Clinical & Biochemical Characterization of Gastroenteropancreatic Neuroendocrine Tumors

KIMBERLY KAMP

CLINICAL & BIOCHEMICAL CHARACTERIZATION OF GASTROENTEROPANCREATIC NEUROENDOCRINE TUMORS

KLINISCHE & BIOCHEMISCHE KARAKTERISERING VAN GASTROENTEROPANCREATISCHE NEUROENDOCRIENE TUMOREN

Kimberly Kamp

COLOFON

The work described in this thesis was conducted at the Section of Endocrinology of the Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands.

Printing of this thesis was kindly supported by:

Biochemical and clinical characterization of gastroenteropancreatic neuroendocrine tumors
ISBN: 978-94-6169-801-8

Cover design: Erwin Timmerman
Layout and printing: Optima Grafische Communicatie, Rotterdam

CLINICAL & BIOCHEMICAL CHARACTERIZATION OF GASTROENTEROPANCREATIC NEUROENDOCRINE TUMORS

Klinische & biochemische karakterisering van gastroenteropancreatische neuroendocriene tumoren

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam

op gezag van de rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
dinsdag 26 april 2016 om 13.30 uur

door

Kimberly Kamp
geboren te Oosterhout

Erasmus University Rotterdam

Erasmus

PROMOTIECOMMISSIE

Promotor	Prof.dr. W.W. de Herder
Overige leden	Prof.dr. L.J. Hofland Prof.dr. D.J. Kwekkeboom Prof.dr. G.D. Valk
Copromotor	Dr. R.A. Feelders

Voor Oma Kamp-Korteweg

CONTENTS

Chapter 1	General Introduction	9
Chapter 2	Is true non-secretion of chromogranin A an unfavorable prognostic factor in ENETS Stage IV gastroenteropancreatic neuroendocrine tumors?	39
Chapter 3	Serum neuron-specific enolase level is an independent predictor of overall survival in patients with gastroenteropancreatic neuroendocrine tumors	51
Chapter 4	Occurrence of second primary malignancies in patients with neuroendocrine tumors of the digestive tract and pancreas	57
Chapter 5	The prevalence and relevance of adrenal masses in patients with sporadic gastroenteropancreatic neuroendocrine tumors	67
Chapter 6	Parathyroid hormone-related peptide (PTHrP) secretion by gastroenteropancreatic neuroendocrine tumors (GEP-NETs): clinical features, diagnosis, management and follow-up	83
Chapter 7	Prevalence and clinical features of the ectopic ACTH syndrome in patients with gastroenteropancreatic and thoracic neuroendocrine tumors	105
Chapter 8	Safety and efficacy of everolimus in gastrointestinal and pancreatic neuroendocrine tumors after ^{177}Lu-octreotate	123
Chapter 9	Sequential everolimus and sunitinib treatment in pancreatic metastatic well-differentiated neuroendocrine tumors resistant to prior treatments	139
Chapter 10	General Discussion	155
Chapter 11	Summary - Samenvatting	165
Chapter 12	List of Publications	177
	Curriculum Vitae	181
	PhD Portfolio	182
	List of Abbreviations	185
	Dankwoord	190

Chapter 1

General Introduction

Clinical overview of GastroEnteroPancreactic Neuroendocrine Tumors

Kimberly Kamp[1], Roxanne C.S. van Adrichem[1], Richard A. Feelders[1], Wouter W. de Herder[1]

[1]*Department of Internal Medicine, Sector of Endocrinology, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands*

Invited Review: ***Netherlands Journal of Medicine***

Manuscript in preparation

GENERAL INTRODUCTION

1. HISTORY OF NEUROENDOCRINE TUMORS (NETs)

Nomenclature

The nomenclature of neuroendocrine tumors (NETs) has changed over time and has often led to confusion. At the beginning of the twentieth century (1907), the German pathologist, Siegfried Oberndorfer, introduced the term "karzinoide" (carcinoid, or carcinoma-like) for submucosal tumors in the small bowel, thereby connecting the carcinoma-like features of these tumors with their relatively benign disease course[1, 2]. The term carcinoid was subsequently connected to the tumor localization of NETs arising from the gut and lesions from the bronchopulmonary system. In addition, the more aggressive, poorly differentiated NETs came to be defined as "atypical carcinoids". "Carcinoid" is also used as a general term to describe the clinical "carcinoid syndrome", which is the result of an overproduction of vasoactive peptides by small intestinal and bronchial NETs. The term "'carcinoid' is currently still widely used and a source of nomenclature confusion. The WHO nomenclature has replaced the term gastrointestinal carcinoid by NET with a classification for the tumor according to the degree of differentiation and proliferation[2-6]. For the bronchopulmonary NETs, the widely used terminology remains "typical" and "atypical carcinoids"[7].

History of functional pancreatic NETs

Insulinoma: In 1869 German pathologist, Paul Langerhans, was the first to describe pancreatic islet cells, named the "Ilots de Langerhans" by the French histologist G. Edouard Laguesse in 1893[8]. The American (US) surgeon Seale Harris was the first to identify a case of endogenous hyperinsulinism in 1924[9], but the association between hyperinsulinism and a functional pancreatic islet cell tumor was established by US physician Russel M. Wilder et al. in a necropsy report in 1927[10]. During the previous year (1926), the US surgeon William J. Mayo had performed an exploratory laparotomy on this same patient who was eventually diagnosed with a metastatic, malignant insulinoma[11].

Glucagonoma: The US physiologists Charles P. Kimball and John R. Murlin were the first to describe glucagon in 1923[12]. In 1942, US dermatologist S. William Becker et al. were the first to describe the typical skin eruption, afterward called "necrolytic migratory erythema" in a patient with a pancreatic glucagonoma[13].

VIPoma: In 1958, US physician John V. Verner and Irish-US pathologist Ashton B. Morrison were the first to describe a watery diarrhea syndrome as "Verner Morrison syndrome"[14]. In 1970, vasoactive intestinal peptide (VIP) was isolated by the Egyptian-American physician Sami Said and Estonian-Swedish biochemist Viktor Mutt[15].

Gastrinoma: Sporadic case reports of patients with peptic ulcer disease and acid hypersecretion in the presence of pancreatic tumors had already appeared prior to 1955. However, US surgeons Robert. M. Zollinger and Edwin. H. Ellison were the first to demonstrate a causal relationship between these findings when they reported upon two cases of gastrinoma in 1955[16]. In 1967, the British physiologist Roderic A. Gregory et al. succeeded in extracting gastrin from a pancreatic NET[17], thereby linking the Zollinger-Ellison syndrome (ZES) to the functional NET called "gastrinoma".

Somatostatinoma: In 1977, the research groups of Lars-Inge Larsson and Jens Rehfeld, and of Om Ganda independently reported the first two cases of pancreatic somatostatinoma[18, 19]. In 1979, the somatostatinoma syndrome in a 52-year-old man with distinct clinical symptoms and excessive somatostatin levels of a tumor of the ampulla of Vater was comprehensively described by the Austrian gastroenterologist Günter Krejs et al[20].

2. EPIDEMIOLOGY OF GASTROENTEROPANCREATIC NEUROENDOCRINE TUMORS (GEP-NETs)

Due to inconsistency in the nomenclature and classification of NETs, precise epidemiology of Gastroenteropancreatic (GEP)-NETs has not been established to date. In the past, national cancer registries considered some GEP-NETs not to be malignant and, therefore, did not record these in their databases. Incidence and prevalence of GEP-NETs have shown a remarkable increase over the past three decades. Whether this is a true increase in NET incidence, the result of improved diagnostic procedures and medical attention of clinicians, the result of better registration, or a combination of all these possible explanations, is still unclear.

Recent epidemiologic studies show that the age-adjusted incidence of all GEP-NETs is 3.65, for pancreatic NETs this is 0.43, for bronchial NETs this varies between 0.2 and 2.0 and for thymic NETs it is 0.4 per 100,000 population per year[21, 22]. In the Netherlands, the incidence of gastro-intestinal (GI)-NETs, in men and women was 1.8 and 1.9 respectively per 100,000 inhabitants (1989-1996)[23]. From 2007 to 2011, the Dutch Cancer Registry reported a strong increase in incidence of NETs with 2,679 new patients (16.4 per 100,000) in 2007 growing to up to 3,144 new patients (19.0 per 100,000) in 2011. (< Dutch National Cancer Registry).

3. DIAGNOSIS

Clinical aspects of GEP- and bronchopulmonary NETs

Historically, NETs were classified according to their localization in the embryologic gut, i.e. the foregut, midgut and hindgut. Foregut tumors included bronchopulmonary, gastric, proximal duodenal and pancreatic NETs, midgut NETs were localized from the distal duodenum up to the ascending colon and hindgut tumors originated from the neuroendocrine cells in the transverse and descending colon and rectum[24]. The clinical presentation of GEP-NETs varies according to the anatomic site of origin, size, functionality and metastatic spread of the primary tumor. Functioning NETs give early hormone-related symptoms, whereas non-functioning NETs present late with symptoms attributable to tumor growth and metastatic spread. Non-functioning NETs are often clinically silent (i.e. not causing a hormonal, or hormone related syndrome) and are frequently discovered as incidental findings[25]. Morbidity and mortality in patients with functioning NETs are mainly caused by the secretion of bioactive peptides and their related biological effects in combination with the effects of tumor expansion, while patients with non-functioning NETs suffer more from tumor expansion with mechanical effects, like bowel obstruction or ischemia[26, 27]. The secretory products (peptides and amines), incidence and clinical features of GEP-NETs are presented in Table 1.

In addition to the production of peptides which are physiologically produced by the neuroendocrine cells of origin, functional NET can also produce hormones, growth factors and cytokines that are usually secreted by specific endocrine glands. The term paraneoplastic syndrome is used to describe this spectrum of symptoms[28]. Humoral paraneoplastic syndromes in GEP-NETs are: hypercalcemia of malignancy due to parathyroid hormone-related protein (PTHrP) secretion, Cushing's syndrome caused by ectopic ACTH or CRH secretion, syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH) due to ectopic secretion of vasopressin (ADH), secretion of atrial natriuretic peptide (ANP), acromegaly secondary to growth hormone-releasing hormone (GHRH)-hypersecretion or production of GH, non-islet cell tumor hypoglycemia (NICTH) secondary to hypersecretion of an insulin growth factor 2 (IGF2) precursor and very rare ectopic luteinizing hormone (LH) production[28].

Table 1. Secretory products (peptides and amines), incidence and clinical features of GEP-NETs according to anatomic site.

SITE	PEPTIDE/AMINE	INCIDENCE	CLINICAL FEATURES
BRONCHOPULMONARY	5-HTP		Cough, hemoptysis, pneumonia, airway obstruction
	ACTH, CRH, GHRH, PTH-related, ADH	0.2 - 2.0	Cushing's syndrome, acromegaly, hypercalcemia, SIADH
	Serotonin (5-HT), tachykinins		Carcinoid syndrome
STOMACH	ECL-cells: histamine		Type 1: Chronic atrophic gastritis - hypergastrinemia
		0.3	Type 2: Zollinger-Ellison Syndrome - hypergastrinemia
			Type 3: Sporadic - incidental finding endoscopy
	G-cells: gastrin, ghrelin, 5-HT		Incidental finding during endoscopy
PANCREAS			
Non-functioning	Pancreatic Polypeptide	0.43	Mass-related effects: abdominal pain, weight loss, nausea, jaundice
Functioning			
Insulinoma	Insulin, proinsulin	1 - 2	Whipple's Triad: low blood glucose levels, symptoms of hypoglycemia, improvement of symptoms after glucose administration
Gastrinoma	Gastrin	1 - 1.5	Zollinger-Ellison Syndrome
VIPoma	VIP	0.1	Verner-Morrison syndrome: secretory diarrhea, hypokalemia, achlorhydria
Glucagonoma	Glucagon	0.01-0.1	Diabetes Mellitus, necrolytic migratory erythema, weight loss
Somatostatinoma	Somatostatin	< 0.1	Cholelithiasis, diarrhea with steatorrhea, diabetes mellitus
Ectopic hormone production	ACTH, GRF, PTHrP	< 0.1	Cushing's Syndrome, acromegaly, hypercalcemia
DUODENUM	5-HT, somatostatin	included	Abdominal pain, nausea, vomiting, jaundice, gastrointestinal bleeding
	gastrin	in	Zollinger-Ellison Syndrome: peptic ulcers, diarrhea, epigastric pain
		ileum	Incidental finding during endoscopy
ILEUM-JEJUNUM	5-HT (5-HIAA), prostaglandins		Carcinoid syndrome: flushing, diarrhea, bronchoconstriction, carcinoid heart disease
	tachykinins, Ghrelin	1.1	Abdominal pain, gastrointestinal bleeding, mesenteric fibrosis: obstruction & ischemia
APPENDIX	Ghrelin, 5-HT,	0.2	Incidental finding during appendectomy, abdominal pain
COLON	Ghrelin	0.3 - 0.4	Abdominal pain, gastrointestinal bleeding, enlarged liver, weight loss
			Incidental finding at screening for colorectal cancer
RECTUM	Ghrelin, 5-HT,	0.8 - 1.0	Rectal bleeding, pain, constipation
			Incidental finding at screening for colorectal cancer

Diagnostic approaches of GEP-NETs: biomarkers, imaging, pathology, staging & grading

In general, patients with clinical signs and symptoms suggestive of NETs should be referred to a center with special interest in, and knowledge of, these malignancies. Accurate diagnosis of GEP-NETs is based on general serum biomarkers (chromograninA – CgA and, neuron-specific enolase – NSE), pathologic elevations of circulating, hypersecreted neuroendocrine hormones or peptides[29], imaging according to international protocols and standards[30, 31], in combination with histological confirmation according to current guidelines[32, 33].

Biomarkers

The general principle of biomarkers is based on the evaluation of a large panel of markers at strategic points in the disease course (e.g. at diagnosis or after relapse/recurrence) in order to identify specific biomarkers for an individual patient. However, some GEP-NETs are known for their variability in produced hormones and biomarker profiles over time.

General biomarkers

Chromogranin A (CgA)

The most important general serum marker, CgA, is a water-soluble acidic glycoprotein with 439 amino acids, which is found in the secretory dense core granules of most neuroendocrine cells[34]. The chromogranin family further consists of CgB, and secretogranin II, sometimes called CgC[35]. Depending on the extent of disease, serum CgA is elevated in >60% of patients with functionally active or non-functional NETs. The highest levels of CgA have been found in patients with metastatic small intestinal NETs and non-functioning pancreatic NETs[29, 36]. CgA levels correlate with tumor volume and biological activity of the tumor, but care should be taken in measuring CgA and interpreting the results. Somatostatin analogs (SSAs) are known to affect blood levels of CgA by blocking the production and release of CgA in addition to reducing tumor mass. Falsely elevated circulating levels of CgA have also been reported in patients using proton pump inhibitors (PPIs) or histamine 2 blockers (H2-blockers), in patients with severe renal or liver failure and in those with chronic atrophic gastritis type A or inflammatory bowel disease[36-38]. A recent meta-analysis demonstrated that the sensitivity and specificity of elevated serum levels of CgA in the diagnosis of patients with NETs are 0.73 and 0.95, respectively[39].

Neuron-specific enolase (NSE)

NSE, a cell-specific isoenzyme of the glycolytic enzyme enolase, is present in the cytoplasm of neurons and neuroendocrine cells. Serum NSE is elevated in 30-50% of patients with GEP-NETs[40-42]. NSE levels can be used as a circulating biomarker for both

non-functioning and functioning GEP-NETs[43, 44]. Several studies restrict the use of NSE as a marker for dedifferentiation of high grade tumors[40].

24-hour urinary excretion of 5-hydroxy indole acetic acid (5-HIAA)

The urinary breakdown metabolite of serotonin is 5-hydroxy indole acetic acid (5-HIAA), which is a clinically relevant marker for NETs with the carcinoid syndrome. Levels of 24-hour urinary 5-HIAA are correlated with severity of carcinoid heart disease and prognosis in patients with the carcinoid syndrome[23, 45]. The sensitivity of urinary 5-HIAA in the presence of a carcinoid syndrome in NETs is 90%. However, certain foods and medications can influence urinary 5-HIAA levels and should be avoided during 24-hour urine collection[36].

Specific hormones

Insulin

The diagnosis of insulinomas is established using the following diagnostic criteria from the most recent consensus report from the US Endocrine Society: endogenous hyperinsulinism documented by the finding of symptoms, signs or both with plasma concentrations of glucose <3.0 mmol/liter (<55 mg/dl), insulin ≥3.0 mU/ml (≥18 pmol/l), C-peptide ≥0.6 ng/ml (≥0.2 nmol/l), and proinsulin ≥5.0 pmol/l[46]. Other consensus papers do not agree with the high cut-off level of blood glucose used in this guideline and prefer a cut-off value amounting to 2.2 mmol/l (40 mg/dl)[47].

Gastrin

In 40% of ZES patients, the diagnosis of gastrinoma or ZES is established by demonstrating elevated fasting serum gastrin (FSG) concentrations (>10x ULN) in combination with a low gastric pH<2. Determination of gastric pH is necessary because some conditions such as atrophic gastritis, pernicious anemia, *Helicobacter pylori* infections or the use of PPIs or H2-blockers cause hypergastrinemia. However, 60% of ZES patients have normal or only mildly elevated (2-10x ULN) FSG concentrations in combination with a gastric pH<2. For these patients, the secretin test is the provocative test of choice. In contrast with the physiological situation where secretin decreases gastrin levels, serum gastrin increases after infusion of secretin in ZES patients[36, 48-51].

Other more rare NET hormones

The diagnosis of VIPoma can be confirmed by demonstrating elevated fasting plasma VIP and peptide histidine-methionine (PHM) concentrations. Elevated plasma glucose levels and elevated fasting plasma somatostatin levels can be measured in patients with somatostatinoma and elevated fasting plasma glucagon levels in patients with glucagonoma[26].

Imaging

In order to obtain tissue for definitive diagnosis, GEP-NETs must first be localized. The various localization techniques, which are able to identify both primary and metastatic tumor, are divided into morphological techniques and functional imaging.

Initial imaging for localization of the primary tumor and for the staging and planning of treatment for the extent of disease generally includes: somatostatin receptor scintigraphy using ^{111}In-pentetreotide scintigraphy (Octreoscan) or ^{68}Ga-DOTA-TATE Positron emission tomography (PET) in combination with CT (PET-CT), computed tomography (CT) and magnetic resonance imaging (MRI) of the chest, abdomen and pelvis[25, 26].

For patients undergoing follow-up after complete resection or with stable disease and evaluation of treatment response, we prefer to perform an Octreoscan, or ^{68}Ga-DOTA-TATE PET once every other year or whenever indicated. For patients with advanced, metastatic disease, we generally prefer CT or MRI for the follow-up of known disease sites[25, 26].

Morphological imaging of GEP-NETs

Transabdominal ultrasound

Transabdominal ultrasound is not considered the primary imaging modality for the localization of GEP-NETs due to low sensitivity as compared to other imaging modalities. It can, however, sometimes be used to guide percutaneous biopsies of metastases or primary lesions. Occasionally a non-functional NET with liver metastases is identified by ultrasonography done for other reasons[31, 52].

Computed Tomography (CT)

Multi-phasic CT is generally recommended for the detection of metastases in the liver or lymph nodes and has a sensitivity of approximately 80-90% for metastatic GEP-NETs. As GEP-NETs are highly vascularized tumors, intravenous contrast enhances the tumor during the early arterial phase of imaging and shows washout during the delayed portal venous phase. An irregular soft tissue mass with calcification, surrounded by radiating strands of fibrosis in the mesenteric fat - resembling spokes in a bicycle wheel – is characteristic for midgut carcinoids[31, 52].

Magnetic Resonance Imaging (MRI)

Generally recommended MRI sequences for the detection of GEP-NETs are T1-, T2-weighted images and multiphasic (arterial, portal venous, and delayed) dynamic MRI. The appearance of GEP-NETs is variable on non-contrast MRI. They can be either hypo- or iso-intense on T1-weighted images, whereas liver metastases typically show high signals on T2- weighted images. Although spatial resolution is lower with MRI than CT,

the better soft tissue contrast of MRI facilitates the detection of small NETs and allows a clearer distinction between hemangiomas and metastases in the liver[31, 52].

Endoscopy, endoscopic ultrasound and video capsule endoscopy

Standard upper and lower gastrointestinal (GI) endoscopic techniques can be used for identification and tissue collection of GEP-NETs. Upper GI endoscopy can identify tumors up to the ligament of Treitz whereas lower GI colonoscopy can detect colon and rectal NETs and some terminal ileal NETs[25]. However, NETs are more often discovered as an incidental finding due to the increased use of endoscopy for other reasons and generally present as intramucosal lesions[53].

Endoscopic ultrasound (EUS) is highly sensitive for the detection of NETs located in the stomach, duodenum and pancreas, and is superior to conventional ultrasound. It can be particularly useful for the detection of small NETs, for determining invasiveness or the presence of pathological lymph nodes. With regard to the preoperative work-up of pancreatic NETs, it clearly visualizes anatomical correlations[25, 53, 54].

Video capsule endoscopy has limited potential for surveillance of the small bowel for NETs and the inability to obtain tissue for diagnosis is one of its major limitations [55].

Somatostatin receptor imaging (SRS)

^{111}In-pentetreotide scintigraphy (OctreoScan®)

^{111}In-pentetreotide has the same receptor-binding profile as the SSAs, octreotide and lanreotide, making it a useful radiopharmaceutical for imaging of somatostatin receptor subtype (sst) 2- (and 5) (sst_2 and sst_5)-positive tumors. Overall sensitivity for NETs is 89%, for small intestinal NETs 86-95% and for pancreatic NET, the overall reported sensitivity varies between 60 to 90%[56]. The yield of this imaging technique can be enhanced by the use of single positron emission CT (SPECT). OctreoScan® is effective for screening the entire body for primary and metastatic lesions, which conventional imaging procedures do not reveal in first instance. OctreoScan® is furthermore used to evaluate whether peptide receptor radiotherapy (PRRT) is a potential treatment option for an individual patient with a progressive GEP-NET[30, 57].

Positron emission tomography (PET)

Recently, the combination of PET and CT, using gallium-68 labelled SSAs (^{68}Ga-DOTA-TATE) has been introduced for somatostatin receptor imaging, achieving higher sensitivity and specificity rates as well as giving results over a much shorter time frame then OctreoScan®[58]. Other tracers for PET, such as β-[(11)C]-5-hydroxy-L-tryptophan (^{11}C-5-HTP) and 6-(18)F-L-3,4-dihydroxyphenylalanine (^{18}F-DOPA) show promise for the detection of pancreatic and GI-NETs respectively. However, these techniques are still not widely available[58, 59].

[^{18}F]-fluorodeoxyglucose (FDG-)PET can be used for poorly differentiated neuroendocrine carcinomas or when OctreoScan® is negative or equivocal, because proliferative activity in NETs is generally lower than in solid tumors[60]. However, recent studies show that FDG-PET positivity in well-differentiated NETs with Ki-67 values of >10%, may indicate a more aggressive disease course[61]. Therefore combinations of OctreoScan® or ^{68}Ga-DOTA-TATE and FDG-PET are increasingly used for well- and moderately differentiated NETs[62, 63].

Pathology

The definitive diagnosis of GEP-NETs is based on pathology. A biopsy of the primary tumor and/or metastases, if feasible, is therefore obligatory. Another possibility is to examine tissue obtained during surgery.

Aside from various types of staining e.g. hematoxylin and eosin (HE) staining, immunostaining for general neuroendocrine markers like CgA and synaptophysin, and sst_2, mitotic count and Ki-67 proliferative index should be assessed. Furthermore, immunostaining for specific hormones such as insulin, glucagon, gastrin or VIP may provide information on hormonal production in functioning NETs. However, these stainings are not routinely indicated for functional GEP-NET diagnosis[33].

Table 2. Grading for GEP-NETs.

	Mitotic count	Ki-67 index*
Grade	**(10 HPF)**	**(%)**
Grade 1	< 2	≤ 2
Grade 2	2 -20	3 - 20
Grade 3	> 20	> 20

10 HPF: high power field = 2mm^2, at least 40 fields (at 40× magnification) evaluated in areas of highest mitotic density.

**MIB-1 antibody; % of 2,000 tumor cells in areas of highest nuclear labeling*

Staging & Grading

Definitions for Staging and Grading systems of GEP-NETs have changed over time. Staging and Grading of GEP-NETs is currently defined according to the 2010 WHO classification with the introduction of the general terms neuroendocrine tumor (NET) and neuroendocrine carcinoma (NEC). Grading is based on mitotic count and Ki-67 proliferative index (Table 2). This Grading system came together with an organ-specific tumor, lymph node, metastases (TNM) Staging system (Supplementary data)[6, 64, 65]. In the near future this Grading system will be expanded with the introduction of the term Grade 3 NET next to Grade 3 NEC[66].

4. TREATMENT

Surgical treatment and interventions

Surgery

Surgery is the only curative treatment option for GEP-NET patients with localized disease[25, 67]. However, patients often present late with symptoms attributable to tumor growth and metastatic disease. Surgery nevertheless can also be an option for patients with advanced stages of GEP-NET. Firstly, debulking of the primary tumor and its limited hepatic or lymph node metastases with subsequent treatment of metastases with resection, embolization or radiofrequency ablation[25, 68, 69]. Liver transplantation is only occasionally considered for patients without extra hepatic metastases[70]. Several retrospective uncontrolled studies demonstrate that resection of the primary tumor is associated with an increased overall survival in GEP-NET patients[67, 71-73]. Secondly, it is possible that surgery is useful for symptom control[25, 68, 69]. In the third place, surgery can be indicated to prevent small bowel obstruction or ischemia caused by the primary GI-NET, or associated mesenterial fibrosis[68, 69]. Fourthly, enucleation or local resection of specific functioning NETs or NETs with ectopic hormone production can be considered. Surgical biadrenalectomy can be a palliative option for patients with the ectopic ACTH syndrome[74]. The majority of patients with localized, non-metastatic insulinomas (85-95%) and gastrinomas (45-65%) is disease-free after surgery[67, 75]. Finally, cardiac valve replacement is more frequently undertaken in patients with carcinoid heart disease and right-sided heart failure due to technical advances as well as an earlier diagnosis resulting in a decreased perioperative mortality and longer survival with a better quality of life (QOL)[76].

Interventional Radiology

Liver metastases are usually hypervascular and the occlusion of branches of the hepatic artery by surgery or embolization can induce tumor necrosis and regression[77, 78]. Sequential transcatheter arterial chemoembolization (TACE) results in symptom control in 63-100% of patients and tumor response rates between 33 and 80%[78]. At present, there seems to be no role for selective chemo-embolization of liver metastases[79]. Radio-embolization with Yttrium-90-, or Holmium-166 labeled microspheres can also induce tumor regression[80]. Other treatment modalities used to treat liver metastases are percutaneous alcohol injection, radiofrequency ablation (RFA) and cryotherapy.

