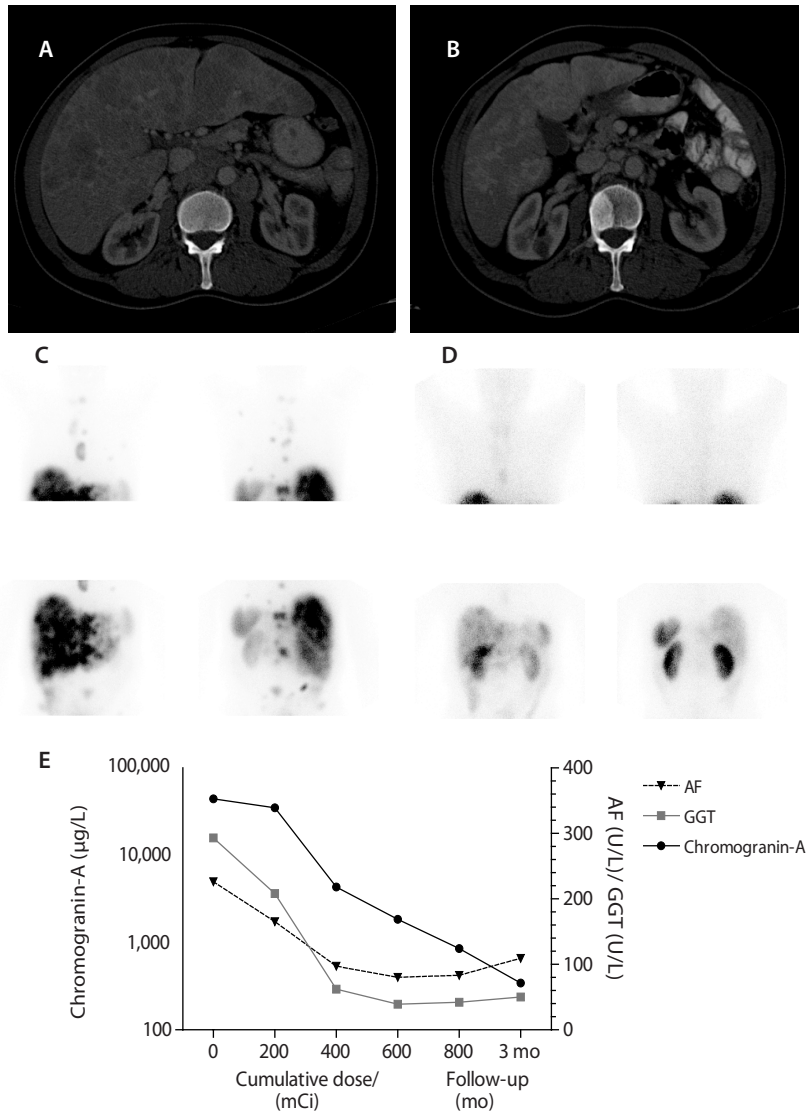

Figure 3. Comparison of CT, [^{111}Indium-DTPA^{0}]octreotide scintigraphy, and tumor-volume–related determinants in serum of patient with a NET of unknown origin with multiple liver and bone metastases before and after treatment with 30.1 GBq (800 mCi) of ^{177}Lu-octreotate. (**A**) CT (bone window; transversal slice) before treatment with ^{177}Lu-octreotate, with no evidence of bone metastases. (**B**) CT (bone window; transversal slice) 6 wk after treatment with ^{177}Lu-octreotate, showing bone metastasis located at L2 and shrinkage (pseudocirrhosis) of liver. (**C**) [^{111}Indium-DTPA^{0}]octreotide scintigraphy (anterior and posterior views) before treatment with ^{177}Lu-octreotate showing uptake in multiple liver and bone metastases. (**D**) [^{111}Indium-DTPA^{0}]octreotide scintigraphy (anterior and posterior views) 4 mo after last treatment with ^{177}Lu-octreotate, showing reduction of liver and bone metastases and shrinkage of liver. (**E**) Serum alkaline phosphatase, γ-glutamyl transpeptidase, and chromogranin A levels in same patient during and 3 mo after treatment with ^{177}Lu-octreotate, showing significant decrease, indicating tumor response.

AF: alkaline phosphatase; GGT: γ-glutamyl transpeptidase.

Table 2. Tumor markers and tumor-volume-related determinants in serum before treatment with ^{177}Lu-octreotate and at time of best response in patients with elevated values at baseline.

Parameter	Reference value	Baseline	Best response	*P*
Chromogranin A (µg/L)	<95	979	514	0.002*
5-HIAA (µmol/L)	<50	746 ± 166	437 ± 126	0.02*
Alkaline phosphatase (U/L)	<120	185	130	0.08
Bone-specific alkaline phosphatase (µg/L)	<20.1 (men), <14.3 (premenopausal women), <22.4 (postmenopausal women)	35 ± 10	29 ± 6	0.43
Bilirubin (µmol/L)	<17	32 ± 6	16 ± 1	0.05
γ-glutamyl transpeptidase (U/L)	<35	220	123	0.05
Aspartate aminotransferase (U/L)	<31	79 ± 23	70 ± 12	0.63
Alanine aminotransferase (U/L)	<31	76 ± 21	63 ± 16	0.38
Lactate dehydrogenase (U/L)	<450	593 ± 108	419 ± 26	0.28

*Significant difference (Wilcoxon signed ranks test for chromogranin A and paired *t* test for 5-HIAA).
Data are mean ± SEM or median.

5-HIAA: 5-hydroxyindoleacetic acid.

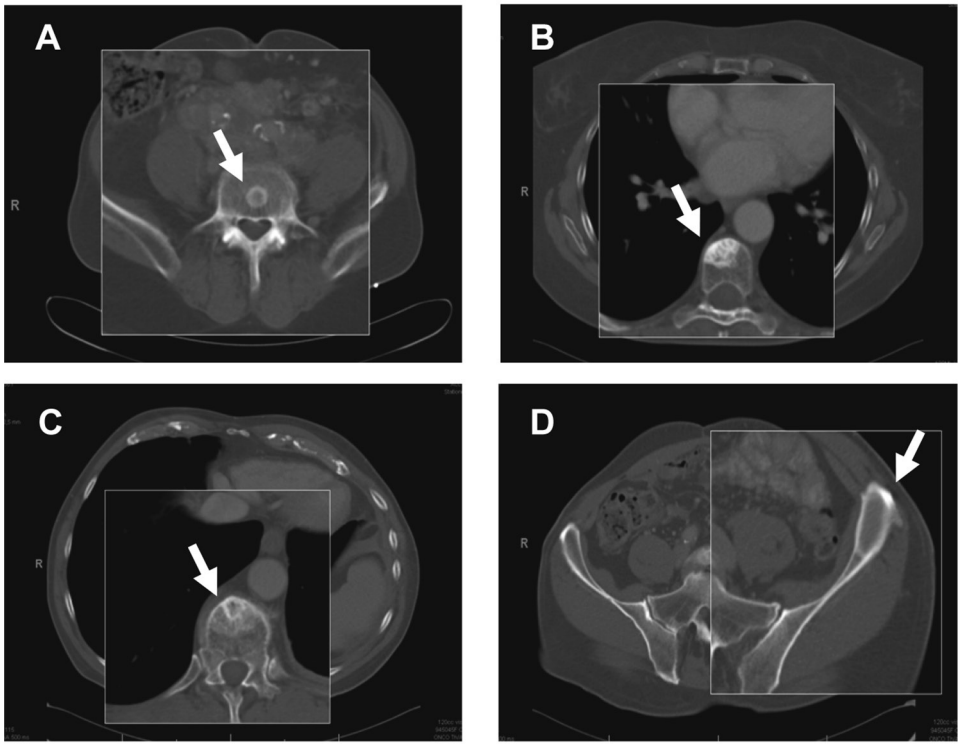

Figure 4. Roentgenologic CT appearance (transversal slices; zoomed in on bone metastases) of gastroenteropancreatic and bronchial NETs. Arrows indicate relevant bone lesion. (**A**) Small sclerotic rim. (**B**) Larger sclerotic area. (**C**) Vague sclerotic area with sclerotic rim inside. (**D**) Lytic lesion with sclerotic rim.

Aspect of Lesions

Sixty bone lesions were sclerotic, whereas 4 were lytic with a sclerotic rim. Cortical boundaries were intact in all lesions. Table 3 lists the various aspects of bone lesions at baseline and at the time point of best response. Figure 4 shows examples of bone lesion aspects. The distribution of these categories was not significantly different according to primary tumor or the presence of elevated 5-HIAA. The presence of a boundary or demarcation of bone lesions was more pronounced after treatment. The mean HUs of bone lesions increased significantly after treatment in the group with elevated 5-HIAA levels at baseline (Table 3). Figure 5 shows the microscopic appearance of a bone metastasis and the reactive changes in bone in one of the patients.

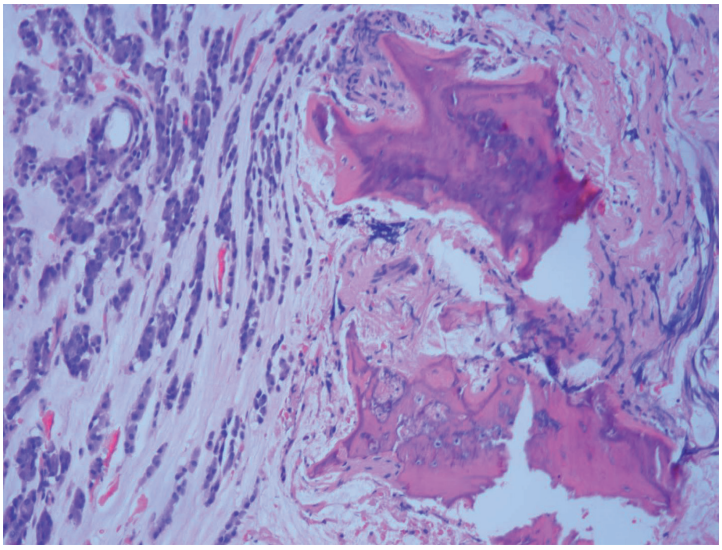

Figure 5. Microscopic appearance (hematoxylin and eosin (HE) staining) of a bone metastasis located at L4-L5 of a bronchial neuroendocrine tumor. The left part of the picture shows the typical epithelial tumor cells, arranged in cords and small nests, whereas on the right bony elements with reactive changes are seen.

One hundred three liver lesions were assessed. Table 3 shows the various aspects of liver lesions at baseline and at the time point of best response. Figure 6 shows examples of liver lesion aspects. The presence of a boundary or demarcation of liver lesions was less pronounced after treatment. The mean HUs of liver lesions decreased significantly after treatment in patients with a PR as the best response, whereas in patients with an SD no difference was observed. The mean HUs also decreased significantly in the group with a primary tumor located in the small intestine or in the colon or rectum. In these 2 groups,

the percentage of patients with a PR was identical to that of the groups with other primary localizations (50% vs. 48%, *P*=1.00, for small intestine; 50% vs. 48%, *P*=1.00, for colon or rectum).

The various aspects of other lesions did not change significantly (Table 3).

Table 3. Characteristics of bone, liver, and other lesions before treatment with ^{177}Lu-octreotate and at time of best response.

Characteristic	Baseline	Best response	*P*
Bone lesions	***n*=64**	***n*=61**	
Aspect			
Small sclerotic rim	29	28	
Larger sclerotic area	29	27	
Vague sclerotic area with sclerotic rim inside	2	2	
Lytic lesion with sclerotic rim	4	4	
Boundary of Lesion *(n = 61)*			
Well demarcated	28	53	<0.001*
Moderately or poorly demarcated	33	8	
HUs (mean ± SEM)	423 ± 28	447 ± 31	0.10
Elevated levels of 5-HIAA at baseline†			
Yes (*n*=40)	412 ± 35	444 ± 37	0.047*
No (*n*=20)	419 ± 44	421 ± 51	0.96
Treatment outcome			
PR (*n*=24)	378 ± 44	392 ± 48	0.54
SD (*n*=32)	456 ± 41	490 ± 45	0.11
PD (*n*=2)	525 ± 83	476 ± 3	0.67
Unknown (*n*=3)	359 ± 68	401 ± 38	0.30
Location of primary tumor			
Lung (*n*=26)	463 ± 42	503 ± 44	0.13
Small intestine (*n*=11)	262 ± 42	271 ± 35	0.66
Colon/rectum (*n*=2)	689 ± 241	740 ± 310	0.60
Other (*n*=8)	569 ± 80	609 ± 87	0.51
Unknown (*n*=14)	354 ± 48	347 ± 53	0.73
Liver lesions	***n*=103**	***n*=78**	
Homogeneous *(n=78)*			
Yes	46	50	0.50
No	32	28	
Aspect heterogeneity			
Small dense area on side	6	3	
Hypodense irregularly shaped area in middle	17	9	
Small hypodense area on side	11	11	
Dense irregularly shaped area in middle	3	5	

Characteristic *(Continued)*	**Baseline**	**Best response**	***P***
Liver lesions	***n*=103**	***n*=78**	
Density of lesion			
Hypodense	88	65	
Hyperdense	5	3	
Isodense	2	1	
Mixed‡	8	9	
Boundary of lesion *(n=78)*			
Well demarcated	60	48	0.04*
Moderately or poorly demarcated	18	30	
HUs (mean ± SEM)	69 ± 2	64 ± 3	0.70
Treatment outcome			
PR (*n*=30)	74 ± 4	61 ± 4	0.03*
SD (*n*=39)	66 ± 3	66 ± 4	1.00
PD (*n*=4)	87 ± 1	73 ± 5	0.05
Unknown (*n*=5)	44 ± 9	52 ± 5	0.18
Location of primary tumor			
Lung (*n*=21)	62 ± 5	70 ± 4	0.08
Small intestine (*n*=17)	80 ± 4	58 ± 6	<0.001*
Colon/rectum (*n*=10)	80 ± 6	50 ± 3	0.001*
Other (*n*=1)	54 (NA)	56 (NA)	NA
Unknown (*n*=29)	63 ± 4	68 ± 5	0.22
Other lesions	***n*=3**	***n*=3**	
Boundary of lesion			
Well demarcated	2	3	1.00
Moderately or poorly demarcated	1	0	
HUs (mean ± SEM)	91 ± 4	91 ± 27	0.99

*Significant difference (McNemar test for boundary lesion, paired *t* test for HUs).
†In 1 patient 5-HIAA level was not determined at baseline.
‡Combination of hypodense, hyperdense, or isodense.
Elevated 5-HIAA is ≥50 mmol/L in 24-h urine collection. Numbers in italics are numbers of patients used for McNemar test (some lesions disappeared at best response, and some lesions were not imaged by CT scan; only paired data were used).

HU: hounsfield unit; SEM: standard error of the mean; 5-HIAA: 5-hydroxyindoleacetic acid; PR: partial response; SD: stable disease; PD: progressive disease; NA: not applicable.

Typical examples of the radiologic response of bone lesions and a liver lesion after treatment with ^{177}Lu-octreotate are shown in Figure 7. Of interest are the increased sclerosis of the bone lesions after treatment and the presence of a fluid–fluid level in the liver metastasis – a finding that is characteristic of a NET metastasis.[22]

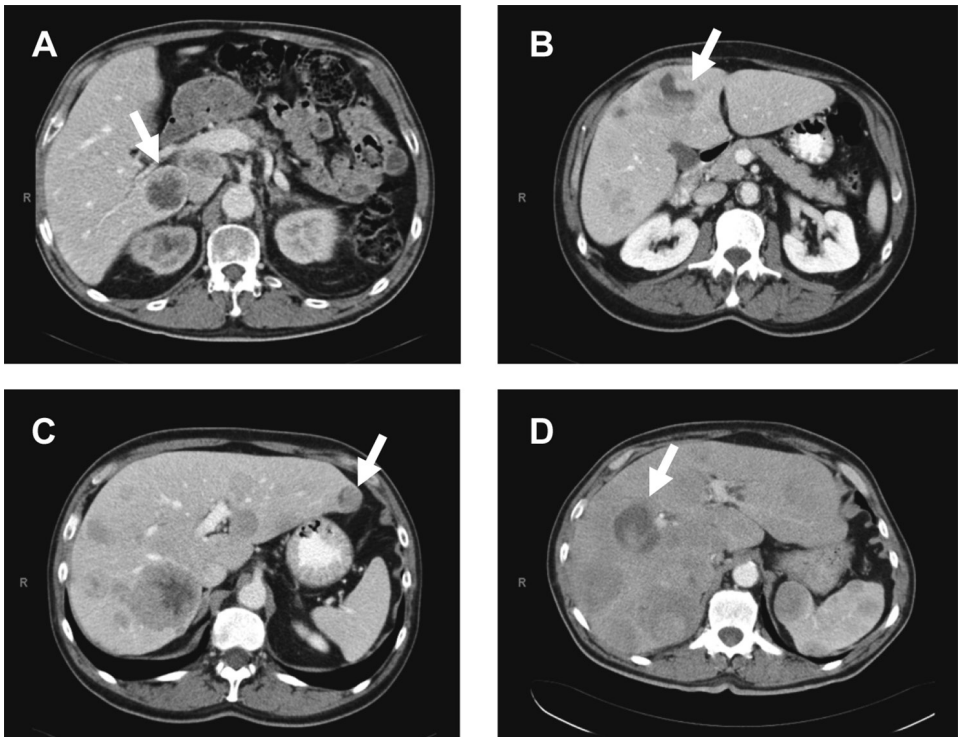

Figure 6. Aspect of heterogeneity on CT (transversal slices) assessed in the venous phase of contrast enhancement of liver metastases in gastroenteropancreatic and bronchial NETs. Arrows indicate relevant liver lesion. (**A**) Small dense area on side. (**B**) Hypodense irregularly shaped area in middle. (**C**) Small hypodense area on side. (**D**) Dense irregularly shaped area in middle.

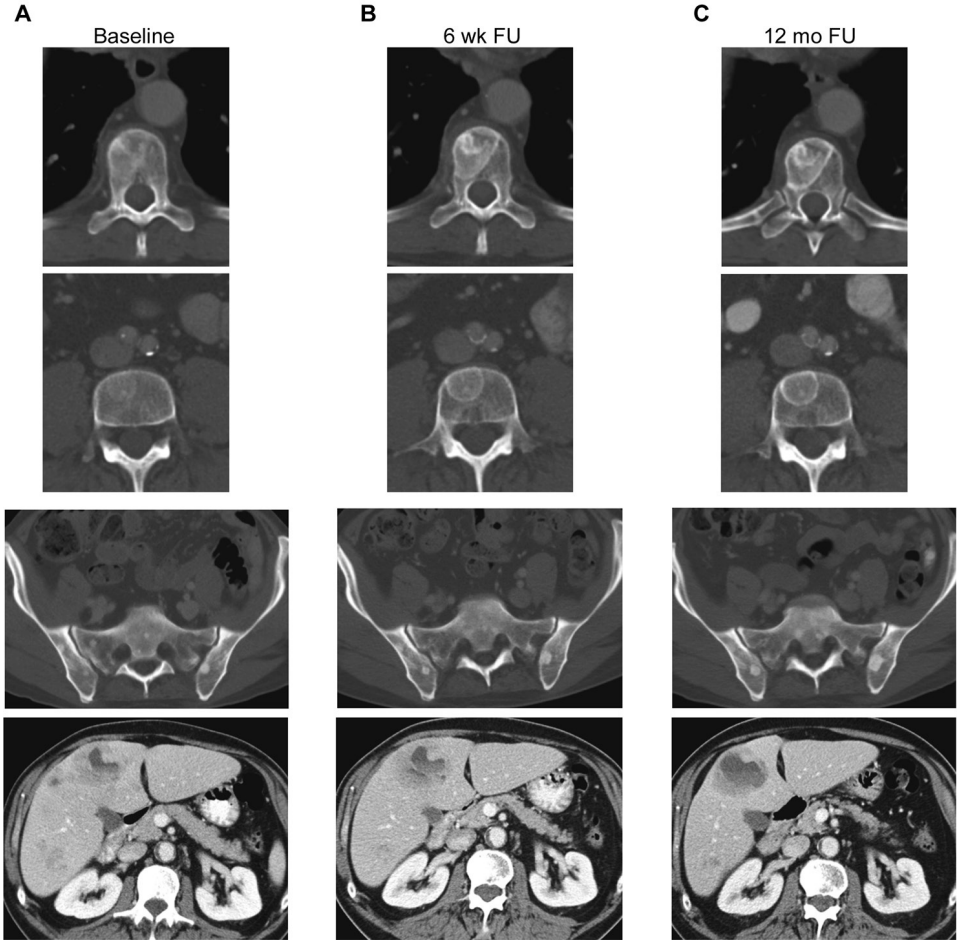

Figure 7. Radiological response of bone and liver lesions after treatment with ^{177}Lu-octreotate. Increased sclerosis of the bone lesions after treatment with ^{177}Lu-octreotate is observed. A fluid-fluid level is observed in the liver metastasis. (**A**) Baseline. (**B**) 6 wk FU. (**C**) 12 mo FU.
FU: follow-up.

DISCUSSION

Peptide receptor radionuclide therapy is a promising treatment modality for NET patients, with high tumor response rates and symptomatic control in most patients.[7, 23-26] We performed this study to explore our observation that, on CT, bone lesions tended to respond differently from soft-tissue lesions to treatment with ^{177}Lu-octreotate.

In this study we found that, on average, bone lesions increased in size on CT after treatment with ^{177}Lu-octreotate, whereas soft-tissue lesions regressed. In 1 patient, bone lesions became visible on CT after treatment, mimicking PD with "new" bone lesions, although

there was an overall treatment response. An additional finding was that bone metastases were not visible on CT before treatment in a considerable number of patients. A difference in visibility of bone lesions on CT for bronchial and small-bowel NETs was seen. Lastly, a change in the HUs of bone or liver lesions in specific patient subgroups was observed after treatment.

The difference in response between bone and soft-tissue lesions on CT as seen in this study poses a clinical dilemma. The apparent progression of bone lesions on CT could be a therapy effect but is, in itself, indistinguishable from PD. Because the intensity or number of bone lesions on SRS declined in 83% of patients, and because the tumor markers chromogranin A and 5-HIAA decreased significantly after treatment, it appears that a real therapy effect is more likely and that the apparent increase in size of bone lesions reflects a healing response to treatment.

The mechanism for this difference in treatment response is not clear. It can be hypothesized that bone lesions had a lower degree of uptake of ^{177}Lu-octreotate than did soft-tissue lesions, resulting in a decreased radiation dose and hence a decreased treatment response. In our study, however, this possibility seems unlikely, because bone metastases were clearly visible on SRS and posttherapy scintigraphy. The intensity or number of bone lesions on SRS also decreased after treatment according to treatment outcome, which supports our hypothesis that there was a true response.

A similar difference in response between bone and other lesions has been described in some case reports[27-28] on patients with bronchial adenocarcinoma treated with epidermal growth factor receptor inhibitors, and in a study[29] describing the response after systemic therapy assessed by ^{18}F-FDG PET in metastatic breast cancer patients. However, these studies could make no distinction between therapy effect and PD.

Moreover, osteoblastic bone flare, defined by an increase in the number or intensity of lesions on bone scintigraphy in the presence of a well-documented response of other tumor sites to treatment, is a well-recognized phenomenon in breast cancer.[30] The phenomenon seen on CT as described in our study could be analogous to this bone flare, since in our study we observed an increased density of bone lesions that probably represents a favorable reaction to therapy.

Several guidelines can be used to assess tumor response. The widely used RECIST and Southwest Oncology Group criteria consider bone lesions as nonmeasurable; new bone lesions are considered PD. The recently revised RECIST criteria (version 1.1)[31] consider lytic or mixed lytic–blastic bone lesions as measurable lesions if they have an identifiable soft-tissue component measuring 10 mm or more on CT. However, osteoblastic lesions, which occur mainly in NETs, remain unmeasurable. Lastly, the M.D. Anderson criteria[32] consider the finding of sclerosis of previously undetected lesions on CT as PR and new lesions as PD. However, the distinction between this sclerosis and new lesions is difficult. When bone metastases are visible on SRS before treatment, the appearance of "new" bone lesions on CT

corresponding to places positive on SRS is most likely "sclerosis of previously undetected lesions," as was demonstrated in 1 patient in our study.

In patients with "new" bone lesions on CT after treatment, although there is an overall treatment response we advise that a new SRS be performed to assess the response of bone metastases, provided that the bone metastases were visible on SRS before treatment. The assumption that these patients have PD based on "new" bone lesions on CT could lead to the erroneous alteration of an effective treatment.

Recently, Ezziddin et al. showed that the response of bone metastases after treatment with ^{177}Lu-octreotate could indeed be evaluated efficiently by [^{111}In-DTPA^{0}]octreotide scintigraphy or ^{68}Ga-DOTA-Tyr^{3}-octreotide PET scan.[33] However, because tumor response evaluation is being performed mainly by CT, we think it is important that clinicians be aware of the difference in treatment response found in this study.

Although bone metastases were not visible on CT before treatment, they were visible on SRS in 11 of 42 patients (26%). This finding is in line with other studies that found bone lesions of NETs to be visualized more often by ^{68}Ga-DOTA-Tyr^{3}-octreotide PET[13] or 6-^{18}F-fluoro-L-DOPA PET[16] than by CT.

Patients with a primary tumor in the small bowel had visible bone lesions on CT before treatment less often than patients with a bronchial NET. A possible explanation for this difference could be that bronchial NETs produce histamine and 5-hydroxytryptophan, the precursor of 5-hydroxytryptamine (serotonin), unlike small-bowel NETs, which produce serotonin.[34-35] It may be hypothesized that CT better visualizes a bone reaction caused by secretion of 5-hydroxytryptophan than that caused by serotonin.

The mean HUs of bone lesions increased significantly after treatment in the group of patients with elevated 5-HIAA levels at baseline; this finding indicates increased sclerosis. It is uncertain whether this sclerosis could be attributed to elevated levels of serotonin or 5-hydroxytryptophan, since 5-HIAA is the breakdown product of both. The mean HUs of liver lesions decreased significantly after treatment in patients with a PR as the treatment outcome. It is difficult to explain this observation. One might hypothesize that although the first step in tumor response to treatment is necrosis, which reduces HUs, the necrosis is followed by fibrosis, which increases HUs. It might be postulated that at the time of best response, lesions consist merely of fibrosis, but our findings did not support this line of thought. The lower HUs at the time of best response might nonetheless be explained by the slow response that NETs usually display, resulting in late tumor-size reductions and perhaps even later signs of fibrosis, not necessarily coinciding with the time of best response.

We recognize that a major inherent limitation of the study is its retrospective design. However, we believe that the study nevertheless gives important and valuable information about the difference in treatment response between bone and soft-tissue lesions in patients with this rare tumor entity.

CONCLUSION

This study demonstrated that bone lesions increased in size on CT after treatment with ^{177}Lu-octreotate even in patients who had a PR as the treatment outcome. Tumor markers and intensity or number of bone lesions on SRS decreased after treatment. Therefore, the apparent increase in the size of bone lesions or the appearance of new bone lesions on CT after treatment with ^{177}Lu-octreotate should be interpreted cautiously, as this finding may be therapy-related rather than indicative of tumor progression.

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Chapter 4

Comparison of Response Evaluation in Patients with Gastroenteropancreatic and Thoracic Neuroendocrine Tumors after Treatment with [^{177}Lu-DOTA^{0},Tyr^{3}] Octreotate

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ABSTRACT

Purpose

Response Evaluation Criteria in Solid Tumors (RECIST) (unidimensional), Southwest Oncology Group (SWOG) solid tumor response criteria (bidimensional), and their modified variants are commonly used in the tumor response assessment after treatment of gastroenteropancreatic and thoracic neuroendocrine tumors (NETs). In the current study, RECIST, SWOG, modified RECIST (mRECIST) and modified SWOG (mSWOG) criteria were compared in patients with NETs treated with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-octreotate).

Methods

Two-hundred sixty eight Dutch patients with NETs who had been treated with ^{177}Lu-octreotate between January 2000 and April 2007 were studied. Computed tomography (CT) or magnetic resonance imaging (MRI) scans were analyzed using RECIST, SWOG, mRECIST and mSWOG criteria (including the tumor response class minor response (MR) (decrease of 13–30% for mRECIST and 25–50% for mSWOG)). The outcomes were correlated with progression-free survival (PFS) and overall survival (OS).

Results

Eleven patients had an unknown tumor response and were excluded. The rates of objective response (OR) (complete response + partial response (+ MR for mRECIST/mSWOG)), stable disease (SD), and progressive disease (PD) were 28%, 49%, and 24%, respectively according to RECIST; 25%, 49%, and 26%, respectively according to SWOG; 44%, 33%, and 24%, respectively according to mRECIST; and 45%, 29%, and 26%, respectively according to mSWOG. In patients who had OR, SD or PD, the median PFS was 26–30, 27–34, and 8 months, respectively, with any of the four response criteria. In patients who had OR, SD or PD, the median OS was 55–57, 56–74, and 11–12 months, respectively, with any of the four response criteria. Subanalyses for patients who had progression before treatment start were comparable.

Conclusion

Patients with PD as treatment outcome had significantly shorter PFS and OS than patients with an OR or SD with all four scoring systems. PFS and OS were comparable for patients with tumor regression and SD. The addition of the response class MR did not improve the correlation with PFS and OS. The four scoring systems gave comparable results in terms of PFS and OS per categorized outcome.

Key Words: neuroendocrine tumor; peptide receptor radionuclide therapy; [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate; treatment response

INTRODUCTION

Gastroenteropancreatic and thoracic neuroendocrine tumors (NETs) are rare neoplasms that usually grow slowly and have a relatively indolent course. Surgery is the only potential for cure. Often, these tumors are metastasized at diagnosis. Treatment options for metastasized disease include somatostatin analogs, chemotherapy, newer targeted therapies such as sunitinib (Sutent; Pfizer Inc, New York, NY), a tyrosine kinase inhibitor[1], or everolimus (Afinitor; Novartis Pharmaceuticals, Basel, Switzerland), an inhibitor of mammalian target of rapamycin (mTOR)[2], peptide receptor radionuclide therapy (PRRT), or liver-directed therapies (in case of predominant liver disease), such as chemoembolization, embolization, or Radiofrequency Ablation (RFA).

PRRT with radiolabeled somatostatin analogs is currently available in a number of, mostly European, centers, and has shown promising results in the treatment of NETs.[3-7]

Tumor response assessment after treatment of NETs is mostly done by imaging with computed tomography (CT) or magnetic resonance imaging (MRI). Several response criteria can be used for this purpose, including the Response Evaluation Criteria in Solid Tumors (RECIST)[8] (unidimensional), the Southwest Oncology Group (SWOG) solid tumor response criteria[9] (bidimensional), and their modified variants. It is not known what criteria correlates best with survival in patients with NETs.

In the current study, RECIST, SWOG, modified RECIST (mRECIST) and modified SWOG (mSWOG) criteria were compared in patients with NETs treated with [^{177}Lu-DOTA^{0},Tyr^{3}] octreotate (^{177}Lu-octreotate).

METHODS

Patients

Dutch patients with gastroenteropancreatic and thoracic NETs who had been treated with ^{177}Lu-octreotate according to protocol in our institution between January 2000 and April 2007 were retrospectively selected. Treatment until April 2007 was used as cutoff date to allow for a sufficient follow-up time. Only Dutch patients were selected, because loss to follow-up is very limited in this patient group. This study is part of the ongoing prospective study in patients with NETs treated with ^{177}Lu-octreotate at the Department of Nuclear Medicine, Erasmus University Medical Center Rotterdam, which was approved by the local medical ethical committee. All patients gave written informed consent to participate in the study.

Treatment

[DOTA^{0},Tyr^{3}]octreotate was obtained from BioSynthema (St Louis, MO, USA). $^{177}LuCl_3$ was distributed by IDB-Holland (Baarle-Nassau, the Netherlands). ^{177}Lu-octreotate was locally prepared as described previously.[10] Granisetron (Kytril®; Roche, Woerden, the Netherlands) 3

mg was injected intravenously 30 minutes before starting the infusion of ^{177}Lu-octreotate. To reduce the radiation dose to the kidneys, an infusion of amino acids (arginine 2.5% and lysine 2.5%, 1 liter) was started 30 minutes before the administration of the radiopharmaceutical and lasted 4 hours. The radiopharmaceutical was co-administered, using a second pump system. Cycle doses were 3.7 or 7.4 GBq, depending on short-term toxicity, injected over 30 minutes. The intended interval between treatments was 6-10 weeks. Patients were treated up to a cumulative intended dose of 22.2–29.6 GBq. If dosimetric calculations indicated that the radiation dose to the kidneys would exceed 23 Gy with a dose of 29.6 GBq, the cumulative dose was reduced to 22.2–27.8 GBq. Routine hematology, liver and kidney function tests were performed before each therapy and at follow-up visits.

Tumor Response

Tumor response assessment was done according to the RECIST criteria[8], to the SWOG criteria[9], and to the mRECIST and mSWOG criteria, in which the tumor response class minor response (MR) was added, pertaining to a decrease of 13–30% for the mRECIST criteria and a decrease of 25–50% for the mSWOG criteria. Table 1 lists the criteria and definitions specified by the RECIST and SWOG criteria.

Tumor lesions had been scored according to the SWOG criteria as part of the ongoing prospective study in patients with NETs treated with ^{177}Lu-octreotate at our institution. For this study, we extracted the longest diameter from the bidimensional measurement for reclassification according to the RECIST criteria. Normally, a maximum of 5 lesions per organ and 10 lesions in total should be used for the RECIST criteria, and a maximum of 3 lesions per organ for the SWOG criteria (total maximum number of lesions is not stated in the SWOG criteria). Because lesions had been scored according to the SWOG criteria as part of the ongoing prospective study, a maximum of 3 lesions per organ was available. Therefore, a maximum of 3 instead of 5 lesions per organ was used for RECIST. Tumor response assessment at 3 months after the last treatment with ^{177}Lu-octreotate was used for this analysis. Tumor response had to be confirmed on a subsequent CT/MRI scan, except for progressive disease (PD). Response categories were: complete response (CR), partial response (PR), stable disease (SD), and PD for the RECIST and SWOG criteria; and CR, PR, MR, SD and PD for the mRECIST and mSWOG criteria. If a patient had only one follow-up scan (and thus no confirmatory scan), and was hereafter lost to follow-up, the tumor response was unknown. However, if a patient died after one follow-up scan, the tumor response was PD. Death or evident clinical progression during treatment or before a CT/MRI scan was made, was defined as PD. Normal follow-up of patients treated with ^{177}Lu-octreotate consists of a CT/MRI at 6 weeks, 3 months, and 6 months after the last treatment, and thereafter every 6 months. Baseline CT/MRI is done within 3 months before start of the treatment with ^{177}Lu-octreotate. Contrast-enhanced CT or gadolinium-enhanced MRI was used for response assessment, unless there was a clinical contra-indication for the use of contrast.

Table 1. Criteria and definitions of response assessment according to the RECIST and SWOG criteria.

	RECIST	SWOG
Disease status	* Measurable lesion: ≥10 mm with spiral CT (longest diameter to be recorded)	* Measurable disease: bidimensionally measurable lesions with clearly defined margins by CT/MRI with both diameters >5 mm
	* Nonmeasurable lesion: all other lesions, including small lesions (<10 mm with spiral CT)	* Evaluable disease: Unidimensionally measurable lesions, masses with margins not clearly defined, lesions with diameters <5 mm, bone disease
	* Truly nonmeasurable lesions: bone lesions, leptomeningeal disease, ascites, pleural/ pericardial effusion, inflammatory breast disease, lymphangitis cutis/ pulmonis, abdominal masses that are not confirmed and followed by imaging techniques, and cystic lesions	* Nonevaluable disease: pleural effusions, ascites, disease documented by indirect evidence only (e.g. by lab values)
	"Target" lesions: all measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total → should be measured at baseline and during follow-up	Maximum of 3 lesions per organ, total maximum number of lesions is not stated
	"Nontarget" lesions: all other lesions (or sites of disease) → no measurements, but presence/ absence should be noted during follow-up	
Response criteria		
Complete Response	Disappearance of all target lesions + disappearance of all nontarget lesions and normalization of tumor marker level	Complete disappearance of all measurable and evaluable disease; no new lesions; no disease related symptoms; no evidence of nonevaluable disease, including normalization of markers and other abnormal lab values
Partial Response	≥30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter	≥50% decrease under baseline in the sum of products of perpendicular diameters of all measurable lesions; no progression of evaluable disease; no new lesions
Stable Disease	Neither sufficient decrease to qualify for PR, nor sufficient increase to qualify for PD, taking as reference the smallest sum longest diameter since start of treatment	Not qualifying for CR/PR/PD

	RECIST	SWOG
Response criteria		
Progressive Disease	≥20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since start of treatment, OR the appearance of a new lesion (target or nontarget lesion), OR the unequivocal progression of existing nontarget lesions*	≥50% increase or an increase of 10 cm^2 (whichever is smaller) in the sum of products of all measurable lesions over smallest sum observed (over baseline if no decrease), OR clear worsening of any evaluable disease, OR reappearance of any lesion which had disappeared, OR appearance of any new lesion/site, OR failure to return for evaluation due to death or deteriorating condition (unless clearly unrelated to this cancer)
Best Response	CR/PR has to be confirmed on a subsequent CT scan†	CR/PR/SD has to be confirmed on a subsequent CT scan

* In contrast to the original RECIST guidelines, we included failure to return for evaluation due to death or deteriorating condition, to the PD group.

† In contrast to the original RECIST guidelines, where a confirmatory CT scan is needed only for CR+PR, for our study SD also had to be confirmed on a subsequent CT scan.

RECIST: Response Evaluation Criteria in Solid Tumors; SWOG: Southwest Oncology Group solid tumor response criteria; CT: computed tomography; MRI: magnetic resonance imaging; PR: partial response; PD: progressive disease; CR: complete response; SD: stable disease.

Statistical Analysis

Progression-free survival (PFS) and overall survival (OS) were calculated. First of January 2010 was used as cutoff date. PFS was defined as the time from the first treatment with ^{177}Lu-octreotate until the time of progression (radiological or clinical) or death from any cause. For PFS analysis, patients were censored in case of no progression at the time of the last tumor assessment by CT/MRI before the cutoff date or lost to follow-up. OS was defined as the time from the first treatment with ^{177}Lu-octreotate until date of death from any cause. For OS analysis, patients were censored if alive at the last date of follow-up before the cutoff date or lost to follow-up. Survival curves were estimated with the Kaplan-Meier method. The different response outcome categories were compared with the Log-rank test.

Intercriterion agreement between the different response criteria was assessed using the Cohen's κ statistics. The intercriterion agreement based on the κ statistic was interpreted as follows: κ of 0.0–0.20, slight agreement; κ of 0.21–0.40, fair; κ of 0.41–0.60, moderate; κ of 0.61–0.80, substantial; κ of 0.81–1.00, almost perfect.[11] The discriminative ability of the response criteria was assessed using the C-index.[12] This index can be seen as a natural extension of the area under the receiver operating characteristic curve for survival analysis. A C index of ≤0.5 indicates prediction no better than chance, and values from 0.5–1.0 (perfect prediction) indicate improvement over chance.[13]

The SPSS (SPSS 15.0, Chicago, IL) and R (Terry Therneau (2012); A Package for Survival Analysis in S; R package version 2.36–14) packages were used. Two-sided p-values are reported. P-values <0.05 were considered to be significant.

RESULTS

Two-hundred eighty-one Dutch patients with gastroenteropancreatic and thoracic NETs had been treated with ^{177}Lu-octreotate according to protocol in our institution between January 2000 and April 2007. Thirteen patients were excluded from this study for the following reasons: only measurable bone lesions (n=5), lesions could not be clearly delineated on CT (n=3), only a written CT report of the CT performed after treatment was available (not the CT images themselves) (n=2), MRI at baseline and follow-up by CT (n=1), baseline images not available (n=1), no measurable lesions on CT (only on [^{111}Indium-DTPA^{0}]octreotide scintigraphy) (n=1).

Thus, 268 patients were evaluated. Baseline characteristics are presented in table 2. There were 138 men and 130 women. Mean age was 59 years (range 23–83). Imaging had been performed with CT in 260 patients, and with MRI in 8 patients. A total of 562 lesions were assessed: 430 liver lesions, 53 primary tumors, 46 lymph nodes, 10 total liver (this was done if single liver lesions could not be measured separately, because all lesions were coalesced), 7 pulmonary lesions, and 16 other soft tissue lesions. All lesions had a baseline longest diameter of ≥10 mm and hence met the definition of a measurable lesion for RECIST.

Tumor Response

Eleven patients, who were all lost to follow-up, had an unknown tumor response and were excluded. The rates of objective response (OR) (CR+PR (+ MR for mRECIST/mSWOG)), SD, and PD were 28% (71/257), 49% (125/257), and 24% (61/257), respectively according to RECIST; 25% (65/257), 49% (125/257), and 26% (67/257), respectively according to SWOG; 44% (112/257), 33% (84/257), and 24% (61/257), respectively according to mRECIST; and 45% (115/257), 29% (75/257), and 26% (67/257), respectively according to mSWOG (Table 3).

Intercriterion Agreement and C Index

Intercriterion agreement by using the Cohen's κ statistics showed good correlation between RECIST and SWOG criteria (κ=0.76 (95% Confidence Interval (CI): 0.69–0.83)), between mRECIST and mSWOG criteria (κ=0.78 (95% CI: 0.71–0.84)), and also between RECIST and mRECIST criteria (κ=0.76 (95% CI: 0.69–0.83)), and between SWOG and mSWOG criteria (κ=0.71 (95% CI: 0.64–0.78)).

The C indices for prediction of PFS were similar for the four response criteria with values of 0.74 (95% CI: 0.70–0.78) for RECIST, 0.72 (95% CI: 0.68–0.76) for SWOG, 0.73 (95% CI:

0.68–0.77) for mRECIST, and 0.72 (95% CI: 0.68–0.76) for mSWOG. Also for OS, the C indices were similar for the four response criteria with values of 0.66 (95% CI: 0.61–0.71) for RECIST, 0.68 (95% CI: 0.63–0.72) for SWOG, 0.66 (95% CI: 0.61–0.71) for mRECIST, and 0.66 (95% CI: 0.61–0.71) for mSWOG. The C indices for PFS were higher than those for OS for all response criteria.

Survival

Eleven patients who had an unknown tumor response were excluded from this analysis. According to RECIST, a total of 206 patients had progression or died (whichever came first). Median PFS for RECIST was 23 months (95% CI: 20–26) for the total group. According to SWOG, a total of 204 patients had progression or died (whichever came first). Median PFS according to SWOG was 23 months (95% CI: 20–26) for the total group. In patients who had OR, SD or PD the median PFS was 26, 33, and 8 months ($p<0.001$, Log-rank test), respectively according to RECIST; 30, 27, and 8 months ($p<0.001$, Log-rank test), respectively according to SWOG; 27, 34, and 8 months ($p<0.001$, Log-rank test), respectively according to mRECIST; and 28, 28, and 8 months ($p<0.001$, Log-rank test), respectively according to mSWOG (Figure 1). The addition of the response class MR did not improve the correlation with PFS.

A total of 145 patients died. Median OS was 51 months (95% CI: 45–57) for the total group. In patients who had OR, SD or PD the median OS was 55, 56, and 11 months ($p<0.001$, Log-rank test), respectively according to RECIST; 57, 63, and 12 months ($p<0.001$, Log-rank test), respectively according to SWOG; 55, 64, and 11 months ($p<0.001$, Log-rank test), respectively according to mRECIST; and 55, 74, and 12 months ($p<0.001$, Log-rank test), respectively according to mSWOG (Figure 2). As for the PFS, the addition of the response class MR did not improve the correlation with OS.

Subanalyses for patients who had progression (based on radiological imaging (not always RECIST or SWOG based) or clinical progression) in the 12 months before treatment with ^{177}Lu-octreotate and those who had not, showed comparable results as for the total group analysis (Figure 3 and 4). Subanalyses for patients with different tumor types showed that in patients with a nonfunctional pancreatic NET, PFS and OS were longest in patients with OR (Figure 5). Especially in patients with midgut NETs longest PFS and OS were observed in patients with SD as tumor outcome (Figure 5).

Table 2. Baseline characteristics of Dutch patients with a gastroenteropancreatic or thoracic neuroendocrine tumor who had been treated with ^{177}Lu-octreotate according to protocol between January 2000 and April 2007 (n=268).

	Yes	No	
Characteristic	**No of patients (%)**	**No of patients (%)**	
Male	138 (52)	130 (49)	
Mean age (years) (range)			59 (23–83)
Primary tumor			
Pancreatic NET	72 (27)		
Nonfunctional	61 (85)		
Functional	11 (15)		
Gastrointestinal or thoracic NET	178 (66)		
Foregut	22 (12)		
Midgut	145 (82)		
Hindgut	11 (6)		
Unknown	18 (7)		
Previous therapy	203 (76)	65 (24)	
Octreotide	142 (53)	126 (47)	
Surgery	118 (44)	150 (56)	
Chemotherapy	26 (10)	242 (90)	
Radiotherapy	10 (4)	258 (96)	
Median administered dose in GBq (range)			29.6 (7.4–30.7)
Liver metastases	237 (88)	31 (12)	
Bone metastases	55 (21)	213 (80)	
Tumor uptake on Octreoscan			
Equal to normal liver	9 (3)		
> Normal liver	194 (72)		
> Kidneys	65 (24)		

NET: neuroendocrine tumor; GBq: gigabecquerel.

Table 3. Tumor response confirmed on 3 months according to RECIST/SWOG/mRECIST/mSWOG (n=257)**.**

	CR	PR	MR	SD	PD	OR
RECIST	3 (1%)	68 (27%)		125 (49%)	61 (24%)	71 (28%)
SWOG	3 (1%)	62 (24%)		125 (49%)	67 (26%)	65 (25%)
mRECIST	3 (1%)	68 (27%)	41 (16%)	84 (33%)	61 (24%)	112 (44%)
mSWOG	3 (1%)	62 (24%)	50 (20%)	75 (29%)	67 (26%)	115 (45%)

RECIST: Response Evaluation Criteria in Solid Tumors; SWOG: Southwest Oncology Group solid tumor response criteria; mRECIST: modified RECIST; mSWOG: modified SWOG; CR: complete response; PR: partial response; MR: minor response; SD: stable disease; PD: progressive disease; OR: objective response (CR + PR (+ MR for mRECIST/mSWOG)).

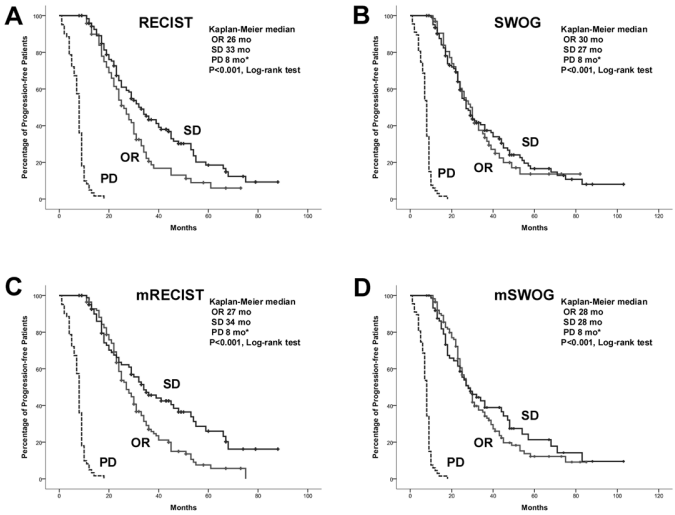

Figure 1. Progression-free survival (PFS) in 257 patients with NETs. A significant difference in the median PFS was observed for patients with OR, SD or PD according to RECIST (**A**), SWOG (**B**), mRECIST (**C**), and mSWOG (**D**).

* Significant difference in the median PFS between PD and either of the other response categories.
mRECIST: modified RECIST; mSWOG: modified SWOG; OR: objective response (complete response + partial response (+ minor response for mRECIST/mSWOG)); PD: progressive disease; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease; SWOG: Southwest Oncology Group solid tumor response criteria.

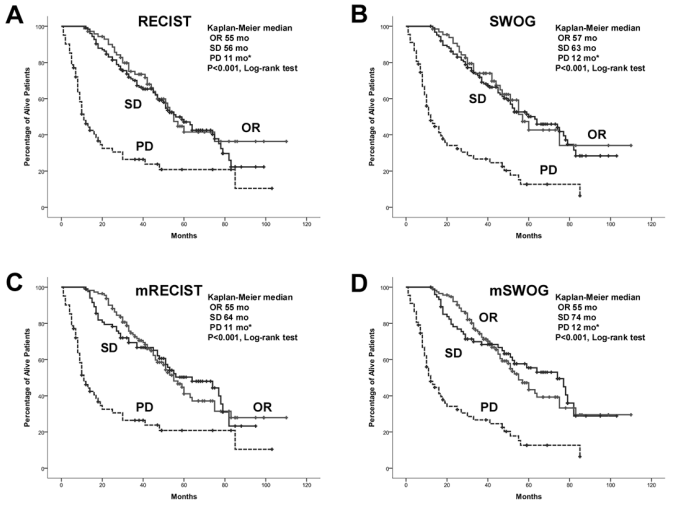

Figure 2. Overall survival (OS) in 257 patients with NETs. A significant difference in the median OS was observed for patients with OR, SD or PD according to RECIST (**A**), SWOG (**B**), mRECIST (**C**), and mSWOG (**D**).

* Significant difference in the median OS between PD and either of the other response categories.
mRECIST: modified RECIST; mSWOG: modified SWOG; OR: objective response (complete response + partial response (+ minor response for mRECIST/mSWOG)); PD: progressive disease; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease; SWOG: Southwest Oncology Group solid tumor response criteria.

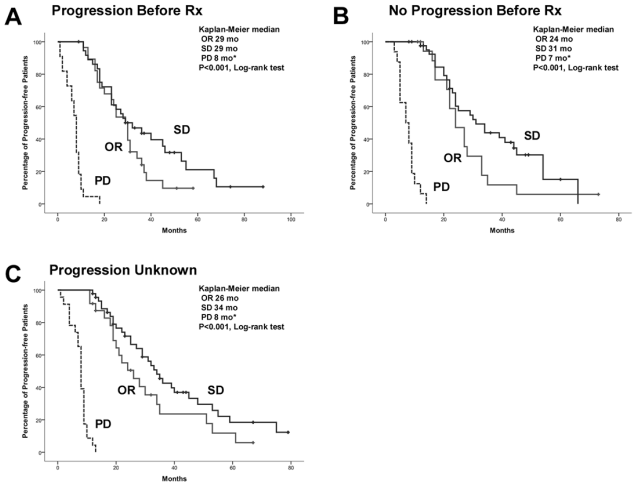

Figure 3. Progression-free survival (PFS) based on RECIST in patients with NETs with progression before treatment (n=87) (**A**), without progression before treatment (n=78) (**B**), and in patients in whom it was unknown if they had progression before treatment (n=92) (**C**). Response categories according to RECIST (OR, SD, and PD). PFS analyses with response categories according to SWOG, mRECIST, and mSWOG, respectively, gave comparable results.

* Significant difference in the median PFS between PD and either of the other response categories. OR: objective response (complete response + partial response (+ minor response for mRECIST/mSWOG)); PD: progressive disease; Rx: treatment; SD: stable disease.

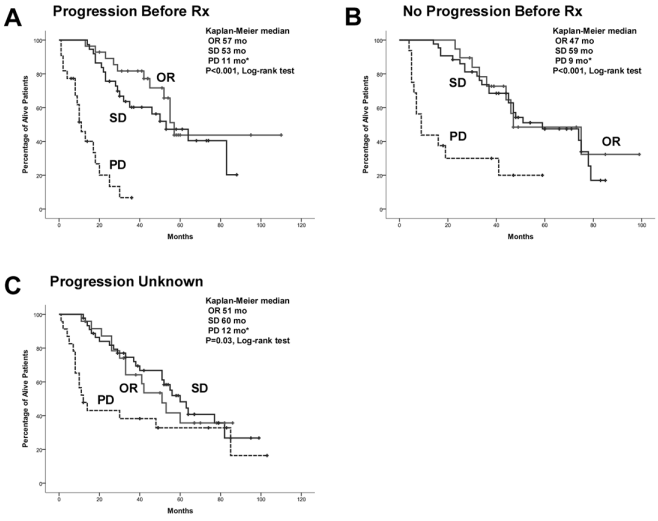

Figure 4. Overall survival (OS) in patients with NETs with progression before treatment (n=87) (**A**), without progression before treatment (n=78) (**B**), and in patients in whom it was unknown if they had progression before treatment (n=92) (**C**). Response categories according to RECIST (OR, SD, and PD). OS analyses with response categories according to SWOG, mRECIST, and mSWOG, respectively, gave comparable results.

* Significant difference in the median OS between PD and either of the other response categories.
OR: objective response (complete response + partial response (+ minor response for mRECIST/mSWOG)); PD: progressive disease; Rx: treatment; SD: stable disease.

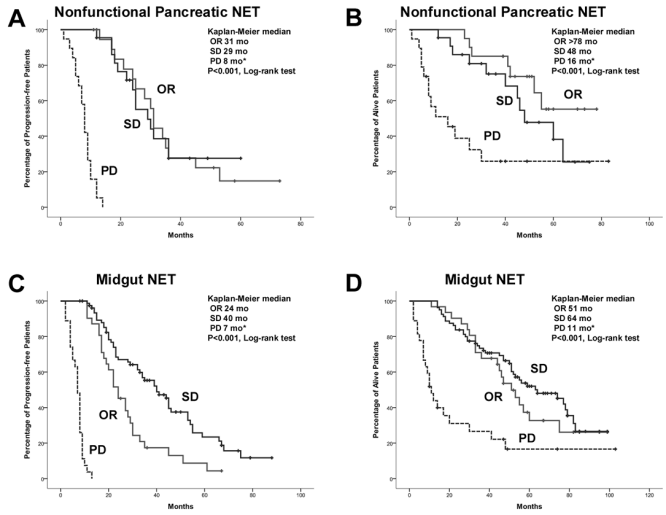

Figure 5. Progression-free survival (PFS) based on RECIST (**A**) and overall survival (OS) (**B**) in 61 patients with a nonfunctional pancreatic NET. A significant difference in the median PFS and OS, respectively, was observed for patients with OR, SD or PD. Patients with OR had the longest PFS and OS, respectively. PFS based on RECIST (**C**) and OS (**D**) in 138 patients with a midgut NET. A significant difference in the median PFS and OS, respectively, was observed for patients with OR, SD or PD. Patients with SD had the longest PFS and OS, respectively. Response categories according to RECIST. Analyses with response categories according to SWOG, mRECIST, and mSWOG, respectively, gave comparable results.