Medical therapy

Somatostatin Analogs (SSAs)

SSAs are the recommended first-line therapy for (non-)functioning well- to moderately differentiated GEP-NETs[25, 81]. GEP-NETs (apart from the majority of non-metastatic insulinomas) express the sst_2 in approximately 90% and 80% of their tumors respectively[82]. The currently available SSAs, octreotide (Sandostatin LAR®) and lanreotide (Somatuline Autogel®), show a high affinity for sst_2 and low to median affinity for sst_3 and sst_5[83]. Therefore, these drugs are effective in inhibiting hormonal hypersecretion, achieving symptom control in up to 71% and biochemical response in up to 51% of GEP-NET patients with minimal adverse effects[84-87]. However, in the long term, some tumors may become desensitized to SSAs which subsequently will lead to a recurrence of symptoms. High-dosed intravenous octreotide results in a rapid reversal of carcinoid crisis, triggered by tumor manipulation (biopsy or surgery) or by anesthesia[88].

The use of SSAs as anti-proliferative agents in patients with GEP-NETs has been established recently in two placebo-controlled, double-blind, prospective, randomized trials. Octreotide LAR resulted in well-differentiated, metastatic small intestinal NETs in a prolongation of time to progression (TTP) from 6 to 14.3 months, as compared to placebo[89]. Lanreotide achieved a significant prolongation of progression-free survival (PFS) in well- and moderately differentiated metastatic, non-functioning, somatostatin receptor-positive GEP-NETs as compared to the placebo group (median PFS not reached versus 18 months)[90]. These inhibitory effects of SSAs on hormone production and tumor growth may partly explain the increase in overall survival of GEP-NET patients since the introduction of SSAs in 1987[22].

Interferon-α & Chemotherapy

Interferon-α (IFN-α) (Intron-A®) binds specifically to surface receptors on the tumor cell and is thereby able to reduce symptoms in patients with GEP-NETs and the carcinoid syndrome. Antiproliferative effects of IFN-α via inhibition of protein synthesis, immunomodulation and inhibition of angiogenesis have been demonstrated in a recent prospective randomized Phase III study. In this study, octreotide LAR was combined with either bevacizumab or IFN-α. Bevacizumab (Avastin®) and IFN-α were both shown to have similar antitumor activity in patients with advanced small intestinal NETs[91]. Nevertheless, this treatment is associated with side effects such as: fever, fatigue, autoimmune disorders and myelosuppression[92]. Another promising type I IFN, IFN-β, has shown a more potent antitumor activity than IFN- α in several experimental studies. IFN-β may therefore be considered a potential and promising new anti-tumor agent for GEP-NETs[93-95].

Chemotherapy is indicated in patients with poorly-differentiated G3 NECs. GI NECs are usually treated with the combination of cisplatinum and etoposide with objective tumor response rates of around 50%[96]. Recent retrospective studies have demonstrated the efficacy of temozolomide, alone or in combination with capecitabine (Xeloda®) and/or bevacizumab as a second-line therapy[97].

In patients with poorly differentiated or rapidly progressive pancreatic NECs, the combination of streptozotocin with 5-fluorouacil (5-FU), or doxorubicin results in objective tumor response rates in 35–40%[96, 98]. Temozolomide-based chemotherapy appears to be promising in pancreatic NETs, either alone or in combination with capecitabine, giving high partial remissions varying from 40 to 70%[99].

Molecular Targeted Therapy

Pasireotide LAR (Signifor LAR®) is multireceptor-targeted SSA with a high affinity for sst_1, sst_2, sst_3 and sst_5[100], but it did not prove superior over octreotide LAR in a recent phase III study, in patients with metastatic GEP-NETs and the carcinoid syndrome[101].

GEP-NETs express vascular endothelial growth factor (VEGF). A phase II study, combining bevacizumab (Avastin®) and interferon alpha-2b (Intron-A®), demonstrated that treatment with the VEGF monoclonal antibody bevacizumab in patients with metastatic NETs decreased tumor blood flow and had tumor stabilizing effects[102]. Several subsequent studies combined bevacizumab with other treatment agents such as 5-FU/streptozocin, or capecitabine (BETTER trials)[103, 104]. These phase II studies showed antitumor activity and a manageable safety profile in the treatment of GEP-NET patients. However, randomized phase III trials are still needed to confirm these findings[103, 104].

The mammalian target of rapamycin (mTOR) is another target for medical treatment of NET[105]. The mTOR pathway is activated in various tumors and plays an important role in the regulation of cell proliferation and angiogenesis[106]. Everolimus (Afinitor®), an oral mTOR inhibitor, is a new, targeted therapy for patients with well-differentiated (WHO Grade 1, 2) GEP-NETs with progressive disease, with a well-established safety profile[107-110]. In the RADIANT-3 trial, which was a double-blind, placebo-controlled, randomized phase III study in patients with progressive pancreatic NETs, everolimus therapy was associated with a 2.4-fold improvement in median PFS as compared to placebo (11.4 months versus 4.6 months)[110]. Everolimus for patients with advanced, non-functional neuroendocrine tumors of the lung or gastrointestinal tract was investigated in the RADIANT-4 trial and results showed that everolimus increased the median progression-free survival from 3.9 months to 11.0 months. Furthermore, everolimus was associated with a 52% reduction in the estimated risk of progression or death[111]. Recently, a large European ENETS randomized open label study (SEQTOR) was initiated. In this study, the efficacy and safety of everolimus followed by chemotherapy with streptozotocin-5-fluorouracilo (STZ-5FU)

upon progression will be compared to the reverse sequence, in advanced progressive pancreatic NETs (NCT02246127).

The tyrosine-kinase inhibitor sunitinib (Sutent®), another targeted therapy, also showed significant antitumor efficacy in patients with advanced, well-differentiated pancreatic NETs. A large randomized, double-blind, placebo-controlled phase III trial of sunitinib demonstrated a median PFS of 11.4 months in the sunitinib group as compared with 5.5 months in the placebo group (95% CI, 0.26-0.66; P<0.001)[112].

Peptide Receptor Radionuclide Therapy (PRRT)

PRRT with radiolabeled SSAs is a promising new treatment modality in patients with inoperable, or metastasized GEP-NETs. The basis of PRRT is the coupling of a radionuclide (^{111}Indium, ^{90}Yttrium or ^{177}Lutetium) to a SSA. After intravenous administration of a radiolabeled somatostatin receptor agonist, this complex binds to the sst_2 receptors and is subsequently internalized by the tumor via receptor-mediated endocytosis resulting in targeted radiation and tumor necrosis[57]. In contrast, radiolabeled somatostatin antagonists have been developed, but these radioligands are not internalized. However, their binding to inactivated somatostatin receptors might give these compounds a therapeutic advantage over the currently used receptor agonists[113]. In a retrospective series, GEP-NET patients were treated with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (Lutathera®), usually four cycles every 6-10 weeks up to a cumulative dose of 27.8-29.6 GBq. Complete remission was achieved in 2%, partial remission (decrease in tumor size > 50 %) in 28% and stable disease in 35 % of patients with a median time to progression of 40 months[114]. Generally PRRT is well tolerated and serious (grade 3) hematological toxicity, MDS, nephrotoxicity and liver toxicity are relatively rare in patients not pre-treated with chemotherapy and occur in less than 1% of the patients[57, 115]. However, high cumulative dosages can cause serious side effects, including kidney failure, cytopenias, or myelodysplastic syndrome[116]. Recently, the results of the first Phase III multicentric, stratified, open, randomized, controlled trial in which ^{177}Lu-octreotate was compared to high dose octreotide LAR in patients with inoperable, progressive, somatostatin receptor positive midgut NETs were presented at international meetings (Strosberg, NANETS, Ruszniewski, European Cancer Congress). In this NETTER-1 study, the median PFS was not reached for ^{177}Lu-octreotate and it was 8.4 months with high dose octreotide LAR. The safety profile observed in this trial was consistent with the safety information generated in the Phase I-II clinical trials. Furthermore, neoadjuvant ^{177}Lu-octreotate therapy made surgery feasible in 9 out of 29 patients with initially unresectable non-functioning pancreatic NETs[117].

AIMS AND OUTLINE OF THIS THESIS

The aims of the studies presented in this thesis are:

1. Evaluation of general biochemical tumor markers and their prognostic value
   a. Chromogranin A (CgA)
   b. Neuron-specific enolase (NSE)
2. Evaluation of the association between GEP-NETs and presence of other neoplastic lesions
   a. Second primary malignancies
   b. Incidental adrenal masses during imaging
3. Evaluation of paraneoplastic syndromes in a large cohort of GEP-NET patients
   a. Cushing's syndrome caused by ectopic ACTH secretion (EAS)
   b. Hypercalcemia of malignancy due to parathyroid hormone-related protein (PTHrP) hypersecretion
4. Evaluation of safety and efficacy of sequential treatments
   a. Everolimus after peptide receptor radionuclide therapy (PRRT) in GEP-NETs
   b. Molecular targeted therapies: everolimus and sunitinib in pancreatic NETs

Chapter 1 gives an overview of the current literature on epidemiological, clinical and biochemical aspects of GEP-NETs as well as diagnostic strategies and different treatment modalities. **Chapter 2** describes the prognostic value of CgA in patients with ENETS TNM Stage IV GEP-NETs with elevated levels of CgA and without elevated levels of CgA ("true non-secretors") and determines whether true non-secretion of CgA is an unfavorable prognostic factor. **Chapter 3** elucidates the role of NSE as a prognostic biomarker in patients with ENETS TNM Stage IV GEP-NETs. In **Chapter 4** we determine whether there is indeed a true, increased risk for a second primary malignancy in a GEP-NET patient group compared with an age- and sex-matched control group of patients with identical malignancies. In **Chapter 5** the prevalence of adrenal lesions incidentally discovered during abdominal imaging of patients with GEP-NETs is estimated, with identification of their radiological features and clinical significance during the course of disease. **Chapter 6** describes metastatic GEP-NET patients who present with symptoms and signs of hypercalcemia of malignancy as a result of the very rare paraneoplastic PTHrP hypersecretion. We aim to evaluate clinical, biochemical, and radiological features, including the evaluation of effective treatment options in a large single-center case series. In **Chapter 7** the prevalence of EAS is assessed in a large cohort of patients with thoracic and GEP-NETs. Furthermore clinical, biochemical, and radiological features; management; and treatment outcome of this EAS patient cohort is described, including a comparison of prognosis between patients with and without EAS. **Chapter 8** compares the safety and efficacy profile of everolimus in GEP-NET patients with documented disease progression

after prior treatment with ^{177}Lu-octreotate radionuclide therapy with the earlier established profile of everolimus. **Chapter 9** describes sequential molecular targeted therapy with everolimus and sunitinib in patients with advanced well-differentiated pancreatic NETs in terms of safety and effect on disease progression and survival. Finally **Chapter 10 and 11** provide a general discussion and a summary of the presented data in this thesis.

REFERENCES

1. **Kloppel G, Dege K, Remmele W, Kapran Y, Tuzlali S, Modlin IM.** Siegfried Oberndorfer: a tribute to his work and life between Munich, Kiel, Geneva, and Istanbul. *Virchows Archiv.* 2007;451 Suppl 1:S3-7.
2. **Oberndorfer S.** Karzinoide tumores des dünnedarms. *Frankf Z Pathol.* 1907;1:426-432.
3. **Kloppel G, Perren A, Heitz PU.** The gastroenteropancreatic neuroendocrine cell system and its tumors: the WHO classification. *Annals of the New York Academy of Sciences.* 2004;1014:13-27.
4. **Pape UF, Jann H, Muller-Nordhorn J, Bockelbrink A, Berndt U, Willich SN, Koch M, Rocken C, Rindi G, Wiedenmann B.** Prognostic relevance of a novel TNM classification system for upper gastroenteropancreatic neuroendocrine tumors. *Cancer.* 2008;113:256-265.
5. **Rindi G, Capella C, Solcia E.** Introduction to a revised clinicopathological classification of neuroendocrine tumors of the gastroenteropancreatic tract. *The quarterly journal of nuclear medicine.* 2000;44:13-21.
6. **Bosman FT, et al. eds.** WHO classification of tumours of the digestive system. 4th ed. Lyon, France: IARC Press; 2010
7. **Caplin ME, Baudin E, Ferolla P, Filosso P, Garcia-Yuste M, Lim E, Oberg K, Pelosi G, Perren A, Rossi RE, Travis WD, participants Ecc.** Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. *Annals of oncology. 2*015;26:1604-1620.
8. **Langerhans P.** Beiträge zur mikroskopischen Anatomie der Bauchspeicheldrüse. Berlin: Gustav Lange, 1869.
9. **Harris S.** Hyperinsulinism, a definite disease entity. Etiology, Pathology, Symptoms, Diagnosis, Prognosis and Treatment of spontaneous insulinogenic hypoglycemia (Hyperinsulinism). *J Am Med Assoc.* 1933; 101:1958-1965.
10. **Wilder RM, Allan FN, Power MH, Robertson HE.** Carcinoma of the islands of the pancreas. *J Am Med Assoc.* 1927; 89:348-355.
11. **van Heerden JA, Churchward MM.** Dr Dickinson Ober Wheelock--a case of sporadic insulinoma or multiple endocrine neoplasia type 1? *Mayo Clinic proceedings.* 1999;74:735-738.
12. **Kimball CP, Murlin JR.** Aqueous extracts of pancreas. III. Some precipitation reactions of insulin. *J Biol Chem.* 1923; 58:337-346.
13. **Becker SW, Kahn D, Rothman S.** Cutaneous manifestations of internal malignant tumours. *Arch Dermatol Syphilol.* 1942; 45:1069-1080.
14. **Verner JV, Morrison AB.** Islet cell tumor and a syndrome of refractory watery diarrhea and hypokalemia. *The American journal of medicine.* 1958;25:374-380.
15. **Said SI, Mutt V.** Potent peripheral and splanchnic vasodilator peptide from normal gut. *Nature.* 1970;225:863-864.
16. **Zollinger RM, Ellison EH.** Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. *Annals of surgery.* 1955;142:709-723; discussion, 724-708.

17. **Gregory RA, Grossman MI, Tracy HJ, Bentley PH.** Nature of the gastric secretagogue in Zollinger-Ellison tumours. *Lancet.* 1967;2:543-544.

18. **Larsson LI, Hirsch MA, Holst JJ, Ingemansson S, Kuhl C, Jensen SL, Lundqvist G, Rehfeld JF, Schwartz TW.** Pancreatic somatostatinoma. Clinical features and physiological implications. *Lancet.* 1977;1:666-668.

19. **Ganda OP, Weir GC, Soeldner JS, Legg MA, Chick WL, Patel YC, Ebeid AM, Gabbay KH, Reichlin S.** "Somatostatinoma": a somatostatin-containing tumor of the endocrine pancreas. *The New England journal of medicine.* 1977;296:963-967.

20. **Krejs GJ, Orci L, Conlon JM, Ravazzola M, Davis GR, Raskin P, Collins SM, McCarthy DM, Baetens D, Rubenstein A, Aldor TA, Unger RH.** Somatostatinoma syndrome. Biochemical, morphologic and clinical features. *The New England journal of medicine.* 1979;301:285-292.

21. **Fraenkel M, Kim MK, Faggiano A, Valk GD.** Epidemiology of gastroenteropancreatic neuroendocrine tumours. *Best practice & research. Clinical gastroenterology.* 2012;26:691-703.

22. **Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB.** One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *Journal of clinical oncology.* 2008;26:3063-3072.

23. **Quaedvlieg PF, Visser O, Lamers CB, Janssen-Heijen ML, Taal BG.** Epidemiology and survival in patients with carcinoid disease in The Netherlands. An epidemiological study with 2391 patients. *Annals of oncology.* 2001;12:1295-1300.

24. **Oberg K.** Neuroendocrine gastrointestinal tumors--a condensed overview of diagnosis and treatment. *Annals of oncology.* 1999;10 Suppl 2:S3-8.

25. **Modlin IM, Oberg K, Chung DC, Jensen RT, de Herder WW, Thakker RV, Caplin M, Delle Fave G, Kaltsas GA, Krenning EP, Moss SF, Nilsson O, Rindi G, Salazar R, Ruszniewski P, Sundin A.** Gastroenteropancreatic neuroendocrine tumours. *The Lancet. Oncology.* 2008;9:61-72.

26. **Kaltsas GA, Besser GM, Grossman AB.** The diagnosis and medical management of advanced neuroendocrine tumors. *Endocrine reviews.* 2004;25:458-511.

27. **Mignon M.** Natural history of neuroendocrine enteropancreatic tumors. *Digestion.* 2000;62 Suppl 1:51-58.

28. **Kaltsas G, Androulakis, II, de Herder WW, Grossman AB.** Paraneoplastic syndromes secondary to neuroendocrine tumours. *Endocrine-related cancer.* 2010;17:R173-193.

29. **O'Toole D, Grossman A, Gross D, Delle Fave G, Barkmanova J, O'Connor J, Pape UF, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: biochemical markers. *Neuroendocrinology.* 2009;90:194-202.

30. **Kwekkeboom DJ, Krenning EP, Scheidhauer K, Lewington V, Lebtahi R, Grossman A, Vitek P, Sundin A, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: somatostatin receptor imaging with (111)In-pentetreotide. *Neuroendocrinology.* 2009;90:184-189.

31. **Sundin A, Vullierme MP, Kaltsas G, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: radiological examinations. *Neuroendocrinology.* 2009;90:167-183.

32. **Klimstra DS, Modlin IR, Coppola D, Lloyd RV, Suster S.** The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. *Pancreas.* 2010;39:707-712.

33. **Kloppel G, Couvelard A, Perren A, Komminoth P, McNicol AM, Nilsson O, Scarpa A, Scoazec JY, Wiedenmann B, Papotti M, Rindi G, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: towards a standardized approach to the diagnosis of gastroenteropancreatic neuroendocrine tumors and their prognostic stratification. *Neuroendocrinology.* 2009;90:162-166.

34. **Deftos LJ.** Chromogranin A: its role in endocrine function and as an endocrine and neuroendocrine tumor marker. *Endocrine reviews.* 1991;12:181-187.

35. **Taupenot L, Harper KL, O'Connor DT.** The chromogranin-secretogranin family. *The New England journal of medicine.* 2003;348:1134-1149.

36. **de Herder WW.** Biochemistry of neuroendocrine tumours. *Best practice & research. Clinical endocrinology & metabolism.* 2007;21:33-41.

37. **Modlin IM, Gustafsson BI, Moss SF, Pavel M, Tsolakis AV, Kidd M.** Chromogranin A--biological function and clinical utility in neuro endocrine tumor disease. *Annals of surgical oncology.* 2010;17:2427-2443.

38. **Yao JC, Pavel M, Phan AT, Kulke MH, Hoosen S, St Peter J, Cherfi A, Oberg KE.** Chromogranin A and neuron-specific enolase as prognostic markers in patients with advanced pNET treated with everolimus. *The Journal of clinical endocrinology and metabolism.* 2011;96:3741-3749.

39. **Yang X, Yang Y, Li Z, Cheng C, Yang T, Wang C, Liu L, Liu S.** Diagnostic value of circulating chromogranin a for neuroendocrine tumors: a systematic review and meta-analysis. *PloS one.* 2015;10:e0124884.

40. **Baudin E, Gigliotti A, Ducreux M, Ropers J, Comoy E, Sabourin JC, Bidart JM, Cailleux AF, Bonacci R, Ruffie P, Schlumberger M.** Neuron-specific enolase and chromogranin A as markers of neuroendocrine tumours. *British journal of cancer.* 1998;78:1102-1107.

41. **Oberg K.** Circulating biomarkers in gastroenteropancreatic neuroendocrine tumours. *Endocrine-related cancer.* 2011;18 Suppl 1:S17-25.

42. **Velayoudom-Cephise FL, Duvillard P, Foucan L, Hadoux J, Chougnet CN, Leboulleux S, Malka D, Guigay J, Goere D, Debaere T, Caramella C, Schlumberger M, Planchard D, Elias D, Ducreux M, Scoazec JY, Baudin E.** Are G3 ENETS neuroendocrine neoplasms heterogeneous? *Endocrine-related cancer.* 2013;20:649-657.

43. **D'Alessandro M, Mariani P, Lomanto D, Carlei F, Lezoche E, Speranza V.** Serum neuron-specific enolase in diagnosis and follow-up of gastrointestinal neuroendocrine tumors. *Tumour biology.* 1992;13:352-357.

44. **Nobels FR, Kwekkeboom DJ, Coopmans W, Schoenmakers CH, Lindemans J, De Herder WW, Krenning EP, Bouillon R, Lamberts SW.** Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. *The Journal of clinical endocrinology and metabolism.* 1997;82:2622-2628.

45. **Zuetenhorst JM, Bonfrer JM, Korse CM, Bakker R, van Tinteren H, Taal BG.** Carcinoid heart disease: the role of urinary 5-hydroxyindoleacetic acid excretion and plasma levels of atrial natriuretic peptide, transforming growth factor-beta and fibroblast growth factor. *Cancer.* 2003;97:1609-1615.

46. **Cryer PE, Axelrod L, Grossman AB, Heller SR, Montori VM, Seaquist ER, Service FJ, Endocrine S.** Evaluation and management of adult hypoglycemic disorders: an Endocrine Society Clinical Practice Guideline. *The Journal of clinical endocrinology and metabolism.* 2009;94:709-728.

47. **de Herder WW, Niederle B, Scoazec JY, Pauwels S, Kloppel G, Falconi M, Kwekkeboom DJ, Oberg K, Eriksson B, Wiedenmann B, Rindi G, O'Toole D, Ferone D, Frascati Consensus C, European Neuroendocrine Tumor S.** Well-differentiated pancreatic tumor/carcinoma: insulinoma. *Neuroendocrinology.* 2006;84:183-188.

48. **Berna MJ, Hoffmann KM, Long SH, Serrano J, Gibril F, Jensen RT.** Serum gastrin in Zollinger-Ellison syndrome: II. Prospective study of gastrin provocative testing in 293 patients from the National Institutes of Health and comparison with 537 cases from the literature. evaluation of diagnostic criteria, proposal of new criteria, and correlations with clinical and tumoral features. *Medicine.* 2006;85:331-364.

49. **Berna MJ, Hoffmann KM, Serrano J, Gibril F, Jensen RT.** Serum gastrin in Zollinger-Ellison syndrome: I. Prospective study of fasting serum gastrin in 309 patients from the National Institutes of Health and comparison with 2229 cases from the literature. *Medicine.* 2006;85:295-330.

50. **Jensen RT, Cadiot G, Brandi ML, de Herder WW, Kaltsas G, Komminoth P, Scoazec JY, Salazar R, Sauvanet A, Kianmanesh R, Barcelona Consensus Conference p.** ENETS Consensus Guidelines for the management of patients with digestive neuroendocrine neoplasms: functional pancreatic endocrine tumor syndromes. *Neuroendocrinology.* 2012;95:98-119.

51. **Jensen RT, Niederle B, Mitry E, Ramage JK, Steinmuller T, Lewington V, Scarpa A, Sundin A, Perren A, Gross D, O'Connor JM, Pauwels S, Kloppel G, Frascati Consensus C, European Neuroendocrine Tumor S.** Gastrinoma (duodenal and pancreatic). *Neuroendocrinology.* 2006;84:173-182.

52. **Rockall AG, Reznek RH.** Imaging of neuroendocrine tumours (CT/MR/US). *Best practice & research. Clinical endocrinology & metabolism.* 2007;21:43-68.

53. **O'Toole D, Palazzo L.** Endoscopy and Endoscopic Ultrasound in Assessing and Managing Neuroendocrine Neoplasms. *Front Horm Res.* 2015;44:88-103.

54. **Anderson MA, Carpenter S, Thompson NW, Nostrant TT, Elta GH, Scheiman JM.** Endoscopic ultrasound is highly accurate and directs management in patients with neuroendocrine tumors of the pancreas. *The American journal of gastroenterology.* 2000;95:2271-2277.

55. **van Tuyl SA, van Noorden JT, Timmer R, Stolk MF, Kuipers EJ, Taal BG.** Detection of small-bowel neuroendocrine tumors by video capsule endoscopy. *Gastrointestinal endoscopy.* 2006;64:66-72.

56. **Toumpanakis C, Kim MK, Rinke A, Bergestuen DS, Thirlwell C, Khan MS, Salazar R, Oberg K.** Combination of cross-sectional and molecular imaging studies in the localization of gastroenteropancreatic neuroendocrine tumors. *Neuroendocrinology.* 2014;99:63-74.

57. **Kwekkeboom DJ, Kam BL, van Essen M, Teunissen JJ, van Eijck CH, Valkema R, de Jong M, de Herder WW, Krenning EP.** Somatostatin-receptor-based imaging and therapy of gastroenteropancreatic neuroendocrine tumors. *Endocrine-related cancer.* 2010;17:R53-73.

58. **Brabander T, Kwekkeboom DJ, Feelders RA, Brouwers AH, Teunissen JJ.** Nuclear Medicine Imaging of Neuroendocrine Tumors. *Front Horm Res.* 2015;44:73-87.

59. **Koopmans KP, de Vries EG, Kema IP, Elsinga PH, Neels OC, Sluiter WJ, van der Horst-Schrivers AN, Jager PL.** Staging of carcinoid tumours with 18F-DOPA PET: a prospective, diagnostic accuracy study. *The Lancet. Oncology.* 2006;7:728-734.

60. **Binderup T, Knigge U, Loft A, Mortensen J, Pfeifer A, Federspiel B, Hansen CP, Hojgaard L, Kjaer A.** Functional imaging of neuroendocrine tumors: a head-to-head comparison of somatostatin receptor scintigraphy, 123I-MIBG scintigraphy, and 18F-FDG PET. *Journal of nuclear medicine.* 2010;51:704-712.

61. **Severi S, Nanni O, Bodei L, Sansovini M, Ianniello A, Nicoletti S, Scarpi E, Matteucci F, Gilardi L, Paganelli G.** Role of 18FDG PET/CT in patients treated with 177Lu-DOTATATE for advanced differentiated neuroendocrine tumours. *European journal of nuclear medicine and molecular imaging.* 2013;40:881-888.

62. **Kayani I, Bomanji JB, Groves A, Conway G, Gacinovic S, Win T, Dickson J, Caplin M, Ell PJ.** Functional imaging of neuroendocrine tumors with combined PET/CT using 68Ga-DOTATATE (DOTA-DPhe1,Tyr3-octreotate) and 18F-FDG. *Cancer.* 2008;112:2447-2455.

63. **Oh S, Prasad V, Lee DS, Baum RP.** Effect of Peptide Receptor Radionuclide Therapy on Somatostatin Receptor Status and Glucose Metabolism in Neuroendocrine Tumors: Intraindividual Comparison of Ga-68 DOTANOC PET/CT and F-18 FDG PET/CT. *Int J Mol Imaging.* 2011;2011:524130.

64. **Rindi G, Kloppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, Erikssson B, Falchetti A, Falconi M, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B, all other Frascati Consensus Conference p, European Neuroendocrine Tumor S.** TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Archiv.* 2006;449:395-401.

65. **Rindi G, Kloppel G, Couvelard A, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B.** TNM staging of midgut and hindgut (neuro) endocrine tumors: a consensus proposal including a grading system. *Virchows Archiv.* 2007;451:757-762.

66. **Heetfeld M, Chougnet CN, Olsen IH, Rinke A, Borbath I, Crespo G, Barriuso J, Pavel M, O'Toole D, Walter T, other Knowledge Network m.** Characteristics and treatment of patients with G3 gastroenteropancreatic neuroendocrine neoplasms. *Endocrine-related cancer.* 2015;22:657-664.

67. **Norton JA.** Surgery for primary pancreatic neuroendocrine tumors. *Journal of gastrointestinal surgery.* 2006;10:327-331.

68. **Akerstrom G, Hellman P, Hessman O, Osmak L.** Management of midgut carcinoids. *Journal of surgical oncology.* 2005;89:161-169.

69. **de Herder WW, Krenning EP, Van Eijck CH, Lamberts SW.** Considerations concerning a tailored, individualized therapeutic management of patients with (neuro)endocrine tumours of the gastrointestinal tract and pancreas. *Endocrine-related cancer.* 2004;11:19-34.

70. **van Vilsteren FG, Baskin-Bey ES, Nagorney DM, Sanderson SO, Kremers WK, Rosen CB, Gores GJ, Hobday TJ.** Liver transplantation for gastroenteropancreatic neuroendocrine cancers: Defining selection criteria to improve survival. *Liver transplantation.* 2006;12:448-456.

71. **Norton JA.** Endocrine tumours of the gastrointestinal tract. Surgical treatment of neuroendocrine metastases. *Best practice & research. Clinical gastroenterology.* 2005;19:577-583.

72. **Roland CL, Bian A, Mansour JC, Yopp AC, Balch GC, Sharma R, Xie XJ, Schwarz RE.** Survival impact of malignant pancreatic neuroendocrine and islet cell neoplasm phenotypes. *Journal of surgical oncology.* 2012;105:595-600.

73. **Tomassetti P, Campana D, Piscitelli L, Casadei R, Santini D, Nori F, Morselli-Labate AM, Pezzilli R, Corinaldesi R.** Endocrine pancreatic tumors: factors correlated with survival. *Annals of oncology.* 2005;16:1806-1810.

74. **Reincke M, Ritzel K, Osswald A, Berr C, Stalla G, Hallfeldt K, Reisch N, Schopohl J, Beuschlein F.** A critical reappraisal of bilateral adrenalectomy for ACTH-dependent Cushing's syndrome. *European journal of endocrinology.* 2015;173:M23-32.

75. **Norton JA, Jensen RT.** Resolved and unresolved controversies in the surgical management of patients with Zollinger-Ellison syndrome. *Annals of surgery.* 2004;240:757-773.

76. **Gustafsson BI, Hauso O, Drozdov I, Kidd M, Modlin IM.** Carcinoid heart disease. *International journal of cardiology.* 2008;129:318-324.

77. **Ruszniewski P, O'Toole D.** Ablative therapies for liver metastases of gastroenteropancreatic endocrine tumors. *Neuroendocrinology.* 2004;80 Suppl 1:74-78.

78. **O'Toole D, Ruszniewski P.** Chemoembolization and other ablative therapies for liver metastases of gastrointestinal endocrine tumours. *Best Pract Res Clin Gastroenterol.* 2005;19:585-594.

79. **Maire F, Lombard-Bohas C, O'Toole D, Vullierme MP, Rebours V, Couvelard A, Pelletier AL, Zappa M, Pilleul F, Hentic O, Hammel P, Ruszniewski P.** Hepatic arterial embolization versus chemoembolization in the treatment of liver metastases from well-differentiated midgut endocrine tumors: a prospective randomized study. *Neuroendocrinology.* 2012;96:294-300.

80. **Cao CQ, Yan TD, Bester L, Liauw W, Morris DL.** Radioembolization with yttrium microspheres for neuroendocrine tumour liver metastases. *Br J Surg.* 2010;97:537-543.

81. **Toumpanakis C, Caplin ME.** Update on the role of somatostatin analogs for the treatment of patients with gastroenteropancreatic neuroendocrine tumors. *Semin Oncol.* 2013;40:56-68.

82. **Reubi JC, Laissue J, Waser B, Horisberger U, Schaer JC.** Expression of somatostatin receptors in normal, inflamed, and neoplastic human gastrointestinal tissues. *Annals of the New York Academy of Sciences.* 1994;733:122-137.

83. **Lamberts SW, van der Lely AJ, de Herder WW, Hofland LJ.** Octreotide. *The New England journal of medicine.* 1996;334:246-254.

84. **Klibanski A, Melmed S, Clemmons DR, Colao A, Cunningham RS, Molitch ME, Vinik AI, Adelman DT, Liebert KJ.** The endocrine tumor summit 2008: appraising therapeutic approaches for acromegaly and carcinoid syndrome. *Pituitary.* 2010;13:266-286.

85. **Modlin IM, Pavel M, Kidd M, Gustafsson BI.** Review article: somatostatin analogues in the treatment of gastroenteropancreatic neuroendocrine (carcinoid) tumours. *Alimentary pharmacology & therapeutics.* 2010;31:169-188.

86. **Oberg K, Kvols L, Caplin M, Delle Fave G, de Herder W, Rindi G, Ruszniewski P, Woltering EA, Wiedenmann B.** Consensus report on the use of somatostatin analogs for the management of neuroendocrine tumors of the gastroenteropancreatic system. *Annals of oncology.* 2004;15:966-973.

87. **Ruszniewski P, Ducreux M, Chayvialle JA, Blumberg J, Cloarec D, Michel H, Raymond JM, Dupas JL, Gouerou H, Jian R, Genestin E, Bernades P, Rougier P.** Treatment of the carcinoid syndrome with the longacting somatostatin analogue lanreotide: a prospective study in 39 patients. *Gut.* 1996;39:279-283.

88. **Kvols LK, Martin JK, Marsh HM, Moertel CG.** Rapid reversal of carcinoid crisis with a somatostatin analogue. *The New England journal of medicine.* 1985;313:1229-1230.

89. **Rinke A, Muller HH, Schade-Brittinger C, Klose KJ, Barth P, Wied M, Mayer C, Aminossadati B, Pape UF, Blaker M, Harder J, Arnold C, Gress T, Arnold R, Group PS.** Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. *Journal of clinical oncology.* 2009;27:4656-4663.

90. **Caplin ME, Pavel M, Cwikla JB, Phan AT, Raderer M, Sedlackova E, Cadiot G, Wolin EM, Capdevila J, Wall L, Rindi G, Langley A, Martinez S, Blumberg J, Ruszniewski P, Investigators C.** Lanreotide in metastatic enteropancreatic neuroendocrine tumors. *The New England journal of medicine.* 2014;371:224-233.

91. **Phan AT.** SWOG S0518: phase III prospective randomized comparison of depot octreotide plus interferon alpha-2b versus depot octreotide plus bevacizumab (NSC #704865) in advanced, poor prognosis carcinoid patients (NCT00569127). *Clin Adv Hematol Oncol.* 2015;13:15-16.

92. **Oberg K.** Interferon in the management of neuroendocrine GEP-tumors: a review. *Digestion.* 2000;62 Suppl 1:92-97.

93. **Booy S, van Eijck CH, Janssen JA, Dogan F, van Koetsveld PM, Hofland LJ.** IFN-beta is a potent inhibitor of insulin and insulin like growth factor stimulated proliferation and migration in human pancreatic cancer cells. *Am J Cancer Res.* 2015;5:2035-2046.

94. **Vitale G, de Herder WW, van Koetsveld PM, Waaijers M, Schoordijk W, Croze E, Colao A, Lamberts SW, Hofland LJ.** IFN-beta is a highly potent inhibitor of gastroenteropancreatic neuroendocrine tumor cell growth in vitro. *Cancer Res.* 2006;66:554-562.

95. **Vitale G, van Koetsveld PM, de Herder WW, van der Wansem K, Janssen JA, Colao A, Lombardi G, Lamberts SW, Hofland LJ.** Effects of type I interferons on IGF-mediated autocrine/paracrine growth of human neuroendocrine tumor cells. *Am J Physiol Endocrinol Metab.* 2009;296:E559-566.

96. **Eriksson B, Annibale B, Bajetta E, Mitry E, Pavel M, Platania M, Salazar R, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: chemotherapy in patients with neuroendocrine tumors. *Neuroendocrinology.* 2009;90:214-219.

97. **Welin S, Sorbye H, Sebjornsen S, Knappskog S, Busch C, Oberg K.** Clinical effect of temozolomide-based chemotherapy in poorly differentiated endocrine carcinoma after progression on first-line chemotherapy. *Cancer.* 2011;117:4617-4622.

98. **Kouvaraki MA, Ajani JA, Hoff P, Wolff R, Evans DB, Lozano R, Yao JC.** Fluorouracil, doxorubicin, and streptozocin in the treatment of patients with locally advanced and metastatic pancreatic endocrine carcinomas. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2004;22:4762-4771.

99. **Strosberg JR, Fine RL, Choi J, Nasir A, Coppola D, Chen DT, Helm J, Kvols L.** First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. *Cancer.* 2011;117:268-275.

100. **Schmid HA.** Pasireotide (SOM230): development, mechanism of action and potential applications. *Molecular and cellular endocrinology.* 2008;286:69-74.

101. **Wolin EM, Jarzab B, Eriksson B, Walter T, Toumpanakis C, Morse MA, Tomassetti P, Weber MM, Fogelman DR, Ramage J, Poon D, Gadbaw B, Li J, Pasieka JL, Mahamat A, Swahn F, Newell-Price J, Mansoor W, Oberg K.** Phase III study of pasireotide long-acting release in patients with metastatic neuroendocrine tumors and carcinoid symptoms refractory to available somatostatin analogues. *Drug Des Devel Ther.* 2015;9:5075-5086.

102. **Yao JC, Phan A, Hoff PM, Chen HX, Charnsangavej C, Yeung SC, Hess K, Ng C, Abbruzzese JL, Ajani JA.** Targeting vascular endothelial growth factor in advanced carcinoid tumor: a random assignment phase II study of depot octreotide with bevacizumab and pegylated interferon alpha-2b. *Journal of clinical oncology.* 2008;26:1316-1323.

103. **Ducreux M, Dahan L, Smith D, O'Toole D, Lepere C, Dromain C, Vilgrain V, Baudin E, Lombard-Bohas C, Scoazec JY, Seitz JF, Bitoun L, Kone S, Mitry E.** Bevacizumab combined with 5-FU/streptozocin in patients with progressive metastatic well-differentiated pancreatic endocrine tumours (BETTER trial)--a phase II non-randomised trial. *European journal of cancer.* 2014;50:3098-3106.

104. **Mitry E, Walter T, Baudin E, Kurtz JE, Ruszniewski P, Dominguez-Tinajero S, Bengrine-Lefevre L, Cadiot G, Dromain C, Farace F, Rougier P, Ducreux M.** Bevacizumab plus capecitabine in patients with progressive advanced well-differentiated neuroendocrine tumors of the gastrointestinal (GI-NETs) tract (BETTER trial)--a phase II non-randomised trial. *European journal of cancer.* 2014;50:3107-3115.

105. **Capdevila J, Salazar R, Halperin I, Abad A, Yao JC.** Innovations therapy: mammalian target of rapamycin (mTOR) inhibitors for the treatment of neuroendocrine tumors. *Cancer metastases reviews.* 2011;30 Suppl 1:27-34.

106. **Wullschleger S, Loewith R, Hall MN.** TOR signaling in growth and metabolism. *Cell.* 2006;124:471-484.

107. **Pavel ME, Hainsworth JD, Baudin E, Peeters M, Horsch D, Winkler RE, Klimovsky J, Lebwohl D, Jehl V, Wolin EM, Oberg K, Van Cutsem E, Yao JC, Group R-S.** Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet.* 2011;378:2005-2012.

108. **Yao JC, Lombard-Bohas C, Baudin E, Kvols LK, Rougier P, Ruszniewski P, Hoosen S, St Peter J, Haas T, Lebwohl D, Van Cutsem E, Kulke MH, Hobday TJ, O'Dorisio TM, Shah MH, Cadiot G, Luppi G, Posey JA, Wiedenmann B.** Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumors after failure of cytotoxic chemotherapy: a phase II trial. *Journal of clinical oncology.* 2010;28:69-76.

109. **Yao JC, Phan AT, Chang DZ, Wolff RA, Hess K, Gupta S, Jacobs C, Mares JE, Landgraf AN, Rashid A, Meric-Bernstam F.** Efficacy of RAD001 (everolimus) and octreotide LAR in advanced low- to intermediate-grade neuroendocrine tumors: results of a phase II study. *Journal of clinical oncology.* 2008;26:4311-4318.

110. **Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Oberg K, Rad001 in Advanced Neuroendocrine Tumors TTSG.** Everolimus for advanced pancreatic neuroendocrine tumors. *The New England journal of medicine.* 2011;364:514-523.

111. **Yao JC, Fazio N, Singh S, Buzzoni R, Carnaghi C, Wolin E, Tomasek J, Raderer M, Lahner H, Voi M, Pacaud LB, Rouyrre N, Sachs C, Valle JW, Fave GD, Van Cutsem E, Tesselaar M, Shimada Y, Oh DY, Strosberg J, Kulke MH, Pavel ME, Rad001 in Advanced Neuroendocrine Tumours FTSG.** Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. *Lancet.* 2015;

112. **Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, Chen JS, Horsch D, Hammel P, Wiedenmann B, Van Cutsem E, Patyna S, Lu DR, Blanckmeister C, Chao R, Ruszniewski P.** Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *The New England journal of medicine.* 2011;364:501-513.

113. **Wild D, Fani M, Fischer R, Del Pozzo L, Kaul F, Krebs S, Fischer R, Rivier JE, Reubi JC, Maecke HR, Weber WA.** Comparison of somatostatin receptor agonist and antagonist for peptide receptor radionuclide therapy: a pilot study. *Journal of nuclear medicine.* 2014;55:1248-1252.

114. **Kwekkeboom DJ, de Herder WW, Kam BL, van Eijck CH, van Essen M, Kooij PP, Feelders RA, van Aken MO, Krenning EP.** Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0,Tyr3]octreotate: toxicity, efficacy, and survival. *Journal of clinical oncology.* 2008;26:2124-2130.

115. **van Essen M, Krenning EP, Kam BL, de Herder WW, Feelders RA, Kwekkeboom DJ.** Salvage therapy with (177)Lu-octreotate in patients with bronchial and gastroenteropancreatic neuroendocrine tumors. *Journal of nuclear medicine.* 2010;51:383-390.

116. **Bergsma H, van Vliet EI, Teunissen JJ, Kam BL, de Herder WW, Peeters RP, Krenning EP, Kwekkeboom DJ.** Peptide receptor radionuclide therapy (PRRT) for GEP-NETs. *Best practice & research. Clinical gastroenterology.* 2012;26:867-881.

117. **van Vliet EI, van Eijck CH, de Krijger RR, Nieveen van Dijkum EJ, Teunissen JJ, Kam BL, de Herder WW, Feelders RA, Bonsing BA, Brabander T, Krenning EP, Kwekkeboom DJ.** Neoadjuvant Treatment of Nonfunctioning Pancreatic Neuroendocrine Tumors with [177Lu-DOTA0,Tyr3] Octreotate. *Journal of nuclear medicine.* 2015;56:1647-1653.

SUPPLEMENTARY DATA

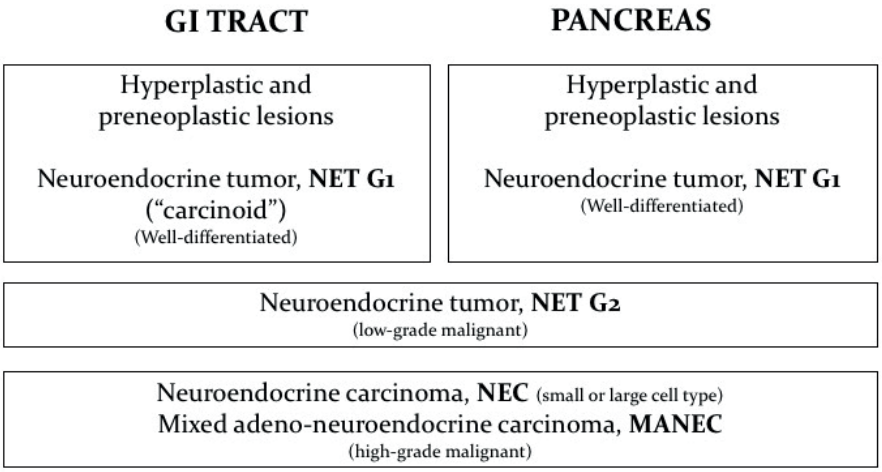
Table 1. Tumor, Lymph Node(s), Metastases (TNM) Staging Systems.

	ENETS	AJCC
	Proposal for a TNM Classification and Disease Staging for Endocrine Tumors	**Definitions of TNM**
	Primary tumor (T)	**Primary tumor (T)**
TX	Tumor cannot be assessed	Tumor cannot be assessed
TO	No evidence of primary tumor	No evidence of primary tumor
T1	Tumor limited to the pancreas and size <2 cm	Tumor limited to the pancreas, ≤2 cm in greatest dimension
T2	Tumor limited to the pancreas and size 2 - 4 cm	Tumor limited to the pancreas, >2 cm in greatest dimension
T3	Tumor limited to the pancreas and size >4 cm or invading duodenum or bile duct	Tumor extends beyond the pancreas but without involvement of the celiac axis or the superior mesenteric artery
T4	Tumor invading adjacent organs (stomach, spleen, colon, adrenal gland) or the wall of celiac axis or superior mesenteric artery	Tumor involves the celiac axis or the superior mesenteric artery (unresectable primary tumor)
	Regional Lymph Nodes (N)	**Regional Lymph Nodes (N)**
NX	Regional lymph node(s) cannot be assessed	Regional lymph node(s) cannot be assessed
N0	No regional lymph node metastases	No regional lymph node metastases
N1	Regional lymph node metastases	Regional lymph node metastases
	Distant Metastases (M)	**Distant Metastases (M)**
MX	Distant metastases cannot be assessed	
M0	No distant metastases	No distant metastases
M1	Distant metastases	Distant metastases

Table 2. TNM Staging Systems.

Stage	T	N	M
I	T1	N0	M0
IIa	T2	N0	M0
IIb	T3	N0	M0
IIIa	T4	N0	M0
IIIb	Any T	N1	M0
IV	Any T	Any N	M1

Figure 1. WHO 2010 Classification.



Chapter 2

Is true non-secretion of chromogranin A an unfavorable prognostic factor in ENETS Stage IV gastroenteropancreatic neuroendocrine tumors?

Kimberly Kamp[1]*, Roxanne C.S. van Adrichem[1]*, Timon Vandamme[1,2], Yolanda B. de Rijke[3], Marc Peeters[2], Richard A. Feelders[1], Wouter W. de Herder[1]

[1]*Department of Internal Medicine, Sector of Endocrinology, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands*

[2]*Center for Oncological Research, University of Antwerp, Antwerp, Belgium*

[3]*Department of Clinical Chemistry, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands*

*joint first authors

Submitted

ABSTRACT

Context: Chromogranin A (CgA) is a widely used biomarker for the work-up of gastroenteropancreatic neuroendocrine tumors (GEP-NETs), correlating with tumor volume and biological activity. During diagnosis and follow-up we found patients with elevated CgA levels and patients without elevated CgA levels (='true non-secretors').

Objective: Determine whether true non-secretion of CgA is an unfavorable prognostic factor in patients with Stage IV GEP-NETs.

Design: Retrospective case study.

Setting: Tertiary referral hospital.

Main outcome measurements: Overall survival (OS) estimated with Kaplan–Meier methods.

Patients: 692 consecutive patients were evaluated with a median follow-up of 61.3 months (25th – 75th percentile: 35.7 – 97.5) and a median OS of 104.6 months (95% CI: 94.4-136.5). After exclusion of patients with concomitant proton pump inhibitors, 616 and 524 patients were included for analysis of baseline and follow-up CgA, respectively. Cut-off values for baseline and follow-up CgA groups were: normal (reference range (RR)), intermediate (≤ 2x upper limit of normal (ULN)), high (2-10x ULN) and very high (>10x ULN).

Results: OS was significantly shorter in patients with high baseline CgA (median 103.9 vs. 222.4 months, $P<0.01$) and very high baseline CgA vs. RR (56.2 vs. 222.4 months, $P<0.0001$). For follow-up CgA, OS was only significant shorter in the very high follow-up CgA vs. RR (62.9 months vs. not reached). This effect remained in multi-variate analysis with Cox proportional hazard models.

Conclusions: True non-secretion of CgA has shown to be a favorable biomarker for OS in patients with Stage IV GEP-NETs, both at first referral as well as during follow-up.

INTRODUCTION

Chromogranin A (CgA), a member of the granin family, is an acidic glycoprotein with 439 amino acids which is present in the secretory dense core granules of most neuroendocrine cells[1]. Immunohistochemistry for CgA is widely used and considered to be the most valuable tissue-based biomarker in the diagnosis of neuroendocrine tumors (NETs)[2]. Elevated levels of serum or plasma CgA can be found in various types of NETs, including gastrointestinal tract NETs, (non-)functioning pancreatic NETs, paragangliomas, pheochromocytomas, medullary thyroid carcinomas, pituitary and parathyroid adenomas and in some patients with small-cell lung cancer[3, 4]. Furthermore CgA has shown to be the best available general serum tumor marker for the work-up of gastroenteropancreatic NETs (GEP-NETs)[3, 5]. A recent meta-analysis demonstrated that the sensitivity and specificity of elevated serum levels of CgA in the diagnosis of patients with NETs are 0.73 and 0.95 respectively[6].

The highest levels of CgA have been found in patients with metastatic small intestine NETs and non-functioning pancreatic NETs[3, 7]. Depending on the extent of the disease, serum CgA is elevated in >60% of patients. CgA levels may correlate with tumor volume, presence of metastases and biological activity in the tumors, but care should be taken in measuring CgA and interpreting the results. Somatostatin analogues (SSAs) are known to affect blood levels of CgA by blocking the production and release of CgA in addition to affecting tumor burden. Falsely elevated levels of CgA have also been reported in patients using proton pump inhibitors (PPIs) or histamine H2 blockers, in patients with renal or liver failure, and in those with chronic atrophic gastritis type A or inflammatory bowel disease[7-9].

Both functionally active NETs and non-functioning NETs can co-secrete CgA with amines and peptides that are present in their neurosecretory granules[3, 7]. During diagnosis and follow-up we found patients with metastatic GEP-NETs that secrete CgA resulting in elevated circulating CgA levels and patients with metastatic GEP-NETs without elevated CgA levels. The latter we have called 'true non-secretors'. The reason why some patients with well-differentiated metastatic GEP-NETs do not show elevated circulating CgA levels is not known. It is well known that poorly differentiated neuroendocrine carcinomas (NECs) lose their expression of CgA[2]. On the other hand expression of CgA in non-endocrine tumors is considered a poor prognostic factor[10]. In our study in neuroendocrine tumor patients, we postulated that these non-secretors would have a poorer prognosis because we considered the lack of secretion of any substance from a GEP-NET to be a sign of further dedifferentiation.

Since the prognostic value of CgA in patients with metastatic NETs has not been confirmed to date[3, 5], this study, conducted in a large single-center cohort, aimed to determine whether true non-secretion of CgA is an unfavorable prognostic factor in patients with metastatic, ENETS/AJCC TNM Stage IV[11-13] GEP-NETs. Finally, we investigated

whether there were any significant differences in patient and tumor characteristics between 'true non-secretors' and patients with CgA secreting GEP-NETs.

PATIENTS AND METHODS

Patients

In this retrospective case study, all patients with metastatic, ENETS/AJCC TNM Stage IV [11-13] GEP-NETs, diagnosed between 1 January 1993 and 31 December 2012 were identified from the Erasmus MC NET database and included.

TNM Stage IV indicates the presence of metastases at any distant anatomical site (including non-regional lymph nodes)[11-13]. The date of diagnosis was defined as the date at which tumor tissue was collected during biopsy or surgery. Follow-up time was determined from the date of diagnosis to the date of death or the last follow-up for survivors. Patients diagnosed with the multiple endocrine neoplasia syndrome type 1 (MEN1) were excluded. In addition, to prevent influence of PPIs on CgA levels, patients with concomitant PPI use at the time of CgA measurement were excluded. Information on age, sex, location of primary tumor, OctreoScan® (SRS) positivity, presence, or absence of bone metastases and concomitant use of PPIs was collected.

Definitions CgA groups

Patient groups were defined by first CgA level at referral or diagnosis (baseline CgA) and highest CgA level measured during follow-up (follow-up CgA). All serum CgA measurements were performed in the Erasmus MC, using an ELISA (CisBio Bioassays, Codolet, Franassay; upper limit of normal (ULN) <94 µg/l).

Four patient groups were defined by both baseline CgA and follow-up CgA levels. Cut-off values for serum CgA were: normal baseline CgA or follow-up CgA (reference range, <94 µg/l), intermediate baseline CgA or follow-up CgA (≤2xULN, 94-88µg/l), high baseline CgA or follow-up CgA (2-10xULN, 188-940 µg/l) and for very high baseline or follow-up CgA (>10xULN, >940 µg/l).

Primary outcome was overall survival, calculated from date of diagnosis to date of death by any cause, or date of last follow-up.

Statistical analysis

Continuous data were described as the mean and standard deviation (SD) and were compared by ANOVA tests. Categorical data were described as counts and percentages and were compared by $\chi 2$ tests. Overall survival was estimated with the Kaplan–Meier method. The hazard ratios (HRs) were estimated using a Cox proportional hazards model. HRs and 95% confidence intervals (CIs) were also calculated. The proportional hazard

assumption (Schoenfeld residuals) was always satisfied. Data analysis was performed using statistical software R version 3.1.3 and is based on the survival-package. A two-sided P-value of <0.05 was considered statistically significant.