* Significant difference in the median PFS, and OS, respectively, between PD and either of the other response categories.
NET: neuroendocrine tumor; OR: objective response (complete response + partial response (+ minor response for mRECIST/mSWOG)); PD: progressive disease; SD: stable disease.

DISCUSSION

In this study we compared four different response criteria, i.e. RECIST, SWOG, mRECIST, and mSWOG, in the tumor response assessment in patients with NETs treated with ^{177}Lu-octreotate. The RECIST and SWOG criteria gave comparable results, with a good correlation as indicated by the Cohen's κ statistic. The same held true for the mRECIST and mSWOG criteria. Patients with PD as treatment outcome had significantly shorter PFS and OS than patients with an OR or SD with all four scoring systems. PFS and OS were comparable for patients with tumor regression and SD.

Tumor response assessment by imaging is regarded as the most objective response assessment available nowadays. In the response assessment of NETs both RECIST[1-2, 7, 14] and SWOG criteria[4-6] are widely used. To our knowledge, this is the first report to compare these two criteria for NETs. Our data indicate that the application of the RECIST or SWOG criteria gives the same results, and predicts PFS and OS in a comparable way. Furthermore, the

modified variants (i.e. mRECIST and mSWOG) did not improve the correlation with PFS and OS.

In other tumor types, modified response criteria were able to predict survival better than the 'classical' criteria. In the response assessment of hepatocellular carcinoma after treatment with Sorafenib (a multikinase inhibitor), the application of modified RECIST criteria, based on the unidimensional measurement of only the contrast-enhanced portion of a hepatic lesion at the arterial phase on CT, resulted in better prediction of OS than with the application of the classical RECIST criteria.[15] Also in the response assessment of hepatocellular carcinoma after chemoembolization, the modified RECIST criteria as explained above and the European Association for the Liver (EASL) criteria, based on the bidimensional measurement of only the contrast-enhanced portion of a hepatic lesion at the arterial phase on CT, resulted in better prediction of OS than the classical criteria.[16]

Other 'new' criteria are the Choi criteria which are developed for the assessment of gastrointestinal stromal tumors (GIST) to treatment with imatinib mesylate.[17] The Choi criteria are based on quantification of change in both tumor size and density on CT. A decrease in tumor size of more than 10% or a decrease in tumor density of more than 15% on CT is defined as good response. Good responders on CT at 2 months had significantly longer time to progression than those who did not respond.[17]

Some of these new criteria have also been applied to NET patients. The EASL criteria have been applied to patients with NETs with liver metastases treated with hepatic arterial chemoembolization with doxorubicin-eluting beads[18-19] or with ^{90}Y radioembolization.[20] The Choi criteria have been applied to a patient with a pancreatic NET treated with Sunitinib and Octreotide LAR. In that patient, a response could be demonstrated by using Choi criteria, but not by RECIST criteria.[21]

For the four response criteria investigated in this study, the patients with SD as treatment outcome had a comparable OS to patients with OR as treatment outcome. For PFS, according to SWOG patients with OR had a longer PFS (30 months) than patients with an SD (27 months); for mSWOG PFS was the same for SD and OR patients. However, RECIST and mRECIST showed better PFS for SD than for OR patients. This is an unexpected finding. We performed subanalyses for patients with and without progression before treatment with ^{177}Lu-octreotate, and for different tumor types to further explore this finding. Subanalyses for patients with and without progression before treatment with ^{177}Lu-octreotate showed comparable results as for the total group analysis. For the nonfunctional pancreatic NETs, OS and, to a lesser extent, PFS, was longer for OR than for SD patients. Subanalyses for functional pancreatic NETs were not reliable, since this group was too small to permit valid comparisons. Also the groups of patients with a foregut NET and with a hindgut NET were too small to permit valid conclusions. Especially in the midgut NET patients, SD patients had a comparable PFS and OS to OR patients. This could be explained by the slow-growing nature of midgut NETs, which can be stable for several years. In this sense, it is questionable

whether these patients have benefited from the treatment with ^{177}Lu-octreotate at all, or that they also would have remained stable without treatment. Patients with SD as treatment outcome may have different patient or tumor characteristics, which could explain the difference in survival. One such tumor characteristic could be the Ki67 proliferative index, which has proven to be an important prognostic factor for survival in patients with midgut NETs.[22-23] However, the Ki67 index was not available for most patients, because it was not determined routinely in our institution before 2007. In a subsequent analysis, carried out in patients treated after April 2007, we determined the distribution of WHO grading[24] (which incorporates the Ki67 index) in patients with a nonfunctional pancreatic NET and those with a midgut NET. Midgut NETs had significantly more often a low proliferation rate than nonfunctional pancreatic NETs (WHO grade 1 (Ki67: 0–2%): 24 patients, WHO grade 2 (Ki67 >2–20%): 18 patients for midgut NETs vs WHO grade 1: 8 patients, WHO grade 2: 30 patients, WHO grade 3 (Ki67 >20%): 2 patients for nonfunctional pancreatic NETs, p=0.001, Fisher's exact test using Monte Carlo method), supporting the hypothesis above.

Next to morphologic assessment, tumor response assessment can also be performed by functional imaging, e.g. by positron emission tomography (PET) imaging. For NETs, PET can be performed with ^{68}Ga-DOTA-Tyr^{3}-octreotide[25], 6-^{18}F-fluoro-L-DOPA[26-27], or ^{11}C-5-hydroxytryptophan[26], amongst others. Combining PET with CT gives anatomic and functional information on tumors in a single examination. This may very well be the future of imaging in NETs. However, PET with some of these radiopharmaceuticals is not widely available.

Furthermore, volumetric evaluation of tumors, i.e. 3D assessment instead of 1D (unidimensional) or 2D (bidimensional) assessment, has been suggested as a better method for evaluating tumor size.[28] However, because there are no standardized response criteria for 3D assessment of tumors and since this application is not available in many centers, 3D assessment of tumors is not integrated in routine clinical practice, to date.

Although the retrospective character is an inherited limit of this study, we feel that the long follow-up time and large number of patients and events, permit valid conclusions to be made.

CONCLUSION

In conclusion, patients with PD as treatment outcome had significantly shorter PFS and OS than patients with an OR or SD with all four scoring systems. PFS and OS were comparable for patients with tumor regression and SD. The addition of the response class MR did not improve the correlation with PFS and OS. The four scoring systems gave comparable results in terms of PFS and OS per categorized outcome.

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Chapter 5

Neoadjuvant Treatment of Nonfunctioning Pancreatic Neuroendocrine Tumors with [^{177}Lu-DOTA^{0},Tyr^{3}]Octreotate

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ABSTRACT

Objective

To describe the neoadjuvant use of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-octreotate) in nonfunctioning pancreatic neuroendocrine tumors (NETs).

Background Data

Pancreatic NETs are rare neoplasms, for which surgery is the only potential for cure. When surgery is not possible due to tumor size and vascular involvement, neoadjuvant treatment with ^{177}Lu-octreotate may be an option.

Methods

We studied 119 Dutch patients with a pathology-proven nonfunctioning pancreatic NET treated with ^{177}Lu-octreotate. Patients were divided into 3 groups: borderline or irresectable pancreatic tumor (group 1); ≤3 liver metastases (group 2); >3 liver metastases/other distant metastases (group 3). Patients in group 1 + 2 were considered neoadjuvant treated patients. Progression-free survival (PFS) was analyzed using the Kaplan-Meier method and Cox proportional hazards modelling.

Results

Successful surgery after ^{177}Lu-octreotate was performed in 10/119 studied patients (8%). In the neoadjuvant treated patients, surgery was performed in 9/29 patients (31%). Seventy-three patients had progression or died. WHO grading was the strongest predictor of progression ($p<0.001$). Surgery after ^{177}Lu-octreotate was associated with a lower risk of progression at univariate analysis ($p=0.02$). Although significance was not achieved for surgery after ^{177}Lu-octreotate at multivariate analysis (hazard ratio 0.31 for progression (95% Confidence Interval: 0.09-1.03), $p=0.06$), results point to a progression-free survival advantage for the operated patients. The median PFS was 69 months for patients in group 1 + 2 with successful surgery, 49 months for the other patients in group 1+2, and 25 months for patients in group 3 ($p=0.01$, Log-rank test).

Conclusions

Neoadjuvant treatment with ^{177}Lu-octreotate is a valuable option for patients with initially irresectable pancreatic NETs. Our data suggest that successful surgery after ^{177}Lu-octreotate is associated with increased PFS.

INTRODUCTION

Pancreatic neuroendocrine tumors (NETs) are rare neoplasms. They account for approximately 1.3–2% of all pancreatic cancers in incidence[1-2], whereas they represent almost 10% of pancreatic cancers in prevalence analyses,[1] due to their slow-growing nature. Despite the fact that these tumors have an 'indolent' nature, survival of patients with metastatic disease is limited with an overall 5-year survival of approximately 35%.[3-4]

In case of metastatic disease, treatment options for pancreatic NETs may include streptozocin-based chemotherapy[5-6], chemotherapy with capecitabine and temozolomide,[7] peptide receptor radionuclide therapy (PRRT), or, in case of predominant liver disease, liver-directed therapies, such as debulking surgery, chemoembolization, embolization, radioembolization, or radiofrequency ablation (RFA). Newer treatment options include sunitinib (Sutent; Pfizer Inc, New York, NY), a tyrosine kinase inhibitor[8], or everolimus (Afinitor; Novartis Pharmaceuticals, Basel, Switzerland), an inhibitor of mammalian target of rapamycin (mTOR).[9]

Still, surgery remains the only potential to cure patients with pancreatic NETs. However, surgery is often not possible due to either vascular involvement or the presence of distant metastases. A few case reports have described the use of PRRT as neoadjuvant treatment in patients with pancreatic NETs.[10-14] Here we describe our experience with treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-octreotate) in a neoadjuvant setting in a large series of patients with nonfunctioning pancreatic NETs with a long follow-up.

METHODS

Patients

From the Dutch patients who had been treated with ^{177}Lu-octreotate in our institution between January 2000 and June 2011, we retrospectively selected patients: 1) with a pathology-proven nonfunctioning pancreatic NET; 2) who had completed the treatment with ^{177}Lu-octreotate, unless the patient died earlier or had evident clinical progressive disease (PD) during treatment; and 3) who had had a minimum of 2 follow-up CTs, unless the patient died earlier or had a PD as treatment outcome. Nonfunctioning pancreatic NETs were defined by the absence of a clinical syndrome caused by hormonal hypersecretion. Only Dutch patients were selected, because loss to follow-up is very limited in this patient group. Patients were divided into 3 groups: borderline or irresectable pancreatic tumor (group 1); tumor with ≤3 liver metastases (group 2); tumor with >3 liver metastases, other distant metastases and/or a Whipple procedure/distal pancreatectomy before ^{177}Lu-octreotate (group 3). Patients in group 2 all had advanced pancreatic NETs with either borderline or irresectable primary tumors. Both group 1 and 2 were regarded as receiving ^{177}Lu-octreotate in a neoadjuvant setting. Patients with a Whipple procedure/distal pancreatectomy before

^{177}Lu-octreotate were included in group 3, because their primary tumor was already resected and hence ^{177}Lu-octreotate was not given in a neoadjuvant setting.
In this study, nonfunctioning pancreatic NETs were staged according to the Varadhachary/Katz CT staging system.[15-16] Briefly, tumors with arterial (superior mesenteric artery, coeliac axis, or common hepatic artery) abutment (<90° contact) and/or venous (portal or superior mesenteric vein) involvement with short segment occlusion with possible reconstruction were considered borderline resectable. Tumors with >90° arterial encasement with no technical option for reconstruction or venous occlusion, and/or tumor thrombus over a long segment were considered irresectable.
This study is part of the ongoing prospective study in patients with gastroenteropancreatic NETs treated with ^{177}Lu-octreotate at the Department of Nuclear Medicine, Erasmus University Medical Center Rotterdam, which was approved by the local medical ethical committee. All patients gave written informed consent to participate in the study.

Treatment

[DOTA^{0},Tyr^{3}]octreotate was obtained from BioSynthema (St Louis, MO, USA). $^{177}LuCl_3$ was distributed by IDB-Holland (Baarle-Nassau, the Netherlands). ^{177}Lu-octreotate was locally prepared as described previously.[17] Granisetron 3 mg was injected intravenously 30 minutes before starting the infusion of ^{177}Lu-octreotate. To reduce the radiation dose to the kidneys, an infusion of amino acids (arginine 2.5% and lysine 2.5%, 1 liter) was started 30 minutes before the administration of the radiopharmaceutical and lasted 4 hours. The radiopharmaceutical was co-administered using a second pump system. Cycle doses were 7.4 GBq, injected over 30 minutes. The intended interval between treatments was 6–10 weeks. Patients were treated up to a cumulative intended dose of 22.2–29.6 GBq. Routine hematology, liver, and kidney function tests were performed before each therapy and at follow-up visits.

In Vivo Measurements

Tumor response assessment was done according to the Southwest Oncology Group (SWOG) solid tumor response criteria[18] with the addition of the tumor response class minor response (MR), pertaining to a decrease of 25–50%. Response categories had to be confirmed on a subsequent CT scan, except for PD.

Grading

Tumors were classified according to the ENETS-WHO 2010 grading system.[19-20] The Ki67 proliferative index was assessed according to standard procedures in 2,000 tumor cells (except in four patients) in areas with the highest nuclear labeling using the MIB1 antibody (Dako, Glostrup, Denmark). Ki67 values were determined on biopsies and resection specimens obtained before the treatment with ^{177}Lu-octreotate. Because these data were not available in 7 patients with successful surgery after ^{177}Lu-octreotate, we decided to use

the Ki67 values on the resection specimens obtained after ^{177}Lu-octreotate in these patients to allow all patients with successful surgery to be included in the Cox proportional hazards analysis.

Statistics

Progression-free survival (PFS) and overall survival (OS) were calculated. First of June 2011 was used as cutoff date. PFS was defined as the time from the first treatment with ^{177}Lu-octreotate until the time of progression (radiological (according to SWOG) or clinical) or death from any cause. For PFS analysis, patients were censored in case of absence of progression at the time of the last tumor assessment by CT/MRI before the cutoff date or lost to follow-up. OS was defined as the time from the first treatment with ^{177}Lu-octreotate until the date of death from any cause. For OS analysis, patients were censored if alive at the last date of follow-up before the cutoff date or lost to follow-up. PFS and OS analyses were performed using the Kaplan-Meier method and the results were compared by the Log-rank test. Univariate and multivariate Cox proportional hazards modelling was conducted to evaluate parameters predictive for PFS.

The comparisons between the patients with and without surgery were carried out using chi-square tests (or, if applicable, Fisher's exact tests) for categorical variables, or Independent *t* tests or Mann-Whitney *U* tests for continuous variables. Two-sided p-values were reported. P-values <0.05 were considered significant. The SPSS statistical package (SPSS 15.0, Chicago, IL) was used.

RESULTS

Two hundred fourteen patients with a nonfunctioning pancreatic NET were treated with ^{177}Lu-octreotate in our institution between January 2000 and June 2011; 95 non-Dutch patients were excluded. So, 119 patients were evaluated. There were 54 men and 65 women. Mean age was 55 years (range 23–85). Group 1 comprised: 15 patients, group 2: 14 patients, and group 3: 90 patients (including 23 patients with a Whipple procedure/ distal pancreatectomy before ^{177}Lu-octreotate). Three patients, all in group 3, had the Multiple Endocrine Neoplasia Type-1 (MEN-1) syndrome; none of these had surgery after ^{177}Lu-octreotate. Eleven patients were also treated with the drug capecitabine (Xeloda®; Roche, Basel, Switzerland), according to a new treatment protocol as part of an ongoing randomized clinical trial as described previously[21]; none of these had surgery after ^{177}Lu-octreotate. None of the patients with successful surgery after ^{177}Lu-octreotate developed a serious delayed toxicity after ^{177}Lu-octreotate.

The tumor response at 3 months after the last treatment with ^{177}Lu-octreotate was: remission (complete response (CR) + partial response (PR) + MR) in 72 patients (61%), stable disease (SD) in 24 patients (20%), and PD in 21 patients (18%). Two patients had an unknown tumor response.

Ki67 values and WHO grading at baseline were available for 77 patients. In four patients, the Ki67 index was assessed in <2,000 tumor cells. Median Ki67 value was 6% (range 1–50). Fifteen patients had a G1 tumor (Ki67: 0–2%), 53 patients a G2 tumor (Ki67 >2–20%), and 9 patients a G3 tumor (Ki67 >20%).

Surgery After ^{177}Lu-octreotate

Successful surgery after treatment with ^{177}Lu-octreotate was performed in 10/119 patients (8%). In the neoadjuvant treated patients (i.e. all patients in group 1 and 2), successful surgery was performed in 9/29 patients (31%). The 10th patient had >3 liver metastases before treatment with ^{177}Lu-octreotate, and thus did not belong to group 1 or 2. There were no significant differences in patient or tumor characteristics prior to ^{177}Lu-octreotate between patients with and without successful surgery after ^{177}Lu-octreotate in the neoadjuvant treated patients (Table 1). All patients with successful surgery after ^{177}Lu-octreotate had a borderline or irresectable pancreatic tumor prior to ^{177}Lu-octreotate due to vascular involvement as judged before treatment start by a surgeon with expertise in pancreatic surgery (CvE) and by a radiologist. All patients were restaged after the treatment with ^{177}Lu-octreotate by the same, abovementioned, surgeon.

In addition to the 10 successfully operated patients, an attempt at resection was made in another patient; however, peroperatively extensive tumor invasion into the caval vein was found. Lastly, another patient, who had a PR after ^{177}Lu-octreotate, and whose tumor was judged resectable after ^{177}Lu-octreotate, refused surgery.

Patient and tumor characteristics of the 10 patients with successful surgery after ^{177}Lu-octreotate are presented in table 2. Six patients had a Whipple procedure (one in combination with a reconstruction of the portal vein with a biograft and one combined with a resection of a para-aortic lymph node and RFA of a liver lesion); two patients had a pylorus preserving pancreaticoduodenectomy (one in combination with a resection of a thrombus in the portal vein with an end-to-end portal anastomosis); and two patients had a distal pancreatectomy and splenectomy (one in combination with a metastasectomy of 3 liver metastases and coagulation of 2 other liver metastases (this patient belonged to group 3)). The median time between the last treatment with ^{177}Lu-octreotate and surgery was 11 months (range 7–33). No surgical complications related to ^{177}Lu-octreotate administration were observed. There was no perioperative mortality.

Pathology characteristics of the surgery specimens are presented in table 2. All resection specimens showed fibrosis/sclerosis or necrosis as a treatment effect of ^{177}Lu-octreotate (Figure 1).

Three patients had a local recurrence and/or developed liver metastases 22, 48, and 56 months, respectively, after surgery. The other seven patients were disease-free with a median follow-up of 7 months (range 0–54) after surgery. Figure 2 shows the clinical course in a patient who had a local recurrence and liver metastases 48 months after surgery. One patient died 33 months after surgery and the other nine patients were alive with a median follow-up of 40 months (range 0–84) after surgery.

Table 1. Baseline characteristics of patients with and without successful surgery after treatment with ^{177}Lu-octreotate in the neoadjuvant treated patients (n=29).

Characteristic	Successful surgery	Irresectable	*P* value
No. of patients	9	20	
Male	5	9	0.70
Mean age (years) (range)	52 (41–71)	56 (32–81)	0.46
Mean baseline longest diameter pancreatic tumor (in millimeter) (range)‡	72 (36–100)	69 (21–120)	0.79
Tumor uptake on octreoscan			
Equal to normal liver	0	2	0.47
> Normal liver	4	11	
> Kidneys	5	7	
Previous therapy	3	5	0.68
Octreotide	0	3	0.53
Surgery	3	4	0.64
Radiotherapy	0	1	1.00
Chemotherapy	0	0	NA
Median total administered dose (GBq) (range)	30.0 (22.3–30.3)	29.8 (11.2–30.2)	0.10
Regression (CR/PR/MR) as treatment outcome¥	8	11	0.11
Mean baseline AF (U/L) (range)¶	470 (210–954)	383 (134–1109)	0.74
Location pancreatic tumor			
Head	8	15	0.63
Body/tail	1	5	

‡ Data on longest diameter pancreatic tumor were available in all patients with successful surgery after ^{177}Lu-octreotate, and in 18/20 patients without successful surgery after ^{177}Lu-octreotate (in 2 patients only liver metastases could be measured); ¥ Treatment outcome pertaining to confirmed tumor response on 3 months after treatment according to the Southwest Oncology Group solid tumor response criteria; ¶ In patients with elevated levels at baseline (normal value alkaline phosphatase: <120 U/L).

NA: not applicable; GBq: gigabecquerel; CR: complete response; PR: partial response; MR: minor response; AF: alkaline phosphatase.

Survival

Seventy-three patients had progression or died. The median PFS in the total group of 119 patients was 30 months (95% Confidence Interval (CI): 23–38). The median PFS was 69 months for patients in group 1 and 2 with successful surgery, 49 months for the other patients in group 1 and 2, and 25 months for patients in group 3 (p=0.01, Log-rank test) (Figure 3). The difference in PFS between the operated patients in group 1 and 2, and the other patients in group 1 and 2 was not significant (p=0.22, Log-rank test). A total of 43 patients died. The median OS in the total group of 119 patients was 63 months (95% CI: 45-81). The median OS was more than 103 months for patients in group 1 and 2 with successful surgery, 60 months for the other patients in group 1 and 2, and 52 months for patients in group 3 (p=0.10, Log-rank test).

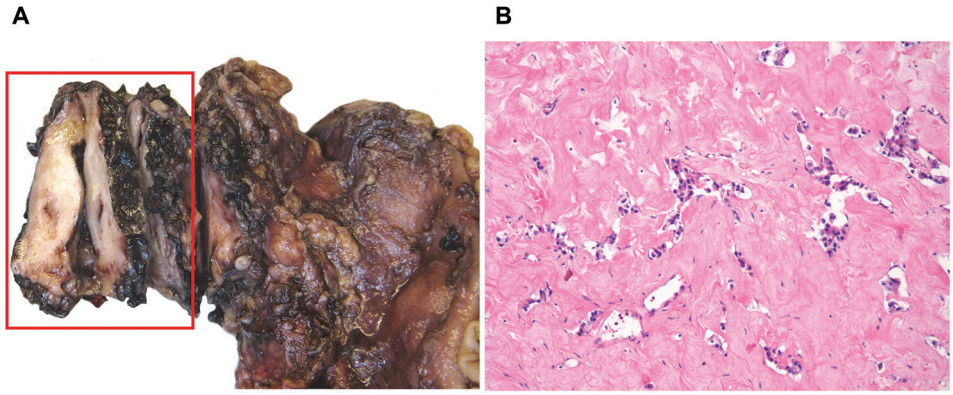

Figure 1. Macroscopic and microscopic appearances of the pancreatic resection specimen of patient 4, showing extensive ^{177}Lu-octreotate treatment effect, including sclerosis and hyalinization of the tumor. (**A**) Resection specimen of a pancreatic neuroendocrine tumor, showing hyalinization of the tumor (red square). (**B**) Hematoxylin and eosin (HE) staining of the same tumor showing extensive degenerative changes, including sclerosis and hyalinization of the tumor, with few remaining viable tumor cells. Magnification x100.

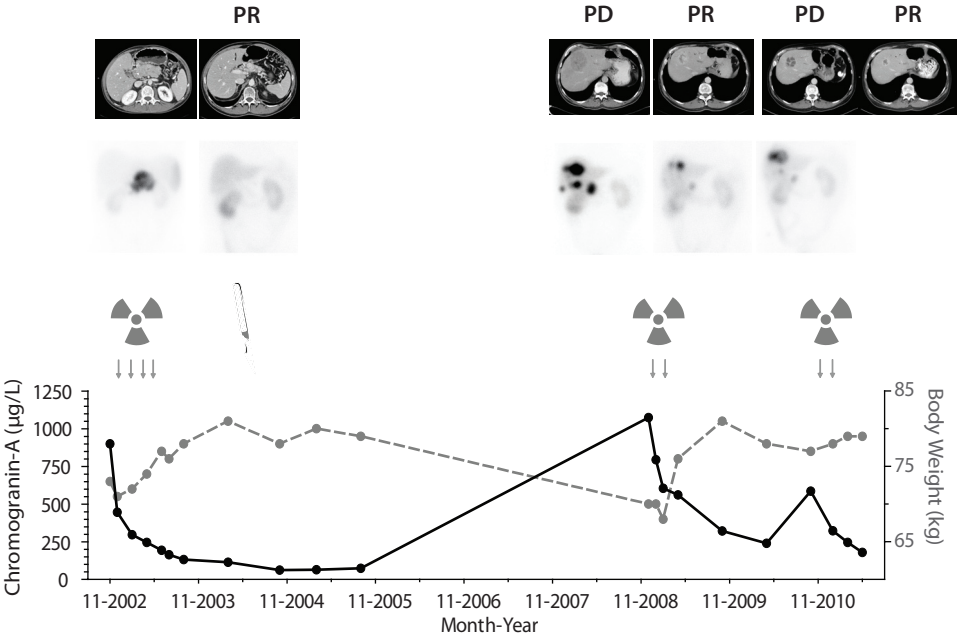

Figure 2. Clinical course in a patient with a neuroendocrine tumor in the pancreatic tail (patient 6), presenting imaging studies (CT scan and [^{111}Indium-DTPA^{0}]octreotide scintigraphy (SRS)), serum Chromogranin A levels and body weight over time. The patient had had a distal pancreatectomy and splenectomy 14 months after regular treatment with ^{177}Lu-octreotate. He had a local recurrence and liver metastases 48 months after surgery, for which he received two additional cycles of ^{177}Lu-octreotate. Two years after that, he had again tumor progression, and he received again two cycles of ^{177}Lu-octreotate. The treatments with ^{177}Lu-octreotate were accompanied by a decrease in Chromogranin A level and an increase in body weight.

Arrows denote treatment cycles with ^{177}Lu-octreotate. PR: partial response; PD: progressive disease.

Table 2. Patient, tumor and pathology characteristics of the patients with successful surgery after ^{177}Lu-octreotate.