RESULTS

Patient inclusion and stratification

In total, after exclusion of 19 MEN1 patients, 692 consecutive patients were evaluated with a median follow-up of 61.3 months (25^{th} – 75^{th} percentile: 35.7 – 97.5 months) and a median overall survival (mOS) of 104.6 months (95% CI: 94.4-136.5). After exclusion of patients with concomitant PPI use, 616 and 524 patients were included for analysis of baseline and follow-up CgA, respectively. Of these patients, 492 (79.9%) had an elevated baseline CgA level (>1xULN) and 465 (88.7%) had an elevated follow-up CgA level (>1xULN). Other clinical conditions which might have caused elevations in CgA were excluded.

Patient characteristics of the different groups for baseline CgA and follow-up CgA measurements can be found in Table 1. Highly significant differences were found for only two parameters: age at diagnosis for both baseline CgA and follow-up CgA measurement, and bone metastases differed only significantly for follow-up CgA measurement.

Baseline CgA and survival

Median time between histological diagnosis of the GEP-NET and measurement of baseline CgA was 3.2 months (25^{th} – 75^{th} percentile: 0.9 – 17.4 months). With regard to the measurement of baseline CgA, survival analysis without concomitant PPI use (N=616) showed a mOS of 222.4 months in the normal baseline CgA group (95% CI: 141.0-not reached (NR)), and 213.0 months in the intermediate baseline CgA group (95% CI: 114.2-NR; Cox-adjusted HR vs. normal CgA: 1.26 [0.79-1.99], P=0.33). Subsequently, mOS was 103.9 months in the high baseline CgA group (95% CI: 90.8-144.8; HR vs. normal CgA: 1.92 [1.29-2.88], P<0.01) and 56.2 months in the very high baseline CgA group (95% CI: 49.1-65.7; HR vs. normal CgA: 3.58 [2.44-5.26], P<0.0001) (Figure 1). Using a Cox proportional hazard model, age at diagnosis (HR 1.02 [1.01-1.03], P<0.0001), bone metastases (HR 1.33 [1.03-1.72], P<0.05), SRS positivity (HR 0.30 [0.18-0.53], P<0.0001) and unknown/other origin of tumor (HR 1.58 [1.18-2.12], P<0.01) had a statistical significance, while sex did not contribute significantly to the model.

In subanalysis, only including the 351 patients with known ENETS/WHO Grading, the same Cox proportional hazard model with ENETS/WHO Grade as an additional parameter was applied and showed that ENETS/WHO Grade 3 significantly contributed (HR vs. ENETS/WHO

Table 1. Characteristics at baseline of patient groups based upon baseline chromogranin A (CgA) measurement and follow-up CgA measurement. Differences are tested by ANOVA for age and through $\chi 2$ for all other variables.

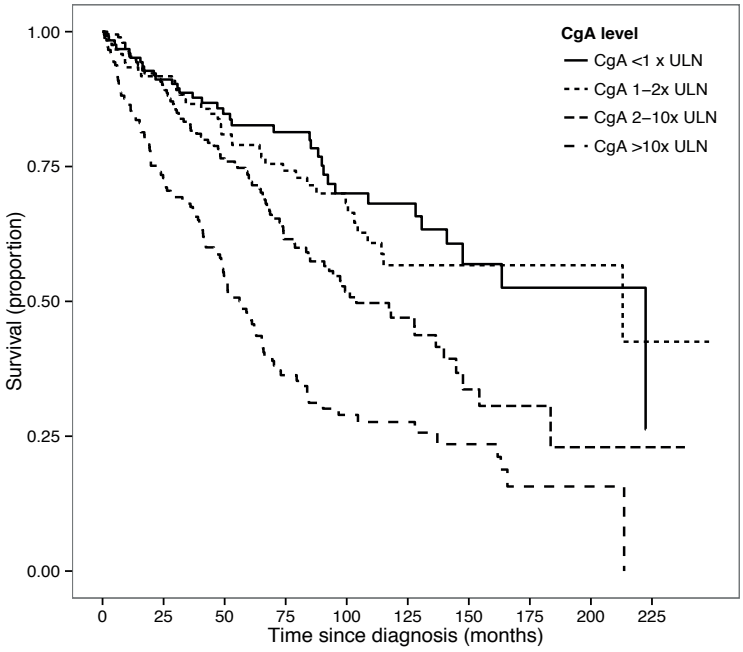
	Normal CgA <1xULN (<94 µg/l)	CgA 1-2xULN (94-188 µg/l)	CgA 2-10xULN (188-940 µg/l)	CgA >10xULN (>940 µg/l)	Significant difference
Baseline CgA measurement					
N	124	121	194	177	
Age at diagnosis (years ± SD)	54.9±11.5	59.05±12.19	59.15±10.81	60.78±10.6	0.0001
Sex – male (%)	52.4	51.2	50.0	57.6	NS
SRS positivity (%)	87.9	93.3	96.3	92.6	NS
Bone metastases (%)	29.0	17.35	29.38	28.81	NS
Primary tumor site					
siNETs (%)	38.7	48.76	49.48	40.67	NS
pancreatic NETs (%)	32.25	30.57	26.80	32.76	NS
Other (%)	29.03	20.66	23.71	26.55	NS
Follow-up CgA measurement					
N	59	86	156	223	
Age at diagnosis (years ± SD)	52.97±11.15	58.12±10.97	59.21±11.89	60.66±10.5	<0.0001
Sex – male (%)	50.8	47.6	50.0	57.4	NS
SRS positivity (%)	86.4	94.1	93.6	93.7	NS
Bone metastases (%)	33.9	15.1	28.8	30.9	0.03
Primary tumor site					
siNETs (%)	50.8	39.53	51.28	45.73	NS
pancreatic NETs(%)	22.03	32.55	26.92	25.56	NS
Other (%)	27.11	27.90	21.79	28.69	NS

CgA=chromogranin A, ULN= upper limit of normal, SRS=OctreoScan®

siNETs=small intestine neuroendocrine tumors,

Primary tumor site "Other" includes: NETs of unknown origin, NETs of stomach, duodenum and colorectal.

Figure 1. Kaplan-Meier estimate of overall survival in normal (<1 x ULN, —) ,intermediate (1-2 x ULN, - - -), high (2-10 x ULN, – –) and very high (>10 x ULN, – ·) baseline serum chromogranin A (CgA) level groups (N=616).



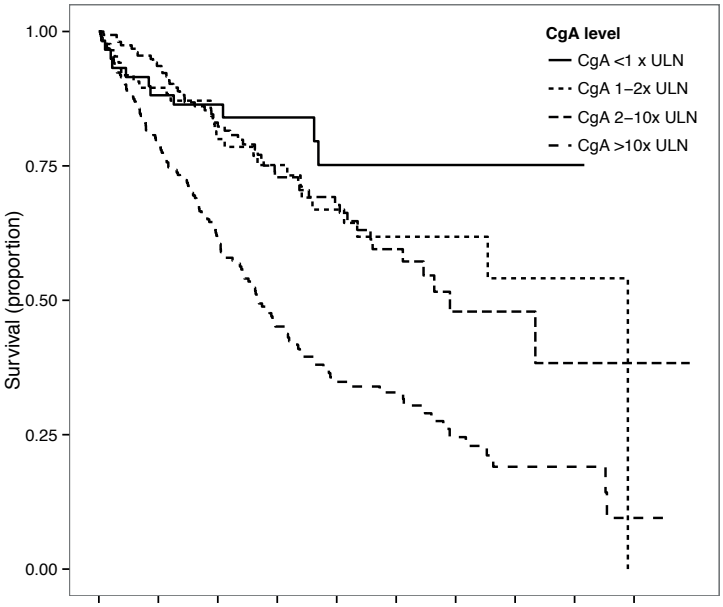
Grade 1: 5.02 [2.92- 8.65], P<0.0001) to the model. However, very high CgA remained independently associated with overall survival (HR vs. normal CgA: 3.54 [2.06- 6.10], P<0.0001).

Follow-up CgA and survival

Follow-up CgA measurement during the course of the disease was used to define four groups: low, intermediate, high and very high follow-up CgA groups. Median time between histological diagnosis of the GEP-NET and measurement of follow-up CgA was 18.6 months (25^{th} – 75^{th} percentile: 3.9 – 52.1).

In the patients without concomitant PPI use (N=524), mOS was not reached in the normal follow-up CgA group, while mOS in the intermediate follow-up CgA group was 222.4 months (95% CI: 163.5-NR; HR vs. normal: 1.58 [0.77-3.24], P=0.21). In the high and very high follow-up CgA group, mOS was 147.6 months (95% CI: 127.8-NR; HR vs. normal: 1.55 [0.80-3.02], P=0.20) and 67.3 months (95% CI: 59.3-83.4; HR vs. normal: 3.70 [1.98-6.91], P<0.001), respectively (Figure 2). Additional significant contributors to the

Figure 2. Kaplan-Meier estimate of overall survival in normal (<1 x ULN, ——) ,intermediate (1-2 x ULN, - - -), high (2-10 x ULN, — —) and very high (>10 x ULN, — · —) follow-up chromogranin A (CgA) level groups (N=524).



Number of patients at risk

CgA <1 x ULN	59	52	39	27	15	11	6	5	2	0
CgA 1–2x ULN	86	76	56	40	27	17	10	5	3	0
CgA 2–10x ULN	156	144	109	67	46	28	13	5	3	2
CgA >10x ULN	223	174	120	69	42	28	16	5	4	2

used Cox proportional hazard model included: bone metastases (HR 1.41 [1.06-1.87], P<0.05), SRS positivity (HR 0.33 [0.17-0.62, P<0.0001) and unknown/other origin of tumor (HR 1.60 [1.17-2.19], P<0.01), while sex did not contribute significantly. In sub-analysis, only including the 302 patients with known ENETS/WHO Grading, the same Cox proportional hazard model with ENETS/WHO Grade as an additional parameter was applied and showed that ENETS/WHO Grade 3 significantly contributed (HR vs. ENETS/WHO Grade 1: 4.19 [2.29-7.64], P<0.0001) to the model. In this extended model, very high CgA remained independently associated with overall survival (HR vs. normal CgA: 2.99 [1.40-6.40], P<0.005).

DISCUSSION

This single-center retrospective study demonstrates that, contrary to our expectations, true non-secretion of CgA is not an unfavorable prognostic factor for patients with ENETS/AJCC TNM Stage IV GEP-NETs, both when measured at first diagnosis as well as when measured at follow-up. Both serum baseline CgA and follow-up CgA levels show a positive correlation with overall survival.

The selected timeframe of 20 years for inclusion in this study was based upon the first availability of the most commonly used imaging techniques and treatment modalities in our institution. This included the introduction of somatostatin receptor imaging with the OctreoScan®, peptide receptor radiotherapy (PRRT)[14] and SSAs[15]. Any possible bias caused by evolving imaging and treatment protocols has therefore been minimized.

Patients in our research population, referred to our hospital for PRRT, mostly have metastatic disease. For CgA measurements in patients with metastatic disease specificities of 100% have been reported[16-19].

Bone metastases in our cohort only influenced follow-up CgA levels, likely because bone metastases were not yet present at the time of diagnosis. Patients who live longer are more likely to develop bone metastases during the course of their disease. This is reflected by the relatively high frequency of bone metastases in our patients with normal CgA levels.

A recent meta-analysis demonstrated that CgA is an efficient biomarker for the diagnosis of NETs with a sensitivity of 73% and specificity of 95%, indicating that serum CgA might be helpful in the clinical management and follow-up of NETs[6]. Another study by Yao and colleagues evaluated the prognostic value of CgA in patients with pancreatic NETs treated with everolimus. They confirmed the prognostic importance of baseline levels of CgA by multivariate analysis, hereby identifying CgA as an independent predictor of overall survival[9]. In line with this study we confirm a significant difference in OS between true non-secretors and different elevated levels of CgA secretion by not only pancreatic NETs, but also by small intestinal and other NETs and, therefore, determination of CgA at first consultation can be used for predicting prognosis in all types of GEP-NETs. Up until the present study the prognostic value of CgA in patients with GEP-NET had not been confirmed[3].

An elevated CgA level at baseline was found in over 80% of the patients, which is in accordance with earlier published data[3, 9, 20, 21].

Current ENETS guidelines state that where possible, PPIs should be interrupted, leaving a clearance of at least three half-lives, prior to CgA plasma sampling[3]. The potential weakness of CgA as a biomarker is that the use of PPIs can frequently cause a significant elevation in CgA levels[7, 8]. Since PPIs are now widely available in drugstores without a doctor's prescription, the value of future studies will likely be affected.

By selecting strict cut-off values to divide the patients in four groups, both at first measurement as highest measurement during follow-up, the impact of relatively small increases in CgA could be studied. Our study demonstrates that patients in high and very high CgA groups clearly have a worse prognosis when compared with those in the normal CgA group. Hence, an increase in CgA indicates a more aggressive disease course. The determination of CgA at first consultation can be used for predicting the prognosis. Also, CgA during the course of the disease provides additional information on tumor aggressiveness. The earlier hypothesis that GEP-NETs tumors may lose CgA expression to incomplete or partial endocrine differentiation is hereby refuted.

For our data collection we did not include information on Tumor Grading, because Ki-67 staining on tumor samples was not routinely used for the diagnostic work-up during the entire follow-up period. After all, the ENETS/WHO Grading system was introduced in 2010 and our inclusion of patients dates back to 1993[12, 13]. We therefore used SRS positivity as a surrogate marker for Tumor Grading, since SRS-positive GEP-NETs are generally well-differentiated, ENETS/WHO Grade 1-2 tumors[22, 23].

However, the assumption that all SRS-positive could have ENETS/WHO Grade 1-2 tumors could be considered a limitation of this study. We therefore studied the subpopulation of patients with known ENETS/WHO 2010 Grading and demonstrated that CgA remains associated with survival.

In conclusion, true non-secretion of CgA has been proven to be an independent biomarker for overall survival in patients with Stage IV well- and moderately differentiated GEP-NETs, both at first referral as well as perhaps more evident at follow-up.

REFERENCES

1. **Deftos LJ.** Chromogranin A: its role in endocrine function and as an endocrine and neuroendocrine tumor marker. *Endocrine reviews.* 1991;12:181-187.
2. **Rindi G, Kloppel G.** Endocrine tumors of the gut and pancreas tumor biology and classification. *Neuroendocrinology.* 2004;80 Suppl 1:12-15.
3. **O'Toole D, Grossman A, Gross D, Delle Fave G, Barkmanova J, O'Connor J, Pape UF, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: biochemical markers. *Neuroendocrinology.* 2009;90:194-202.
4. **Sobol RE, O'Connor DT, Addison J, Suchocki K, Royston I, Deftos LJ.** Elevated serum chromogranin A concentrations in small-cell lung carcinoma. *Annals of internal medicine.* 1986;105:698-700.
5. **Oberg K.** Circulating biomarkers in gastroenteropancreatic neuroendocrine tumours. *Endocrine-related cancer.* 2011;18 Suppl 1:S17-25.
6. **Yang X, Yang Y, Li Z, Cheng C, Yang T, Wang C, Liu L, Liu S.** Diagnostic value of circulating chromogranin a for neuroendocrine tumors: a systematic review and meta-analysis. *PloS one.* 2015;10:e0124884.
7. **de Herder WW.** Biochemistry of neuroendocrine tumours. *Best practice & research. Clinical endocrinology & metabolism.* 2007;21:33-41.
8. **Modlin IM, Gustafsson BI, Moss SF, Pavel M, Tsolakis AV, Kidd M.** Chromogranin A--biological function and clinical utility in neuro endocrine tumor disease. *Annals of surgical oncology.* 2010;17:2427-2443.
9. **Yao JC, Pavel M, Phan AT, Kulke MH, Hoosen S, St Peter J, Cherfi A, Oberg KE.** Chromogranin A and neuron-specific enolase as prognostic markers in patients with advanced pNET treated with everolimus. *The Journal of clinical endocrinology and metabolism.* 2011;96:3741-3749.
10. **Grabowski P, Schonfelder J, Ahnert-Hilger G, Foss HD, Heine B, Schindler I, Stein H, Berger G, Zeitz M, Scherubl H.** Expression of neuroendocrine markers: a signature of human undifferentiated carcinoma of the colon and rectum. *Virchows Archiv.* 2002;441:256-263.
11. **Kulke MH, Anthony LB, Bushnell DL, de Herder WW, Goldsmith SJ, Klimstra DS, Marx SJ, Pasieka JL, Pommier RF, Yao JC, Jensen RT, North American Neuroendocrine Tumor S.** NANETS treatment guidelines: well-differentiated neuroendocrine tumors of the stomach and pancreas. *Pancreas.* 2010;39:735-752.
12. **Rindi G, Kloppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, Erikssson B, Falchetti A, Falconi M, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B, all other Frascati Consensus Conference p, European Neuroendocrine Tumor S.** TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Archiv.* 2006;449:395-401.
13. **Rindi G, Kloppel G, Couvelard A, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B.** TNM staging of midgut and hindgut

(neuro) endocrine tumors: a consensus proposal including a grading system. *Virchows Archiv.* 2007;451:757-762.

14. **Bergsma H, van Vliet EI, Teunissen JJ, Kam BL, de Herder WW, Peeters RP, Krenning EP, Kwekkeboom DJ.** Peptide receptor radionuclide therapy (PRRT) for GEP-NETs. *Best practice & research. Clinical gastroenterology.* 2012;26:867-881.

15. **Lamberts SW, van der Lely AJ, de Herder WW, Hofland LJ.** Octreotide. *The New England journal of medicine.* 1996;334:246-254.

16. **Eriksson B, Arnberg H, Oberg K, Hellman U, Lundqvist G, Wernstedt C, Wilander E.** A polyclonal antiserum against chromogranin A and B--a new sensitive marker for neuroendocrine tumours. *Acta endocrinologica.* 1990;122:145-155.

17. **O'Connor DT, Pandlan MR, Carlton E, Cervenka JH, Hslao RJ.** Rapid radioimmunoassay of circulating chromogranin A: in vitro stability, exploration of the neuroendocrine character of neoplasia, and assessment of the effects of organ failure. *Clinical chemistry.* 1989;35:1631-1637.

18. **Sobol RE, Memoli V, Deftos LJ.** Hormone-negative, chromogranin A-positive endocrine tumors. *The New England journal of medicine.* 1989;320:444-447.

19. **Zatelli MC, Torta M, Leon A, Ambrosio MR, Gion M, Tomassetti P, De Braud F, Delle Fave G, Dogliotti L, degli Uberti EC, Italian CromaNet Working G.** Chromogranin A as a marker of neuroendocrine neoplasia: an Italian Multicenter Study. *Endocrine-related cancer.* 2007;14:473-482.

20. **Janson ET, Holmberg L, Stridsberg M, Eriksson B, Theodorsson E, Wilander E, Oberg K.** Carcinoid tumors: analysis of prognostic factors and survival in 301 patients from a referral center. *Annals of oncology.* 1997;8:685-690.

21. **Nobels FR, Kwekkeboom DJ, Coopmans W, Schoenmakers CH, Lindemans J, De Herder WW, Krenning EP, Bouillon R, Lamberts SW.** Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. *The Journal of clinical endocrinology and metabolism.* 1997;82:2622-2628.

22. **van Vliet EI, de Herder WW, de Rijke YB, Zillikens MC, Kam BL, Teunissen JJ, Peeters RP, Krenning EP, Kwekkeboom DJ.** Hypocalcaemia after treatment with [177Lu-DOTA 0,Tyr3]octreotate. *European journal of nuclear medicine and molecular imaging.* 2013;40:1843-1852.

23. **Kwekkeboom DJ, de Herder WW, Kam BL, van Eijck CH, van Essen M, Kooij PP, Feelders RA, van Aken MO, Krenning EP.** Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0,Tyr3]octreotate: toxicity, efficacy, and survival. *Journal of clinical oncology.* 2008;26:2124-2130.

Chapter 3

Serum neuron-specific enolase level is an independent predictor of overall survival in patients with gastroenteropancreatic neuroendocrine tumors

Roxanne C.S. van Adrichem[1]*, Kimberly Kamp[1]*, Timon Vandamme[1,2], Marc Peeters[2], Richard A. Feelders[1], Wouter W. de Herder[1]

[1]*Department of Internal Medicine, Sector of Endocrinology, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands*

[2]*Center for Oncological Research, University of Antwerp, Antwerp, Belgium*

*joint first authors

***Annals of Oncology*, Epub December 2015.**

LETTER TO THE EDITOR

Serum neuron-specific enolase (NSE) is considered a tumor marker in patients with gastroenteropancreatic neuroendocrine tumors (GEP-NETs)[1]. It is elevated in 30-50% of GEP-NET patients and correlates with tumor size[2, 3]. NSE has a sensitivity of 38% and specificity of 73% for GEP-NET detection[2]. The prognostic role of serum NSE as a biomarker for GEP-NETs patients' survival is poorly studied[4].

We retrospectively studied 592 patients with sporadic (non-familial) ENETS TNM Stage IV GEP-NETs. Median follow-up was 58.7 months (25^{th}-75^{th} percentile: 34.0-92.9). Serum NSE was measured at first consultation, using enzyme immunoassay (NSE Cobas E602, Roche Diagnostics, Mannheim, Germany).

Cut-off values for serum NSE were: NSE ≤1xULN (≤16.2 µg/l), NSE 1-3xULN (16.2-48.6 µg/l) and NSE >3xULN (48.6 µg/l).

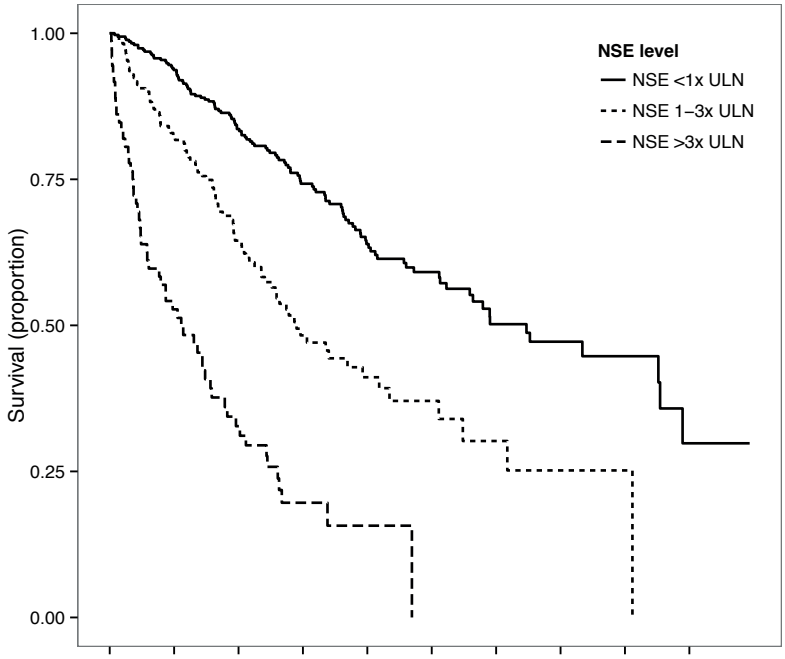
Primary outcome was overall survival, calculated from date of diagnosis to date of death by any cause, or date of last follow-up. Using statistical software R version 3.1.3 "survival" package, overall survival was estimated with the Kaplan–Meier method. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated with Cox proportional hazards models including age at diagnosis, OctreoScan® (SRS) positivity (Krenning Scale ≥2 in all lesions), primary tumor site, sex, and bone metastases.

242 (41%) of GEP-NET patients had an elevated NSE (>1xULN). NSE >3xULN were seen in pancreatic NETs.

Median overall survival (mOS) across all groups was 103.9 months (95% CI: 92.8-137.1). mOS was 161.8 months in the NSE ≤1xULN group (95% confidence interval (CI): 130.7-not reached (NR)) and 72.5 months in the NSE 1-3xULN group (95% CI: 60.2-108.6; Cox proportional hazard-adjusted HR vs. NSE ≤1xULN: 1.96 [1.45-2.63], P<0.001). In the NSE >3xULN group, mOS was 27.8 months (95% CI: 15.2-44.7; HR vs. NSE ≤1xULN: 6.15 [4.36-8.69], P<0.001) (Figure 1). Significant contributors to our model included: age at diagnosis (HR 1.03 [1.02-1.04], P<0.001) and SRS positivity (HR 0.48 [0.28-0.83], P<0.001).

The ENETS/WHO Grading system using Ki-67 staining was introduced in 2010[5]. Therefore, we used SRS positivity as a surrogate marker for ENETS/WHO Tumor Grading, since SRS-positive GEP-NETs are generally well-differentiated, ENETS/WHO Grade 1-2 tumors. However, the assumption that all SRS-positive could have ENETS/WHO Grade 1-2 tumors could be considered a limitation of this study. We therefore studied the subpopulation of 367 patients with known ENETS/WHO 2010 Grading (62% of all patients). In this population, the same Cox proportional hazard model with ENETS/WHO Grade as an additional parameter was applied and showed that higher ENETS/WHO Grade significantly contributed (P<0.001) to the model, but that NSE remained independently associated with overall survival (P<0.001). Multivariate analysis data is shown (Supplementary Table 1).

Figure 1. Kaplan-Meier estimate of overall survival in normal (≤1 x ULN,—), intermediate (1-3 x ULN, - - -) and high (>3 x ULN, – –) level groups of first serum neuron-specific enolase measurement by referral (NSE).



Number of patients at risk

NSE <1x ULN	350	325	239	159	104	64	37	21	13	4
NSE 1–3x ULN	170	138	90	41	24	13	7	2	1	0
NSE >3x ULN	72	36	20	7	1	0	0	0	0	0

This study demonstrates that NSE is a biomarker for overall survival in ENETS TNM Stage IV GEP-NET patients. Our study cohort had a median follow-up of almost 5 years and a mOS of over 8.5 years across all groups. Elevated NSE was found in over 40% of patients, confirming published data[2, 3]. Elevated serum NSE indicates a more aggressive disease course and determination of NSE at first consultation could, therefore, have prognostic implications.

REFERENCES

1. **Nobels FR, Kwekkeboom DJ, Coopmans W, Schoenmakers CH, Lindemans J, De Herder WW, Krenning EP, Bouillon R, Lamberts SW.** Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. *The Journal of clinical endocrinology and metabolism.* 1997;82:2622-2628.

2. **Baudin E, Gigliotti A, Ducreux M, Ropers J, Comoy E, Sabourin JC, Bidart JM, Cailleux AF, Bonacci R, Ruffie P, Schlumberger M.** Neuron-specific enolase and chromogranin A as markers of neuroendocrine tumours. *British journal of cancer.* 1998;78:1102-1107.