							Characteristics resection specimen			Treatment effects of ^{177}Lu-octreotate in resection specimen		
Patient	Age (yrs); Sex	Best Response	Time between last ^{177}Lu-octreotate treatment and surgery (mo)	Type surgery	Complications surgery	Follow-up	Margins free yes/no	Lymph nodes positive (…/… lymph nodes)	Ki67	Necrosis	Fibrosis/ Sclerosis	Other
1.	60; Male	SD	8.6	Whipple	Line sepsis	Local recurrence 22 mo after Whipple; 31 mo after Whipple extensive lymphadenopathy and liver metastases, SRS negative; died 33 mo after surgery	Yes	4/4	1%	+	+	Hemorrhage, perineural infiltration
2.	41; Female	MR	6.5	PPPD + thrombectomy portal vein and end-to-end portal anastomosis	None	Disease-free at 0 mo after surgery; alive at 0 mo after surgery	No	0/7	50%	-	+	-
3.	42; Male	MR	20.3	Whipple	Urinary tract infection	Disease-free at 32 mo after surgery; alive at 40 mo after surgery	Yes	0/0	8%	+	-	-
4.	71; Female	PR	24.1	PPPD	None	Disease-free at 0 mo after surgery*; alive at 4 mo after surgery	Yes	0/14	1%	-	+	-
5.	54; Male	PR	10.4	Whipple	None	Disease-free at 51 mo after surgery; alive at 58 mo after surgery	Yes	0/11	1%	-	+	-
6.	52; Male	PR	13.7	Distal pancreatectomy + splenectomy	None	Local recurrence + liver metastases 48 mo after surgery; 2 extra cycles of ^{177}Lu-octreotate, again PR; 2 yrs later again PD and again 2 extra cycles of ^{177}Lu-octreotate, again PR; alive at 84 mo after surgery	No	0/3	1%	-	+	-

							Characteristics resection specimen			Treatment effects of ^{177}Lu-octreotate in resection specimen		
Patient	Age (yrs); Sex	Best Response	Time between last ^{177}Lu-octreotate treatment and surgery (mo)	Type surgery	Complications surgery	Follow-up	Margins free yes/no	Lymph nodes positive (…/… lymph nodes)	Ki67	Necrosis	Fibrosis/ Sclerosis	Other
7.	43; Male	PR	7.3	Whipple and reconstruction portal vein with a biograft	Venous bypass occlusion, wound infection, intra-abdominal abcess, and ileus	Liver- and lymph node metastases 56 mo after surgery; 2 extra cycles of ^{177}Lu-octreotate, MR; thereafter potential curative RFA of a remaining liver metastasis; alive at 70 mo after surgery	Yes	0/4	3%	-	+	Hemorrhage
8.	60; Male	PR	10.3	Distal pancreatectomy + splenectomy, metastasectomy liver (3x), coagulation liver metastasis (2x), 1 liver metastasis in situ (this liver metastasis was not visible on CT/SRS during FU up to 54 mo after surgery)	Urinary tract infection	Disease-free at 54 mo after surgery; alive at 59 mo after surgery	No	0/6	3%	-	+	-
9	43; Female	MR	11.8	Whipple + resection para-aortic lymph node + RFA liver lesion	Intra-abdominal abcess, infected thrombus jugular vein (central venous line for total parental nutrition)	Disease-free at 7 mo after surgery; alive at 7 mo after surgery	No	4/10	1%	-	+	-

Patient	Age (yrs); Sex	Best Response	Time between last ^{177}Lu-octreotate treatment and surgery (mo)	Type surgery	Complications surgery	Follow-up	Characteristics resection specimen			Treatment effects of ^{177}Lu-octreotate in resection specimen		
							Margins free yes/no	Lymph nodes positive (…/… lymph nodes)	Ki67	Necrosis	Fibrosis/ Sclerosis	Other
10	60; Female	PR	33.2	Whipple	Atrial fibrillation and fever due to fluid collection behind left liver lobe, wound infection	Disease-free at 0 mo after surgery*; alive at 2 mo after surgery	Yes	1/5	30%	-	+	Vasoinvasion, perineural growth

* No imaging has yet been done after surgery.

SD: stable disease; SRS: [^{111}Indium-DTPA^{0}]octreotide scintigraphy; MR: minor response; PPPD: pylorus preserving pancreaticoduodenectomy; PR: partial response; FU: follow-up; RFA: radiofrequency ablation.

Table 3. Risk factors for progression in progression-free survival at univariate and multivariate analysis (n=119).

Variable		Univariate Analysis			Multivariate Analysis		
	Median PFS (95% CI) in mo	**Hazard Ratio**	**95% CI**	***P* value**	**Hazard Ratio**	**95% CI**	***P* value**
Age (continuous variable)		1.00	0.98–1.02	0.78			
WHO grading*							
1	54 (18–90)	1.00			1.00		
2	30 (27–34)	1.78	0.82–3.85	0.15	1.48	0.67–3.28	0.33
3	12 (5–19)	7.93	2.77–22.67	<0.001	6.83	2.37-19.72	<0.001
Group pancreatic tumor*							
1	67 (52–82)	1.00					
2	49 (20–78)	1.77	0.59–5.27	0.31			0.83
3	25 (21–29)	3.09	1.33–7.19	0.01			0.54
AF baseline elevated*							
no	38 (17–59)	1.00					
yes	24 (18–31)	2.28	1.41–3.67	0.001			0.05
Successful surgery after ^{177}Lu-octreotate*							
no	25 (21–30)	1.00					
yes	69 (NA)	0.24	0.08–0.76	0.02			0.06
Sex							
female	25 (21–30)	1.00					
male	36 (28–44)	0.79	0.49–1.26	0.32			
Ki67 (continuous variable)		1.05	1.03–1.08	<0.001			
Baseline AF (U/L)							
<120	38 (17–59)	1.00					
120–500	25 (20–30)	2.09	1.26–3.44	0.004			
>500	13 (2–24)	3.46	1.66–7.23	0.001			
Baseline CgA (µg/L)*							
<150	53 (26–79)	1.00					
150–1000	29 (17–40)	1.70	0.91–3.16	0.10			0.46
>1000	23 (17–29)	2.89	1.48–5.63	0.002			0.54

* Pertaining to variables included in the multivariate analysis.

NA: not applicable; AF: alkaline phosphatase; CgA: chromogranin A.

Potential risk factors for progression and death in the total group of patients at univariate and multivariate analysis are listed in table 3. WHO grade 3 was the strongest predictor for progression at univariate analysis (Figure 3). This was confirmed by multivariate analysis. Successful surgery after ^{177}Lu-octreotate did not reach statistical significance at multivariate analysis (hazard ratio 0.31 (95% CI: 0.09–1.03), p=0.06). Analyzing only group 1 and 2, age was the only factor associated with progression or death at univariate analysis (table 4). A multivariate analysis was not performed, since age was the only variable which would be included in the model.

Table 4. Risk factors for progression in progression-free survival at univariate analysis group 1 and 2 (n=29).

Variable	Median PFS (95% CI) in mo	Hazard Ratio	95% CI	*P* value
Age (continuous variable)		1.05	1.01–1.10	0.03
WHO grading				
1	54 (18–90)	1.00		
2/3	55 (38–72)	0.81	0.23–2.88	0.74
AF baseline elevated				
no	69 (48–90)	1.00		
yes	49 (18–81)	1.96	0.65–5.86	0.23
Successful surgery after ^{177}Lu-octreotate				
no	49 (28–70)	1.00		
yes	69 (NA)	0.44	0.12–1.66	0.23
Sex				
female	49 (10–89)	1.00		
male	69 (64–74)	0.34	0.09–1.27	0.11
Ki67 (continuous variable)		0.95	0.79–1.14	0.58
Baseline AF (U/L)				
<120	69 (48–90)	1.00		
120–500	37 (16–57)	2.01	0.64–6.31	0.23
>500	49 (NA)	1.68	0.19–14.68	0.64
Baseline CgA (µg/L)				
<150	55 (36–75)	1.00		
150–1000	67 (20–113)	1.20	0.33–4.31	0.79
>1000	23 (NA)	7.57	0.56–103.10	0.13

No multivariate analysis was performed because only 1 variable (i.e. age) had a p-value ≤0.10, and would be included in the model.
NA: not applicable; AF: alkaline phosphatase; CgA: chromogranin A.

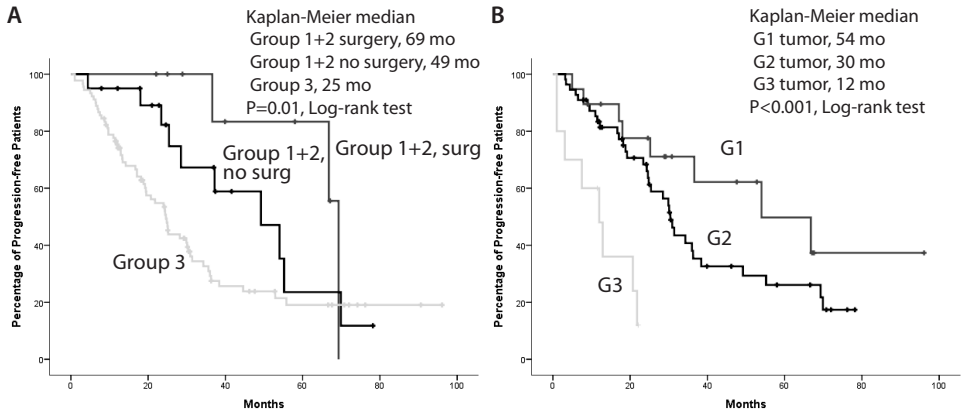

Figure 3. (**A**) Progression-free survival (PFS) in 119 patients with nonfunctioning pancreatic neuroendocrine tumors. A significant difference in the median PFS was observed for patients in group 1 and 2 with successful surgery after ^{177}Lu-octreotate, patients in group 1 and 2 without successful surgery after ^{177}Lu-octreotate, and patients in group 3. (**B**) Progression-free survival (PFS) in 119 patients with nonfunctioning pancreatic neuroendocrine tumors. A significant difference in the median PFS was observed for patients with a G1 tumor, patients with a G2 tumor, and patients with a G3 tumor.

DISCUSSION

In this study, we found an encouraging rate of successful surgery in 9/29 neoadjuvant treated patients with a pancreatic NET (31%). There were no significant differences in baseline characteristics, which could potentially select patients for surgery, between the operated and non-operated patients in the neoadjuvant treated group. Although we realize that some surgeons might deem most of our patients resectable upfront, we feel it is important that every patient who can be treated with PRRT in a neoadjuvant manner is evaluated by a surgeon for assessment of (potential) resectability of the tumor. Even patients with an SD as response outcome may be eligible for surgery, as was demonstrated in one patient in our series. This patient had a tumor size decrease of 22% after ^{177}Lu-octreotate. Although this was not enough to be recorded as MR, it was sufficient to resolve the vascular involvement, which caused the tumor to be considered irresectable before ^{177}Lu-octreotate treatment.

None of the patients considered for surgery showed progressive disease. In most patients there was an obvious response to PRRT and no vascular resection was necessary anymore during pancreaticoduodenectomy. Patients with extensive vascular involvement or venous portal/mesenteric thrombosis prior to PRRT developed sufficient venous collaterals during treatment. In most cases this was through the inferior mesenteric vein. These patients underwent resection with reconstruction of the portal and partial mesenteric vein leaving the collateral circulation intact. Surgery after PRRT could be safely performed in all patients.

PRRT as neoadjuvant treatment in patients with pancreatic NETs has been described in a few case reports. Three patients received ^{90}Y-based somatostatin analogs[10-12], and two patients ^{177}Lu-based somatostatin analogs.[13-14] Although these case reports demonstrate the potential of PRRT in a neoadjuvant setting, follow-up after surgery was limited and ranged between 2 and 22 months. The present study describes a group of patients with surgery after PRRT with a long follow-up, allowing us to report on survival.

The median PFS in our total patient group was 30 months. Patients with successful surgery after ^{177}Lu-octreotate had a significantly better PFS than patients who were not operated after ^{177}Lu-octreotate (69 months versus 25 months, p=0.009). However, the non-operated patients also included patients with extensive disease, who generally have a poor prognosis. Although significance was not reached for successful surgery after ^{177}Lu-octreotate at multivariate analysis (hazard ratio 0.31 for progression (95% CI: 0.09–1.03), p=0.06), the results point to a progression-free survival advantage for the operated patients. This is in line with various previous reports that demonstrated that resection of the primary tumor was associated with improved survival in patients with pancreatic NETs.[22-24] The difference in PFS between operated and non-operated patients in the neoadjuvant treated group in this study did not reach statistical significance, which might be explained by the small patient numbers and small number of events.

Due to the retrospective nature of this study, an effect of selection bias of the operated patient group can not be excluded. For example, in patients who have a co-morbidity which precludes surgery, this co-morbidity may be the reason of death leading to a worse survival

in the non-operated patients. In our patient group, however, this seems unlikely, since none of the non-operated patients had been refused for surgery because of co-morbidities. However, since resection was undertaken at a median time of 11 months after the last treatment, a better prognostic group might have been selected by inherent behaviour of the cancer. To demonstrate an effect on survival, ideally, a prospective study should be undertaken, in which patients who are eligible for surgery after ^{177}Lu-octreotate are randomized between surgery and no surgery. However, we deem such a study not ethical.

This study showed that the main risk factor for progression and death in PFS analysis was WHO grading. This is in accordance with previous studies.[25-26] Also, a trend was observed for an association between elevated baseline alkaline phosphatase levels and increased risk of progression and death. This is in line with a previous study.[27]

Neoadjuvant treatment with fluorouracil-based or gemcitabine-based chemoradiation is widely being performed in patients with pancreatic adenocarcinoma (reviewed in[28]). In contrast, to our knowledge, only four patients with a pancreatic NET have been described who had a curative resection of their tumor after chemotherapy.[6] This small number of patients is puzzling to us, given the high response rates between 39–70% after varying types of chemotherapy for (metastasized) pancreatic NETs.[5-7, 29] If more widespread use of chemotherapy in a neoadjuvant setting would be performed, this could lead to more curative resections in patients with initially irresectable pancreatic NETs.

A limitation of this study is its retrospective nature. We are aware that because the cases were identified retrospectively, and definitions of 'resectable' and 'irresectable' are often subjective and surgeon-dependent, some cases may have been considered resectable in other centers, even without ^{177}Lu-octreotate. However, the patients described in this study, were all deemed irresectable before treatment with ^{177}Lu-octreotate by a surgeon with expertise in pancreatic surgery, and would not have been operated on in our center. Furthermore, this study showed that the approach of first ^{177}Lu-octreotate and then surgery can be safely performed, and can be considered in patients with a nonfunctioning pancreatic NET with limited tumor load. The treatment with ^{177}Lu-octreotate might have led to less extensive surgery, possibly resulting in lower morbidity. We feel that our observations provide justification for a prospective study on the neoadjuvant use of ^{177}Lu-octreotate in patients with nonfunctioning pancreatic NETs, in which predefined criteria of tumor resectability should be incorporated.

CONCLUSION

Surgery can be considered after treatment with ^{177}Lu-octreotate in patients with initially irresectable pancreatic NETs, as our data suggest that successful surgery after ^{177}Lu-octreotate is associated with increased PFS. Our observations provide justification for a prospective study on the neoadjuvant use of ^{177}Lu-octreotate in patients with nonfunctioning pancreatic NETs.

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Chapter 6

Hypocalcaemia after Treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]Octreotate

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ABSTRACT

Purpose

The aim of this study was to explore the possible mechanisms of an observed decline in serum calcium level in patients with neuroendocrine tumours (NETs) treated with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-octreotate).

Methods

Fourty-seven NET patients who were normocalcaemic at baseline were prospectively analysed regarding serum calcium, albumin, creatinin, alkaline phosphatase, gamma GT, magnesium, phosphate, and 25-hydroxy vitamin D, at baseline and up to 6 months after treatment. Parathyroid hormone (PTH), 1.25-Dihydroxyvitamin D_3, type 1 amino-terminal propeptide of procollagen (PINP), bone-specific alkaline phosphatase, carboxy-terminal cross-linking telopeptide of bone collagen (CTX), collagen type I cross-linked N-telopeptide (NTX); and creatinin and calcium in 24-hour urine collection, were evaluated at baseline and at 3/6 months follow-up. Another 153 NET patients were retrospectively analysed to estimate the occurrence of hypocalcaemia in a larger patient group.

Results

In the prospectively analysed patients, the mean serum calcium level decreased significantly after treatment (2.31 ± 0.01 to 2.26 ± 0.02 mmol/l, p=0.02). Eight patients (17%) had a marked decrease in serum calcium levels with a nadir in serum calcium level of ≤2.10 mmol/l. In 5 patients (11%), calcium substitution therapy was prescribed. PTH increased significantly (5.9 ± 0.6 to 6.7 ± 0.8 pmol/l, p=0.02), presumably in response to the decreasing serum calcium levels. 25-hydroxy vitamin D remained stable after treatment. Creatinin levels increased significantly (73 ± 3 to 77 ± 3 µmol/l, p=0.01), however not enough to explain the hypocalcaemia. Phosphate levels remained unaffected. In the retrospectively analysed patients, the mean serum calcium level decreased significantly from 2.33 ± 0.01 at baseline to a nadir of 2.24 ± 0.01 mmol/l at 18 months after treatment (p<0.001). Thirty-three patients (22%) had a nadir in serum calcium level of ≤2.10 mmol/l. Eleven patients (7%) received calcium substitution therapy.

Conclusions

The mean serum calcium level decreased significantly after treatment with ^{177}Lu-octreotate, resulting in a mild hypocalcaemia in ± 20% of patients. The cause of the hypocalcaemia after ^{177}Lu-octreotate observed in this study remains unknown, after we excluded several potential causes of hypocalcaemia. Serum calcium levels should be monitored after peptide receptor radionuclide therapy, and calcium substitution therapy should be initiated where appropriate.

Key Words: neuroendocrine tumour, [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, side effects, hypocalcaemia

INTRODUCTION

Peptide receptor radionuclide therapy (PRRT) with radiolabelled somatostatin analogues is a relatively novel treatment modality in patients with metastasized or inoperable somatostatin receptor-positive tumours, including neuroendocrine tumours (NETs) of bronchial, intestinal, or pancreatic origin, thyroid carcinomas, and paragangliomas. Response rates after PRRT are encouraging. Complete and partial responses obtained after treatment with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide are in the same range as after treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-octreotate) (i.e. 10-30%).[1-5] The acute side effects of treatment with ^{177}Lu-octreotate consist of transient nausea after 25% of administrations, vomiting after 10%, and abdominal pain after 10%.[5] Severe late side effects such as renal insufficiency and myelodysplastic syndrome occur in less than 1% of patients.[5] Endocrine side effects of ^{177}Lu-octreotate are modest, and include a transient decrease in inhibin B levels, with a concomitant rise in follicle-stimulating hormone (FSH) levels, a decrease of total testosterone and sex hormone binding globulin, and a transient increase in luteinizing hormone (LH), in men.[6] In postmenopausal women, a modest decrease in FSH and LH levels is found.[6] The main side effect of treatment with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide is renal toxicity.[7-9] In our clinical practice we noted that some patients developed a hypocalcaemia after treatment with ^{177}Lu-octreotate, sometimes requiring calcium substitution therapy. Therefore, we performed a prospective study to study the frequency of hypocalcaemia as well as the average drop in serum calcium levels after ^{177}Lu-octreotate. To explore possible mechanisms causing this hypocalcaemia, several factors that influence serum calcium levels, like albumin, creatinin, alkaline phosphatase, gamma GT, magnesium, phosphate, 25-hydroxy vitamin D, parathyroid hormone (PTH), 1.25-Dihydroxyvitamin D_3, type 1 amino-terminal propeptide of procollagen (PINP), bone-specific alkaline phosphatase, carboxy-terminal cross-linking telopeptide of bone collagen (CTX), and collagen type I cross-linked N-telopeptide (NTX), were measured.

METHODS

Patients

We performed two substudies in all Dutch patients receiving ^{177}Lu-octreotate. The first study (group 1) was a prospective study. Inclusion criteria for this study were: 1) treatment with ^{177}Lu-octreotate according to protocol; 2) a baseline serum calcium value of ≥2.15 mmol/l (≥8.6 mg/dl). Exclusion criteria were: 1) calcium substitution therapy at baseline; 2) a history of thyroid/parathyroid surgery; 3) a history of neck surgery; 4) a history of external radiation to the neck; 5) a history of treatment with radioactive iodine; 6) patients with the Multiple Endocrine Neoplasia Type-1 (MEN-1) syndrome; 7) patients with a creatinin clearance <50 ml/min (measured in 24-hour urine collection). The inclusion period was between October 2010 and December 2011. The end date of the study was first of October 2012.

The second study (group 2) was a retrospective study, including all patients treated with ^{177}Lu-octreotate in the time period of July 2006 until October 2010. The same inclusion and exclusion criteria as described above were applied to these patients. This group was analysed to estimate the occurrence of hypocalcaemia after treatment with ^{177}Lu-octreotate in a larger patient group with a long follow-up.
All patients gave written informed consent to participate in the study, which was approved by the hospital's medical ethical committee.

Treatment

[DOTA^0,Tyr^3]octreotate was obtained from BioSynthema (St Louis, MO, USA). $^{177}LuCl_3$ was distributed by IDB-Holland (Baarle-Nassau, the Netherlands). ^{177}Lu-octreotate was locally prepared as described previously.[10] Granisetron (Kytril®; Roche, Woerden, the Netherlands) 3 mg was injected intravenously 30 minutes before starting the infusion of ^{177}Lu-octreotate. To reduce the radiation dose to the kidneys, an infusion of amino acids (arginine 2.5% and lysine 2.5%, 1 litre) was started 30 minutes before the administration of the radiopharmaceutical and lasted 4 hours. The radiopharmaceutical was co-administered using a second pump system. Cycle doses were 7.4 GBq, injected over 30 minutes. The intended interval between treatments was 6–10 weeks. Patients were treated up to a cumulative intended dose of 29.6 GBq. Routine haematology, liver and kidney function tests were performed before each therapy and at follow-up visits.

Biochemical analysis

The patients in the prospective study (group 1) were evaluated for serum calcium, albumin, creatinin, alkaline phosphatase, gamma GT, magnesium, phosphate, and 25-hydroxy vitamin D, at baseline, 4 weeks after each treatment (normally patients have 4 treatment cycles), and 6 weeks, 3 months and 6 months after the last treatment with ^{177}Lu-octreotate. PTH, 1.25-Dihydroxyvitamin D_3, PINP, bone-specific alkaline phosphatase, CTX, NTX; and creatinin and calcium in 24-hour urine collection (to calculate the creatinin clearance in ml/min and excreted calcium/24 hours, respectively), were evaluated at baseline and at 6 months after the last treatment with ^{177}Lu-octreotate.
Blood samples were stored at -20^{o}C until assayed (in one run).
When serum albumin was decreased (i.e. below 35 g/l), serum calcium values were corrected for serum albumin using the following formula: corrected serum calcium = serum calcium + (0.02 × (40 – serum albumin)). When calcium substitution therapy was initiated, patients were censored for all laboratory measurements from the date of substitution onwards. When vitamin D substitution was initiated, patients were censored for 25-hydroxy vitamin D measurements, but not for the other laboratory values. Patients who had had a nadir in serum calcium level of ≤2.10 mmol/l were identified, because normally this is the threshold for calcium substitution therapy in our institution.

The patients in the retrospective study (group 2) were evaluated for only serum calcium (corrected for serum albumin in case of decreased serum albumin, as described above), which was performed as part of their routine blood work performed before each therapy with ^{177}Lu-octreotate and at follow-up visits. Patients were censored when calcium substitution therapy was initiated.

Various biochemical bone parameters in serum were measured. Bone-specific alkaline phosphatase and PINP were measured as markers of bone formation, and NTX and CTX as markers of bone resorption. Bone-specific alkaline phosphatase was measured by an immunoenzymetric assay using the Ostase BAP kit (IDS, Frankfurt am Main, Germany) with an inter-assay coefficient of variation (CV) of <7.5%. PINP was measured by RIA (Orion Diagnostica Oy, Espoo, Finland) with intra-assay and interassay CV of 13.7% and <6.4%, respectively. NTX was measured in serum by ELISA (Osteomark, Ostex International, Seattle, Wash., USA) with an intra-assay and interassay CV of 4.6% and 4.5%, respectively. CTX was measured in serum by sandwich electrochemiluminescence immunoassay (Cobas 8000, Roche Diagnostics) with an interassay CV of 1%.

1.25-Dihydroxyvitamin D_3 was assessed by radioimmunoassay (Immunodiagnostic Systems; Boldon, UK); intra- and interassay CVs were 8% and 10%, respectively. PTH was measured by a chemiluminescence assay on a Vitros ECi system (Ortho Clinical Diagnostics, Rochester, NY)) with an interassay CV of <6%.

Statistics

Data are expressed as mean ± standard error of the mean (SEM), unless otherwise indicated. Repeated measurement analysis was performed using a Linear Mixed Model with an unstructured covariance matrix with time as a categorical variable, and with Bonferroni correction for multiple comparisons. Blood and urine values before and after treatment were compared with paired *t* tests or Wilcoxon signed ranks tests. The correlation between the administered dose in GBq and a nadir in serum calcium level of ≤2.10 mmol/l was tested with Mann-Whitney *U* tests. The SPSS (SPSS version 20.0; IBM) package was used. Two-sided p-values are reported. P-values <0.05 were considered to be significant.

RESULTS

Group 1

One hundred and ten Dutch patients were evaluated between October 2010 and December 2011 at our outpatient clinic for potential treatment with ^{177}Lu-octreotate. In 22 patients, treatment with ^{177}Lu-octreotate was not started (yet); 14 patients received treatment off-protocol due to various reasons; 9 patients had a baseline serum calcium value <2.15 mmol/l; in 8 patients no calcium determination in 24-hour urine collection at baseline was performed; in 2 patients no blood samples for the additional calcium-related measurements (like PTH), at baseline were performed; 3 patients had calcium substitution

therapy at baseline; 3 patients had a history of thyroid surgery; and 2 patients had a history of neck surgery. So, 47 patients were included in this study. Baseline characteristics of these patients are presented in table 1.

Table 1. Baseline characteristics of Dutch patients who had been treated with ^{177}Lu-octreotate according to protocol and who were studied for possible mechanisms of hypocalcaemia after treatment with ^{177}Lu-octreotate (*n*=47).

Characteristic	No of patients (%)	
Male	28 (60)	
Female	19 (40)	
Mean age (years) (range)		63 (42–80)
Primary tumour		
Jejuno-Ileal NET	14 (30)	
Duodenal NET	1 (2)	
Bronchial NET	3 (6)	
Unknown primary	14 (30)	
Pancreas NF	10 (21)	
Glucagonoma	2 (4)	
Insulinoma	1 (2)	
Glomus tumour	1 (2)	
Paraganglioma	1 (2)	
Previous therapy		
Octreotide	31 (66)	
Surgery	20 (43)	
Chemotherapy	4 (9)	
Radiotherapy	2 (4)	
Interferon	2 (4)	
Liver embolisation/chemoembolisation	1 (2)	
Liver RFA	2 (4)	
Liver metastases	36 (77)	
Bone metastases	7 (15)	
WHO grading*		
G1 (Ki67: 0-2%)	12 (31)	
G2 (Ki67 >2-20%)	24 (62)	
G3 (Ki67 >20%)	3 (8)	
Median Ki67* (range)		5 (1–50)
Concomitant capecitabine	7 (15)	
Median total administered dose in GBq (range)		29.7 (11.2–30.4)
Tumour uptake on octreoscan		
Equal to normal liver	6 (13)	
> Normal liver	26 (55)	
> Kidneys	15 (32)	

* Data on Ki67 and WHO grading were available in 39 patients.
NET: neuroendocrine tumour; GBq: gigabecquerel.

Because of lower than expected recruitment of patients, blood samples for the additional calcium-related measurements (like PTH), and creatinin and calcium in 24-hour urine collection were performed on 3 months instead of 6 months after treatment, in 17 patients. Eight of 47 patients (17%) had had a nadir in serum calcium level of ≤2.10 mmol/l at any time during or after treatment. Calcium substitution during or up to 6 months after treatment with ^{177}Lu-octreotate was prescribed in 5/47 patients (11%) (in one patient calcium was prescribed because of a L1 lumbar spine fracture at the place of a bone metastasis (serum calcium was 2.37 mmol/l)). The median total administered dose was not significantly different between the patients with and without a nadir in serum calcium level of ≤2.10 mmol/l (29.6 versus 29.7 GBq, respectively, p=0.50).

One patient who had magnesium substitution therapy at baseline was censored for magnesium analyses; another patient who had vitamin D substitution therapy at baseline was censored for 25-hydroxy vitamin D analyses. One patient was excluded for gamma GT analyses, because of an extreme outlier in gamma GT (gamma GT value at baseline of 39x upper limit of normal). Four patients were started on vitamin D substitution therapy while on-study, and were censored for 25-hydroxy vitamin D analyses, from the date of substitution onwards.

In 5 patients, no laboratory parameters after treatment were available because of death in 2 patients and progressive disease in 3 patients. Four patients, who were started on calcium substitution therapy while on-study, were censored from the date of substitution onwards. In the 5th patient who received calcium substitution therapy, calcium substitution was prescribed based on the serum calcium level at 6 months after treatment. Because the analyses were done until 6 months after treatment, no censoring was needed for this patient.

Table 2 shows the results of the repeated measurement analyses. The mean serum calcium level decreased significantly from 2.29 ± 0.01 mmol/l to a nadir of 2.24 ± 0 .01 mmol/l at 6 weeks after treatment (p=0.02) (figure 1). Magnesium showed a slight decrease from 0.83 ± 0.01 mmol/l at baseline to a nadir of 0.80 ± 0.01 mmol/l at 6 weeks after treatment (p=0.001). Creatinin first showed a non-significant decrease from 74 ± 3 µmol/l at baseline to 71 ± 3 µmol/l after the first treatment (p=0.39). At 6 months after treatment, however, creatinin was significantly higher than at baseline (79 ± 3 versus 74 ± 3 µmol/l, p=0.007).

The comparisons between various laboratory parameters at baseline and after treatment are presented in table 3. The mean serum calcium level decreased significantly from 2.31 ± 0.01 mmol/l at baseline to 2.26 ± 0.02 mmol/l after treatment (p=0.02). As expected from the longitudinal analyses, mean magnesium level decreased and mean creatinin level increased after treatment. Mean PTH level increased from 5.9 ± 0.6 pmol/l at baseline to 6.7 ± 0.8 pmol/l after treatment (p=0.02). The bone resorption markers NTX and CTX increased significantly after treatment. Calcium in 24-hour urine decreased significantly. The other laboratory parameters did not change significantly after treatment.

Table 2. Laboratory values before, during and after treatment with ^{177}Lu-octreotate.

Laboratory value	Baseline	After Rx1	After Rx2	After Rx3	After Rx4	FU 6 Weeks	FU 3 Months	FU 6 Months
Calcium (mmol/l)	2.29 ± 0.01	2.26 ± 0.01	2.27 ± 0.02	2.27 ± 0.01	2.26 ± 0.01	2.24 ± 0.01*	2.25 ± 0.01*	2.25 ± 0.02
Phosphate (mmol/l)	1.05 ± 0.03	1.00 ± 0.03	0.98 ± 0.03	1.00 ± 0.03	0.96 ± 0.03*	1.00 ± 0.03	1.04 ± 0.03	1.05 ± 0.03
Magnesium (mmol/l)	0.83 ± 0.01	0.82 ± 0.01	0.81 ± 0.01	0.81 ± 0.01*	0.82 ± 0.01	0.80 ± 0.01*	0.81 ± 0.01	0.81 ± 0.01
Albumin (g/l)	44 ± 0.4	43 ± 1	44 ± 1	44 ± 1	44 ± 0.5	44 ± 0.5	45 ± 1	44 ± 1
Alkaline phosphatase (U/l)	117 ± 15	131 ± 23	98 ± 10	97 ± 10	100 ± 11	100 ± 11	101 ± 12	102 ± 11
Gamma GT (U/l)	125 ± 27	131 ± 25	120 ± 25	123 ± 24	133 ± 28	128 ± 28	140 ± 33	117 ± 35
Creatinin (µmol/l)	74 ± 3	71 ± 3	72 ± 3	72 ± 3	72 ± 3	73 ± 3	75 ± 3	79 ± 3*
25-hydroxy vitamin D (nmol/l)	70 ± 6	68 ± 5	59 ± 5	62 ± 5	60 ± 5	64 ± 5	68 ± 6	64 ± 5

Various calcium-related markers in serum before, during and after treatment with ^{177}Lu-octreotate.
Results are expressed as mean ± standard error of the mean (SEM).
* $p<0.05$ when compared with laboratory value at baseline.
Normal values: Calcium, 2.20–2.65 mmol/l; Phosphate, 0.80-1.40 mmol/l; Magnesium, 0.70-1.05 mmol/l; Albumin, 35–50 g/l; Alkaline phosphatase, <115 U/l (male), <98 U/l (female); Gamma GT, <55 U/l (male), <38 U/l (female); Creatinin, 65–115 µmol/l (male), 55-90 µmol/l (female); 25-hydroxy vitamin D, 50–120 nmol/l.

Gamma GT: gamma glutamyl transpeptidase; FU: follow-up; Rx: treatment.

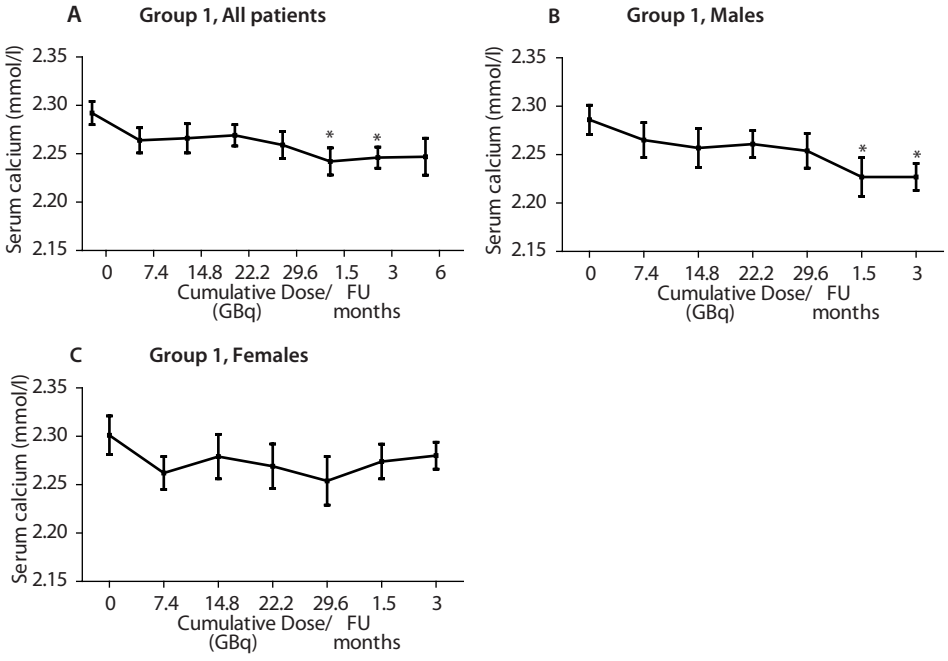

Figure 1. (**A**) Repeated measurement analysis of mean (± SEM) serum calcium levels in patients with neuroendocrine tumours in group 1 treated with ^{177}Lu-octreotate. A significant decrease of 2.29 ± 0.01 mmol/l at baseline to a nadir of 2.24 ± 0.01 at 6 weeks after treatment was observed, *p<0.05. (**B**+**C**) Subanalyses for males (**B**) and females (**C**) showed a significant decline in mean serum calcium levels for males, but not for females, *p<0.05.

We also evaluated other endocrine markers (which were performed as part of the routine blood work performed before each therapy with ^{177}Lu-octreotate and at follow-up visits), performed subanalyses for males and females, and performed subanalyses for patients with and without bone metastases. Because only 2 females were premenopausal, and 17 females were postmenopausal, we decided to analyse all females together. The concentrations of hormones are presented in table 3. There were no significant changes in thyroid-related hormones. Mean LH and FSH in females decreased significantly after treatment. Mean oestradiol levels remained unchanged. Mean FSH levels in males increased significantly after treatment. Testosterone showed a non-significant decrease from 15.28 ± 2.14 nmol/l at baseline to 11.26 ± 0.92 after treatment (p=0.09).

Table 3. Additional laboratory values before and after treatment.

Laboratory value	Baseline	After treatment	p-value
Calcium (mmol/l)	2.31 ± 0.01	2.26 ± 0.02	0.02*
Phosphate (mmol/l)	1.04 ± 0.04	1.06 ± 0.03	0.74
Magnesium (mmol/l)	0.83 ± 0.01	0.80 ± 0.01	0.045*
Albumin (g/l)	45 ± 0.5	45 ± 1	0.76
Alkaline phosphatase (U/l)	122 ± 18	109 ± 15	0.26
Gamma GT (U/l)	46	45	0.92
Creatinin (µmol/l)	73 ± 3	77 ± 3	0.01*
25-hydroxy vitamin D (nmol/l)	75 ± 7	75 ± 6	0.95
1.25-Dihydroxyvitamin D_3 (pmol/l)	161 ± 7	155 ± 6	0.36
PTH (pmol/l)	5.9 ± 0.6	6.7 ± 0.8	0.02*
PINP (µg/l)	39	45	0.51
BAP (µg/l)	14.3 ± 1.4	17.6 ± 2.0	0.05
NTX (nM BCE)	13.7 ± 0.6	15.8 ± 1.2	0.03*
CTX (µg/l)	0.17	0.29	0.005*
Creatinin clearance in 24-hour urine (ml/min)	111 ± 5	109 ± 5	0.51
Calcium in 24-hour urine (mmol/24hr)	2.59 ± 0.27	2.09 ± 0.23	0.02*
TSH (mU/l)	1.31 ± 0.10	1.28 ± 0.09	0.71
fT4 (pmol/l)	16.5 ± 0.4	16.3 ± 0.5	0.72
T3 (nmol/l)	1.89 ± 0.04	1.94 ± 0.05	0.34
LH (male) (U/l)	5.6 ± 0.8	6.3 ± 0.8	0.32
LH (female) (U/l)	30.6 ± 5.2	21.3 ± 2.5	0.008*
FSH (male) (U/l)	10.0 ± 1.8	21.1 ± 2.0	<0.001*
FSH (female) (IU/l)	65.1 ± 7.2	55.5 ± 4.6	0.03*
Testosterone (nmol/l)	15.28 ± 2.14	11.26 ± 0.92	0.09
Oestradiol (pmol/l)	40 ± 8	46 ± 7	0.40

Various calcium-related markers in serum and urine (creatinin clearance in 24-hour urine and calcium in 24-hour urine) before and after treatment with ^{177}Lu-octreotate.

Results are expressed as mean ± standard error of the mean (SEM) or median (median for Gamma GT, PINP, and CTX).

Results may differ slightly from the results presented in table 2, because in table 3 only paired data were used.

* Significant difference (Paired *t* test for calcium, magnesium, creatinin, PTH, NTX, calcium in 24-hour urine, LH (female), FSH (male), FSH (female); Wilcoxon signed ranks test for CTX).

Normal values: Calcium, 2.20–2.65 mmol/l; Phosphate, 0.80–1.40 mmol/l; Magnesium, 0.70–1.05 mmol/l; Albumin, 35–50 g/l; Alkaline phosphatase, <115 U/l (male), <98 U/l (female); Gamma GT, <55 U/l (male), <38 U/l (female); Creatinin, 65–115 µmol/l (male), 55–90 µmol/l (female); 25-hydroxy vitamin D, 50–120 nmol/l; 1.25-Dihydroxyvitamin D_3, 38–183 pmol/l; PTH, 1.4–7.3 pmol/l; PINP, 22–87 µg/l (male),16–75.8 µg/l (female, premenopausal), 16–96 µg/l (female, postmenopausal); BAP, <20.1 µg/l (male), <14.3 µg/l (female premenopausal), <22.4 µg/l (female postmenopausal); NTX, 6.2–19.0 nm BCE (male), 5.4–24.2 nM BCE (female); CTX, <0.58 µg/l (male 30-50 years), <0.70 µg/l (male 51–71 years), <0.85 µg/l (male >70 years), <0.57 µg/l (female premenopausal), <1.01 µg/l (female postmenopausal); Calcium in 24-hour urine, 2.5–7.5 mmol/24hr; TSH, 0.4–4.3 mU/l; fT4, 11-25 pmol/l; T3, 1.4–2.5 nmol/l; LH, 1.5–8.0 U/L (male), 2.0–8.0 U/l (female follicular), 10-55 U/l (female ovulatory), 2.0–7.0 U/l (female luteal), 15-90 U/l (female postmenopausal); FSH, 2–7 IU/l (male), 2–8 IU/l (female follicular), 3–15 IU/l (female LH peak), 1–6 IU/l (female luteal), 35–150 IU/l (female postmenopausal); Testosterone, 10–30 nmol/l; Oestradiol, 50–250 pmol/l (female follicular early), 250–1000 pmol/l (female follicular late), 400–1500 pmol/l (female LH peak), 250–1000 pmol/l (female luteal mid), 150–250 pmol/l (female luteal late), <50 pmol/l (female postmenopausal).

BAP: bone-specific alkaline phosphatase; BCE: bone collagen equivalents; CTX: carboxy-terminal cross-linking telopeptide of bone collagen; FSH: follicle-stimulating hormone; fT4: free thyroxine; gamma GT: gamma glutamyl transpeptidase; LH: luteinizing hormone; NTX: collagen type I cross-linked N-telopeptide; PINP: type 1 amino-terminal propeptide of procollagen; PTH: parathyroid hormone; T3: triiodothyronine; TSH: thyroid-stimulating hormone.

Subanalyses for males and females (table 4 and figure 1) showed that whereas serum calcium showed a significant decrease in males, it showed no significant change in females. Furthermore, creatinin increased significantly in males, whereas it was stable in females. The increase in NTX and CTX was significant only in females. Serum calcium levels before and after treatment for each patient for the total group, and separately for males and females, are shown in figure 2. In the total group, as well as for males and females separately, there was an overall tendency of decreasing serum calcium levels.

Subanalyses for patients with bone metastases were unreliable, since only 7 patients had bone metastases, and 2 of them received calcium substitution therapy after ^{177}Lu-octreotate and were therefore censored. The patients without bone metastases had similar outcomes as the total patient group (*data not shown*).

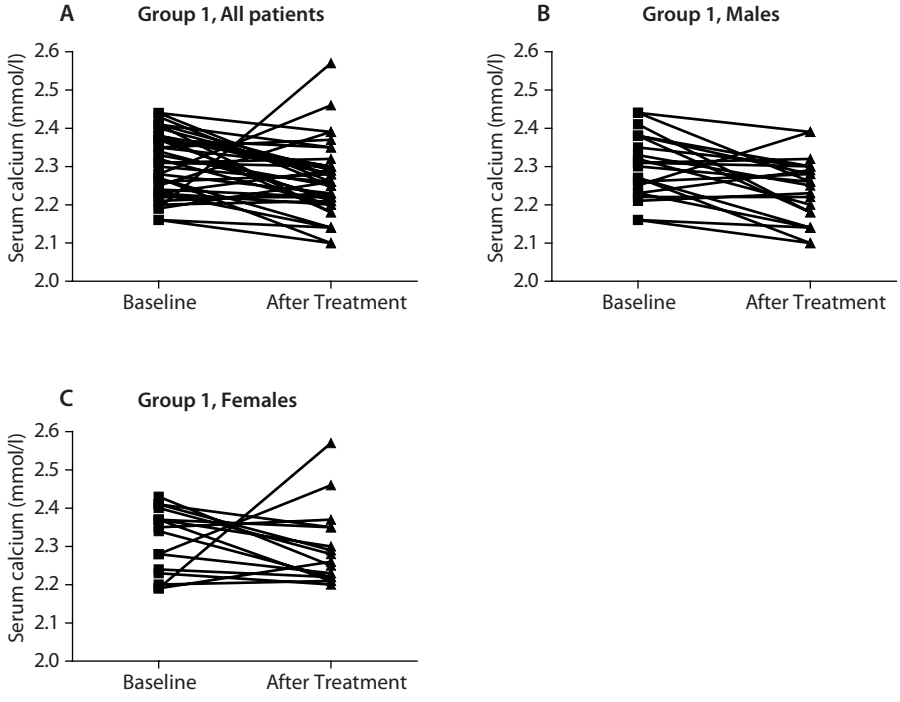

Figure 2. (**A**) Mean serum calcium level before and after treatment with ^{177}Lu-octreotate in patients with neuroendocrine tumours in group 1. An overall tendency of decreasing serum calcium levels is observed. Two outliers with an obvious increase in serum calcium level are observed. Each line connects one individual patient. (**B**) Mean serum calcium level before and after treatment in males. An overall tendency of decreasing serum calcium levels is observed. Each line connects one individual patient. (**C**) Mean serum calcium level before and after treatment in females. An overall tendency of decreasing serum calcium levels is observed, with 2 outliers with an obvious increase in serum calcium level. Each line connects one individual patient.

Table 4. Additional laboratory values before and after treatment according to gender.

	Male			Female		
Laboratory value	**Baseline**	**After treatment**	**p-value**	**Baseline**	**After treatment**	**p-value**
Calcium (mmol/l)	2.30 ± 0.02	2.24 ± 0.02	0.004*	2.32 ± 0.02	2.30 ± 0.03	0.61
Phosphate (mmol/l)	0.95 ± 0.05	1.01 ± 0.05	0.33	1.18 ± 0.04	1.13 ± 0.03	0.21
Magnesium (mmol/l)	0.82 ± 0.01	0.79 ± 0.02	0.07	0.83 ± 0.02	0.82 ± 0.02	0.41
Albumin (g/l)	44 ± 1	45 ± 1	0.53	45 ± 1	45 ± 1	0.75
Alkaline phosphatase (U/l)	128 ± 29	123 ± 25	0.71	113 ± 17	91 ± 6	0.19
Gamma GT (U/l)	47	39	0.65	123 ± 48	88 ± 22	0.24
Creatinin (µmol/l)	80 ± 3	87 ± 3	0.006*	63 ± 4	63 ± 4	0.94
25-hydroxy vitamin D (nmol/l)	71 ± 7	69 ± 7	0.63	79 ± 13	84 ± 12	0.65
1.25-Dihydroxyvitamin D_3 (pmol/l)	163 ± 7	154 ± 9	0.34	160 ± 14	157 ± 9	0.81
PTH (pmol/l)	5.9 ± 0.9	7.1 ± 1.1	0.03*	5.8 ± 0.8	6.2 ± 1.0	0.39
PINP (µg/l)	38	41	0.95	50 ± 6	59 ± 7	0.33
BAP (µg/l)	14.2 ± 2.4	18.3 ± 3.3	0.13	14.3 ± 1.1	16.6 ± 1.5	0.20
NTX (nM BCE)	12.8	13.7	0.21	13.3 ± 0.8	15.9 ± 0.7	0.02*
CTX (µg/l)	0.21 ± 0.03	0.28 ± 0.05	0.15	0.23 ± 0.04	0.36 ± 0.06	0.03*
Creatinin clearance in 24-hour urine (ml/min)	121 ± 6	120 ± 7	0.86	98 ± 7	92 ± 6	0.37
Calcium in 24-hour urine (mmol/24hr)	2.40 ± 0.31	2.01 ± 0.29	0.09	2.89 ± 0.48	2.22 ± 0.39	0.09
TSH (mU/l)	1.49 ± 0.14	1.41 ± 0.12	0.49	1.05 ± 0.14	1.09 ± 0.13	0.66
fT4 (pmol/l)	16.3 ± 0.5	16.0 ± 0.5	0.55	16.7 ± 0.8	16.7 ± 0.9	0.90
T3 (nmol/l)	1.91 ± 0.05	1.92 ± 0.06	0.91	1.86 ± 0.08	1.96 ± 0.09	0.20
LH (U/l)	5.6 ± 0.8	6.3 ± 0.8	0.32	30.6 ± 5.2	21.3 ± 2.5	0.008*
FSH (U/l)	10.0 ± 1.8	21.1 ± 2.0	<0.001*	65.1 ± 7.2	55.5 ± 4.6	0.03*
Testosterone (nmol/l)	15.28 ± 2.14	11.26 ± 0.92	0.09	NA		
Oestradiol (pmol/l)	NA			40 ± 8	46 ± 7	0.40

Various calcium-related markers in serum and urine (creatinin clearance in 24-hour urine and calcium in 24-hour urine) before and after treatment with ^{177}Lu-octreotate according to gender.
Results are expressed as mean ± standard error of the mean (SEM) or median (median for Gamma GT (male), PINP (male), and NTX (male)).
* Significant difference (Paired *t* test for calcium (male), creatinin (male), PTH (male), NTX (female), CTX (female), LH (female), FSH (male), FSH (female)).
Normal values: See Table 3.

BAP: bone-specific alkaline phosphatase; BCE: bone collagen equivalents; CTX: carboxy-terminal cross-linking telopeptide of bone collagen; FSH: follicle-stimulating hormone; fT4: free thyroxine; Gamma GT: gamma glutamyl transpeptidase; LH: luteinizing hormone; NA: not applicable; NTX: collagen type I cross-linked N-telopeptide; PINP: type 1 amino-terminal propeptide of procollagen; PTH: parathyroid hormone; T3: triiodothyronine; TSH: thyroid-stimulating hormone.

Table 5. Baseline characteristics of Dutch patients who had been treated with ^{177}Lu-octreotate according to protocol between July 2006 until October 2010 (*n*=153).

Characteristic	No of patients (%)	
Male	86 (56)	
Female	67 (44)	
Mean age (years) (range)		60 (32–80)
Primary tumour		
Jejuno-Ileal NET	30 (20)	
Duodenal NET	2 (1)	
Bronchial NET	6 (4)	
Unknown primary	45 (29)	
Pancreas NF	47 (31)	
Glucagonoma	2 (1)	
Insulinoma	2 (1)	
Glomus tumour	1 (1)	
Paraganglioma	1 (1)	
Colorectal NET	10 (7)	
Gastric NET	3 (2)	
Meningeoma	3 (2)	
Gastrinoma	1 (1)	
Previous therapy		
Octreotide	73 (48)	
Surgery	65 (43)	
Chemotherapy	2 (1)	
Radiotherapy	8 (5)	
Interferon	6 (4)	
Liver embolisation/chemoembolisation	4 (3)	
Liver RFA	4 (3)	
Liver metastases	126 (82)	
Bone metastases	29 (19)	
WHO grading*		
G1 (Ki67: 0-2%)	33 (34)	
G2 (Ki67 >2-20%)	60 (63)	
G3 (Ki67 >20%)	3 (3)	
Median Ki67* (range)		4.5 (1–30)
Concomitant capecitabine	30 (20)	
Median total administered dose in GBq (range)		29.9 (7.5–30.7)
Tumour uptake on octreoscan		
Equal to normal liver	19 (12)	
> Normal liver	76 (50)	
> Kidneys	58 (38)	

* Data on Ki67 and WHO grading were available in 96 patients.

NET: neuroendocrine tumour; GBq: gigabecquerel.

Group 2

Two hundred and forty-seven patients were treated with ^{177}Lu-octreotate between July 2006 until October 2010. Thirty-nine patients received treatment off-protocol; 12 patients had a baseline serum calcium value <2.15 mmol/l; 9 patients had calcium substitution therapy at baseline; 6 patients had no serum calcium measurement performed at baseline; 6 patients had the MEN-1 syndrome; 13 patients had a history of thyroid surgery (whether or not in combination with radioactive iodine); 4 patients had a history of neck surgery; 1 patient had received external radiation to the neck; and 1 patient had a history of treatment with radioactive iodine (without thyroid surgery). Another three patients were excluded for other reasons. So, 153 patients were included in the retrospective analysis. Baseline characteristics of these patients are presented in table 5.

Thirty-three of 153 patients (22%) had had a nadir in serum calcium level of ≤2.10 mmol/l at any time during or after treatment. Calcium substitution during or up to 36 months after treatment with ^{177}Lu-octreotate was prescribed in 11/153 patients (7%) (in one patient calcium was prescribed because of a spinal compression fracture (serum calcium was 2.16 mmol/l)). The median total administered dose was not significantly different between the patients with and without a nadir in serum calcium level of ≤2.10 mmol/l (29.8 versus 29.9 GBq, respectively, p=0.31).

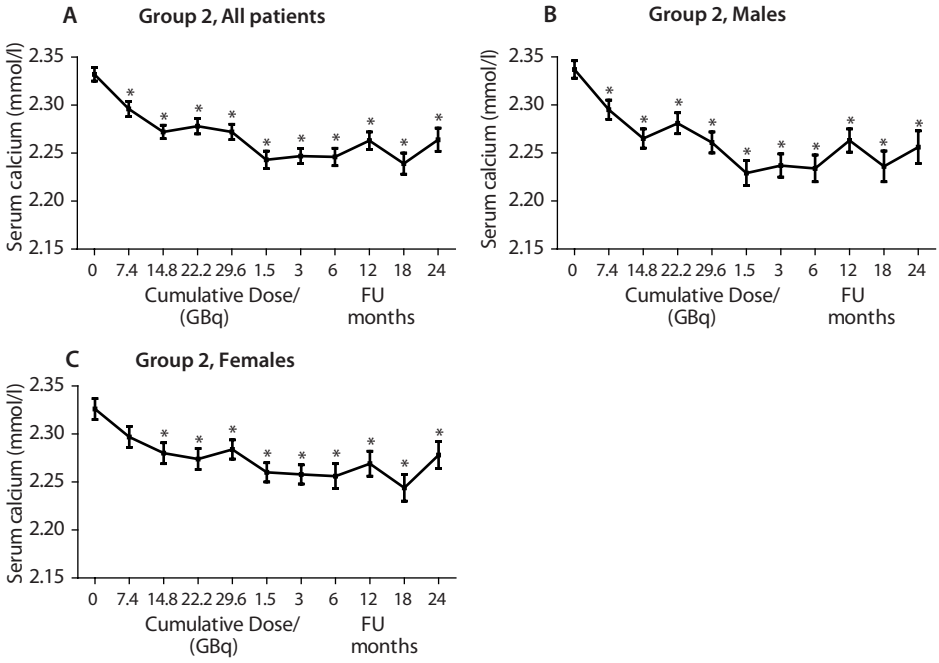

Figure 3. (**A**) Repeated measurement analysis of mean (± SEM) serum calcium levels in patients with neuroendocrine tumours in group 2 treated with ^{177}Lu-octreotate. A significant decrease of 2.33 ± 0.01 mmol/l at baseline to a nadir of 2.24 ± 0.01 at 18 months after treatment was observed, *p<0.05. (**B**+**C**) Subanalyses for males (**B**) and females (**C**) showed a significant decline in mean serum calcium levels for both males and females, *p<0.05.