3. **Oberg K.** Circulating biomarkers in gastroenteropancreatic neuroendocrine tumours. *Endocrine-related cancer.* 2011;18 Suppl 1:S17-25.

4. **Yao JC, Pavel M, Phan AT, Kulke MH, Hoosen S, St Peter J, Cherfi A, Oberg KE.** Chromogranin A and neuron-specific enolase as prognostic markers in patients with advanced pNET treated with everolimus. *The Journal of clinical endocrinology and metabolism.* 2011;96:3741-3749.

5. **Rindi G.** The ENETS guidelines: the new TNM classification system. *Tumori.* 2010;96:806-809.

SUPPLEMENTARY DATA

Table 1. Multivariate analysis model with hazard ratios (HRs) estimated trough Cox proportional hazards model. P-values determined with Wald test for HR in Cox proportional hazards regression 95% CI.

Variable	Hazard ratio	95% Confidence interval	P-value
	All patients (n=592)		
NSE <1x ULN	1.00		
NSE 1-3x ULN	1.96	1.45-2.63	<0.00001
NSE >3x ULN	6.15	4.36-8.69	<0.00001
Age at diagnosis	1.03	1.02-1.04	<0.00001
SRS positivity	0.48	0.28-0.83	0.003
Presence of bone metastases	1.20	0.91-1.56	NS
Sex (female)	0.85	0.67-1.10	NS
Small intestinal primary	1.00		
Pancreatic primary	1.12	0.84-1.51	NS
Other primary site	1.14	0.83-1.56	NS
	Patients with known WHO/ENETS Grading (n=367)		
NSE <1x ULN	1.00		
NSE 1-3x ULN	2.32	1.51-3.56	0.0001
NSE >3x ULN	10.23	6.08-17.23	<0.00001
Age at diagnosis	1.04	1.02-1.04	<0.00001
SRS positivity	1.07	0.46-2.52	NS
Presence of bone metastases	1.21	0.82-1.79	NS
Sex (female)	0.97	0.67-1.40	NS
Small intestinal primary	1.00		
Pancreatic primary	1.01	0.65-1.58	NS
Other primary site	1.14	0.69-1.71	NS
WHO/ENETS Grade 1	1.00		
WHO/ENETS Grade 2	1.52	1.01-2.29	0.04
WHO/ENETS Grade 3	5.42	3.07-9.57	<0.00001

Primary tumor site "Other" includes: NETs of unknown origin, stomach, duodenum & colorectal NETs.

Chapter 4

Occurrence of second primary malignancies in patients with neuroendocrine tumors of the digestive tract and pancreas

Kimberly Kamp[1], Ronald A.M. Damhuis[2], Richard A. Feelders[1], Wouter W. de Herder[1]

[1]*Department of Internal Medicine, Sector of Endocrinology, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands,*
[2]*Comprehensive Cancer Centre Netherlands, Rotterdam, the Netherlands*

Endocrine-Related Cancer **(2012) 19, 95-99.**

ABSTRACT

An increased association between neuroendocrine tumors of the gastrointestinal tract and pancreas (GEP-NET) and other second primary malignancies has been suggested. We determined whether there is indeed an increased risk for second primary malignancies in GEP-NET patients compared with an age- and sex-matched control group of patients with identical malignancies. The series comprised 243 men and 216 women, diagnosed with a GEP-NET between 2000 and 2009 in a tertiary referral center. The timeline, before-at-after diagnosis, and the type of other malignancies were studied using person-year methodology. Poisson distributions were used for testing statistical significance. All data were cross-checked with the Dutch National Cancer Registry. Out of 459 patients with GEP-NET, 67 (13.7%) had a second primary cancer diagnosis: 25 previous cancers (5.4%), 13 synchronous cancers (2.8%), and 29 metachronous cancers (6.3%). The most common types of second primary cancer were breast cancer (n=10), colorectal cancer (n=8), melanoma (n=6), and prostate cancer (n=5). The number of patients with a cancer history was lower than expected, although not significant (n=25 vs. n=34.5). The diagnosis of synchronous cancers, mainly colorectal tumors, was higher than expected (n=13 vs. n=6.1, $P<0.05$). Metachronous tumors occurred as frequent as expected (n=29 vs. n=25.2, NS). In conclusion, our results are in contrast to previous studies and demonstrate that only the occurrence of synchronous second primary malignancies, mainly colorectal cancers, is increased in GEP-NET patients compared with the general population.

INTRODUCTION

Neuroendocrine tumors (NETs) are a heterogeneous group of tumors with various clinical manifestations and biological behavior[1].

The primary localizations of the majority of metastatic NETs are the gastrointestinal (GI) and bronchopulmonary tracts, and pancreas. In addition, these tumors can also be found in other more rare primary localizations such as ovaries, liver, and kidneys.

NETs that originate from cells of the diffuse neuroendocrine system of the GI-tract and the pancreas, gastroenteropancreatic NETs (GEP-NETs), are considered to be relatively rare tumors. However, more recent studies on NET epidemiology have demonstrated an increasing GEP-NET incidence and prevalence over the past 30 years. According to the United States Surveillance, Epidemiology, and End Results (SEER) database and several other European databases, current estimates of GEP-NET incidence vary between 2.5 and 5 cases per 100 000 population[2-7]. It is not yet evident whether this is a true increase in NET incidence, or the result of an increased use of diagnostic procedures, or a combination of both.

Previously published studies have reported an association between GEP-NETs and second primary malignancies[8-10]. Unfortunately, these studies were either small case series or autopsy studies[8-10]. Most studies also did not differentiate between previous, synchronous and metachronous lesions. The absence of age and sex correlations between the investigated populations and National Cancer Registries is also a major drawback in the reported series. Etiologic explanations ranged from incidental discovery to stimulation of cancer growth by neuroendocrine factors.

The aim of this study was to determine whether there was indeed a true increased risk for a second primary malignancy in a GEP-NET patient group compared with an age- and sex-matched control group of patients with identical malignancies.

PATIENTS AND METHODS

Patients

Patients with GEP-NETs were identified from the Erasmus MC NET database. Patients diagnosed with the multiple endocrine neoplasia type 1 (MEN1) syndrome were excluded from the study. The medical histories of 459 (non-MEN1) patients with GEP-NET, evaluated between 2000 and 2009 in the Erasmus MC, Rotterdam, The Netherlands, were reviewed. All GEP-NET patients treated in the Erasmus MC, Rotterdam (as described in this study) gave written informed consent before inclusion in the study, which was approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam. Data were collected from medical records and cross-checked with the Dutch National Cancer

Registry. The diagnosis of associated second primary malignancies was made by chart review – including pathology reports – the medical history, physical examination of the patient, clinical notes and the correspondence of the referring physician, documenting and cross-checking the previous diagnosis of malignancy.

Associated malignancies were assigned as 'previous' (diagnosed >6 months before GEP-NET diagnosis), 'synchronous' (diagnosed within 6 months before or after GEP-NET diagnosis), or 'metachronous' second primary malignancies (diagnosed >6 months after GEP-NET diagnosis).

Noninvasive, benign tumors (adenomas), carcinoma *in situ* of the cervix, and non-melanoma tumors of the skin (basaliomas and basal cell cancers) were excluded from this study.

Statistical analysis

The expected number of second primary malignancies was calculated with age- and sex-specific reference tables, using actuarial calculations[11]. Confidence intervals were constructed using Poisson tables for the observed number of malignancies.

For previous cancers, the age- and sex-specific distribution of the NET cohort was multiplied with a prevalence table, derived from the Dutch National Cancer Registry. The prevalence table describes the proportion of patients living with a previous diagnosis of cancer at a given age and stratified by sex.

For synchronous tumors, person-years at risk were calculated in a similar fashion up to 6 months after diagnosis, and then multiplied by two. The expected number of tumors was obtained by multiplying these person-years at risk with corresponding age- and sex-specific incidence rates for the Dutch population, derived from the Dutch National Cancer Registry.

For metachronous tumors, person-years at risk were calculated from 6 months after the date of diagnosis of the first GEP-NET until the censored date of metachronous cancer, date of death or end of the follow-up (01-01-2010). For the total number of previous, synchronous, and metachronous tumors, differences between the observed and expected numbers were tested for significance using Poisson tables. To avoid *post-hoc* bias, subgroup analyses were only performed for the most prevalent previous, synchronous, or metachronous second primary malignancies (n>3 per group).

RESULTS

From 2000 to 2009, 459 consecutive patients – 243 men and 216 women (female-to-male ratio, 1.1:1) – with GEP-NETs were evaluated at the Erasmus MC, Rotterdam, the Netherlands. The median age of the patients at the time of the GEP-NET diagnosis was

62.3 years (range 23.8–89.1 years). The mean follow-up of the study population was 44 months (range 0.4–118.6 months). Table 1 shows the clinical characteristics of the individuals in the analysis. Metastases were demonstrated in 432 patients (94.1%). The great majority of patients (88.2%) were diagnosed with ENETS Stage IV disease[12, 13] (Table 1).

Table 1. Characteristics of 459 consecutive patients with gastroenteropancreatic neuroendocrine tumors (GEP-NETs), diagnosed from 2000-2009 in the Erasmus MC, Rotterdam, the Netherlands.

		N	%
Total		459	100
Gender	Male	243	52.9
	Female	216	47.1
Age	< 50	63	13.7
	50-69	272	59.3
	> 70	124	27.0
Primary localization	**Pancreas**	**166**	**36.2**
	Non-functioning	130	28.3
	Insulinoma	25	5.4
	Glucagonoma	1	0.2
	Gastrinoma	5	1.1
	VIPoma	5	1.1
	Small intestine	**140**	**30.5**
	Colorectal	**57**	**12.4**
	Stomach	**12**	**2.6**
	Appendix	**6**	**1.3**
	CUP (carcinoid unknown primary)	**78**	**17.0**
ENETS Stage	I-IIIa	28	6.1
	IIIB	26	5.7
	IV	405	88.2

Sixty-three (13.7%) GEP-NET patients had 67 second primary cancers. Table 2 shows the occurrence of the most prevalent second primary malignancies in 459 patients diagnosed with GEP-NETs divided into previous, synchronous, and metachronous cancers.

The 67 second primary malignancies could be divided over 25 previous cancers (5.4%), 13 synchronous cancers (2.8%), and 29 metachronous cancers (6.3%). The most common types of second primary cancer were breast cancer (n=10), colorectal cancer (n=8), melanoma (n=6), and prostate cancer (n=5). Other second primary malignancies tumors, which are not included in the table, because of their small numbers, were: bronchial carcinoma (n=2), small intestinal carcinoma (n=2), renal cell carcinoma (n=4), lung

carcinoma (n=2), gynecological malignancies (n=3), myelodysplastic syndromes (n=2), and leukemia (n=2).

The number of patients with a cancer history was lower than expected but not significantly (n=25 vs. n=34.5). Diagnosis of synchronous cancers was higher than expected (n=13 vs. n=6.1, P<0.05). Synchronous cancers were colorectal cancer (n=4), small intestinal cancer (n=2), bronchial carcinoma (n=2), renal cell cancer (n=2), breast cancer (n=1), prostate cancer (n=1), and bladder cancer (n=1). Metachronous tumors occurred as frequent as expected (n=29 vs. n=25.2, NS) (Table 2).

Table 2. Occurrence of second primary malignancies in 459 patients with gastroenteropancreatic neuroendocrine tumors (GEP-NETs), diagnosed from 2000-2009 in the Erasmus MC, Rotterdam, the Netherlands.

		Observed	Expected	95% CI	SIR (O/E)	95% CI (SIR)
Prev	**Total**	25	34.5	16.2-36.9	0.72	0.47-1.07
	Prostate	5	6.7	1.6-11.7	0.75	0.24-1.75
	Breast	5	7.5	1.6-11.7	0.67	0.21-1.56
	Melanoma	4	2.3	1.1-10.2	1.74	0.48-4.43
Synchr	**Total**	13	6.1	6.9-22.2*****	2.13	1.13-3.64
	Colorectal	4	0.9	1.1-10.2*****	4.44	1.22-11.33
Metachr	**Total**	29	25.2	19.4-41.7	1.15	0.77-1.65
	Breast	5	2.6	1.6-11.7	1.92	0.62-4.50
	Colorectal	4	4.0	1.1-10.2	1.00	0.28-2.55
Total		**67**	**65.8**	**51.9-85.0**	**1.02**	**0.79-1.29**

Prev (previous second primary malignancies), diagnosed >6 months before GEP-NET diagnosis; Synchr (synchronous second primary malignancies), diagnosed within 6 months before or after GEP-NET diagnosis; Metachr (metachronous second primary malignancies), diagnosed >6 months after GEP-NET diagnosis; CI, confidence interval; SIR, standardized incidence ratio.

**P<0.05.*

DISCUSSION

We have evaluated the occurrence of second primary malignancies in a large cohort of patients with GEP-NETs, who were followed in a single, academic, tertiary referral institution. We have only found a significant increased risk of synchronous second primary malignancies, mainly colorectal cancers, in patients with GEP-NET.

We have chosen not to use GEP-NET data from a National Registry since it occurred to us that the GEP-NET registration in the Dutch National Cancer Registry is incomplete. Reasons for this decision were: some GEP-NETs were not considered to be malignant and, therefore, not reported. Variability in the GEP-NET nomenclature occurred over time. Also variability in classification systems was noted over time. In our study group,

the great majority of patients were diagnosed with ENETS Stage IV disease. Patients were not randomized.

The Netherlands has an estimated population of 16.6 million people. Our center covers approximately one fifth of this population. Until now, there is no national GEP-NET registry in the Netherlands. Therefore, we cannot give an impression on the proportion of Dutch GEP-NET patients who are treated in our center.

In historical series, the incidence of second primary malignancies in patients with GI-NETs (carcinoids) ranged from 12 to 46%, with an average of 17%[9]. In our series, the incidence of second primary malignancies in patients with GEP-NETs is 13.7%, which is in line with the findings in these historical GI-NET series.

A different distribution of GI-NETs (carcinoids) was noted in Taiwanese patients. In comparison with Western patients with GI-NETs, the Taiwanese patients presented with significantly more carcinoid tumors located in the rectum[14]. This study showed that Taiwanese GI-NET patients had a high probability of developing associated, non-carcinoid tumors mainly in the GI-tract, lungs, and the genitourinary system. However, a statistical quantification of risk using a national reference group was not performed[14].

It still remains questionable whether there is a true increased incidence of second primary malignancies in GEP-NET patients. The historical series did not correct for age, sex, period of diagnosis, and time from diagnosis and did not provide standardized incidence/ mortality ratios, nor used data obtained from national cancer registries for comparison. Population-based cancer registries can provide high-quality, long-term, national data, with histological confirmation in the majority of cases[15]. The major strength of our study is the use of an age and sex national reference group by using linkage to the Dutch National Cancer Registry.

In a study on NETs (carcinoids) and adenocarcinomas of the small intestine, Zar et al. corrected their analyses for sex, age, period of diagnosis, and time from diagnosis. These authors concluded that second primary malignancies were generally diagnosed within the first year after the diagnosis of a tumor in the small intestine. This was possibly due to the extensive clinical work-up and follow-up of their patients[16].

In a study with a similar design in patients with primary lung carcinoids, Cote et al. reported an increased risk of breast cancer in females within the first 5 years after the diagnosis of the lung carcinoid. However, after that period, the risk of breast cancer was lower than expected[17]. These authors also reported on increased risks of breast and prostate cancer in males who had an earlier diagnosis of a lung carcinoid. In these studies, other types of second primary malignancies in lung carcinoid patients were not more prevalent than in the general population[17].

Statistical quantification of risk using a population-based reference group has not yet been used for analyzing second primary cancer risks in GEP-NET patients. Therefore, we have conducted an analysis in this group of patients, using the same methodology

as the study in patients with lung carcinoids[17]. Our methodology was also similar to the methodologies used in two large studies analyzing second primary cancer risks in patients with Merkel cell carcinomas, which are neuroendocrine skin tumors[18, 19].

In conclusion, our results are refining conclusions obtained in previous studies and demonstrate that mainly the occurrence of synchronous second primary (intestinal) malignancies is increased in GEP-NET patients compared with the general population. This is probably due to incidental findings obtained at radiological or surgical examination, or gastroenterology work-up. Surveillance bias after diagnosis should always be considered as an explanation for excess risk of second primary malignancies, as medical attention is intensified immediately after a cancer diagnosis.

Owing to the rarity of GEP-NET and the diversity of the other cancer types, collaborative international studies will be required to study this issue in further detail. This study does not support extensive screening programs for second primary malignancies in GEP-NET patients.

REFERENCES

1. **Modlin IM, Oberg K, Chung DC, Jensen RT, de Herder WW, Thakker RV, Caplin M, Delle Fave G, Kaltsas GA, Krenning EP, Moss SF, Nilsson O, Rindi G, Salazar R, Ruszniewski P, Sundin A.** Gastroenteropancreatic neuroendocrine tumours. *The Lancet. Oncology.* 2008;9:61-72.

2. **Halfdanarson TR, Rabe KG, Rubin J, Petersen GM.** Pancreatic neuroendocrine tumors (PNETs): incidence, prognosis and recent trend toward improved survival. *Annals of oncology.* 2008;19:1727-1733.

3. **Halfdanarson TR, Rubin J, Farnell MB, Grant CS, Petersen GM.** Pancreatic endocrine neoplasms: epidemiology and prognosis of pancreatic endocrine tumors. *Endocrine-related cancer.* 2008;15:409-427.

4. **Lawrence B, Gustafsson BI, Chan A, Svejda B, Kidd M, Modlin IM.** The epidemiology of gastroenteropancreatic neuroendocrine tumors. *Endocrinology and metabolism clinics of North America.* 2011;40:1-18, vii.

5. **Niederle MB, Hackl M, Kaserer K, Niederle B.** Gastroenteropancreatic neuroendocrine tumours: the current incidence and staging based on the WHO and European Neuroendocrine Tumour Society classification: an analysis based on prospectively collected parameters. *Endocrine-related cancer.* 2010;17:909-918.

6. **Yao JC, Eisner MP, Leary C, Dagohoy C, Phan A, Rashid A, Hassan M, Evans DB.** Population-based study of islet cell carcinoma. *Annals of surgical oncology.* 2007;14:3492-3500.

7. **Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB.** One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *Journal of clinical oncology.* 2008;26:3063-3072.

8. **Gerstle JT, Kauffman GL, Jr., Koltun WA.** The incidence, management, and outcome of patients with gastrointestinal carcinoids and second primary malignancies. *Journal of the American College of Surgeons.* 1995;180:427-432.

9. **Habal N, Sims C, Bilchik AJ.** Gastrointestinal carcinoid tumors and second primary malignancies. *Journal of surgical oncology.* 2000;75:310-316.

10. **Schneider C, Wittekind C, Kockerling F.** [An unusual incidence of carcinoid tumors and secondary malignancies]. *Der Chirurg; Zeitschrift fur alle Gebiete der operativen Medizen.* 1995;66:607-611.

11. **Breslow NE, Day NE.** Statistical methods in cancer research. Volume II--The design and analysis of cohort studies. *IARC scientific publications.* 1987:1-406.

12. **Rindi G, Kloppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, Erikssson B, Falchetti A, Falconi M, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B, all other Frascati Consensus Conference p, European Neuroendocrine Tumor S.** TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Archiv.* 2006;449:395-401.

13. **Rindi G, Kloppel G, Couvelard A, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B.** TNM staging of midgut and hindgut

(neuro) endocrine tumors: a consensus proposal including a grading system. *Virchows Archiv.* 2007;451:757-762.

14. **Li AF, Hsu CY, Li A, Tai LC, Liang WY, Li WY, Tsay SH, Chen JY.** A 35-year retrospective study of carcinoid tumors in Taiwan: differences in distribution with a high probability of associated second primary malignancies. *Cancer.* 2008;112:274-283.

15. **Tulinius H, Storm HH, Pukkala E, Andersen A, Ericsson J.** Cancer in the Nordic countries, 1981-86. A joint publication of the five Nordic Cancer Registries. *APMIS. Supplementum.* 1992;31:1-194.

16. **Zar N, Garmo H, Holmberg L, Hellman P.** Risk of second primary malignancies and causes of death in patients with adenocarcinoma and carcinoid of the small intestine. *European journal of cancer.* 2008;44:718-725.

17. **Cote ML, Wenzlaff AS, Philip PA, Schwartz AG.** Secondary cancers after a lung carcinoid primary: a population-based analysis. *Lung cancer.* 2006;52:273-279.

18. **Bzhalava D, Bray F, Storm H, Dillner J.** Risk of second cancers after the diagnosis of Merkel cell carcinoma in Scandinavia. *British journal of cancer.* 2011;104:178-180.

19. **Kaae J, Hansen AV, Biggar RJ, Boyd HA, Moore PS, Wohlfahrt J, Melbye M.** Merkel cell carcinoma: incidence, mortality, and risk of other cancers. *Journal of the National Cancer Institute.* 2010;102:793-801.

Chapter 5

The prevalence and relevance of adrenal masses in patients with sporadic gastroenteropancreatic neuroendocrine tumors

George Kanakis[1]*, Kimberly Kamp[2]*, Konstantinos Tsiveriotis[1], Richard A. Feelders[2], Alexandra Zormpala[3], Wouter W. de Herder[2]**, Gregory Kaltsas[1]**

[1]*Department of Pathophysiology (Endocrine Unit), University of Athens Medical School, Athens, Greece,*
[2]*Department of Internal Medicine, Sector of Endocrinology, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands*
[3]*Department of Radiology, Laikon General Hospital, Athens, Greece*

*joint first authors
**joint last authors

Clinical Endocrinology **(2013) 78, 950–956.**

ABSTRACT

Objective: The widespread application of abdominal computerized tomography (CT) imaging has revealed that 0.98–4.0% of individuals harbor adrenal lesions (incidentalomas). There is, however, paucity of information regarding the prevalence of adrenal lesions in patients with gastroenteropancreatic neuroendocrine tumors (GEP-NETs). Purpose of this study was to estimate the prevalence of adrenal lesions in patients with GEP-NETs and identify their radiological features and clinical significance.

Design: The prevalence of adrenal lesions was estimated retrospectively in 438 patients with GEP-NETs who underwent abdominal imaging. Secretory status and changes in size were documented during subsequent follow-up. MEN1 patients and ectopic ACTH-secreting tumors were excluded.

Results: Adrenal lesions were detected in 32 (8.4%) of 383 patients included. The majority (22 patients – 69%) were located at the left adrenal gland and the mean size was 23.6 mm. In two patients, one with a well and another with a poorly differentiated tumor, clinicopathological features suggested adrenal metastases. During a mean follow-up period of 69.5 months, no subsequent growth of any adrenal lesion was observed. Endocrine evaluation documented subclinical glucocorticoid hypersecretion in 4 cases (14%). The presence of adrenal lesions did not correlate to distant metastases, however, they were observed more frequently in patients with G3 tumors.

Conclusion: The prevalence of adrenal lesions in patients with GEP-NETs was found to be higher than the general population and mostly represent benign adrenal adenomas (except patients with G3 tumors). Nevertheless, individualized assessment of imaging characteristics should still be considered.

INTRODUCTION

The widespread application of modern imaging to detect abdominal pathology has revealed an increased prevalence of inadvertently discovered adrenal masses, named adrenal incidentalomas (AI). The prevalence of AI among patients who underwent abdominal computerized tomography (CT) is reported to be between 0.98% and 4.0%, with a trend to increase among the elderly[1-4]. In addition, autopsy studies have demonstrated that the mean prevalence of adrenal masses in 87,065 cases is 6.0%, ranging from 1% to 32%[5-7]. Although these lesions are mostly unilateral, approximately 10–15% of cases can be bilateral,[5] and in the vast majority (~80%), AI are proven to be benign adrenal adenomas[8]. In oncologic patients, adrenal masses are detected more frequently as autopsy series have revealed an overall prevalence of 27%[9-11]; this prevalence may increase up to 35% and 39% in the case of lung and breast cancer, respectively[12].

Gastroenteropancreatic neuroendocrine tumors (GEP-NETS) comprise a heterogeneous group of relatively rare neoplasms that exhibit a more indolent biological behavior compared to epithelial neoplasms[13]. As a result of their relatively slow progression and in the absence of a clinical syndrome, GEP-NETs are frequently metastatic at the time of diagnosis[14], mainly to the liver (85%), peritoneal cavity (18%), bones (8%), other intra-abdominal sites (6%) and lungs (4%)[15]. However, there is currently no data regarding the prevalence of metastases to the adrenal glands in patients with GEP-NETs. Therefore, the detection of adrenal lesions during abdominal imaging in such patients is a diagnostic challenge since their metastatic origin needs to be excluded.

The aim of our study was to estimate the prevalence of adrenal masses discovered during imaging of patients with GEP-NETs, define their radiological features and demonstrate their clinical significance during the course of the disease.

PATIENTS AND METHODS

We retrospectively studied 438 consecutive patients with GEP-NETs, evaluated at two institutions in Greece and the Netherlands between 1990 and 2011. All included patients fulfilled the prerequisite of having been routinely surveyed at least at 6-month intervals with abdominal imaging [CT or Magnetic Resonance Imaging (MRI)] and gave written informed consent before inclusion in the studies, which were approved by the Medical Ethics Committees in both institutions.

Exclusion criteria were as follows: the presence of the disease in the context of a familial syndrome, as such patients develop adrenal lesions with a higher prevalence than the general population[16]. In addition, patients with GEP-NETs and Cushing's syndrome due to ectopic adrenocorticotrophin (ACTH) secretion were excluded from the study, as adrenal enlargement could be related to the excessive and continuous ACTH hyperse-

cretion. Demographical data, the localization of the primary GEP-NET(s) as well as the presence of metastatic disease to lymph nodes and/or distant organs, were recorded. Biological behavior of the primary tumor(s) was assessed based on the Ki-67 cellular proliferation index, and Grading was performed according to the Grading system proposed by the European Neuroendocrine Tumor Society (ENETS). Using this classification system, Grade 1 (G1 Ki-67 ≤ 2%) and Grade 2 (G2 Ki-67 > 2%) tumors are regarded as well-differentiated tumors, whereas Grade 3 (G3, Ki-67 > 20%) tumors are regarded as poorly differentiated carcinomas. In addition, the recently introduced TNM system for the classification of such tumors was also considered[17, 18].

Abdominal imaging of the patients that met the inclusion criteria were reviewed and evaluated for the presence of adrenal abnormalities by a single physician at each participating institution (R.A.F. & A.Z). When adrenal lesions were detected, their maximum size and radiological features were recorded and somatostatin receptor scintigraphy scans (SRS) with ^{111}In-pentetreotide were reviewed for the presence of uptake at the adrenal region. Additionally, growth potential of the adrenal lesions was evaluated by monitoring the lesion's size during subsequent follow-up using established criteria (RECIST)[19].