In this group, the mean serum calcium level decreased significantly from 2.33 ± 0.01 mmol/l to a nadir of 2.24 ± 0.01 mmol/l at 18 months after treatment ($p<0.001$) (figure 3). Subanalyses for males and females showed a same pattern in serum calcium decline after treatment for both sexes (figure 3).

DISCUSSION

In this study, a significant decline in serum calcium level after treatment with ^{177}Lu-octreotate was observed in a prospectively studied patient group, in which we aimed to find mechanisms explaining the decrease in serum calcium levels. A marked decrease in serum calcium levels to a nadir of ≤2.10 mmol/l at any time during or after treatment was observed in 8/47 patients (17%). In five patients (11%), calcium substitution therapy was prescribed. The decline in serum calcium levels after treatment was confirmed in a larger, retrospectively analysed, patient group, in which 22% of patients developed a serum calcium of ≤2.10 mmol/l.

Several factors were analysed to study the cause of the hypocalcaemia, such as hypoparathyroidism, vitamin D deficiency, renal insufficiency, pseudohypoparathyroidism, intestinal malabsorption, and low calcium intake. One possible explanation could be that indirect irradiation ('cross-fire') of the parathyroid glands coming from direct irradiation of the thyroid glands by ^{177}Lu-octreotate might cause hypoparathyroidism, leading to hypocalcaemia. The parathyroid glands are anatomically located in the region of the thyroid gland. Somatostatin receptors are expressed in the thyroid, as evidenced by in vitro studies and by the physiological uptake in vivo during somatostatin receptor scintigraphy.[11-12] The thyroid glands will therefore always receive some irradiation during treatment with ^{177}Lu-octreotate. Hypoparathyrodisim and associated hypocalcaemia has been observed after ^{131}Iodine (^{131}I) treatment for thyroid cancer.[13-14] However, the significant reduction in mean serum calcium was not accompanied by a reduction in serum PTH. In contrast, mean PTH increased significantly after treatment pointing towards an adequate response of the parathyroid glands to the decrease in serum calcium levels.

Mean 25-hydroxy vitamin D levels were unaffected after treatment with ^{177}Lu-octreotate. Also, the repeated measurement analyses showed no significant change in 25-hydroxy vitamin D levels during and after treatment. Serum creatinin after treatment was significantly higher than before treatment. However, whereas serum calcium levels already decreased after the first treatment with ^{177}Lu-octreotate, serum creatinin levels only increased at 6 months follow-up. Creatinin clearance as calculated by 24-hour urine collection before and after treatment was unchanged. Furthermore, in case of renal-induced hypocalcaemia, serum phosphate and 24-hour urinary excretion of calcium should be increased, and this was not observed in our study. Therefore, renal insufficiency seems not to have caused the observed decline in serum calcium levels. Pseudohypoparathyroidism, in which there is a peripheral

resistance to PTH, is also unlikely, since this is also associated with elevated phosphate levels. We addressed intestinal absorption by investigating complaints of diarrhoea. All but one patient had a decrease in diarrhoea, or had no diarrhoea at all. Therefore, it is unlikely that the intestinal absorption of calcium has caused the observed hypocalcaemia after treatment with ^{177}Lu-octreotate. We did not perform dietary questionnaires to assess calcium intake of patients. Instead, we used weight as a surrogate. Weight increased or was stable in most patients after treatment. Four patients had a reduction in weight of ≥3 kg after treatment; none of these patients had a decline in serum calcium level. Therefore, as far as we can assess, a decrease in calcium intake does not seem to be the cause of the observed hypocalcaemia. One could hypothesise that radiation caused a change in set point of the calcium sensing receptor in the parathyroid glands. However, an activation of this receptor leading to lower serum calcium levels would not be expected to be accompanied by increasing levels of serum PTH, as we observed.

We found a decrease in LH and FSH levels in females, and an increase in FSH levels in males. This is in line with a previous study reporting on the endocrine side effects of treatment with ^{177}Lu-octreotate.[6] The mean oestradiol level was unaffected, and the mean testosterone levels, although slightly lower after treatment, were still above the lower limit of normal (i.e. >10 nmol/l). Cancer treatment induced hypogonadism can cause bone loss, however this is not associated with changes in serum calcium levels.[15]

In group 1, we found a difference in serum calcium decline after treatment for males and females. Whereas males showed a significant reduction in serum calcium levels after treatment, mean serum calcium levels in females remained unchanged. When plotting the serum calcium level before and after treatment of every patient separately, it became clear that there were 2 female outliers. There were no clear patient characteristics in these 2 patients possibly causing hypercalcaemia (such as bone metastases or the production of PTH-related peptide by the tumour). Excluding the outlier with the largest increase in serum calcium showed a reduction in serum calcium levels in females. In the analysis of group 2, males and females both had a decline in serum calcium levels after treatment. Therefore, the observed stable serum calcium levels in females in group 1 are probably caused by 2 outliers, and do not reflect a true gender difference.

There are several limitations to this study. First, we did not measure ionized serum calcium levels. However, in the setting of normal serum albumin levels (as was the case in the vast majority of our patients), total serum calcium levels correspond well to the concentration of ionized serum calcium levels. Second, we did not perform dietary questionnaires to assess the calcium intake of patients. Nonetheless, the observed significant decline in serum calcium levels after treatment with ^{177}Lu-octreotate warrants close monitoring of these levels during and after PRRT.

CONCLUSION

In conclusion, the mean serum calcium level decreased significantly after treatment with ^{177}Lu-octreotate. Eight of 47 patients (17%) had a marked decrease with a nadir in serum calcium level of ≤2.10 mmol/l at any time during or after treatment. The decline in serum calcium levels was confirmed in a larger, retrospectively analysed, patient group, in which 22% of patients developed a hypocalcaemia. The cause of the hypocalcaemia observed after ^{177}Lu-octreotate is yet unknown, after we excluded hypoparathyroidism, vitamin D deficiency, renal insufficiency, pseudohypoparathyroidism, intestinal malabsorption, and low calcium intake as potential causes of hypocalcaemia. However, the findings are clinically relevant and suggest that the normal calcium homeostasis failed in several patients. We recommend that serum calcium levels should be closely monitored during and after PRRT, and that calcium substitution therapy should be initiated where appropriate.

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Chapter 7

Improved Control of Severe Hypoglycemia in Patients with Malignant Insulinomas by Peptide Receptor Radionuclide Therapy

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ABSTRACT

Context

Insulinomas are relatively rare neuroendocrine tumors of the pancreas. Only 10% are considered malignant. Control of insulin hypersecretion and hypoglycemia in patients with malignant insulinomas may be extremely difficult. Different medications and chemotherapy schedules have been used.

Patients

Five patients with metastatic insulinomas and severe, poorly controllable, hypoglycemia are described. These patients required continuous glucose infusion to control severe hypoglycemia, which were induced by the high levels of insulin secretion. Conventional medications, such as diazoxide, or streptozotocin-based chemotherapies had been used to control hypoglycemia but were ineffective and/or produced adverse effects. All patients were treated with sc octreotide.

Intervention

Peptide receptor radionuclide therapy with radiolabeled somatostatin analogs was used.

Results

After the start of radiolabeled somatostatin analog therapy, the five patients with metastatic insulinomas had stable disease for a mean period of 27 months. During these months, the patients were without any hypoglycemic episodes. Finally, three of five patients died because of progressive disease.

Conclusions

Radiolabeled somatostatin analog therapy can stabilize tumor growth and can be very successful in further controlling severe hypoglycemia in malignant insulinomas. In our series, this eventually resulted in improved survival outside the hospital setting.

INTRODUCTION

Insulinoma is a rare neuroendocrine tumor, exclusively localized in the pancreas, which uncontrolled produces excessive amounts of insulin. In approximately 10% of cases, multiple tumors are found, mainly in genetic poly-endocrine syndromes like multiple endocrine neoplasia type I. According to the World Health Organization (WHO), the only criterion of malignancy is the presence of metastases. Patients with insulinomas suffer from severe hypoglycemia due to inappropriately increased circulating plasma insulin levels. The diagnosis can be made by demonstrating nonsuppressed plasma insulin, proinsulin, and/or C-peptide levels in the presence of hypoglycemia. Factitious use of oral blood glucose-lowering drugs should always be excluded.[1-5] In specific cases, a 72-h fast, which is considered the gold standard, might be necessary.[6] Localization of metastatic disease in patients with insulinomas can be achieved by using transabdominal or endoscopic ultrasound, computed tomography (CT), magnetic resonance imaging, and somatostatin receptor scintigraphy. The preferred therapy for insulinoma is curative surgical resection. However, in metastatic disease, surgery can only be used to debulk the tumor, thus resulting in reduction of insulin hypersecretion. Controlling hypoglycemia in patients with malignant insulinomas remains a challenge for the clinician. These patients often suffer from severe long-lasting hypoglycemia and may need continuous iv glucose or feeding through a gastric tube during the night. Traditional drugs, like diazoxide, yield only a temporary effect and may cause side effects.[7] Conventional chemotherapy for neuroendocrine carcinomas of the pancreas consists of streptozotocin in combination with 5-fluorouracil (5-FU) or doxorubicin.[8] In addition, somatostatin analogs, such as octreotide and lanreotide, can be used to control the hypoglycemia.[9]

Recently, successful control of hypoglycemic hyperinsulinism in patients with metastatic insulinomas using the mammalian target of rapamycin (mTOR) inhibitors rapamycin[10] or everolimus[11] has been reported.

In the last decade, a novel therapy for inoperable, metastatic, neuroendocrine tumors has been introduced. This therapy uses [^{111}In]octreotide, [^{90}Y]octreotide, [^{90}Y]lanreotide, or [^{177}Lu]octreotate.[12-13] We present five cases of patients with inoperable malignant insulinomas and severe hypoglycemia in whom treatment with [^{111}In]octreotide or [^{177}Lu]octreotate not only (temporarily) controlled the tumor growth but also was very successful in controlling the severe hypoglycemia.

PATIENTS AND METHODS

General inclusion criteria for peptide receptor radiotherapy (PRRT) using [^{111}In]octreotide or [^{177}Lu]octreotate were tumor uptake during [^{111}In]diethylenetriaminepentaacetate (DTPA)^{0}-octreotide scintigraphy (OctreoScan; Mallinckrodt, Petten, The Netherlands) preceding the therapy that was at least as high as that in normal liver tissue, no previous treatment with

other radiolabeled somatostatin analogs, serum hemoglobin at least 6 mmol/liter, white blood cell count at least 2×10^9/liter, platelet count at least 75×10^9/liter, serum creatinine concentration no higher than 150 μmol/liter or creatinine clearance at least 40 ml/min, and Karnofsky performance status at least 50.

All patients treated with PRRT in the Erasmus Medical Center, Rotterdam (including the five patients described in the present manuscript), gave written informed consent before inclusion in the PRRT studies, which were approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam.

$^{111}InCl_3$ and DTPA-octreotide were obtained from Mallinckrodt (St. Louis, MO), and labeling was performed in accordance with the package insert. The cycle dosages were 10–11 GBq. The interval between the [^{111}In]DTPA-octreotide treatments was 4 wk. Routine hematology, liver, and kidney function tests were performed before each therapy as well as at follow-up visits. 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)^0-[Tyr^3]-octreotate was obtained from Mallinckrodt. $^{177}LuCl_3$ was obtained from NRG (Petten, The Netherlands) and Missouri University Research Reactor and was distributed by IDB-Holland (Baarle-Nassau, The Netherlands). [^{177}Lu]octreotate was locally prepared as described before.[14]

In the patients treated with [^{177}Lu]octreotate, 3 mg granisetron or 8 mg ondansetron was injected iv, and an infusion of amino acids (2.5% lysine, 2.5% arginine in 1 liter 0.9% NaCl; 250 ml/h) was started 30 min before the administration of the radiopharmaceutical and lasted 4 h. Cycle dosages varied between 100 mCi (3.7 GBq) and 200 mCi (7.4 GBq), injected in 30 min. The interval between treatments was 6–10 wk. Routine hematology, liver, and kidney function tests were performed before each therapy as well as at follow-up visits.

RESULTS

Case 1

A 29-yr-old female presented with cognitive disturbances, occasionally accompanied by hypoglycemia. She complained of fatigue, pain in the right upper quadrant of the abdomen, nausea, and a weight gain of approximately 17 kg in the previous year. The laboratory results supported the diagnosis of endogenous hyperinsulinemic hypoglycemia [blood glucose was 18 mg/dl (1.0 mmol/liter), and plasma insulin was 488.4 pmol/liter; fasting reference = 0–100 pmol/liter].

CT of the abdomen demonstrated multiple multilobar liver metastases and enlarged periaortic lymph nodes. A primary pancreatic tumor was located at the corpus-tail area of the pancreas. An [^{111}In]pentetreotide scan demonstrated intense uptake of this radioligand that corresponded with the known metastases in the liver and periaortic lymph nodes and in a supraclavicular lymph node. The primary pancreatic tumor could not be visualized. Histological examination of a supraclavicular lymph node biopsy revealed a poorly differentiated neuroendocrine carcinoma with a Ki-67 index of 25% and positive

immunohistochemistry for the somatostatin receptor subtype 2a (sst_{2a}). Insulin could not be detected immunohistochemically, probably due to dedifferentiation of the metastatic tumor. A malignant insulinoma was diagnosed. Because of the hypoglycemic episodes, diazoxide treatment was started. However, a dose reduction was necessary because of the development of severe edema. Thereafter, hypoglycemia recurred. The patient needed frequent meals with short intervals to maintain euglycemia, and octreotide sc was started. With this therapy regimen, the frequency of the hypoglycemic episodes was reduced from four times daily to none. Treatment with [^{111}In]pentetreotide therapy was given 8 months after initial diagnosis because of progressive disease and recurrence of hypoglycemic episodes with a frequency of once every 4 d. Common Terminology Criteria for Adverse Events grade 1–2 leucopenia (with spontaneous recovery) and grade 1–2 anemia developed after the seventh cycle of high-dose [^{111}In]pentetreotide. After nine cycles of high-dose [^{111}In]pentetreotide at 4-wk intervals up to a total administered activity of 92.8 GBq, CT showed stable disease. Hypoglycemia remained absent. Nonreversible grade 2 thrombocytopenia developed after the ninth administration of [^{111}In]pentetreotide. One month after the last cycle of [^{111}In]pentetreotide, 18 months after the initial diagnosis, progressive disease was diagnosed, and ascites had developed. The patient's clinical condition rapidly deteriorated. At this stage, she presented with hyperglycemia. This might be explained by progressive disease and probably also by dedifferentiation of the tumor. The tumor might have stopped producing insulin and/or might have started producing hyperglycemic peptides like glucagon or somatostatin. Unfortunately, this was not tested. The patient's general condition was considered too poor for chemotherapy. Palliative treatment was initiated. The patient died 20 months after the initial diagnosis because of progressive malignant insulinoma (Table 1).

Case 2

A 48-yr-old female was diagnosed with atypical Alzheimer's disease, presenting as a cognitive disorder with lack of concentration and loss of memory. At presentation, she had intermittent collapses with loss of consciousness. She complained of fatigue and reduced appetite, but her body weight remained stable. Fasting laboratory tests revealed blood glucose of 39.6 mg/dl (2.2 mmol/liter), plasma C-peptide of 3.09 ng/ml (1.03 nmol/liter) [fasting reference = 1.5–2.4 ng/ml (0.5–0.8 nmol/liter)], plasma proinsulin of 812 pmol/liter (fasting reference = 2–5 pmol/liter), and plasma insulin of 288 pmol/liter. This was consistent with the diagnosis of endogenous hyperinsulinemic hypoglycemia. CT of the abdomen demonstrated diffuse hepatic metastases and a mass in the tail of the pancreas. An [^{111}In]pentetreotide scan demonstrated intense radiotracer uptake in multifocal liver lesions, multiple skeletal lesions, and a lesion in the pancreatic tail (Figure 1). Histological examination of a liver biopsy revealed a moderately differentiated, WHO2010 grade 2, neuroendocrine tumor with a Ki-67 index of 5–10%. (Immunohistochemistry for insulin was

not performed.) Based on the histological and radiological imaging, a metastatic pancreatic insulinoma with liver and bone metastases was diagnosed. The patient was recommended to have food intake at regular intervals; however, she also needed continuous infusions of 5% glucose and diazoxide treatment. Still, the blood glucose levels remained poorly controllable. The diazoxide treatment had to be stopped because of nausea. The patient was started on sc octreotide therapy. With this therapy, the frequency of the hypoglycemic episodes was significantly reduced from three to four times daily to two to three times weekly, and the iv glucose could be discontinued. Because of the favorable response to sc octreotide, it was decided to treat her with [^{177}Lu]octreotate. She underwent treatment with four cycles of [^{177}Lu]octreotate at 6- to 8-wk intervals up to a total administered activity of 30.2 GBq (Figure 1). No hematological or renal toxicities were recorded during this therapy. Since the start of this treatment, hypoglycemia did not recur, and slowly the cognitive disturbances improved (Figure 2). Twenty months since the initial diagnosis, the disease was still stable (Figure 3), and the patient was still euglycemic (Table 1). At recent analysis, 26 months after the initial diagnosis, there is evidence of growth of liver metastases and new liver metastases have developed.

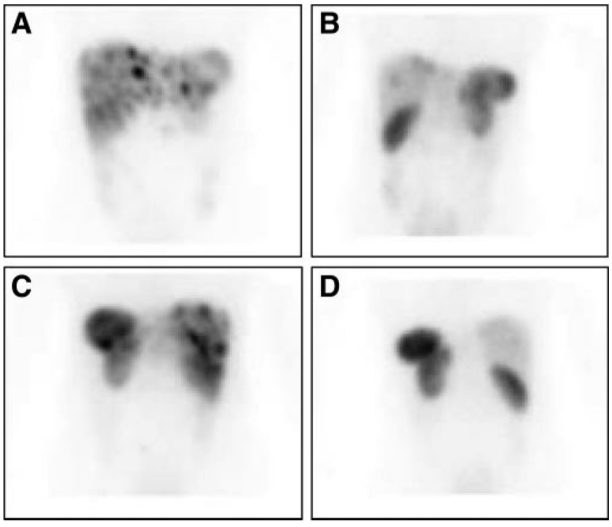

Figure 1. Comparison of baseline (**A** and **C**) [^{111}In]pentetreotide scan of the abdomen and [^{111}In] pentetreotide scan performed 10 months after initial diagnosis (**B** and **D**) in a 48-yr-old female with a metastatic malignant insulinoma treated with [^{177}Lu]octreotate demonstrating diminished uptake in the liver lesions, suggesting shrinkage of the liver metastases (case 2).

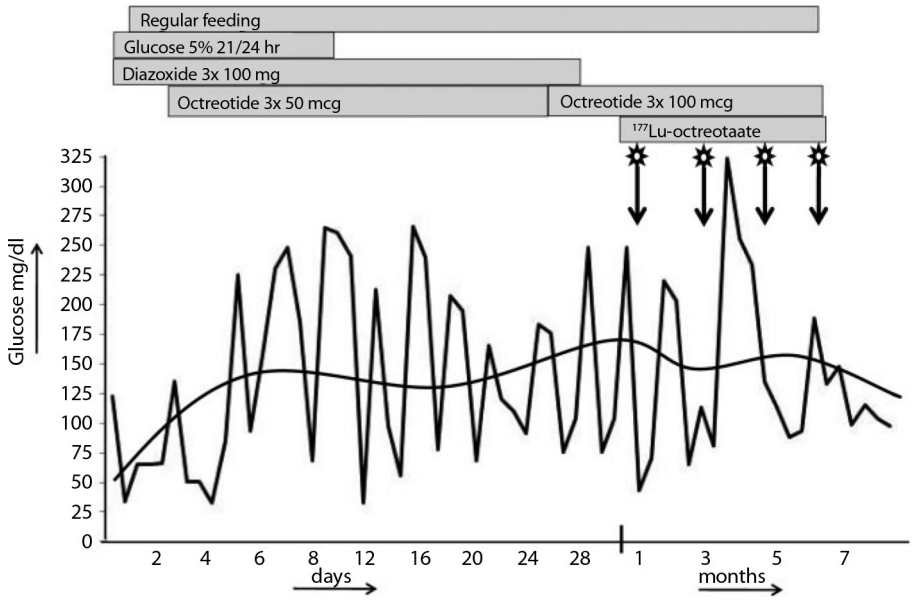

Figure 2. Timeline of treatment interventions (including iv glucose, diazoxide, sc octreotide, and [^{177}Lu] octreotate) with values for blood glucose levels in a 48-yr-old female with a malignant insulinoma (case 2).

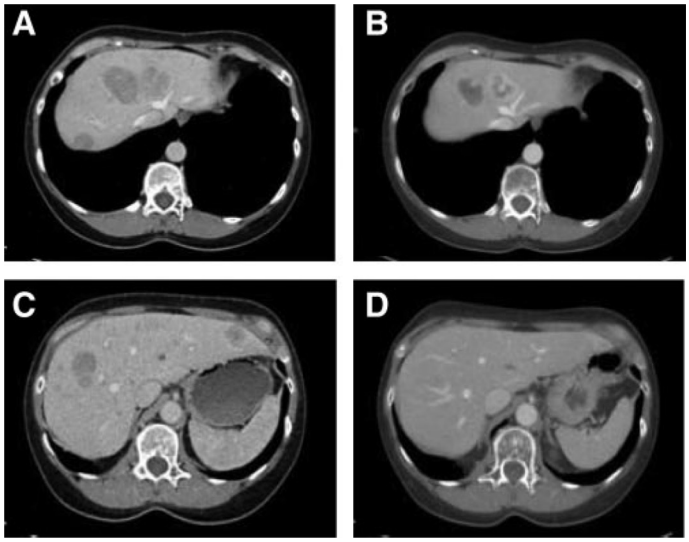

Figure 3. Comparison of baseline (**A** and **C**) CT scans of the abdomen and CT scans performed 10 months after initial diagnosis (**B** and **D**) in a 48-yr-old female with a malignant insulinoma treated with [^{177}Lu]octreotate showing partial response of the liver metastases (case 2).

Table 1. Successful control of severe hypoglycemia with PRRT.

Case	Age (yr),sex	Diagnosis	Pathology	Immunohistochemistry
1	29, female	Insulinoma pancreas with liver and lymph node metastases	Supraclavicular lymph node metastasis grade 3 NET, Ki-67 25%, liver metastasis NET	Cg and synaptophysin positive, insulin negative, sst_{2a} positive
2	48, female	Insulinoma pancreas with liver and bone metastases	Liver metastasis grade 2 NET, Ki-67 5–10%	Cg and synaptophysin positive, insulin and sst_{2a} not performed
3	55, female	Insulinoma pancreas with liver metastases	Pancreas grade 1 NET, Ki-67 <2%, liver metastasis grade 1 NET	Cg and synaptophysin positive, insulin positive, sst_{2a} positive
4	50, male	Insulinoma pancreas with liver metastases	No pathology, diagnosis made on clinical grounds	
5	34, male	Insulinoma pancreas with lymph node, liver, and bone metastases	Supraclavicular lymph node metastasis grade 2 NET, Ki-67 2–15%	Cg and synaptophysin positive, insulin positive, sst_{2a} positive

Diazoxide	Octreotide	Surgery	Chemotherapy	PRRT	Survival after initial diagnosis
Yes	Yes	No	No	[^{111}In]Octreotide (9x/4 wk; total administered activity = 92.8 GBq)	20 months[a]
Yes	Yes	No	No	[^{177}Lu]Octreotate (4x/6–8 wk; total administered activity = 30.2 GBq)	26 months, tumor progression
Yes	Yes	Yes	No	[^{177}Lu]Octreotate (4x/6–8 wk; total administered activity = 29.6 GBq)	20 months, remission
Yes	Yes	No	Yes	[^{177}Lu]Octreotate (4x/6–8 wk + 2x/8 wk; total administered activity = 45.0 GBq)	68 months[a]
Yes	Yes	No	No	[^{177}Lu]Octreotate (4x/6 wk + 2x/8 wk; total administered activity = 43.4 GBq)	31 months[a]

[a] Died.
NET: neuroendocrine tumor; Cg: chromogranin; sst2a: somatostatin receptor subtype 2a.

Case 3

A 55-yr-old female had a collapse, and at the time of the collapse, a fasting plasma glucose level of 43.3 mg/dl (2.4 mmol/liter), insulin of 401 pmol/liter, and C-peptide of 3.33 ng/ml (1.11 nmol/liter) were measured. CT of the abdomen demonstrated multiple liver metastases and a mass in the tail of the pancreas. An [^{111}In]pentetreotide scan demonstrated intense radiotracer uptake by multifocal liver lesions and the lesion in the pancreatic tail. A malignant insulinoma was diagnosed. The patient was initially treated with diazoxide and iv glucose. Later, diazoxide had to be stopped because of adverse events such as nausea, and octreotide sc was started.

The patient kept suffering from severe long-lasting hypoglycemia and needed continuous iv glucose. In an attempt to reduce the insulin hypersecretion, the patient underwent debulking surgery. Histological examination of the pancreas mass revealed a well differentiated, WHO2010 grade 1, neuroendocrine tumor with a Ki-67 index of less than 2% and positive immunohistochemistry for insulin. The iv glucose could be stopped, but the patient needed frequent meals with short intervals and octreotide to maintain a euglycemic state. Still, she incidentally reported hypoglycemia at weekly intervals. The patient was subsequently treated with four cycles of [^{177}Lu]octreotate, at 6- to 8-wk intervals up to a total administered activity of 29.6 GBq. During these cycles of [^{177}Lu] octreotate, the patient no longer suffered from hypoglycemic episodes (Figure 4). Twelve months after the first cycle of [^{177}Lu]octreotate and 16 months after the debulking surgery, CT of the abdomen demonstrated regression of the liver metastases and no tumor recurrence in the pancreas (Figure 5).

Since the start of the [^{177}Lu]octreotate, the hypoglycemia did not recur. Twenty months after the initial diagnosis, the disease is still in remission, and the patient is still in a euglycemic state (Table 1).

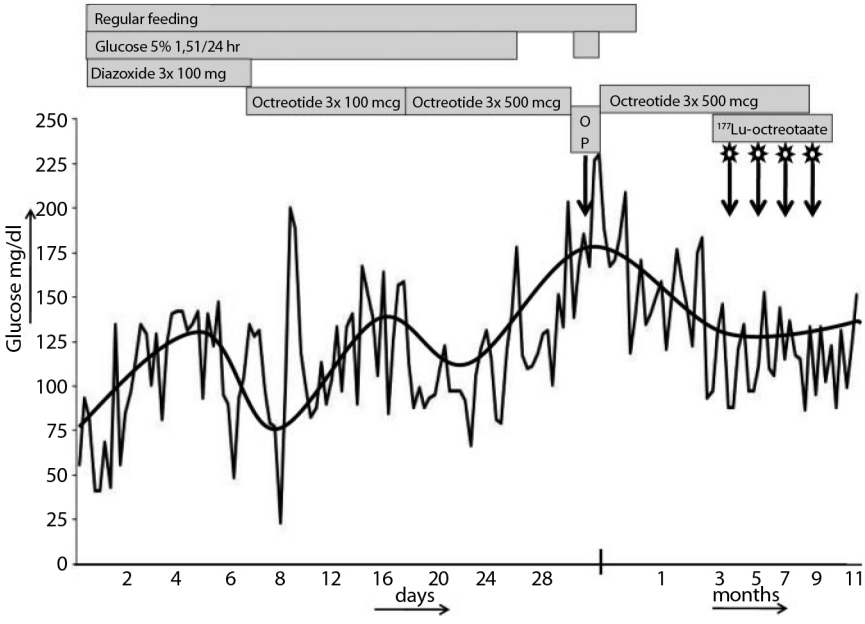

Figure 4. Timeline of treatment interventions (including iv glucose, diazoxide, sc octreotide, and [^{177}Lu] octreotate) with values for blood glucose levels in a 55-yr-old female with a malignant insulinoma (case 3).

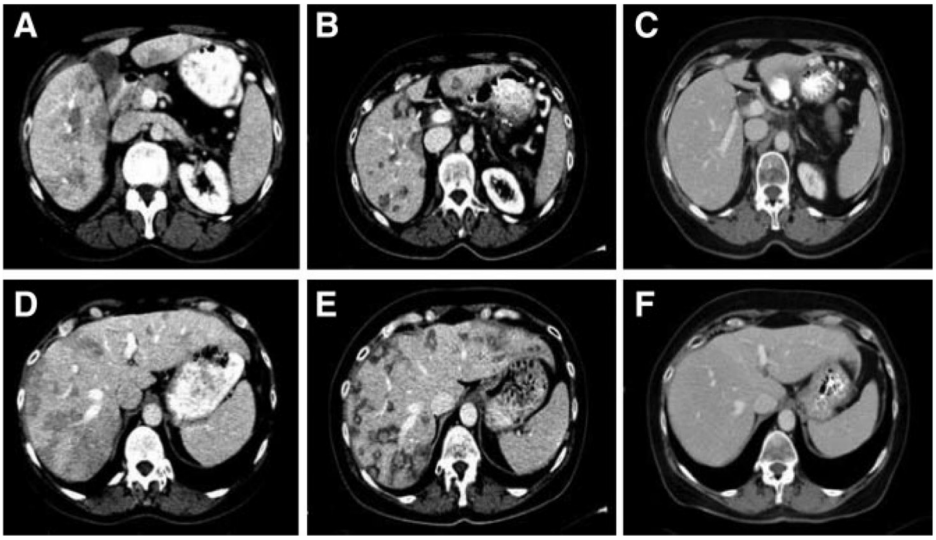

Figure 5. Comparison of baseline (**A** and **D**) CT scans of the abdomen and CT scans after surgery (**B** and **E**) and CT scans performed 15 months after initial diagnosis (**C** and **F**) in a 55-yr-old female with a malignant insulinoma treated with [^{177}Lu]octreotate showing remission of the liver metastases (case 3).

Case 4

A 50-yr-old male presented with hypoglycemic coma after a short period of fasting before a planned gastroduodenoscopy for hematemesis. At gastroduodenoscopy, gastritis and duodenitis were diagnosed, which were probably caused by the use of carbasalate calcium. He had started using this therapy after a transient ischemic attack, which was diagnosed the previous year. Already at the time of the transient ischemic attack, a random fasting plasma glucose level of 48.6 mg/dl (2.7 mmol/liter) was measured, but no action was undertaken. The fasting laboratory tests were plasma glucose of 18 mg/dl (1.0 mmol/ liter), insulin of 173 pmol/liter, and C-peptide of 4.65 ng/ml (1.55 nmol/liter). CT revealed a pancreatic mass with diffuse liver metastases. An [^{111}In]pentetreotide scan demonstrated areas of intense tracer uptake in the liver and pancreas, which corresponded with the CT findings. A malignant insulinoma was diagnosed. Histological examination of the pancreatic mass or liver metastases was not performed, because the clinical presentation with hypoglycemia in combination with the CT/[^{111}In]pentetreotide scan images was found to be pathognomonic. Octreotide sc was started followed by two cycles of chemotherapy with streptozotocin and 5-FU. The severity and frequency of hypoglycemia diminished. After two cycles of chemotherapy, additional treatment with [^{177}Lu]octreotate was given as four cycles at 8-wk intervals up to a total administered activity of 30.0 GBq. After four cycles of radiolabeled somatostatin analog therapy, partial remission was demonstrated, and the hypoglycemia

was well controlled. No hematological or renal toxicities were recorded during this therapy. Two years after the last cycle of [^{177}Lu]octreotate, he was diagnosed with progression of the liver metastases, but the hypoglycemia did not recur. Additional treatment with two cycles of [^{177}Lu]octreotate at 6- to 8-wk intervals up to a total administered activity of 45.0 GBq followed. Again, partial remission of the disease was induced and hypoglycemia still controlled. Seven months after the last cycle of [^{177}Lu]octreotate, progression of the liver metastases recurred. Since the first treatment with [^{177}Lu]octreotate, the patient had had no hypoglycemic episodes for 43 months. Palliative treatment was initiated. The patient died 68 months after the initial diagnosis of malignant insulinoma (Table 1).

Case 5

A 34-yr-old male was referred to our hospital because of an insulinoma in the head of the pancreas with multiple lymph node metastases. The patient was initially treated with diazoxide and iv glucose. The diazoxide had to be stopped because of side effects, and octreotide sc was started. Subsequently, iv glucose infusion could be stopped, and the patient no longer suffered from hypoglycemic episodes. An [^{111}In]pentetreotide scan demonstrated intense radiotracer localization in the pancreatic head and the locoregional and distant lymph nodes. Histological examination of a supraclavicular lymph node biopsy revealed a moderately differentiated, WHO2010 grade 2, neuroendocrine tumor with a Ki-67 index of 2–15%. A positive immunohistochemical staining for insulin was observed. Based on the proven metastasis of an insulin producing neuroendocrine tumor, a malignant insulinoma was diagnosed. The patient was treated with four cycles of [^{177}Lu]octreotate at 6- to 8-wk intervals up to a total administered activity of 28.7 GBq. No hematological or renal toxicities were recorded during this therapy. The disease remained stable for 17 months after the last [^{177}Lu]octreotate treatment, which was 21 months after the initial diagnosis. During and after these cycles of [^{177}Lu]octreotate, the patient no longer suffered from hypoglycemic episodes. Subsequently, the patient developed liver metastases and progressive lymph node metastases. Two additional cycles of [^{177}Lu]octreotate at 8-wk intervals up to a total administered activity of 43.4 GBq were administered. Euglycemia was maintained for another 6 months. Hereafter, he again had progressive disease with progression of the liver and lymph node metastases, and new bone metastases were diagnosed. Palliative treatment was initiated. The patient died 31 months after the initial diagnosis because of progressive malignant insulinoma (Table 1).

DISCUSSION

We present five patients with a history of severe, life-threatening hypoglycemia with malignant, insulin-producing, neuroendocrine tumors of the pancreas with metastases in the liver and/or lymph nodes and/or bone. Different treatments were unsuccessfully

introduced to achieve and/or maintain euglycemia. Continuous iv glucose treatments required hospitalization in all patients. Our goals of treatment were to achieve long-lasting euglycemia in an outpatient setting, to induce progression-free survival, and to maintain an acceptable quality of life.

Diazoxide is a well-known drug that is used to control hypoglycemia in patients with insulinoma. It is an antihypertensive benzothiadiazine derivative that produces hyperglycemia as a side effect.[15] It inhibits the release of insulin by pancreatic β-cells by opening ATP-sensitive potassium channels.[16-17] All our patients received diazoxide, but most suffered from serious adverse events such as nausea and edema, and hence either the diazoxide dose had to be reduced or the drug had to be stopped. Chemotherapy for pancreatic neuroendocrine carcinomas consists of streptozotocin in combination with 5-FU and/or doxorubicin.[13, 18] Streptozotocin is an alkylating nitrosourea compound, but the exact mechanism of cytotoxicity is not known. This drug is well known for producing diabetes mellitus in experimental animals.[19] 5-FU inhibits thymidylate synthetase, causing depletion of thymidine and subsequently leading to cell death. Doxorubicin binds to DNA and inhibits DNA and RNA synthesis. One of our patients had been treated with chemotherapy and became euglycemic.

Insulinomas express somatostatin receptors.[20-22] Five different subtypes of somatostatin receptors, named sst_{1-5}, are currently known. Bertherat and co-workers[20] found that sst_2 and sst_5 are the most frequently expressed somatostatin receptors in insulinomas. In most of the tumors they investigated, higher expression of sst_2 than sst_5 was observed, but a subgroup of tumors presented higher expression of sst_5 than of sst_2. Vezzosi and co-workers[21] found that octreotide sc was an effective treatment of hypoglycemia in more than 50% of patients with insulinoma. All their patients had sst_2 positive benign insulinomas. The sst-subtype expression differs between benign and malignant insulinomas. Portela-Gomes and co-workers[22] found that sst_4 was the most frequently expressed sst in both benign and malignant insulinomas. The malignant tumors, but none of the benign tumors, also expressed sst_5. In this series, all other receptor subtypes were expressed in low numbers, and no difference between benign and malignant insulinomas was found. The sst expression is very important for the clinical benefit of somatostatin analog treatment of malignant insulinomas. Lanreotide, octreotide, and the radiolabeled somatostatin analogs predominately bind with a high affinity to sst_2. The somatostatin analog octreotide can increase, but paradoxically also lower, blood glucose levels in insulinoma patients.[23] Aggravation of hypoglycemia after administration of octreotide in patients with metastatic insulinomas can probably be attributed to the inhibition of insulin-antagonistic hormones such as GH and glucagon.[24] Therefore, the blood glucose levels should always be monitored carefully after administrating octreotide for the first time in insulinoma patients. In two of our five patients, hypoglycemia completely disappeared with octreotide (cases 1 and 5), and in three patients, the frequency and/or severity of the hypoglycemic episodes improved with

this therapy. One patient (case 1) even developed hyperglycemia at the end of her disease course and after PRRT (case 1). All five patients had intense uptake of [^{111}In]pentetreotide at the sites of the primary tumors, except in one patient (case 1), and its metastases (all cases). PRRT using ^{111}In-, ^{90}Y-, or ^{177}Lu-labeled somatostatin analogs has become increasingly available in many centers worldwide. Initially, studies were performed using [^{111}In]DTPA^{0}-octreotide, which emits Auger electrons and has a maximum tissue penetration range of only 10 µm. In recent years, [^{90}Y-DOTA^{0},Tyr^{3}]octreotide, [^{90}Y-DOTA]lanreotide, [^{90}Y-DOTA^{0},Tyr^{3}] octreotate, and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate have become the radiopharmaceuticals of first choice. ^{90}Y has a longer tissue penetration range (12 mm) than ^{177}Lu (2 mm). An additional advantage of ^{177}Lu over ^{90}Y is that this radionuclide also emits γ-radiation apart from β-radiation, thus enabling better dosimetry. It has been shown that the cytotoxic effects of ^{177}Lu can at least continue until 6–12 months after the administration of the last dose, thus resulting in additional tumor reductions. PRRT using radiolabeled somatostatin analogs is currently considered as one of the most successful therapies for inoperable, metastatic pancreatic neuroendocrine tumors expressing somatostatin receptors.[25-26] All five patients were treated with PRRT using radiolabeled somatostatin analogs. One patient was treated with cycles of [^{111}In]octreotide, because [^{177}Lu]octreotate was not yet available at that time. The other four patients were treated with [^{177}Lu]octreotate. The time period of maintaining stable disease varied from 18–50 months. [^{177}Lu]octreotate was successful in controlling further tumor progression in four of our patients (patients 2–5). In all patients, euglycemia was achieved and maintained. This illustrates that PRRT added to the standard octreotide course by inducing or maintaining euglycemia. Of particular interest, hypoglycemia did not recur despite tumor progression. This phenomenon cannot be easily explained. However, in a study in neuroendocrine tumors of the digestive tract (carcinoid tumors) refractory to octreotide, Bushnell and co-workers[27] made similar observations using [^{90}Y]octreotide. Apart from tumor responses, these authors also observed improvement of symptoms, even in patients who did not show any tumor regression.[27] Adverse reactions observed after PRRT can be divided into direct side effects and more delayed effects of radiotoxicity. Direct effects include nausea, vomiting, and abdominal pain. In general, these side effects occur in a minority of patients and can be easily treated with antiemetics or pain medication. Subacute, hematological toxicity, WHO grade 3 or 4, occurs in up to 9.5% of patients. Temporary hair loss (WHO grade 1; no baldness) occurred in 62% of patients. In less than 2% of patients, delayed serious toxicities especially to the bone marrow resulting in myelodysplastic syndrome and/or leukemia and to the kidneys resulting in renal insufficiency and liver failure occurred.[28] None of our patients developed serious hematological, liver, or renal toxicities with [^{177}Lu] octreotate or [^{111}In]octreotide treatment.

Ong and co-workers[29] have described two cases with malignant insulinomas treated with [^{177}Lu]octreotate. However, one of their cases was also cotreated with everolimus (RAD001). Everolimus is an mTOR inhibitor. mTOR functions downstream of phosphatidylinositol

3-kinase and AKT and is activated in 15% of pancreatic neuroendocrine tumors.[30] Inhibitors of mTOR, like everolimus, can produce regression of neuroendocrine tumors.[31] Depending on the dose and schedule of mTOR inhibitors, insulin secretion may either be suppressed or increased. It is also possible that these drugs induce peripheral insulin resistance. Furthermore, these drugs might reduce insulin production by reducing the insulin-producing tumor mass. Hyperglycemia is a well-known side effect of treatment with mTOR inhibitors. Bourcier and co-workers[10] have shown successful control of hypoglycemia using rapamycin (sirolimus) in an elderly patient with a metastatic pancreatic insulinoma. Kulke and co-workers[11] have also demonstrated successful control of hypoglycemia in four patients with metastatic insulinomas using everolimus. All four of these patients had received treatment with depot octreotide, and three patients continued treatment with this drug.[11] Our patients did not receive treatment with mTOR inhibitors, because these were not yet available at the time of admission or not yet approved for use in pancreatic neuroendocrine tumors at the time of admission, or clinical trials with these drugs were not ongoing in our center at the time of admission.

Here we presented five patients with inoperable malignant insulinomas and severe hypoglycemia in whom treatment with [^{111}In]octreotide or [^{177}Lu]octreotate in combination with sc octreotide not only controlled tumor growth but also successfully contributed to improved control of severe hypoglycemia. In addition, the patients did not experience hypoglycemia during a mean follow-up of 25.6 months. In three patients, palliative treatment was initiated because of further tumor progression but not because of recurrence of severe, uncontrollable, hypoglycemia. Two patients are still alive and do not suffer from hypoglycemic episodes, and one of them is still progression free.

CONCLUSION

In conclusion, apart from somatostatin analogs, PRRT using radiolabeled somatostatin analogs is a very effective treatment to control severe hypoglycemia induced by over-production of endogenous insulin by malignant, metastatic insulinomas. It does so by controlling the tumor mass and potentially also by influencing tumor (de)differentiation.

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Chapter 8

Summary and General Discussion

Neuroendocrine tumors (NETs) are rare neoplasms with an incidence of 2–5 per 100,000 inhabitants.[1-3] The majority of NETs abundantly express somatostatin receptors and these tumors can be visualized in patients using the radiolabeled somatostatin analog [^{111}Indium-DTPA^{0}]octreotide (^{111}In-octreotide) (OctreoScan®; Covidien, Petten, the Netherlands).[4] A logical next step to this tumor visualization *in vivo* was to also try to treat these patients with radiolabeled somatostatin analogs. Early studies in the 1990s used high activities of the Auger electron emitting ^{111}In-octreotide for Peptide Receptor Radionuclide Therapy (PRRT). These treatments often resulted in symptom relief in patients with metastasized NETs, but objective tumor responses were rare.[5-6] The use of a different chelator, DOTA instead of DTPA, allowed stable binding of somatostatin analogs with the β-emitting radionuclides ^{90}Yttrium (^{90}Y) and ^{177}Lutetium (^{177}Lu). Since 2000, PRRT in Rotterdam is being performed with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-octreotate or ^{177}Lu-DOTATATE). [^{90}Y-DOTA^{0},Tyr^{3}]octreotide is being used by other centers.

In this thesis, several treatment effects of ^{177}Lu-octreotate are evaluated. **Chapter 1** is a general introduction of NETs (including epidemiology and diagnosis), of the different therapeutic modalities for these tumors, and also provides information on how to measure treatment effects, including tumor response, tumor markers, survival, and quality of life measurement.

Chapter 2 is a review of the literature on PRRT. The results and side effects of therapy with the radiolabeled somatostatin analogs ^{111}In-octreotide, [^{90}Y-DOTA^{0},Tyr^{3}]octreotide, and ^{177}Lu-octreotate, were discussed. Objective tumor responses observed after ^{111}In-octreotide were rare. Complete and partial responses obtained after treatment with [^{90}Y-DOTA^{0},Tyr^{3}] octreotide are in the same range as after treatment with ^{177}Lu-octreotate (i.e. 10–30%). Several options to improve PRRT, such as combining different radiolabeled somatostatin analogs; the administration of locoregional, i.e. intra-arterial, radiolabeled somatostatin analogs for liver metastases; or the use of radiosensitizing drugs in combination with PRRT; were discussed. Special emphasis was given to the limitations of PRRT, of which nephrotoxicity is the most important one, especially when ^{90}Y is used.

Chapter 3 describes the difference in treatment response of bone and soft-tissue lesions after treatment with ^{177}Lu-octreotate in 42 patients with gastroenteropancreatic and bronchial NETs. The first finding was that, although all patients had positive bone lesions on [^{111}Indium-DTPA^{0}]octreotide scintigraphy (SRS) before PRRT, bone lesions were not visible on CT before PRRT in 26% of patients. The second finding was that whereas bone lesions increased in size after treatment with ^{177}Lu-octreotate, soft-tissue lesions regressed. The difference in percent change of bone and soft-tissue lesions was significant ($p<0.001$). Tumor markers and intensity and/or number of bone lesions on SRS decreased after

treatment. Also, in one patient 'new' bone lesions became visible on CT after treatment. This was not interpreted as being progressive disease, because this patient had a good treatment response, reflected by a partial response of the liver metastases and a significant decline of the tumor marker chromogranin A. We concluded that the apparent increase in size of bone lesions or the appearance of new bone lesions on CT after treatment with ^{177}Lu-octreotate should be interpreted very cautiously. Recognition of this phenomenon is important to prevent mislabeling of these patients as having progressive disease.

In **Chapter 4**, we compared four different response criteria (i.e. RECIST, SWOG, mRECIST, and mSWOG) in the tumor response assessment of 268 Dutch patients with gastroenteropancreatic and thoracic NETs treated with ^{177}Lu-octreotate. The rates of an objective response, stable disease, and progressive disease were comparable for the RECIST and SWOG criteria; and for the mRECIST and mSWOG criteria. The four scoring systems gave comparable results in terms of progression-free survival (PFS) and overall survival (OS) per categorized outcome. As expected, patients with progressive disease as treatment outcome had significantly shorter PFS and OS than the other patients. Since both the RECIST and SWOG criteria are used to assess response after treatment of NETs in different studies, it is important to know that both criteria give comparable results in terms of categorized outcomes and in prediction of survival. However, it was obvious that the addition of the response class "minor response" (MR) did not improve the correlation with PFS and OS.

In **Chapter 5**, we describe the neoadjuvant application of ^{177}Lu-octreotate in patients with nonfunctioning pancreatic NETs. This is a very important application, since surgery is the only potential for cure in these patients. Patients were divided into 3 groups: locally advanced tumor (group 1); ≤3 liver metastases (group 2); >3 liver metastases/other distant metastases (group 3). Patients in group 1+2 were considered neoadjuvant treated patients. Successful surgery after ^{177}Lu-octreotate was performed in 10/119 patients with a nonfunctioning pancreatic NET (8%). In the neoadjuvant treated patients, surgery was performed in an encouraging rate of 9/29 patients (31%). The 10th operated patient had more than 3 liver metastases, and thus did not belong to group 1 or 2 (i.e. the neoadjuvant treated patients). Surgery after PRRT could be safely performed in all 10 patients. The median PFS was 69 months for patients in group 1+2 with successful surgery, 49 months for the other patients in group 1+2, and 25 months for patients in group 3 (p=0.01). Surgery should be considered after treatment with ^{177}Lu-octreotate in patients with initially irresectable pancreatic NETs, as our data suggest that successful surgery after ^{177}Lu-octreotate is associated with increased PFS. We concluded that our observations provide justification for a prospective study on the neoadjuvant use of ^{177}Lu-octreotate in patients with nonfunctioning pancreatic NETs.

In **Chapter 6**, we aimed to investigate potential mechanisms of an observed decline in serum calcium levels after treatment with ^{177}Lu-octreotate. Forty-seven patients with NETs, who all were normocalcemic before the treatment with ^{177}Lu-octreotate, were prospectively analyzed, with regard to various laboratory parameters in serum and urine. The mean serum calcium level decreased significantly from 2.29 ± 0.01 mmol/l to a nadir of 2.24 ± 0.01 mmol/l at 6 weeks after treatment (p=0.02). A marked decrease in serum calcium levels to a nadir of ≤2.10 mmol/l at any time during or after treatment was observed in 8/47 patients (17%). The decline in serum calcium levels was confirmed in a larger, retrospectively analyzed, patient group. Several potential causes of hypocalcemia were excluded, such as primary hypoparathyroidism, vitamin D deficiency, renal insufficiency, pseudohypoparathyroidism, intestinal malabsorption, and low calcium intake, leaving the cause of the hypocalcemia observed after ^{177}Lu-octreotate in this study unknown. We concluded that serum calcium levels should be monitored during and after PRRT, and calcium substitution therapy should be initiated where appropriate.

Chapter 7 describes 5 patients with metastatic insulinomas and severe, poorly controllable, hypoglycemia, requiring continuous glucose infusion. One patient was treated with high doses of ^{111}In-octreotide, and the other four patients with ^{177}Lu-octreotate. After these interventions, disease was stable for a mean period of 27 months. During this period, hypoglycemic episodes did not occur in any of the patients. Eventually, three patients died because of progression. Hypoglycemia in the setting of metastatic insulinomas is often very hard to treat, and can be life threatening. We concluded that the application of PRRT with ^{111}In-octreotide or ^{177}Lu-octreotate provides an excellent method to reduce symptoms of hypoglycemia induced by overproduction of endogenous insulin in patients with metastatic insulinomas.

FUTURE PERSPECTIVES

Treatment with ^{177}Lu-octreotate is a valuable treatment option for patients with metastasized or inoperable NETs. It results in an objective tumor response or stable disease in 80% of patients.[7] Severe late side effects such as renal insufficiency and myelodysplastic syndrome occur in 2–3% of patients. The median time to progression is 40 months from start of treatment with ^{177}Lu-octreotate. Overall median survival compared favourably to historical controls, indicating a survival benefit of 3–6 years. However, these results are based on retrospective data.

There is a lack of prospective, randomized clinical trials involving PRRT. Prospective, randomized clinical trials are regarded as the most convincing level of evidence, and effort must be undertaken to initiate such trials. In our center, a randomized clinical trial is ongoing, in which patients are randomized to either treatment with ^{177}Lu-octreotate or treatment

with ^{177}Lu-octreotate in combination with the chemotherapeutic drug capecitabine (Xeloda®; Roche, Basel, Switzerland).[8]

Very importantly, in 2012, a multicenter, prospective, randomized clinical trial in patients with inoperable, progressive midgut NETs, has started, in which patients are randomized between treatment with ^{177}Lu-octreotate + Octreotide LAR 30 mg and treatment with high dose (60 mg) Octreotide LAR (www.clinicaltrials.gov; ClinicalTrials.gov Identifier: NCT01578239).

Given the recent advances in treatment of patients with pancreatic NETs with everolimus[9] or with sunitinib[10], a randomized clinical trial in patients with pancreatic NETs randomizing between treatment with ^{177}Lu-octreotate and treatment with everolimus, or a trial randomizing between treatment with ^{177}Lu-octreotate and treatment with sunitinib, is very much of interest and must be given priority. Due to the infrequency of NETs, these trials likely need to be multicenter trials. Such a trial in patients with pancreatic NETs is currently in preparation.

Another important application of ^{177}Lu-octreotate is as neoadjvant treatment in patients with initially irresectable pancreatic NETs. As successful surgery after ^{177}Lu-octreotate appears to be associated with increased PFS, surgery should be considered after treatment with ^{177}Lu-octreotate in all patients with initially irresectable pancreatic NETs. Surgery may also be considered after an objective response obtained with chemotherapy in such patients. We feel that a prospective study on the neoadjuvant use of ^{177}Lu-octreotate in patients with nonfunctioning pancreatic NETs should be initiated.

Results from animal experiments[11] and retrospective data in patients with NETs[12-13] suggest that the combination of ^{90}Y-labeled somatostatin analogs and ^{177}Lu-labeled somatostatin analogs is more effective in tumor control than the use of these compounds alone. A prospective randomized clinical trial comparing the combination of ^{90}Y-labeled somatostatin analogs and ^{177}Lu-labeled somatostatin analogs with either compound alone, should be initiated in patients with NETs to confirm these data. In such a study, it should also be investigated whether the combination treatment results in improved survival and improved quality of life when compared with the single treatment.