Adrenal masses measuring <4 cm were considered to be benign, whereas lesions >6 cm were suspicious of harboring malignancy. For lesions with a size between 4 and 6 cm, additional radiological features were utilized to maximize the diagnostic accuracy, such as heterogeneous density, irregular shape, the presence of calcifications or necrosis of the lesion and the presence of invasion to adjacent structures[20]. In addition, low attenuation values (≤10 Hounsfield Units) in unenhanced CT and rapid enhancement combined with rapid wash out of contrast medium in contrast-enhanced CT were suggestive for a benign adrenal adenoma. Similarly in chemical shift MRI, lesions with low-signal intensity on out-of-phase images compared with in-phase images were consistent with benign adenomas, whereas malignant lesions tended to retain signal[21]. An increase in the lesion's size ≥20% was considered as significant according to RECIST criteria[19].

Assessment of the functional status of the adrenal lesions was performed when applicable. Screening for glucocorticoid hypersecretion was based on low-dose (1 mg) overnight dexamethasone suppression test (DST) [using a cortisol cut-off value of 1.8 μg/dl (50 nM) to obtain maximum sensitivity] and the assessment of 24-h urinary cortisol excretion[22]. Confirmation of diagnosis was then achieved using the 2-day low-dose DST (0.5 mg of dexamethasone administered orally four times a day for 2 days – blood sample for cortisol taken on the third day at 9:00 a.m; 6 h after the last dose of dexamethasone – cut-off cortisol value: 50 nM). When appropriate, autonomous aldosterone secretion was evaluated in hypertensive patients using the ratio of ambulatory plasma aldosterone concentration (PAC) to plasma renin activity (PRA). A PAC/PRA ratio of 20 or greater (PAC expressed in ng/dl and PRA in ng/ml/h) was considered as indicative of autonomous aldosterone excretion requiring further investigations. Androgen secretion

was assessed by measuring plasma dehydroepiandrosterone sulphate (DHEA-S) levels. To rule out possible catecholamine hypersecretion from an adrenal pheochromocytoma, 24-h total catecholamines and total metanephrines were measured.

Statistical analysis

The prevalence of adrenal abnormalities in patients with NETs was estimated, and patients with adrenal lesions were compared to those without, regarding various clinicopathological parameters. Comparisons between the two groups were performed with unpaired *t* test for numerical data and chi-square test for categorical data. Level of statistical significance was set to 0.05. Calculations were performed using Statistical Package for Social Sciences (SPSS) software V.13.0 (Chicago, IL, USA).

RESULTS

From the total cohort of 438 patients, 425 had adequate imaging studies to evaluate the presence of adrenal lesions (Figure 1). The mean follow-up period was 69.5 months (range: 7–253). Thirty-six patients with GEP-NETs associated with multiple endocrine neoplasia 1 (MEN1) syndrome were excluded as well as 6 patients with GEP-NETs and ectopic ACTH secretion. Among the remaining 383 patients that met the study criteria, 205 (54%) were female and the mean age of diagnosis of the primary GEP-NET was 57.0 ± 12.4 years (range: 16–81). The majority of these tumors originated from the pancreas (127 patients – 33%), followed in order of frequency by the small intestine (95–25%), colon (29–8%), stomach (25–7%), appendix (20–5%), duodenum (13–3%) and rectum (12–3%). In 62 (16%) patients the primary tumor remained unknown until the completion of the study. Tumor staging could be applied to 379 patients; 47 patients (12%) had tumors confined at the organ of origin (Stages I–IIIA), whereas in 41 (11%) patients, the disease was extended to locoregional lymph nodes (Stage IIIB). In 291 patients (76%), the tumor had already metastasized to distant organs at the time of diagnosis (Stage IV). Information on Tumor Grading was available in 263 patients: 142 (54%) were G1, 112 (43%) G2 and 9 (3%) G3 (Table 1).

Evaluation of the abdominal imaging revealed adrenal lesions in 32 patients, corresponding to a prevalence of 8.4%. The majority of these lesions were located at the left adrenal gland (22 patients – 69%), whereas in three patients (9%), the lesion was located at the right adrenal gland; in 7 (22%) bilateral involvement was observed. The mean diameter of the adrenal lesions was 23.6 ± 14.3 mm, measuring 17.4 ± 5.2 mm (range: 10–24) at the right adrenal and 25.5 ± 16 mm (range: 11–58) at the left one. For comparison reasons, we also assessed the corresponding prevalence of adrenal lesions among the 36 MEN1 patients and detected lesions in 14 of them (38.9%).

Figure 1. Flow diagram reporting the initial number of evaluated individuals, the number of those excluded and the number of those eligible for the study. The results of adrenal evaluation are also summarized.

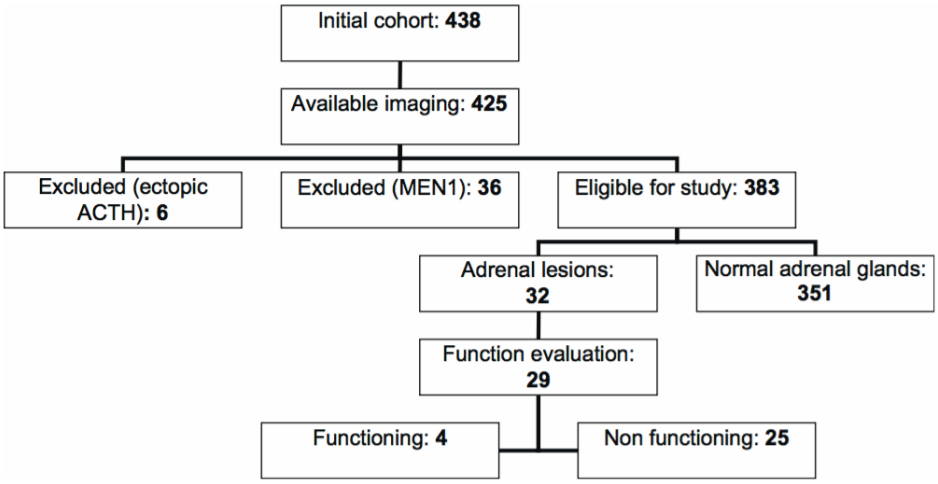


Table 1. Clinico-pathological characteristics of 383 patients with GEP-NETs evaluated for the presence of adrenal lesions. Group analysis has been conducted between patients with and without adrenal lesions.

	ALL PATIENTS (n=383)		ADRENAL LESION (n=32)		NO ADRENAL LESION (n=351)		*P*
Sex	n	%	n	%	n	%	
Male /	205	53.5	15	46.9	190	54.1	0.463
Female	178	46.5	17	53.1	161	45.9	
Age at diagnosis (yrs± SD)	57.0 ± 12.4		58.0 ± 9.0		56.9 ± 12.6		0.964
Localization of primary tumor							
Pancreas	127	33.2%	11	34.4%	116	33.0%	0.847
Small Intestine	95	24.8%	12	37.5%	83	23.6%	0.090
Colon	29	7,6%	0	0%	29	8,3%	0.150
Gastric	25	6.5%	3	9.4%	22	6.3%	0.453
Appendix	20	5.2%	1	3.1%	19	5.4%	1.000
Duodenum	13	3.4%	0	0%	13	3.7%	0.613
Rectum	12	3.1%	0	0%	12	3.4%	0.610
Unknown	62	16.2%	5	15.6%	57	16.2%	1.000
Stage							
Locoregional (Stage I-IIIB)	88	23.2%	11	34,4%	77	22,2%	0,180
Distant (Stage IV)	291	76.8%	21	65.6%	270	77.8%	
Grade							
1 - 2	254	96.6%	21	87.5%	233	97.2%	0.039
3	9	3.4%	3	12.5%	6	2.5%	

Endocrine evaluation was available for 29 patients harboring adrenal lesions and revealed an abnormal response to 1 mg DST, confirmed by a 2-day DST in 4 cases (14%). None of these patients presented with the classical clinical signs of Cushing's syndrome or elevated 24-h urinary cortisol excretion, fitting the diagnosis of subclinical glucocorticoid hypersecretion (SGH; Table 2). In one patient with SGH, the adrenal lesion was excised at the time of diagnosis, whereas repeated hormonal evaluation of the remaining three patients confirmed the diagnosis of SGH without evidence of progression to overt Cushing's disease. In total, three patients underwent adrenalectomy and in all of these cases, the histological examination revealed a benign adrenocortical adenoma. In one patient with Stage IV/Grade 3 pancreatic NET and bilateral adrenal involvement, postmortem histological evaluation revealed bilateral metastatic disease.

The imaging characteristics of the adrenal lesions were suggestive of benign adrenocortical disease in all patients except one patient with a Grade 2 pancreatic NET, who had a left sited adrenal lesion measuring 5.8 cm with heterogeneous density on abdominal CT; this patient also exhibited increased uptake on SRS. Although the lesion was not removed since the patient had already Stage IV inoperable disease, it is highly probable that it represents metastatic adrenal disease. SRS did not reveal any uptake in the adrenal glands of the remaining 31 patients. During subsequent follow-up imaging, none of the adrenal lesions presented growth greater than 20% of its maximal dimension to be considered as significant according to RECIST criteria.

Further on, the patients were divided in two groups: patients who had normal abdominal imaging and those with adrenal lesions. Comparisons were performed between these two groups regarding the patient's sex, age at diagnosis, localization of the primary GEP-NET and the presence of distant metastases. No significant difference was observed in any of these parameters, whereas patients with G3 tumors presented adrenal lesions more frequently than those with well-differentiated NETs (33% vs. 8%, P=0.04).

Table 2. Characteristics of the 32 patients with adrenal lesions.

ID	Localization of primary tumor	Stage	Grade	SRS[a] primary	SRS[b] adrenal	Side	R adrenal imaging findings	R size (mm)	L adrenal imaging findings	L size (mm)	Function	PA
15	PANCREAS	T3N1M1	1	positive	negative	L	normal		diffusely enlarged		NF	no PA
23	SMALL INTESTINE	T2N1M1	1	positive	negative	L	normal		nodular enlargement		NF	no PA
28	PANCREAS	T4N1M1	3	NA	NA	BAI	diffusely enlarged		diffusely enlarged		NF	metastases
32	UNKNOWN	T1N1M1	2	positive	negative	BAI	diffusely enlarged		diffusely enlarged		NF	no PA
35	PANCREAS	T3N1M0	1	positive	negative	L	normal		lesion <10mm		NF	adenoma
62	SMALL INTESTINE	T2N1M0	1	positive	negative	BAI	lesion <10mm		adenoma	11	NF	no PA
64	GASTRIC CARCINOID	T1M1N1	NA	positive	negative	BAI	diffusely enlarged		adenoma	11	NF	no PA
74	SMALL INTESTINE	TxN0M1	NA	positive	negative	BAI	adenoma	16	adenoma	17	NF	no PA
79	SMALL INTESTINE	T2N1M1	1	positive	negative	L	normal		diffusely enlarged		NF	no PA
109	SMALL INTESTINE	T2N1M0	1	positive	negative	L	normal		adenoma	14	NF	no PA
117	PANCREAS	T3N1M1	3	positive	negative	L	normal		adenoma	44	NF	no PA
137	SMALL INTESTINE	T2N1M1	1	positive	negative	R	adenoma	17	normal		NF	no PA
147	SMALL INTESTINE	T3N1M1	NA	positive	negative	L	normal		diffusely enlarged		NF	no PA
166	SMALL INTESTINE	T2N1M0	NA	negative	negative	L	normal		adenoma	24	NF	no PA
186	PANCREAS	T4N1M1	1	positive	negative	L	normal		adenoma	49	NF	no PA
200	UNKNOWN	TxN1M1	NA	positive	negative	BAI	nodular enlargement		nodular enlargement		NF	no PA
201	PANCREAS	T3N1M1	2	positive	positive	L	normal		suspicious	58	NF	no PA
205	UNKNOWN	TxN1M1	NA	positive	negative	L	normal		diffusely enlarged		NF	no PA
233	PANCREAS	T3N1M1	3	positive	negative	L	normal		adenoma	12	NF	no PA
245	UNKNOWN	TXN1M1	1	positive	negative	L	normal		nodular enlargement		NF	no PA
255	SMALL INTESTINE	T2N1M1	1	positive	negative	L	normal		diffusely enlarged		NF	no PA
264	PANCREAS	T2N0M1	NA	positive	negative	BAI	adenoma	24	adenoma	27	NF	no PA

Table 2. Characteristics of the 32 patients with adrenal lesions. (continued).

ID	Localization of primary tumor	Stage	Grade	SRS[a] primary	SRS[b] adrenal	Side	R adrenal imaging findings	R size (mm)	L adrenal imaging findings	L size (mm)	Function	PA
266	PANCREAS	T2N1M1	2	positive	negative	L	normal		lesion <10mm		SCS	no PA
281	SMALL INTESTINE	T2N1M1	1	positive	negative	L	normal		adenoma	20	NF	no PA
G024	SMALL INTESTINE	T3N1M0	1	NA*	negative	L	normal		nodular enlargement		NF	no PA
G025	APPENDIX	TxN0M0	1	NA*	NA	L	normal		adenoma	11	NA	no PA
G044	GASTRIC	T1N0M0	1	NA*	negative	L	normal		adenoma	50	SCS	adenoma
G046	GASTRIC	T1N0M0	NA	NA*	negative	R	adenoma	10	normal		NA	no PA
G067	PANCREAS	T3N1M0	2	positive	negative	L	normal		adenoma	20	SCS	no PA
G076	UNKNOWN	TxN1M0	2	positive	negative	L	normal		adenoma	25	SCS	no PA
G089	PANCREAS	T3N1M0	2	positive	negative	R	adenoma	20	normal		NA	adenoma
G092	SMALL INTESTINE	T3N1M1	1	NA*	negative	L	normal		adenoma	15	NF	no PA

SRS: somatostatin receptor scintigraphy, R: right, L.: left, NA: non available, BAI: bilateral adrenal involvement, NF: non-functioning, SGH: subclinical glucocorticoid hypersecretion, PA: pathology assessment

** SRS performed postoperatively, [a] SRS uptake of the primary NET, [b] SRS uptake of the adrenal glands*

DISCUSSION

In this retrospective study, we found that the prevalence of adrenal lesions discovered during imaging in patients with sporadic GEP-NETs was 8.4%. This figure is higher than that reported in recent studies regarding the general population (0.98 –4.0%)[1-3] (Table 3), but significantly lower than the prevalence observed in patients with other malignancies which is 27%[23]. In such patients, the possibility that adrenal lesions are metastatic is almost 50%[24]. On the contrary, among the 32 patients of the present study with adrenal lesions, only 2 (6.25%) were considered to have metastatic disease. This is in accordance with epidemiological data showing that the liver remains the main metastatic site of GEP-NETs and also highlights the indolent biological behavior and lower metastatic potential of these tumors compared to other malignancies.

Table 3. Comparison of the prevalence of adrenal incidentalomas in patients with GEP-NETs with that reported in the general population.

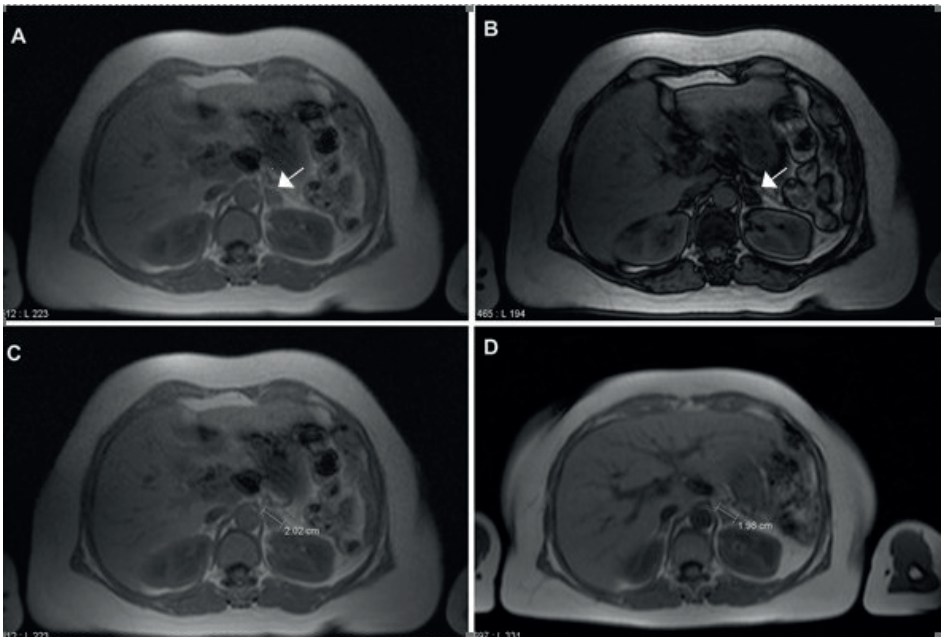
	Year	Total number of patients	Patients with AI	Percent %	$\chi 2$	*P*
Ferreira *et al.*	2005	3382	83	2.5	36.41	< 0.0001
Bovio *et al.*	2006	520	21	4.2	5.25	0.02
Davenport *et al.*	2011	2227	22	0.98	79.97	< 0.0001
Our study	2012	382	32	8.4	-	-

AI, adrenal incidentaloma

The findings of the present study also provide evidence that the distinction between benign and malignant adrenal masses in GEP-NETs can be made using their imaging features. The vast majority of patients demonstrated imaging and scintigraphic features suggestive of benign adrenal adenomas rather than metastatic disease. Follow-up with subsequent scans did not show any significant change at the lesion's size or morphology (Figure 2). As all these findings were not consistent with metastases, fine needle aspiration biopsy was not justified in any of the patients, considering the high rates of perioperative adverse events[25].

The mean size of the adrenal lesions in this study was 23.6 mm (17.4 mm at the right adrenal and 25.5 mm at the left one), somewhat smaller compared to that reported in other studies (30–35 mm)[5, 26]. This finding is in favor of the benign nature of the lesions, because size is related to the probability of malignancy. Hence, in adrenal lesions smaller than 30 mm, the benign-to-malignant ratio can be estimated more than 5:1[27]. Biochemical evaluation of the adrenal tumors revealed that four patients (14%) had SGH, a prevalence that is within the range observed in incidentalomas in the general population (1–29%, average 9%)[27, 28], although more recent reviews estimate this prevalence to 6.4%[8].

Figure 2. Abdominal MRI of case G067 (Table 2).



a: in-phase T1-weighted image depicting a low-signal lesion measuring 20 mm at the right adrenal gland (white arrow), consistent with a cortical adenoma.
b: the same lesion shows signal attenuation in out-of-phase T1-weighted image.
The dimensions of the lesion remain relatively stable as is evident in MRI imaging between panel c: (20.2 mm) and panel d: (19.8 mm) performed 1 year later.

The presence of adrenal lesions in patients with GEP-NETs was not related to distant metastases, further supporting the suggestion that they most probably represent benign adrenal adenomas rather than metastatic disease. Patients with poorly differentiated tumors harbored adrenal lesions more frequent than those with well-differentiated GEP-NETs and the only patient proven histologically to have adrenal metastases had a poorly differentiated (G3) pancreatic NET. These data suggest that aggressive biological behavior of the GEP-NET may predict the possibility that a concurrent adrenal lesion is metastatic.

The higher frequency of adrenal lesions found in patients with GEP-NETs compared to that in general population needs to be further investigated. In such patients, a higher prevalence of synchronous other types of neoplasias has been reported[29]. This co-existence of neoplasias could possibly be explained by inherited defects regarding tumor suppressor genes or oncogenes. Such association has already been described between familial adenomatous polyposis and adrenal neoplasia, resulting from a mutation in the tumor suppressor gene APC[30]. Another possible explanation is that growth and

mitogenic factors secreted by these multipotent tumors could exert tumorigenic effects in other tissues. Potential candidates could be the insulin-like growth factor 1 (IGF1), as its presence has been demonstrated in GEP-NETs at mRNA and protein level[31, 32]. Similar results have been reported regarding the actions of serotonin in carcinoid tumors either directly via its receptors or mediated by connective tissue growth factor (CTGF)[33, 34]. This constitutional tumorigenic predisposition might elucidate the higher frequency of bilateral adrenal lesions observed in this study (22%) compared to bilateral incidentalomas in the general population (10–15%)[5].

According to a recent retrospective analysis in patients with GEP-NETs, the prevalence of metastatic disease (either locoregional or distant) was 86% and the most frequent site of distant metastases was the liver (85%). These figures are similar to the findings of the present study[15]. Involvement of the liver seems to be an independent dismal prognostic factor. Such data do not exist for adrenal involvement. To our knowledge, there are only isolated case reports that describe the presence of adrenal metastases in patients with poorly differentiated NETs[35].

In conclusion, the prevalence of adrenal lesions in GEP-NETs was found to be higher than in the general population, but significantly lower than that encountered in patients with other malignancies and mostly represent benign adrenal adenomas. Our findings therefore suggest that the presence of such lesions in patients with GEP-NETs, even in patients with Stage IV disease, may represent adrenal adenomas rather than metastatic disease (except patients with poorly differentiated GEP-NETs). Nevertheless, individualized assessment of imaging characteristics should still be considered.

REFERENCES

1. **Bovio S, Cataldi A, Reimondo G, Sperone P, Novello S, Berruti A, Borasio P, Fava C, Dogliotti L, Scagliotti GV, Angeli A, Terzolo M.** Prevalence of adrenal incidentaloma in a contemporary computerized tomography series. *Journal of endocrinological investigation.* 2006;29:298-302.
2. **Davenport C, Liew A, Doherty B, Win HH, Misran H, Hanna S, Kealy D, Al-Nooh F, Agha A, Thompson CJ, Lee M, Smith D.** The prevalence of adrenal incidentaloma in routine clinical practice. *Endocrine.* 2011;40:80-83.
3. **Ferreira EV, Czepielewski MA, Faccin CS, Accordi MC, Furtado AP.** [Prevalence of adrenal incidentaloma at computed tomography (chest and abdominal) in a general hospital in Brazil]. *Arquivos brasileiros de endocrinologia e metabologia.* 2005;49:769-775.
4. **Young WF, Jr.** Clinical practice. The incidentally discovered adrenal mass. *The New England journal of medicine.* 2007;356:601-610.
5. **Barzon L, Sonino N, Fallo F, Palu G, Boscaro M.** Prevalence and natural history of adrenal incidentalomas. *European journal of endocrinology.* 2003;149:273-285.
6. **Kloos RT, Gross MD, Francis IR, Korobkin M, Shapiro B.** Incidentally discovered adrenal masses. *Endocrine reviews.* 1995;16:460-484.
7. **Mansmann G, Lau J, Balk E, Rothberg M, Miyachi Y, Bornstein SR.** The clinically inapparent adrenal mass: update in diagnosis and management. *Endocrine reviews.* 2004;25:309-340.
8. **Cawood TJ, Hunt PJ, O'Shea D, Cole D, Soule S.** Recommended evaluation of adrenal incidentalomas is costly, has high false-positive rates and confers a risk of fatal cancer that is similar to the risk of the adrenal lesion becoming malignant; time for a rethink? *European journal of endocrinology.* 2009;161:513-527.
9. **Abrams HL, Spiro R, Goldstein N.** Metastases in carcinoma; analysis of 1000 autopsied cases. *Cancer.* 1950;3:74-85.
10. **Bullock WK, Hirst AE, Jr.** Metastatic carcinoma of the adrenal. *The American journal of the medical sciences.* 1953;226:521-524.
11. **Lam KY, Lo CY.** Metastatic tumours of the adrenal glands: a 30-year experience in a teaching hospital. *Clinical endocrinology.* 2002;56:95-101.
12. **Lumb G, Mackenzie DH.** The incidence of metastases in adrenal glands and ovaries removed for carcinoma of the breast. *Cancer.* 1959;12:521-526.
13. **Kaltsas GA, Besser GM, Grossman AB.** The diagnosis and medical management of advanced neuroendocrine tumors. *Endocrine reviews.* 2004;25:458-511.
14. **Modlin IM, Oberg K, Chung DC, Jensen RT, de Herder WW, Thakker RV, Caplin M, Delle Fave G, Kaltsas GA, Krenning EP, Moss SF, Nilsson O, Rindi G, Salazar R, Ruszniewski P, Sundin A.** Gastroenteropancreatic neuroendocrine tumours. *The Lancet. Oncology.* 2008;9:61-72.
15. **Pape UF, Berndt U, Muller-Nordhorn J, Bohmig M, Roll S, Koch M, Willich SN, Wiedenmann B.** Prognostic factors of long-term outcome in gastroenteropancreatic neuroendocrine tumours. *Endocrine-related cancer.* 2008;15:1083-1097.

16. **Skogseid B, Rastad J, Gobl A, Larsson C, Backlin K, Juhlin C, Akerstrom G, Oberg K.** Adrenal lesion in multiple endocrine neoplasia type 1. *Surgery.* 1995;118:1077-1082.

17. **Klimstra DS, Modlin IR, Coppola D, Lloyd RV, Suster S.** The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. *Pancreas.* 2010;39:707-712.

18. **Rindi G, de Herder WW, O'Toole D, Wiedenmann B.** Consensus guidelines for the management of patients with digestive neuroendocrine tumors: why such guidelines and how we went about It. *Neuroendocrinology.* 2006;84:155-157.

19. **Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J.** New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European journal of cancer.* 2009;45:228-247.

20. **Dunnick NR, Korobkin M.** Imaging of adrenal incidentalomas: current status. *AJR. American journal of roentgenology.* 2002;179:559-568.

21. **Sahdev A, Reznek RH.** The indeterminate adrenal mass in patients with cancer. *Cancer imaging.* 2007;7 Spec No A:S100-109.

22. NIH state-of-the-science statement on management of the clinically inapparent adrenal mass ("incidentaloma"). *NIH consensus and state-of-the-science statements.* 2002;19:1-25.

23. **Mitchell IC, Nwariaku FE.** Adrenal masses in the cancer patient: surveillance or excision. *The oncologist.* 2007;12:168-174.

24. **Lenert JT, Barnett CC, Jr., Kudelka AP, Sellin RV, Gagel RF, Prieto VG, Skibber JM, Ross MI, Pisters PW, Curley SA, Evans DB, Lee JE.** Evaluation and surgical resection of adrenal masses in patients with a history of extra-adrenal malignancy. *Surgery.* 2001;130:1060-1067.

25. **Mody MK, Kazerooni EA, Korobkin M.** Percutaneous CT-guided biopsy of adrenal masses: immediate and delayed complications. *Journal of computer assisted tomography.* 1995;19:434-439.

26. **Herrera MF, Grant CS, van Heerden JA, Sheedy PF, Ilstrup DM.** Incidentally discovered adrenal tumors: an institutional perspective. *Surgery.* 1991;110:1014-1021.