Lastly, the administration of intra-arterial radiolabeled somatostatin analogs for neuroendocrine liver metastases (as discussed in **Chapter 2**) is very much of value in patients with a predominant tumor load in the liver. The mean standardized uptake value of liver metastases in NET patients was higher after intra-arterial infusion of ^{68}Ga-DOTATOC than after intravenous administration.[14] Objective responses after intra-arterial PRRT in NET patients are very encouraging and are reported to be around 60%.[15-16]

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Chapter 9

Samenvatting en Algemene Discussie

Neuroendocriene tumoren (NETs) zijn zeldzame neoplasmata met een incidentie van 2-5 per 100.000 inwoners.[1-3] De meerderheid van NETs brengt ruimschoots somatostatine receptoren tot expressie en deze tumoren kunnen in patiënten afgebeeld worden met behulp van het radioactief gelabelde somatostatine analoog [^{111}Indium-DTPA^{0}]octreotide (^{111}In-octreotide) (OctreoScan®; Covidien, Petten, Nederland).[4] Een logische volgende stap na deze tumor visualisatie *in vivo* was om ook te proberen om patiënten te behandelen met radioactief gelabelde somatostatine analoga. De eerste studies in de jaren '90 van de vorige eeuw gebruikten hoge doses van het Auger electron uitzendende ^{111}In-octreotide voor Peptide Receptor Radionuclide Therapie (PRRT). Deze behandelingen resulteerden vaak in symptoom verbetering in patiënten met gemetastaseerde NETs, echter een objectieve tumorrespons vond zelden plaats.[5-6] Het gebruik van een andere chelator, DOTA in plaats van DTPA, maakte een stabiele binding van somatostatine analoga met de β-straling uitzendende radionucliden ^{90}Yttrium (^{90}Y) en ^{177}Lutetium (^{177}Lu) mogelijk. Sinds 2000 wordt PRRT in Rotterdam verricht met [^{177}Lu-DOTA^{0},Tyr^{3}]octreotaat (^{177}Lu-octreotaat of ^{177}Lu-DOTATAAT). [^{90}Y-DOTA^{0},Tyr^{3}]octreotide wordt gebruikt in andere centra.

In dit proefschrift worden verschillende therapie effecten van ^{177}Lu-octreotaat beschreven. **Hoofdstuk 1** is een algemene inleiding over NETs (inclusief epidemiologie en diagnose), over de verschillende behandelmodaliteiten voor deze tumoren, en beschrijft daarnaast hoe therapie effecten, zoals tumorrespons, tumormarkers, overleving, en kwaliteit van leven, te meten zijn.

Hoofdstuk 2 geeft een overzicht van de literatuur over PRRT. De resultaten en bijwerkingen van behandeling met de radioactief gelabelde somatostatine analoga ^{111}In-octreotide, [^{90}Y-DOTA^{0},Tyr^{3}]octreotide, en ^{177}Lu-octreotaat worden beschreven. Objectieve tumorresponsen na behandeling met ^{111}In-octreotide traden zelden op. Complete en partiële responsen na behandeling met [^{90}Y-DOTA^{0},Tyr^{3}]octreotide treden ongeveer even vaak op als na behandeling met ^{177}Lu-octreotaat (d.w.z. 10–30%). Verscheidene opties om PRRT te verbeteren worden beschreven, zoals het combineren van verschillende radioactief gelabelde somatostatine analoga; de toediening van locoregionale, d.w.z. intra-arteriële, radioactief gelabelde somatostatine analoga voor levermetastasen; of het gebruik van radiosensitiserende geneesmiddelen in combinatie met PRRT. Speciale aandacht wordt gegeven aan de beperkingen van PRRT, waarvan niertoxiciteit de belangrijkste is, vooral wanneer ^{90}Y wordt gebruikt.

Hoofdstuk 3 beschrijft het verschil in de therapierespons tussen bot- en weke delen laesies na behandeling met ^{177}Lu-octreotaat in 42 patiënten met gastroenteropancreatische en bronchiale NETs. De eerste bevinding was dat botlaesies niet zichtbaar waren op CT scan voor PRRT in 26% van de patiënten, terwijl alle patiënten zichtbare botlaesies hadden

op [^{111}Indium-DTPA^{0}]octreotide scintigrafie (SRS) voor PRRT. De tweede bevinding was dat botlaesies groter werden na behandeling met ^{177}Lu-octreotaat, terwijl weke delen laesies kleiner werden. Het verschil in procentuele verandering tussen bot- en weke delen laesies was significant ($p<0.001$). Tumormarkers en de intensiteit van botlaesies en/of het aantal botlaesies op SRS namen af na de behandeling. Daarnaast werd een patiënt beschreven waarin 'nieuwe' botlaesies zichtbaar werden op CT na behandeling. Dit werd niet geïnterpreteerd als progressieve ziekte, omdat deze patiënt een goede reactie op de behandeling had, hetgeen zichtbaar was door een partiële respons van de levermetastasen en een significante daling van de tumormarker chromogranine A. Wij concludeerden dat de ogenschijnlijke toename in grootte van botlaesies of het verschijnen van nieuwe botlaesies op CT na behandeling met ^{177}Lu-octreotaat zeer voorzichtig geïnterpreteerd dient te worden. Het herkennen van dit fenomeen is belangrijk om te voorkomen dat deze patiënten ten onrechte de uitkomst "progressieve ziekte" wordt toebedeeld.

In **Hoofdstuk 4** worden vier verschillende respons criteria (RECIST, SWOG, mRECIST, en mSWOG) vergeleken in de beoordeling van de tumorrespons in 268 Nederlandse patiënten met gastroenteropancreatische en thoracale NETs die behandeld waren met ^{177}Lu-octreotaat. De aantallen van een objectieve respons, stabiele ziekte, en progressieve ziekte waren vergelijkbaar voor de RECIST en SWOG criteria; en voor de mRECIST en mSWOG criteria. De vier scoringssystemen gaven vergelijkbare resultaten wat betreft progressievrije overleving en algemene overleving per gecategoriseerde respons groep. Zoals te verwachten was, hadden patiënten met progressieve ziekte als therapie uitkomst een significant kortere progressievrije overleving en algemene overleving dan de andere patiënten. Omdat zowel de RECIST als de SWOG criteria gebruikt worden voor het beoordelen van de therapierespons van NETs in verschillende studies, is het belangrijk om te weten dat beide criteria vergelijkbare resultaten gaven wat betreft gecategoriseerde respons uitkomst en in de voorspelling van overleving. Het was echter duidelijk dat de toevoeging van de respons categorie "mineure respons" de correlatie met progressievrije overleving en algemene overleving niet verbeterde.

In **Hoofdstuk 5** wordt de neoadjuvante toepassing van ^{177}Lu-octreotaat in patiënten met een niet-functionerende NET van de pancreas beschreven. Dit is een zeer belangrijke toepassing, omdat chirurgie de enige mogelijkheid is om deze patiënten te genezen. De patiënten werden in 3 groepen onderverdeeld: tumor met beperkte, lokale, uitbreiding (groep 1), ≤3 levermetastasen (groep 2); >3 levermetastasen/andere metastasen op afstand (groep 3). Patiënten in groep 1+2 werden beschouwd als neoadjuvant behandelde patiënten. Tien van 119 patiënten met een niet-functionerende NET van de pancreas (8%) werden succesvol geopereerd na behandeling met ^{177}Lu-octreotaat. Van de 29 patiënten die neoadjuvant behandeld werden, werd een bemoedigend aantal van 9 patiënten (31%)

geopereerd. De tiende geopereerde patiënt had meer dan 3 levermetastasen en behoorde dus niet tot groep 1 of 2 (d.w.z. de patiënten die neoadjuvant behandeld werden). Bij de 10 geopereerde patiënten kon chirurgie na PRRT veilig worden uitgevoerd. De mediane progressievrije overleving was 69 maanden voor de succesvol geopereerde patiënten in groep 1+2, 49 maanden voor de overige patiënten in groep 1+2, en 25 maanden voor patiënten in groep 3 (p=0.01). Chirurgie na behandeling met ^{177}Lu-octreotaat dient overwogen te worden in patiënten met een initieel irresectabele NET van de pancreas, omdat onze resultaten erop duiden dat succesvolle chirurgie na ^{177}Lu-octreotaat geassocieerd is met een langere progressievrije overleving. Wij concludeerden dat onze resultaten een prospectieve studie naar de neoadjuvante behandeling met ^{177}Lu-octreotaat van patiënten met een niet-functionerende NET van de pancreas rechtvaardigen.

In **Hoofdstuk 6** worden potentiële oorzaken onderzocht van een geobserveerde daling in serum calciumwaarden na behandeling met ^{177}Lu-octreotaat. Zeven en veertig patiënten met NETs, die allen een normaal serum calciumgehalte voor het starten van de behandeling met ^{177}Lu-octreotaat hadden, werden prospectief geanalyseerd. Bij hen werden verschillende laboratoriumwaarden in serum en urine bepaald. Het gemiddelde serum calciumgehalte daalde significant van 2.29 ± 0.01 mmol/l naar 2.24 ± 0.01 mmol/l op 6 weken na therapie (p=0.02). Een duidelijke afname in het serum calciumgehalte tot een laagste punt van ≤2.10 mmol/l tijdens of na behandeling werd gezien bij 8 van 47 patiënten (17%). De daling in serum calciumgehalte werd tevens gevonden in een grotere groep patiënten, die retrospectief geanalyseerd werd. Verscheidene oorzaken van hypocalciëmie, zoals primaire hypoparathyreoïdie, vitamine D deficiëntie, nierinsufficiëntie, pseudohypoparathyreoïdie, malabsorptie van calcium door de darm, en onvoldoende calciuminname, werden uitgesloten. De oorzaak van de geobserveerde hypocalciëmie na ^{177}Lu-octreotaat, die in deze studie werd gevonden, bleef onverklaard. Wij concludeerden dat het serum calciumgehalte gecontroleerd dient te worden gedurende en na PRRT, en dat, indien nodig, calcium substitutie voorgeschreven moet worden.

Hoofdstuk 7 beschrijft 5 patiënten met een gemetastaseerd insulinoom en ernstige, zeer slecht te controleren hypoglycemieën, waarvoor zij continue glucose infusies nodig hadden. Eén patiënt werd behandeld met hoge doses ^{111}In-octreotide, en de andere vier patiënten met ^{177}Lu-octreotaat. Na deze behandelingen was de ziekte stabiel met een gemiddelde duur van 27 maanden. Daarnaast bleven bij alle patiënten hypoglycemieën uit gedurende deze periode. Uiteindelijk overleden drie patiënten vanwege progressie. Hypoglycemie veroorzaakt door een gemetastaseerd insulinoom is vaak zeer moeizaam te behandelen, en kan levensbedreigend zijn. Wij concludeerden dat PRRT met ^{111}In-octreotide of ^{177}Lu-octreotaat een uitstekende methode kan zijn om de symptomen van hypoglycemieën

die veroorzaakt worden door endogene insuline overproductie bij gemetastaseerde insulinomen, te verminderen.

TOEKOMSTPERSPECTIEVEN

Behandeling met ^{177}Lu-octreotaat is een waardevolle behandeloptie voor patiënten met gemetastaseerde of inoperabele NETs. Het leidt tot een objectieve tumorrespons of stabiele ziekte in 80% van de patiënten.[7] Ernstige bijwerkingen op langere termijn, zoals nierinsufficiëntie of een myelodysplastisch syndroom, komen voor in 2–3% van de patiënten. De mediane tijd tot progressie vanaf het starten van de behandeling met ^{177}Lu-octreotaat is 40 maanden. De mediane algemene overleving is gunstig in vergelijking met historische controles, met aanwijzingen voor een overlevingsvoordeel van 3 tot 6 jaar. Deze resultaten zijn echter gebaseerd op retrospectieve data.

Er is een gebrek aan prospectieve, gerandomiseerde klinische onderzoeken, over PRRT. Prospectieve, gerandomiseerde klinische onderzoeken worden gezien als studies met het hoogste bewijsniveau, en getracht moet worden om zulke onderzoeken te initiëren. Een gerandomiseerde klinische trial waarin patiënten gerandomiseerd worden tussen behandeling met ^{177}Lu-octreotaat en behandeling met ^{177}Lu-octreotaat in combinatie met het chemotherapeuticum capecitabine (Xeloda®; Roche, Basel, Zwitserland), is momenteel gaande op de afdeling Nucleaire Geneeskunde van het Erasmus MC.[8]

Ook interessant is om te vermelden dat in 2012 een multicenter, prospectieve, gerandomiseerde klinische trial gestart is waarin patiënten met inoperabele, progressieve midgut NETs, gerandomiseerd worden tussen behandeling met ^{177}Lu-octreotaat + Octreotide LAR 30 mg en behandeling met hoge dosis Octreotide LAR (60 mg) (www.clinicaltrials.gov; ClinicalTrials.gov Identifier: NCT01578239).

Gezien de recente ontwikkelingen in de behandeling van patiënten met een NET van de pancreas met everolimus[9] of met sunitinib[10], zou een gerandomiseerde klinische trial in patiënten met een NET van de pancreas, waarin patiënten gerandomiseerd worden tussen behandeling met ^{177}Lu-octreotaat en behandeling met everolimus, of gerandomiseerd worden tussen behandeling met ^{177}Lu-octreotaat en behandeling met sunitinib, zeer belangrijk zijn. Gezien de lage incidentie van pancreas NETs, dienen zulke trials multicenter trials te zijn. Zo een studie bij patiënten met een NET van de pancreas is nu in voorbereiding.

Een andere belangrijke toepassing van ^{177}Lu-octreotaat is als neoadjuvante behandeling in patiënten met een initieel irresectabele NET van de pancreas. Omdat succesvolle chirurgie na ^{177}Lu-octreotaat geassocieerd lijkt te zijn met een langere progressievrije overleving,

dient chirurgie na behandeling met ^{177}Lu-octreotaat in alle patiënten met een initieel irresectabele NET van de pancreas overwogen te worden. Chirurgie kan ook overwogen worden in zulke patiënten na een objectieve respons op chemotherapie. Een prospectieve studie met betrekking tot de neoadjuvante toepassing van ^{177}Lu-octreotaat in patiënten met een niet-functionerende NET van de pancreas dient geïnitieerd te worden.

Uit dierexperimenten[11] en uit retrospectieve studies bij patiënten met een NET[12-13] bleek dat de combinatie van ^{90}Y-gelabelde somatostatine analoga en ^{177}Lu-gelabelde somatostatine analoga effectiever was m.b.t. tumorverkleining dan bij het gebruik van deze behandelingen alleen. Een prospectieve gerandomiseerde klinische trial, waarin de combinatie van ^{90}Y-gelabelde somatostatine analoga en ^{177}Lu-gelabelde somatostatine analoga vergeleken wordt met deze behandelingen alleen, dient geïnitieerd te worden bij patiënten met een NET om de bovenbesproken data te bevestigen. In zo een studie dient ook onderzocht te worden of de combinatie behandeling in een langere overleving resulteert en leidt tot een betere kwaliteit van leven in vergelijking met deze behandelingen alleen.

Als laatste, de toediening van intra-arteriële radioactief gelabelde somatostatine analoga bij levermetastasen van een NET (zoals besproken in **Hoofdstuk 2**) is van grote waarde voor patiënten met vooral levermetastasen. De gemiddelde opname van radioactiviteit in levermetastasen van patiënten met een NET was hoger na intra-arteriële toediening van ^{68}Ga-DOTATOC dan na intraveneuze toediening.[14] Objectieve tumorresponsen behaald na intra-arteriële toediening van PRRT zijn zeer bemoedigend, en werden in ongeveer 60% van de patiënten met een NET gezien.[15-16]

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APPENDICES

Dankwoord
Curriculum Vitae
List of Publications
PhD Portfolio

DANKWOORD

Promoveren doe je niet alleen. Graag wil ik een aantal mensen bedanken voor het tot stand komen van dit proefschrift.

Allereerst wil ik de patiënten en hun families hartelijk danken voor hun deelname aan onze studie. Van heinde en verre kwamen patiënten om in het Erasmus MC behandeld te worden. Zonder hen was er geen onderzoek mogelijk geweest.

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Graag wil ik de overige leden van de commissie bedanken:
Prof. dr. W.W. de Herder. Beste Wouter, via jou ben ik aanvankelijk bij dit promotie onderzoek terecht gekomen, waarvoor hartelijk dank. Bedankt voor de input bij verscheidene van mijn artikelen. Ik waardeer je betrokkenheid bij de patiënten zeer, evenals je humor. Ik vond het erg leuk om bij jouw oratie aanwezig te zijn. Hopelijk zullen we in de toekomst nog veel samenwerken.

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Dr. M.A.J. de Ridder, beste Maria, een statisticus die statistiek kan uitleggen zodat een medicus het begrijpt, is goud waard. Jij bent er zo één. Hartelijk dank voor de samenwerking

en wellicht dat we in de toekomst ook nog samen zullen werken. Leuk om je ook nog in het Albert Schweitzer ziekenhuis tegen te komen.

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Liesbeth, mijn andere zusje, jij was me net een maand voor met jouw promotie. Het is toch goed dat je de arts-patiënt communicatie onderzocht hebt, alhoewel wij artsen er niet altijd even goed uit kwamen. Ik vind het jammer dat ik je nu zo weinig zie, omdat je nu in Londen woont, maar het is een heel fijn logeeradres daar! Ik zal zeker heel vaak langskomen. Ik ben supertrots op je dat je je promotie zo goed afgerond hebt, en dat je nu een mooie postdoc plek hebt bij een zeer gerenommeerd Londens instituut. Ik ben heel blij dat jij mijn paranimf wilt zijn op deze dag. Pim, een betere schoonbroer kun je je niet wensen. Have a good one in London.

Lieve mama, ik ben heel trots op je, wat je allemaal voor elkaar hebt gekregen. Ik ben jou en papa erg dankbaar dat jullie me in alles gesteund hebben, altijd achter me stonden, en me altijd vrij hebben gelaten om zelf mijn dingen te doen. Ik ben wat ik nu ben, door jullie. Helaas kan papa niet bij deze dag zijn, maar ik weet zeker dat hij het allemaal prachtig had gevonden. Hij kijkt zeker vanaf een wolkje naar beneden, en als er vandaag een mooie zonnestraal naar beneden komt, dan weet je van wie hij is. Bedankt voor alles.

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CURRICULUM VITAE

Esther Irene van Vliet was born on December 26, 1981 in Sindelfingen, Germany. As a child, she lived with her parents for 3 years in Zambia, after which they moved to the Netherlands. She attended the first 3 years of high school at the Gymnasium Baudartius College in Zutphen, and the last 3 years at the Gymnasium Zandvliet College in The Hague, where she graduated in 2000 (cum laude). After that, she started her medical training at the Medical Faculty of the Erasmus University in Rotterdam. During this period, she did a clinical elective in Pediatric Surgery in Vienna. In August 2005, she completed her Master thesis on 'Early childhood growth, early childhood infectious diseases and type 1 diabetes' at the department of Pediatric Diabetes of the Erasmus MC – Sophia's Children Hospital, supervised by Prof. dr. G.J. Bruining. Before starting with her internships, she travelled in South America and Africa for 3 months. During her internships, she did an elective internship in Tropical Medicine at the Holy Family Mission Hospital in the rural area of Phalombe in Malawi. In January 2008, she obtained her medical degree (cum laude). After that, she travelled for 3 months in Australia, after which she worked for 8 months as a medical physician at the department of Internal Medicine at the Albert Schweitzer Hospital in Dordrecht. In February 2009, she started her clinical research project at the Department of Nuclear Medicine at the Erasmus MC, Rotterdam, under supervision of Prof. dr. E.P. Krenning and Dr. D.J. Kwekkeboom. The research performed during this period is described in this thesis. In January 2013, she started her clinical residency in Internal Medicine at the Albert Schweitzer Hospital in Dordrecht.
She is married to Bas van Splunder and they live happily in Rotterdam.

LIST OF PUBLICATIONS

van Vliet EI, de Herder WW, de Rijke YB, Zillikens MC, Kam BLR, Teunissen JJM, Peeters RP, Krenning EP, Kwekkeboom DJ. Hypocalcaemia after treatment with [^{177}Lu-DOTA^{0},Tyr^{3}] octreotate. *Submitted*

van Vliet EI, Krenning EP, Teunissen JJ, Bergsma H, Kam BL, Kwekkeboom DJ. Comparison of Response Evaluation in Patients with Gastroenteropancreatic and Thoracic Neuroendocrine Tumors after Treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]Octreotate. *Submitted*

van Vliet EI, van Eijck CHJ, de Krijger RR, Nieveen van Dijkum EJ, Teunissen JJM, Kam BLR, de Herder WW, Feelders RA, Bonsing BA, Krenning EP, Kwekkeboom DJ. Neoadjuvant Treatment of Nonfunctioning Pancreatic Neuroendocrine Tumors with [^{177}Lu-DOTA^{0},Tyr^{3}]Octreotate. *Submitted*

Bergsma H, **van Vliet EI**, Teunissen JJM, Kam BLR, de Herder WW, Peeters RP, Krenning EP, Kwekkeboom DJ. Peptide Receptor Radionuclide Therapy (PRRT) for GEP-NETs. Best Practice & Research Clinical Gastroenterology. *Accepted*

van Vliet EI, Teunissen JJM, Kam BLR, de Jong M, Krenning EP, Kwekkeboom DJ. Treatment of Gastroenteropancreatic Neuroendocrine Tumors with Peptide Receptor Radionuclide Therapy. Neuroendocrinology. 2013;97(1):74-85.

van Vliet EI, Hermans JJ, de Ridder MA, Teunissen JJ, Kam BL, de Krijger RR, Krenning EP, Kwekkeboom DJ. Tumor Response Assessment to Treatment with [^{177}Lu-DOTA^{0},Tyr^{3}] Octreotate in Patients with Gastroenteropancreatic and Bronchial Neuroendocrine Tumors: Differential Response of Bone Versus Soft Tissue Lesions. Journal of Nuclear Medicine. 2012 Sep;53(9):1359-66.

Kam BL, Teunissen JJ, Krenning EP, de Herder WW, Khan S, **van Vliet EI**, Kwekkeboom DJ. Lutetium-labelled peptides for therapy of neuroendocrine tumours. Eur J Nucl Med Mol Imaging. 2012 Feb;39 Suppl 1:103-12.

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van Vliet EI, van Ouwerkerk BM. Hypocalcaemia as presenting symptom of velocardiofacial syndrome. Neth J Med. 2009 Mar;67(3):105-6.

BOOK

van Vliet EI, Kam BLR, Teunissen JJM, de Jong M, Krenning EP, Kwekkeboom DJ. Somatostatin analogs and radionuclides used in therapy. In: Somatostatin analogues, from research to clinical practice. Editors: Hubalewska-Dydejczyk A, de Jong M, Signore A, Dierckx RAJO, van de Wiele C, Buscombe J. John Wiley and sons, Inc. ISBN 9781118521533. *In Press*

PHD PORTFOLIO

Summary of PhD training and teaching

Erasmus MC Department:	Nuclear Medicine
Research School:	Molecular Medicine
PhD period:	February 2009 - December 2012
Promotor(s):	Prof.dr. E.P. Krenning
Supervisor:	Dr. D.J. Kwekkeboom

General courses	**Year**
Nihes Course 'Study Design'	2011
Biomedical English Writing and Communication	2011
Nihes Course 'Survival Analysis for Clinicians'	2011
Course 'Literatuurzoeken in diverse databanken' Medical Library	2010
Course 'Inleiding Literatuurzoeken' Medical Library	2010
Nihes Course 'Repeated Measurements in Clinical Studies'	2010
Nihes Course 'Regression Analysis for Clinicians'	2010
Nihes Course 'Biostatistics for Clinicians'	2010
Nihes Course 'Introduction to Clinical Research'	2010
Nihes Course 'Courses for the Quantitative Researcher'	2009
BROK ('Basiscursus Regelgeving Klinisch Onderzoek') Course	2009
Course 'End Note' Medical Library	2009

Specific courses	
Research Meetings, Department of Nuclear Medicine	2009-2012
Course 'Feedback Geven'	2011
Introduction to Statistics and Survival Analysis for MDs	2010
Course 'Research Management'	2010
Course 'Geavanceerde beeldvormende technieken voor dokters'	2009
Course 'Stralingsbescherming Niveau 5B'	2009

Seminars, workshops	
Interactieve nascholingscursus Pancreas NET Rotterdam	2012
Schildkliersymposium Rotterdam	2009
INKEP Meeting Rotterdam	2009

(Inter)national conferences

ENETS Congress Barcelona, Spain (oral presentation & poster presentation)	2013
NVNG Wetenschappelijke Vergadering, UMC St Radboud, Nijmegen (oral presentation)	2012
EANM Congress Milan, Italy (oral presentation)	2012
SNM Congress Miami, USA (poster presentation)	2012
ENETS Congress Copenhagen, Denmark (poster presentation)	2012
Scientific Exchange Program Meeting GEPNET Study Group, Gothenburg, Sweden (oral presentation) (and chair of the session 'Diagnosis/therapy via radionuclides/peptide receptors')	2012
ENETS Congress Lissabon, Portugal	2011
NETWork Symposium, Uppsala, Sweden (oral presentation)	2011
Klinisch Oncologische Samenwerking (KOS) Limburg (oral presentation)	2010
ENETS Congress Berlin, Germany	2010

Teaching activities

For guests from other hospitals at several occasions: Principles and effects of peptide receptor radionuclide therapy	2009-2012

Stellingen behorende bij het proefschrift

^{177}Lu-octreotate in Neuroendocrine Tumors: Treatment Effects

1. Neoadjuvante behandeling met lutetium-177-octreotaat, een radioactief gemerkt somatostatine analoog, is een zeer waardevolle optie voor patiënten met initieel irresectabele niet-functionele neuroendocriene tumoren van de pancreas, en dient bij zulke patiënten altijd overwogen te worden. (*dit proefschrift*)
2. Het unidimensionaal meten (RECIST criteria) of het bidimensionaal meten (SWOG criteria) van tumoren na behandeling met lutetium-177-octreotaat in patiënten met gastroenteropancreatische of thoracale neuroendocriene tumoren geeft vergelijkbare tumor uitkomsten en voorspelt op eenzelfde manier de overleving van deze patiënten. (*dit proefschrift*)
3. De toevoeging van de respons categorie 'minor response' in de gemodificeerde SWOG en gemodificeerde RECIST criteria draagt niet bij aan een betere voorspelling van de overleving van patiënten met gastroenteropancreatische of thoracale neuroendocriene tumoren die behandeld zijn met lutetium-177-octreotaat. (*dit proefschrift*)
4. De computertomografie (CT) scan is geen geschikte methode om de respons van botmetastasen van neuroendocriene tumoren na behandeling met lutetium-177-octreotaat te beoordelen. (*dit proefschrift*)
5. Serum calcium waarden moeten vervolgd worden tijdens en na behandeling met lutetium-177-octreotaat bij patiënten met neuroendocriene tumoren, daar er in deze patiënten een significante daling in serum calcium spiegels optreedt gedurende en na behandeling. (*dit proefschrift*)
6. Rare diseases are not so rare. (Wästfelt M, et al. J Intern Med. 2006;260:1-10)
7. Education is a human right with immense power to transform. On its foundation rest the cornerstones of freedom, democracy and sustainable human development. (Kofi Annan)
8. Een goede basis Interne Geneeskunde is onontbeerlijk tijdens de opleiding Nucleaire Geneeskunde.
9. De beste bondgenoten zijn deze – Tijd en Geduld. (Leo Tolstoy, Oorlog en Vrede)
10. Een 'gezond blozend gelaat' gaat helaas maar zelden op voor patiënten met het carcinoid syndroom.
11. The greatest glory in living lies not in never falling, but in rising everytime we fall. (Nelson Mandela, Long Walk to Freedom)

Esther van Vliet

29 mei 2013