27. **Mantero F, Terzolo M, Arnaldi G, Osella G, Masini AM, Ali A, Giovagnetti M, Opocher G, Angeli A.** A survey on adrenal incidentaloma in Italy. Study Group on Adrenal Tumors of the Italian Society of Endocrinology. *The Journal of clinical endocrinology and metabolism.* 2000;85:637-644.

28. **Flecchia D, Mazza E, Carlini M, Blatto A, Olivieri F, Serra G, Camanni F, Messina M.** Reduced serum levels of dehydroepiandrosterone sulphate in adrenal incidentalomas: a marker of adrenocortical tumour. *Clinical endocrinology.* 1995;42:129-134.

29. **Kamp K, Damhuis RA, Feelders RA, de Herder WW.** Occurrence of second primary malignancies in patients with neuroendocrine tumors of the digestive tract and pancreas. *Endocrine-related cancer.* 2012;19:95-99.

30. **Smith TG, Clark SK, Katz DE, Reznek RH, Phillips RK.** Adrenal masses are associated with familial adenomatous polyposis. *Diseases of the colon and rectum.* 2000;43:1739-1742.

31. **Nilsson O, Wangberg B, Theodorsson E, Skottner A, Ahlman H.** Presence of IGF-I in human midgut carcinoid tumours--an autocrine regulator of carcinoid tumour growth? *International journal of cancer. Journal international du cancer.* 1992;51:195-203.

32. **Wulbrand U, Remmert G, Zofel P, Wied M, Arnold R, Fehmann HC.** mRNA expression patterns of insulin-like growth factor system components in human neuroendocrine tumours. *European journal of clinical investigation.* 2000;30:729-739.

33. **Kaltsas GA, Cunningham JL, Falkmer SE, Grimelius L, Tsolakis AV.** Expression of connective tissue growth factor and IGF1 in normal and neoplastic gastrointestinal neuroendocrine cells and their clinico-pathological significance. *Endocrine-related cancer.* 2011;18:61-71.

34. **Lesurtel M, Soll C, Graf R, Clavien PA.** Role of serotonin in the hepato-gastroIntestinal tract: an old molecule for new perspectives. *Cellular and molecular life sciences : CMLS.* 2008;65:940-952.

35. **Ochiai T, Komiyama S, Ikoma H, Kubota T, Nakanishi M, Ichikawa D, Kikuchi S, Fujiwara H, Sakakura C, Kokuba Y, Sonoyama T, Otsuji E.** A case report of metastatic neuroendocrine carcinoma of the right adrenal gland successfully treated with chemotherapy and surgery. *International journal of clinical oncology.* 2010;15:423-427.

Chapter 6

Parathyroid hormone-related peptide (PTHrP) secretion by gastroenteropancreatic neuroendocrine tumors (GEP-NETs): clinical features, diagnosis, management and follow-up

Kimberly Kamp[1], Richard A. Feelders[1], Roxanne C. S. van Adrichem[1], Yolanda B. de Rijke[2], Francien H. van Nederveen[3], Dik J. Kwekkeboom[4], Wouter W. de Herder[1]

[1]Department of Internal Medicine, Sector of Endocrinology, [2]Department of Clinical Chemistry, [3]Department of Pathology and [4]Department of Nuclear Medicine, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands

***The Journal of Clinical Endocrinology and Metabolism* (2014) 99, 3060-3069.**

ABSTRACT

Context: Only a small number of case reports has been published on patients with PTHrP-hypersecreting metastatic gastroenteropancreatic (GEP) neuroendocrine tumors (NETs).

Objective: The objective of this study was to evaluate the clinical, biochemical, and radiological features, management, and treatment outcome of patients with PTHrP-hypersecreting GEP-NETs.

Design: Retrospective case series.

Setting: Tertiary referral hospital.

Main Outcome Measures: Clinical, biochemical, and radiological features were measured, as well as response to therapy and survival.

Patients: Ten patients with PTHrP-secreting GEP-NETs (nine pancreatic and one unknown primary) with a median age of 50.4 years (range, 38.3– 61.1) were studied. Multiple endocrine neoplasia type 1 patients were excluded.

Results: The median follow-up was 57.2 months (range, 11.6 –204.5 mo). Median overall survival was 86.0 months. In total, 51 different treatment interventions and combinations were applied. In seven of the 10 patients, somatostatin analog (SSA) treatment resulted in a temporary normalization of serum calcium levels with a long-term response observed in two patients (up to 35.2 mo). Peptide receptor radiotherapy (PRRT) with radiolabeled SSAs induced long-term responses ranging from 9.0–49.0 months in four of six patients treated with PRRT.

Conclusions: Hypersecretion of PTHrP by metastatic GEP-NETs is very rare and seems to be exclusively associated with metastatic pancreatic NETs. PTHrP production has major clinical impact because poorly controllable hypercalcemia is associated with increased morbidity and mortality. The most successful treatment options for PTHrP-producing GEP-NETs are SSAs and PRRT using radiolabeled SSAs. Isotonic saline and bisphosphonates can be considered as supportive therapies.

INTRODUCTION

Hypercalcemia is a well-known paraneoplastic manifestation in patients with metastatic malignancies[1]. Two types of hypercalcemia can be distinguished in patients with metastatic gastroenteropancreatic (GEP) and thoracic neuroendocrine tumors (NETs): local osteolytic hypercalcemia, and humoral hypercalcemia of malignancy (HHM)[2-5].

The first is the result of increased bone resorption by osteoclasts mediated by (metastatic) tumor cells, which are in direct contact with bone. The second is associated with the hypersecretion of PTH or PTHrP into the circulation by tumor cells[2-5]. Increased extrarenal conversion of 25-hydroxyvitamin D_3 (calcifediol, calcidiol, 25-hydroxycholecalciferol [25(OH)D]) to 1,25 dihydroxyvitamin D_3 (calcitriol, 1,25-dihydroxycholecalciferol [$1,25(OH)_2D$]), as a result of increased activity of the enzyme 25(OH)D-1α-hydroxylase, generally does not occur in GEP-NETs[2].

Bone resorption by osteoclasts may be stimulated by PTH, PTHrP, and $1,25(OH)_2D$ and can subsequently cause hypercalcemia. A number of cytokines (such as IL-1α, IL-1β, IL-6, TNF, lymphotoxin, and TGF-α) also stimulate octeoclastic bone resorption either alone or in combination with PTHrP. Some of these cytokines have been linked to HHM. Besides stimulating osteoclast-mediated bone resorption, both PTH and PTHrP increase the reabsorption of calcium from the distal tubule, thus interfering with the ability of the kidneys to clear the filtered calcium load. Furthermore, PTH and PTHrP also increase $1,25(OH)_2D$ synthesis, which further contributes to a hypercalcemic state[2].

PTH and PTHrP show amino acid sequence homology at the amino terminus, where eight of the first 13 amino acids are identical. The consequent activation of the shared PTH/PTHrP receptor explains the ability of PTHrP to resemble PTH as an inducer of bone resorption, renal phosphate wasting, and hypercalcemia[6, 7].

There is reasonable doubt about whether PTHrP has an important role in the daily maintenance of calcium homeostasis. Under physiological conditions, circulating levels of PTHrP are considerably lower than PTH levels. Nevertheless, PTHrP is essential for the development of adult tissues and has a number of physiological functions. PTHrP is widely expressed in mesenchymal tissues—including cartilage, many epithelial tissues, skeletal and heart muscle, distal renal tubules, hair follicles, brain, and placenta[6, 7].

PTHrP hypersecretion may be associated with highly malignant tumors (such as squamous cell carcinomas, breast carcinomas, renal cortical carcinomas, and the adult T-cell leukemia syndrome) but also with a variety of less aggressive NETs[6-8].

In patients with primary pancreatic, lung, or thymus NETs presenting with hypercalcemia in combination with elevated circulating PTH levels, primary hyperparathyroidism as part of multiple endocrine neoplasia type 1 (MEN1) syndrome should be considered[9].

Only a few case reports of non-MEN1 patients with metastatic GEP-NETs presenting with hypercalcemia as a result of PTHrP hypersecretion have been described in the

literature. These show that investigational protocols, treatment management, and management of unusual complications vary considerably between patients and institutions[9-15].

We have, therefore, analyzed in a tertiary referral center the clinical, biochemical, and radiological features in all metastatic GEP-NET patients with PTHrP hypersecretion who presented with symptoms and signs of HHM.

PATIENTS AND METHODS

Patients

We studied the medical records of 10 patients with PTHrP-hypersecreting GEP-NETs who were treated between 1986 and 2013 in the Erasmus University Medical Center (Erasmus MC), Rotterdam, the Netherlands. Patients diagnosed with the MEN1 syndrome were excluded.

All GEP-NET patients treated in the Erasmus MC, Rotterdam (as described in the present manuscript), gave written informed consent before inclusion in the studies, which were approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam.

Analysis of clinical and pathological data, laboratory parameters, and imaging findings was performed, and information on surgical and nonsurgical treatments was collected.

During this study, the PTH assay had changed over time (see Assays below). Also, new treatment options were introduced, such as the clinical availability of different radiolabeled somatostatin analogs (SSAs) and the introduction of denosumab. The development and clinical introduction of multireceptor subtypespecific SSAs, like pasireotide, has potentially improved efficacy of SSA treatment in GEP-NET patients[16-18].

Diagnosis of GEP-NET

Diagnosis of GEP-NET was made on the basis of serological markers (chromogranin A [CgA]; neuron-specific enolase [NSE]), pathological elevations of circulating, hypersecreted neuroendocrine hormones or peptides[19], and imaging according to international protocols and standards[20, 21] in combination with histological confirmation according to current guidelines[22, 23].

Diagnosis of PTHrP hypersecretion

The diagnosis of PTHrP-hypersecreting NETs was based on persistent hypercalcemia and (almost) completely suppressed plasma PTH levels in combination with elevated plasma PTHrP levels. Patients with low but detectable PTH levels, absent data on PTHrP levels, and bone metastases were excluded from the analysis. Patients taking pharmacological doses of 25(OH)D or $1,25(OH)_2D$ at baseline were excluded as well.

Assays

PTH and PTHrP were measured in all patients. Assays were performed in the endocrine laboratory of the Erasmus MC, Rotterdam, the Netherlands. PTHrP was measured in EDTA-plasma containing aprotinin using the PTHrP IRMA Kit (Mitsubishi Kagaku Iatron, Inc). After centrifugation, plasma was stored at - 80°C until assayed. The Immulite 2000XPi (Siemens Diagnostics) was used until 2012, and afterward the Vitros ECIQ (Ortho Clinical Diagnostics) was used for measurements of plasma PTH levels. CgA and NSE assays were performed in the Department of Clinical Chemistry of the Erasmus MC. CgA in serum was measured using a solid-phase, two-site IRMA assay (Cisbio Bioassays). NSE in serum was measured using an electrochemiluminescence immunoassay on an immunoassay analyzer (Roche Diagnostics).

Disease progression and response to therapy

Disease progression and response to therapy of hypercalcemia were assessed using three parameters: 1) radiological documentation of progressive disease according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0[24]; 2) progression of clinical symptoms (such as weight loss and symptoms related to hypercalcemia); and 3) worsening of relevant biochemical markers (for example, uncontrolled hypercalcemia, not responsive to treatment).

Statistical analysis

Overall survival was analyzed using Kaplan-Meier methods. Log-rank testing was used to determine whether there was a statistically significant difference between the mortality in the normocalcemic pancreatic NET patient group and the group of hypercalcemic GEP-NET patients with PTHrP hypersecretion. Calculations were performed using Statistical Package for Social Sciences software, version 21.0 (SPSS Inc).

RESULTS

Between 1986 and 2013, after exclusion of MEN1 patients, 895 new patients with GEP-NETs were seen in our center. This series included 295 patients with pancreatic NETs. Eighteen patients presented with, or developed, hypercalcemia.

Baseline data and presenting symptoms

Between 1986 and 2013, a total of 10 patients (six men and four women) were diagnosed with a PTHrP-hypersecreting GEP-NETs. The demographic and biochemical characteristics of these 10 patients are listed in Table 1. Patients had a median age of 50.4 years

(range, 38.3– 61.1 years) at diagnosis of the GEP-NETs. The median follow-up was 57.2 months (range, 11.6–204.5 mo).

Almost all patients (90%) had primary pancreatic PTHrP-hypersecreting NETs, and only one patient was diagnosed with a metastatic GEP-NET of unknown primary. All patients had developed ENETS/NANETS Stage IV disease (Table 1)[25, 26]. Main tumor metastatic localizations were liver (100%), lymph nodes (40%), and bone (10%). Tumor Grading was available in six of 10 GEP-NETs (60%): four GEP-NETs (40%) were classified as well-differentiated (Grade 1), one GEP-NET (10%) was classified as moderately differentiated (Grade 2), and one GEP neuroendocrine carcinoma (NEC) (10%) was classified as poorly differentiated (Grade 3).

All patients had OctreoScan-positive lesions. Currently, three of the 10 patients are still alive and in active follow-up. The primary causes of death included disease progression with uncontrollable hypercalcemia (n=4), cause of death unknown/referred to another hospital (n=2), and septicemia probably caused by cholangitis (n=1).

Interventions

Information regarding therapeutic interventions for PTHrP hypersecretion, treatment response, and duration is listed in Table 2.

A total of 51 different therapeutic regimens, including combinations, were administered. Twenty-seven therapeutic interventions were able to normalize serum calcium levels at some stage. A decrease of serum calcium levels could be achieved with 16 therapeutic interventions, and with eight therapeutic interventions no serum calcium response was observed.

Short-term responses were obtained with iv isotonic saline (0.1–0.2 mo), bisphosphonates (0.2–2.3 mo), and embolization of liver metastases (0.1–1.1 mo).

Peptide receptor radiotherapy (PRRT) with radiolabeled SSAs had favorable effects on the hypercalcemia and plasma PTHrP levels with long-term responses ranging from 9.0 to 49.0 months in four of six patients. However, two of the six patients had worsening of hypercalcemia while still undergoing PRRT or directly after finalization of the PRRT.

In seven of 10 patients, SSA treatment resulted in a temporary normalization of serum calcium levels (<7.7 mo). Two patients obtained a long-term response (up to 49 mo) with a combination of PRRT and SSA.

In the five patients who underwent PRRT with ^{177}Lu-octreotate, tumor stabilization (stable disease [SD]) ranged from 10 to 51 months. This SD was paralleled by normalization of serum calcium levels in three of five patients. In one patient who underwent PRRT with ^{177}Lu-octreotate (Table 1, patient 5), SD was 48 months, whereas the biochemical response lasted 15 months. In one patient (Table 1, patient 4), PRRT with ^{111}In-pentetreotide or ^{90}Yt-octreotide resulted in SD of 7 and 8 months, respectively, but this was not paralleled by a biochemical response. In the only patient treated with sunitinib

Table 1. Patient demographics and biochemical results in 10 patients with PTHrP-hypersecreting NETs.

Case	Age (yr)	Sex	Primary	Metas-tases	Tumor Grade	ENETS Stage	Presen-tation	Symptoms	Calcium* (mmol/l)	PTHrP (pmol/l)	25(OH)D (nmol/l)	$1,25(OH)_2D$ (pmol/l)	CgA (ug/l)	NSE (ug/l)
Reference range									*2.20-2.65*	*0.0-1.5*	*50-120*	*38-183*	*27-94*	*0.0-16.2*
1	41.1	M	Pancreas	L	ID	IV	Syn	A, N	2.99	2.6 – 6.0	118	87	ID	6.5
2	58.3	M	Pancreas	L, LN	1	IV	Meta	A, N, V, PA, O, F, D	3.64	2.9 – 5.2	77	104	19445	222.2
3	40.0	F	Pancreas(VIP)	L, LN	ID	IV	Syn	A, N, V, F, P	3.33	2.3	40	133.9	63	20.5
4	52.9	M	Pancreas	L, B	3	IV	Syn	PD, PU, F, C	3.35	2.0 – 2.6	52	263.9	8486	15.2
5	61.1	M	Pancreas	L	1-2	IV	Syn	A, PU, F	2.89	5.4 – 16.3	60	150.7	377	12.1
6	60.8	M	Unknown	L	2	IV	Syn	A, N, V, PA, PD, PU, F, C	3.42	2.5	29	> 220	ID	14.3
7	38.3	M	Pancreas	L	1	IV	Syn	A, N, V, PA	2.67	2.5	ID	ID	96	28.2
8	42.2	F	Pancreas	L, LN	ID	IV	Syn	A, PD, PU, F, MW, C	3.53	3.1	95	275.2	259	15.8
9	51.3	F	Pancreas	L	ID	IV	Syn	A, V, N	2.93	3.4	65	268.7	53	9.4
10	49.5	F	Pancreas	L, LN	1	IV	Meta	A, N, PA, PD, PU, F	3.57	1.8	24	299.5	49	7.3

Abbreviations: L = liver; B = bone; LN = lymph nodes; Meta = Metachronous ; Syn = Synchronous; PTHrP = parathyroid hormone-related peptide; 25(OH)D = 25 hydroxyvitamin D_3; $1,25(OH)_2D$ = 1,25-dihydroxyvitamin D_3; CgA = chromogranin A; NSE = neuron-specific enolase; ID = insufficient data.

A = anorexia; N= nausea; V = vomiting; PA = poor appetite; O = obstipation; PU = polyuria; PD = polydipsia; F = fatigue; MW = muscle weakness; C = cerebral symptoms; P = palpitations.

No patients showed symptoms or signs of dehydration, ileus, bone pain and hypertension.

** = Serum calcium values were corrected for albumin.*

Table 2. Therapeutic interventions (n=51) after diagnosis of a PTHrP-hypersecreting NET, including response and duration of response (n=10).

Therapeutic interventions (TI) after diagnosis of PTHrP-hypersecretion	N	N	Calcium Response (N)			Response Duration (Months)	
	pts	TI	Normalization	Decrease	None	Normalization	Decrease
Total patients	10	51	27	16	8	0.1-49.0	0.03-1.5
NaCl 0.9%							
24-h	3	3		2	1		0.1-0.2
12-h (intervals)	1	1			1		
Somatostatin analog (SSA)							
Octreotide LAR 20mg/4wk *	1	1	1			35.2	
Octreotide IR	6	7	6	1		1.0-7.7	0.5
Bisphosphonates (B)							
Single short iv infusion	2	4	1	2	1	2.3	0.2-0.4
Repetitive iv infusions	1	1	1			1.6	
Surgery (cytoreduction)							
Primary + Metastases	1	1	1			28.2	
Embolization Liver Metastases	1	3	3			0.1-1.1	
Ethanol injections Liver Metastases	1	2	1		1	0.3	
PRRT							
^{177}Lu-Octreotate (4 cycl.)	2	2			2		
^{177}Lu-Octreotate + Capecitabine (4 cycl.) **	1	1	1			9.0	
^{90}Yt-Octreotide (5 cycl.)	1	1			1		
^{111}In-Pentetreotide (4 cycl.)	1	1	1			0.5	
Glucocorticoids (G)	1	1		1			0.3
Sunitinib (S)	1	1	1			5.2	
Denosumab (D)	1	1		1			0.1
Combination Therapies							
NaCl 0.9% + SSA IR	1	1		1			0.2
NaCl 0.9% + B iv	4	8	2	5	1	0.8-2.7	0.03-1.3
NaCl 0.9% + SSA IR + B iv	2	2	1	1		0.5	0.1
NaCl 0.9% + B oral + B iv repetitive	1	1	1			10.0	
SSA IR + B iv repetitive	1	1	1			1.2	
SSA LAR + B iv repetitive	1	1		1			1.5
SSA LAR + B iv + G	1	1	1				0.5
S + NaCl 0.9%	1	1	1			3.3	
D + NaCl 0.9% + SSA IR	1	1		1			1.5
PRRT ^{177}Lu-Octreotate (4 cycl.) + SSA IR	1	1	1			14.5	
PRRT ^{177}Lu-Octreotate (6 cycl.) + SSA LAR	2	2	2			33.3-49.0	

Abbreviations: TI: Therapeutic intervention; B = bisphosphonate; G = glucocorticoid; S = sunitinib; D = denosumab; IR: immediate release; PRRT: peptide receptor radiotherapy

** 1 patient switched from Octreotide LAR 20 mg/4wk to Octreotide LAR 30 mg/2wk*

*** Capecitabine 1650 mg / m^2*

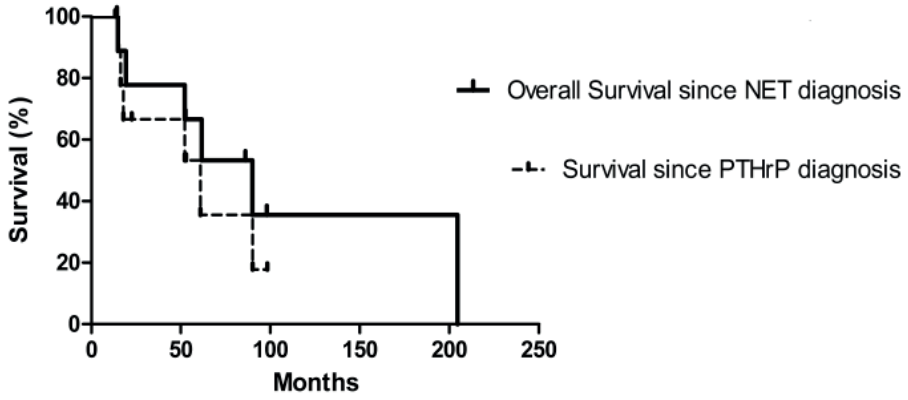
(±isotonic saline) (Table 1, patient 5), SD was 13 months, and this was paralleled by normalization of serum calcium levels for 8 months.

Prognosis

Median overall survival of our normocalcemic patients with pancreatic NETs (n=277) was 161.8 months. Median overall survival of our 10 patients since the first diagnosis of GEP-NET was 86.0 months, and median survival of our 10 patients since the diagnosis of PTHrP hypersecretion was 52.2 months (Figure 1). The overall survival in the patients with PTHrP hypersecretion was significantly shorter (P=0.002) than in the group with normocalcemia.

Seven of 10 patients (70%) finally developed fatal progressive disease according to RECIST version 1.0[24] in combination with uncontrollable elevated serum calcium levels and elevated plasma PTHrP levels.

Figure 1. Survival Kaplan-Meier curve of 10 patients with plasma PTHrP-hypersecreting NETs: overall survival and survival since the diagnosis of PTHrP-hypersecretion.



Median Overall Survival: 86.0 months
Median Survival since PTHrP diagnosis: 52.2 months

CASE REPORTS

This paper now reports in more detail two patients with pancreatic NETs with hypercalcemia due to excessive PTHrP hypersecretion. Patient 5 was diagnosed with a pancreatic NET with synchronous PTHrP secretion, and patient 10 was diagnosed with a pancreatic NET with metachronous PTHrP secretion. For both patients, more than one PTHrP measurement was available.

Patient 5 (Figure 2A)

A 61-year-old man was referred to the urologist for prostate hyperplasia (Table 1, patient 5). Subsequent imaging with computed tomography revealed pathological lesions in the pancreas and liver. The past medical history included type 2 diabetes and exocrine pancreatic insufficiency for which he received oral medication. Symptoms related to the hypercalcemia were involuntary weight loss, fatigue, and polyuria. His clinical condition was WHO-1.

An inoperable well-differentiated (Grade 1) NET in the pancreatic body with multilobar liver metastases was diagnosed. Serum calcium levels were elevated, serum phosphate levels were reduced, and serum creatinine levels were within the reference range. Plasma PTH concentrations were undetectable, whereas plasma PTHrP levels were elevated (Table 1, patient 5). Serum levels of CgA were elevated, and serum levels of NSE were within the reference range (Table 1, patient 5). ^{111}In-pentetreotide scintigraphy (OctreoScan) showed a scan-positive lesion in the pancreas and multiple liver lesions.

The patient started treatment with Octreotide IR, 50 µg three times a day (t.i.d.) sc, which was converted to octreotide long-acting repeatable (LAR) (20 mg/4 wk im). PRRT ^{177}Lu-octreotate (cumulative dose, 43.9 GBq) was given in six cycles. The liver metastases showed regression (partial response), and the pancreatic lesion remained stable. This therapeutic approach also resulted in normalization of serum calcium levels. The octreotide therapy in combination with PRRT was capable of maintaining calcium levels within the reference range for a total period of 7 years.

Seven years after the initial diagnosis of a PTHrP-secreting NET, tumor progression in combination with recurrence of hypercalcemia occurred. The patient was treated with iv bisphosphonates (zoledronic acid, 4 mg), and serum calcium levels normalized again for almost 2 months. Subsequently, treatment with sunitinib at a dose of 37.5 mg/d was started. Serum calcium levels normalized again for another 5 months. At 91 months after the initial diagnosis, the serum calcium levels increased again, iv isotonic saline was given in combination with sunitinib at a dose of 37.5 mg/d, and serum calcium levels normalized for 3 months. After 94 months, all other treatment modalities were ineffective to normalize calcium levels. Intravenous isotonic saline and denosumab (60 mg sc) were both only able to lower the serum calcium levels slightly, and no response was seen after the renewed iv administration of 4 mg zoledronic acid. Also, the combination of iv isotonic saline and denosumab and octreotide IR (500 µg t.i.d. sc) was only able to slightly reduce, but not normalize, the serum calcium levels. Meanwhile, the pancreatic NET and its metastases increased in size and number. Additionally, the patient underwent two cycles of ^{177}Lu-octreotate (data not shown); he is still alive, 100 months after the initial diagnosis, and presently has slightly increased serum calcium levels. His current clinical condition is WHO-2.

Patient 10 (Figure 2B)

A 49-year-old woman was diagnosed with a well-differentiated (Grade 1) NET in the pancreatic head (Table 1, patient 10). Her past medical history was uneventful. She underwent a pancreaticoduodenectomy with curative intent. She was disease-free for 10 years. She subsequently developed lymph node and multilobar liver metastases for which she was treated with metastasectomy and ^{177}Lu-octreotate (44.3 GBq, given in six fractions).

Fifteen years after the diagnosis of the primary pancreatic NET, routine laboratory monitoring showed highly elevated calcium levels. Three months after the first diagnosis of hypercalcemia, the patient was admitted to our hospital with involuntary weight loss as well as nausea, poor appetite, polyuria, polydipsia, and persistent fatigue. Her clinical condition at that time was WHO-2.

Serum calcium concentrations were elevated, whereas serum phosphate and creatinine levels were within the reference range (Table 1, patient 10). Plasma PTH concentrations were undetectable, whereas plasma PTHrP levels were elevated (Table 1, patient 10). Serum levels of CgA and NSE were within the reference range (Table 1, patient 10). ^{99m}Tc bone scintigraphy showed no bone metastases.

The patient was treated with Octreotide LAR (20 mg/4 wk im) in combination with a single short iv infusion of bisphosphonates and dexamethasone. This combination therapy was only able to decrease serum calcium levels for 0.5 month.

After recurrence of the hypercalcemia in combination with elevated plasma PTHrP levels, monthly infusions with bisphosphonates were started in combination with Octreotide LAR (20 mg/4 wk im). This resulted again in a decrease in serum calcium levels for almost 2 months.

Five months after the initial diagnosis of the PTHrP hypersecretion, hepatic arterial embolization was performed because of tumor progression and recurrent hypercalcemia. The hypercalcemia normalized only for a short period of time (0.1–1.1 mo) after three hepatic arterial embolizations. The last treatment option tried in this patient was nightly iv isotonic saline in combination with furosemide and 4 mg zoledronic acid iv/4 wk. Nine months later, 18 months after the initial diagnosis of the PTHrP hypersecretion, the patient died of progressive disease and uncontrollable hypercalcemia.

Figure 2. Serum calcium (corrected for albumin) and plasma PTHrP levels in the follow-up of two patients with PTHrP-hypersecreting pancreatic neuroendocrine tumors.

Figure 2A. Patient 5, pancreas NET with synchronous PTHrP secretion.

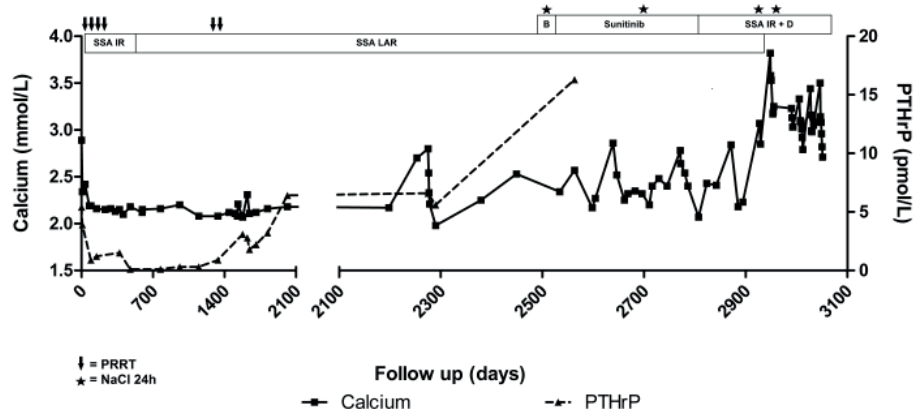
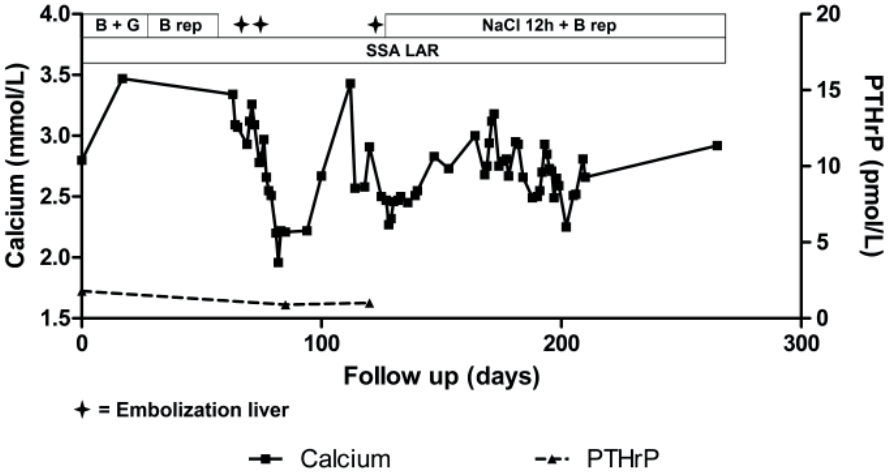


Figure 2B. Patient 10, pancreas NET with metachronous PTHrP secretion.



SSA = somatostatin analog; LAR = long-acting repeatable; B = bisphosphonate; B rep = bisphosphonate repetitive iv infusions; G = glucocorticoids; PTHrP = parathyroid hormone-related peptide; D = denosumab

DISCUSSION

This study presents the clinical, endocrine, and laboratory features of a group of 10 successive patients with PTHrP-hypersecreting GEP-NETs who were evaluated and treated in a single tertiary referral center.

GEP-NETs represent a heterogeneous group of relatively rare neoplasms with a distinct biological behavior[27]. Recent epidemiological reviews show that the age-adjusted incidence of all GEP-NETs is 3.65, and for pancreatic NETs it is 0.43[28]. Our series of 895 new patients with GEP-NETs (295 patients with pancreatic NETs) included only 18 patients with hypercalcemia (2%) and 10 patients (1.1%) with proven PTHrP production, which demonstrates that PTHrP-hypersecreting GEP-NETs are extremely rare.

Only seven studies, reporting a total of 20 patients with PTHrP-hypersecreting pancreatic NETs, have been published[9-15]. Our series is, therefore, the largest published single-center case series.

In our study, we have only included patients with low to undetectable PTH levels. Although very rare, we might, therefore, have missed patients presenting with combined ectopic PTH and PTHrP hypersecretion. Ectopic PTH secretion by NETs and NECs has been described in only a few case reports[29-31]. Combined ectopic PTH and PTHrP hypersecretion has been suggested in a patient with a non-neuroendocrine lung carcinoma[32], but a patient with a pancreatic NEC described by VanHouten et al[31] presented with both elevated PTH and elevated PTHrP levels.

Our management of patients with PTHrP-hypersecreting GEP-NETs was aimed at long-term control and achieving normalization of serum calcium, preferably also paralleled by tumor stabilization or reduction, and finally prolongation of (progression-free) survival.

Resection of the primary pancreatic NET and its metastases was only feasible in one patient; this was followed by a decrease in plasma PTHrP and resulted in normalization of serum calcium levels for more than 2 years. However, in two other patients, hepatic artery embolization and ethanol injections of liver metastases with the intent to obtain significant tumor debulking were successful for a very limited period of time (less than 2 mo) or were unsuccessful.

Alternatively, hypercalcemia can be controlled by medical therapies such as iv isotonic saline, bisphosphonates, glucocorticoids, and SSAs.

The iv administration of isotonic saline corrects possible volume depletion due to hypercalcemia-induced urinary salt wasting and, in some cases, vomiting. Hypovolemia can exacerbate hypercalcemia by impairing the renal clearance of calcium[33]. Our results show that iv isotonic saline should be considered as the standard supportive therapy in patients with PTHrP-hypersecreting NETs and hypercalcemia, but as monotherapy it has only limited effectiveness.

Bisphosphonates inhibit calcium release by interfering with the osteoclast-mediated bone resorption[34, 35]. Because of the ability of PTHrP as an inducer of bone resorption, renal phosphate wasting, and elevated distal tubular reabsorption of calcium, bisphosphonates should theoretically be able to control serum calcium levels in patients with PTHrP-hypersecreting tumors and hypercalcemia[6, 7]. A systematic review of bisphosphonates for HHM showed that, in general, bisphosphonates can normalize serum

calcium levels in >70% patients; however, approximately 25% of cases with HHM are still resistant to bisphosphonate therapy[36]. In our patients, bisphosphonates were only able to decrease, or normalize, serum calcium levels for a relatively short period of time (maximum, 2.3 mo), and therefore the clinical effectiveness of bisphosphonate monotherapy was limited.

As already stated, increased 25(OH)D-1α-hydroxylase activity has not been reported in GEP-NET patients. The activity of this enzyme can be successfully inhibited by glucocorticoids. It is, therefore, not surprising that glucocorticoid administration was ineffective in the control of hypercalcemia in our patients.

Gastrointestinal NETs express the somatostatin receptor subtype 2 (sst_2) in approximately 90% and pancreatic NETs (with the exception of nonmetastatic insulinomas) in approximately 80% of the tumors[37].

The currently available commercial octapeptide SSAs show a high affinity for sst_2 and low-median affinities for somatostatin receptor subtypes 3 and 5 (sst_3 and sst_5)[38]. These drugs are effective therapies for symptom control and control of tumoral hormone secretion in patients with GEP-NETs, achieving symptom control in up to 71% of patients and biochemical response in up to 51%[39-42].

In the present study, SSA treatment resulted in a temporary normalization of serum calcium levels in seven of 10 patients. Two patients obtained a long-term response (up to 35.2 mo).

The use of SSAs as antiproliferative agents in patients with GEP-NETs has been recently established. Sandostatin LAR (30 mg/mo im) resulted in a prolongation of time to progression from 8 to 16.3 months, as compared to placebo, in patients with metastatic NETs of the small intestinal tract[43]. Lanreotide Autogel (120 mg/mo sc) in patients with GEP-NETs resulted in a prolongation of progression-free survival over placebo. Median progression-free survival was not reached with this drug vs. 18 months with placebo[44].

PRRT with ^{177}Lu-octreotate can not only result in a reduction in tumor size and prolongation of overall and progression-free survival, but can also lead to an improvement in symptoms. PRRT with ^{177}Lu-octreotate resulted in complete and partial remissions of metastatic GEP-NETs in 2% and 28% of patients, respectively. Also, symptoms improved in 40–70% of patients[45-47].

In patients treated with PRRT using ^{177}Lu-octreotate, mean serum calcium levels decreased significantly[48]. However, the underlying mechanism for this process could not be elucidated[48]. Hypoparathyroidism, 25(OH)D deficiency, renal insufficiency, pseudohypoparathyroidism, and low calcium intake could be excluded[48]. The potential decrease of serum calcium levels in patients with PTHrP-hypersecreting tumors occurring after PRRT with ^{177}Lu-octreotate is, therefore, an extra advantage of this therapy.

In our series, PRRT also had favorable effects on the hypercalcemia and plasma PTHrP levels, with long-term responses ranging from 9.0 – 49.0 months in four of six patients.

However, two of six patients had worsening of hypercalcemia while still undergoing PRRT, or directly after finalization of the PRRT. Tumor stabilization with PRRT was paralleled by normalization of serum calcium values in three of five patients treated with ^{177}Lu-octreotate.

Seven of the 10 patients developed progressive disease, and this was paralleled by an increase of plasma PTHrP and serum calcium levels. However, not only did the hypercalcemia worsen; the intervals between the treatments and treatment responses shortened as well. Worsening of hypercalcemia, therefore, generally reflected disease progression.

A possibly effective new treatment option for PTHrP-induced hypercalcemia might be the sc administration of denosumab. The human monoclonal antibody denosumab specifically binds the receptor activator of nuclear factor κB (RANK) ligand, blocks the binding of RANK ligand to RANK, and thereby reduces the formation, function, and survival of osteoclasts, which results in decreased bone resorption[35].

In one of our patients, monotherapy with denosumab was not very effective, and calcium levels only slightly decreased for 0.1 month. The combination of denosumab with iv isotonic saline and Octreotide IR (500 µg t.i.d. sc) was able to reduce serum calcium levels for a longer period of time (1.5 mo).

New antitumor treatment options are needed for patients with metastatic GEP-NET presenting with, or developing, PTHrP hypersecretion to prevent recurrence of hypercalcemia and thereby to improve survival of patients with metastatic PTHrP-hypersecreting GEP-NETs.

Clinical trials testing two new targeted antitumor therapies, everolimus and sunitinib, have subsequently led to the recent approval of these two drugs by the US Food and Drug Administration and the European Medicines Agency for the treatment of inoperable, progressive, Grade 1 and 2 pancreatic NETs[49, 50].

In one of our patients, monotherapy with sunitinib (37.5 mg/d) resulted in normalization of serum calcium levels for 5.2 months. After recurrence of hypercalcemia, iv isotonic saline was given in combination with sunitinib, and normocalcemia was achieved for another 3.3 months.

A study with anti-PTH immunotherapy in a patient with metastatic parathyroid carcinoma induced tumor shrinkage accompanied by hormonal, biochemical, and clinical improvements[51]. Anti-PTHrP immunotherapy could be explored as another potentially interesting treatment option in patients with PTHrP-hypersecreting GEP-NETs.

A large international cohort study looked at survival and TNM staging for pancreatic NETs. A cumulative survival of approximately 83% at 5 years and 74% at 10 years was shown[52]. In our study, the 5- and 10-year survival of patients with PTHrP-hypersecreting pancreatic NETs was approximately 70% and <40%, respectively. This suggests that PTHrP hypersecretion is associated with a worse survival. This is most probably due to

complications of HHM in combination with tumor progression and/or suggests that PTHrP production occurs in a subset of GEP-NET patients with a worse clinical course.

We conclude that, although increased hypersecretion of PTHrP by metastatic GEP-NETs is very rare, it has major clinical impact because, apart from the poorly controllable hypercalcemia, it is also a bad prognostic sign. Paraneoplastic PTHrP production in patients with GEP-NETs seems to be exclusively associated with metastatic pancreatic NETs. The most successful treatment options for PTHrP-producing GEP-NETs are SSAs and PRRT using radiolabeled SSAs. Isotonic saline and bisphosphonates are generally used and can be recommended as a supportive therapy.

REFERENCES

1. **Barakat MT, Ashrafian H, Todd JF, Meeran K, Williams GR.** Severe hypercalcaemia from secretion of parathyroid hormone-related peptide. *The Lancet. Oncology.* 2004;5:633-635.
2. **Dawson-Hughes B.** Calcium and Vitamin D. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 7th ed. Washington, DC: American Society for Bone and Mineral Research.* 2008;231–233.
3. **Rosol TJ, Capen CC.** Mechanisms of cancer-induced hypercalcemia. *Laboratory investigation.* 1992;67:680-702.
4. **Stewart AF, Horst R, Deftos LJ, Cadman EC, Lang R, Broadus AE.** Biochemical evaluation of patients with cancer-associated hypercalcemia: evidence for humoral and nonhumoral groups. *The New England journal of medicine.* 1980;303:1377-1383.
5. **Wysolmerski JJ, Broadus AE.** Hypercalcemia of malignancy: the central role of parathyroid hormone-related protein. *Annual review of medicine.* 1994;45:189-200.
6. **Wysolmerski JJ.** Parathyroid Hormone-Related Protein. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 7th ed. Washington, DC: American Society for Bone and Mineral Research***;** 2008;127–133.
7. **Martin TJ, Moseley JM, Williams ED.** Parathyroid hormone-related protein: hormone and cytokine. *The Journal of endocrinology.* 1997;154 Suppl:S23-37.
8. **Strewler GJ.** The parathyroid hormone-related protein. *Endocrinology and metabolism clinics of North America.* 2000;29:629-645.
9. **Srirajaskanthan R, McStay M, Toumpanakis C, Meyer T, Caplin ME.** Parathyroid hormone-related peptide-secreting pancreatic neuroendocrine tumours: case series and literature review. *Neuroendocrinology.* 2009;89:48-55.
10. **Kanakis G, Kaltsas G, Granberg D, Grimelius L, Papaioannou D, Tsolakis AV, Oberg K.** Unusual complication of a pancreatic neuroendocrine tumor presenting with malignant hypercalcemia. *The Journal of clinical endocrinology and metabolism.* 2012;97:E627-631.
11. **Matsen SL, Yeo CJ, Hruban RH, Choti MA.** Hypercalcemia and pancreatic endocrine neoplasia with elevated PTH-rP: report of two new cases and subject review. *Journal of gastrointestinal surgery.* 2005;9:270-279.
12. **Mitlak BH, Hutchison JS, Kaufman SD, Nussbaum SR.** Parathyroid hormone-related peptide mediates hypercalcemia in an islet cell tumor of the pancreas. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme.* 1991;23:344-346.
13. **Mussig K, Petersenn S, Wehrmann M, Horger M, Vierling P, Haring HU, Gallwitz B.** Somatostatin receptor expression in a parathyroid hormone-related peptide-secreting pancreatic neuroendocrine tumour causing severe hypercalcaemia. *European journal of gastroenterology & hepatology.* 2007;19:719-723.
14. **Papazachariou IM, Virlos IT, Williamson RC.** Parathyroid hormone-related peptide in pancreatic neuroendocrine tumours associated with hypercalcaemia. *HPB.* 2001;3:221-225.

15. **Wu TJ, Lin CL, Taylor RL, Kvols LK, Kao PC.** Increased parathyroid hormone-related peptide in patients with hypercalcemia associated with islet cell carcinoma. *Mayo Clinic proceedings.* 1997;72:1111-1115.

16. **Feelders RA, de Herder WW, Neggers SJ, van der Lely AJ, Hofland LJ.** Pasireotide, a multi-somatostatin receptor ligand with potential efficacy for treatment of pituitary and neuroendocrine tumors. *Drugs of today.* 2013;49:89-103.

17. **Kvols LK, Oberg KE, O'Dorisio TM, Mohideen P, de Herder WW, Arnold R, Hu K, Zhang Y, Hughes G, Anthony L, Wiedenmann B.** Pasireotide (SOM230) shows efficacy and tolerability in the treatment of patients with advanced neuroendocrine tumors refractory or resistant to octreotide LAR: results from a phase II study. *Endocrine-related cancer.* 2012;19:657-666.

18. **Wolin EM, Hu K, Hughes G, Bouillaud E, Giannone V, Resendiz KH.** Safety, tolerability, pharmacokinetics, and pharmacodynamics of a long-acting release (LAR) formulation of pasireotide (SOM230) in patients with gastroenteropancreatic neuroendocrine tumors: results from a randomized, multicenter, open-label, phase I study. *Cancer chemotherapy and pharmacology.* 2013;72:387-395.

19. **O'Toole D, Grossman A, Gross D, Delle Fave G, Barkmanova J, O'Connor J, Pape UF, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: biochemical markers. *Neuroendocrinology.* 2009;90:194-202.

20. **Kwekkeboom DJ, Krenning EP, Scheidhauer K, Lewington V, Lebtahi R, Grossman A, Vitek P, Sundin A, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: somatostatin receptor imaging with (111)In-pentetreotide. *Neuroendocrinology.* 2009;90:184-189.

21. **Sundin A, Vullierme MP, Kaltsas G, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: radiological examinations. *Neuroendocrinology.* 2009;90:167-183.

22. **Klimstra DS, Modlin IR, Coppola D, Lloyd RV, Suster S.** The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. *Pancreas.* 2010;39:707-712.

23. **Kloppel G, Couvelard A, Perren A, Komminoth P, McNicol AM, Nilsson O, Scarpa A, Scoazec JY, Wiedenmann B, Papotti M, Rindi G, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: towards a standardized approach to the diagnosis of gastroenteropancreatic neuroendocrine tumors and their prognostic stratification. *Neuroendocrinology.* 2009;90:162-166.

24. **Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG.** New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *Journal of the National Cancer Institute.* 2000;92:205-216.

25. **Kulke MH, Anthony LB, Bushnell DL, de Herder WW, Goldsmith SJ, Klimstra DS, Marx SJ, Pasieka JL, Pommier RF, Yao JC, Jensen RT, North American Neuroendocrine Tumor S.** NANETS treatment guidelines: well-differentiated neuroendocrine tumors of the stomach and pancreas. *Pancreas.* 2010;39:735-752.

26. **Rindi G, Kloppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, Erikssson B, Falchetti A, Falconi M, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B, all other Frascati Consensus Conference p, European Neuroendocrine Tumor S.** TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Archiv.* 2006;449:395-401.

27. **Modlin IM, Oberg K, Chung DC, Jensen RT, de Herder WW, Thakker RV, Caplin M, Delle Fave G, Kaltsas GA, Krenning EP, Moss SF, Nilsson O, Rindi G, Salazar R, Ruszniewski P, Sundin A.** Gastroenteropancreatic neuroendocrine tumours. *The Lancet. Oncology.* 2008;9:61-72.

28. **Fraenkel M, Kim M, Faggiano A, de Herder WW, Valk GD, Knowledge N.** Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocrine-related cancer.* 2014;21:R153-163.

29. **Arps H, Dietel M, Schulz A, Janzarik H, Kloppel G.** Pancreatic endocrine carcinoma with ectopic PTH-production and paraneoplastic hypercalcaemia. *Virchows Archiv. A, Pathological anatomy and histopathology.* 1986;408:497-503.

30. **Kandil E, Noureldine S, Khalek MA, Daroca P, Friedlander P.** Ectopic secretion of parathyroid hormone in a neuroendocrine tumor: a case report and review of the literature. *International journal of clinical and experimental medicine.* 2011;4:234-240.

31. **VanHouten JN, Yu N, Rimm D, Dotto J, Arnold A, Wysolmerski JJ, Udelsman R.** Hypercalcemia of malignancy due to ectopic transactivation of the parathyroid hormone gene. *The Journal of clinical endocrinology and metabolism.* 2006;91:580-583.

32. **Uchimura K, Mokuno T, Nagasaka A, Hayakawa N, Kato T, Yamazaki N, Kobayashi T, Nagata M, Kotake M, Itoh M, Tsujimura T, Iwase K.** Lung cancer associated with hypercalcemia induced by concurrently elevated parathyroid hormone and parathyroid hormone-related protein levels. *Metabolism: clinical and experimental.* 2002;51:871-875.

33. **Hosking DJ, Cowley A, Bucknall CA.** Rehydration in the treatment of severe hypercalcaemia. *The Quarterly journal of medicine.* 1981;50:473-481.

34. **Green JR.** Bisphosphonates: preclinical review. *The oncologist.* 2004;9 Suppl 4:3-13.

35. **Loftus LS, Edwards-Bennett S, Sokol GH.** Systemic therapy for bone metastases. *Cancer control.* 2012;19:145-153.

36. **Saunders Y, Ross JR, Broadley KE, Edmonds PM, Patel S, Steering G.** Systematic review of bisphosphonates for hypercalcaemia of malignancy. *Palliative medicine.* 2004;18:418-431.

37. **Reubi JC, Laissue J, Waser B, Horisberger U, Schaer JC.** Expression of somatostatin receptors in normal, inflamed, and neoplastic human gastrointestinal tissues. *Annals of the New York Academy of Sciences.* 1994;733:122-137.

38. **Lamberts SW, van der Lely AJ, de Herder WW, Hofland LJ.** Octreotide. *The New England journal of medicine.* 1996;334:246-254.

39. **Klibanski A, Melmed S, Clemmons DR, Colao A, Cunningham RS, Molitch ME, Vinik AI, Adelman DT, Liebert KJ.** The endocrine tumor summit 2008: appraising therapeutic approaches for acromegaly and carcinoid syndrome. *Pituitary.* 2010;13:266-286.

40. **Modlin IM, Pavel M, Kidd M, Gustafsson BI.** Review article: somatostatin analogues in the treatment of gastroenteropancreatic neuroendocrine (carcinoid) tumours. *Alimentary pharmacology & therapeutics.* 2010;31:169-188.

41. **Oberg K, Kvols L, Caplin M, Delle Fave G, de Herder W, Rindi G, Ruszniewski P, Woltering EA, Wiedenmann B.** Consensus report on the use of somatostatin analogs for the management of neuroendocrine tumors of the gastroenteropancreatic system. *Annals of oncology.* 2004;15:966-973.

42. **Ruszniewski P, Ducreux M, Chayvialle JA, Blumberg J, Cloarec D, Michel H, Raymond JM, Dupas JL, Gouerou H, Jian R, Genestin E, Bernades P, Rougier P.** Treatment of the carcinoid syndrome with the longacting somatostatin analogue lanreotide: a prospective study in 39 patients. *Gut.* 1996;39:279-283.

43. **Rinke A, Muller HH, Schade-Brittinger C, Klose KJ, Barth P, Wied M, Mayer C, Aminossadati B, Pape UF, Blaker M, Harder J, Arnold C, Gress T, Arnold R, Group PS.** Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. *Journal of clinical oncology.* 2009;27:4656-4663.

44. **van Schaik E, van Vliet EI, Feelders RA, Krenning EP, Khan S, Kamp K, Valkema R, van Nederveen FH, Teunissen JJ, Kwekkeboom DJ, de Herder WW.** Improved control of severe hypoglycemia in patients with malignant insulinomas by peptide receptor radionuclide therapy. *The Journal of clinical endocrinology and metabolism.* 2011;96:3381-3389.

45. **Bergsma H, van Vliet EI, Teunissen JJ, Kam BL, de Herder WW, Peeters RP, Krenning EP, Kwekkeboom DJ.** Peptide receptor radionuclide therapy (PRRT) for GEP-NETs. *Best practice & research. Clinical gastroenterology.* 2012;26:867-881.

46. **Khan S, Krenning EP, van Essen M, Kam BL, Teunissen JJ, Kwekkeboom DJ.** Quality of life in 265 patients with gastroenteropancreatic or bronchial neuroendocrine tumors treated with [177Lu-DOTA0,Tyr3]octreotate. *Journal of nuclear medicine.* 2011;52:1361-1368.

47. **Kwekkeboom DJ, de Herder WW, Kam BL, van Eijck CH, van Essen M, Kooij PP, Feelders RA, van Aken MO, Krenning EP.** Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0,Tyr3]octreotate: toxicity, efficacy, and survival. *Journal of clinical oncology.* 2008;26:2124-2130.

48. **van Vliet EI, de Herder WW, de Rijke YB, Zillikens MC, Kam BL, Teunissen JJ, Peeters RP, Krenning EP, Kwekkeboom DJ.** Hypocalcaemia after treatment with [177Lu-DOTA 0,Tyr3]octreotate. *European journal of nuclear medicine and molecular imaging.* 2013;40:1843-1852.

49. **Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, Chen JS, Horsch D, Hammel P, Wiedenmann B, Van Cutsem E, Patyna S, Lu**

DR, Blanckmeister C, Chao R, Ruszniewski P. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *The New England journal of medicine.* 2011;364:501-513.

50. **Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Oberg K, Rad001 in Advanced Neuroendocrine Tumors TTSG.** Everolimus for advanced pancreatic neuroendocrine tumors. *The New England journal of medicine.* 2011;364:514-523.

51. **Betea D, Bradwell AR, Harvey TC, Mead GP, Schmidt-Gayk H, Ghaye B, Daly AF, Beckers A.** Hormonal and biochemical normalization and tumor shrinkage induced by anti-parathyroid hormone immunotherapy in a patient with metastatic parathyroid carcinoma. *The Journal of clinical endocrinology and metabolism.* 2004;89:3413-3420.

52. **Rindi G, Falconi M, Klersy C, Albarello L, Boninsegna L, Buchler MW, Capella C, Caplin M, Couvelard A, Doglioni C, Delle Fave G, Fischer L, Fusai G, de Herder WW, Jann H, Komminoth P, de Krijger RR, La Rosa S, Luong TV, Pape U, Perren A, Ruszniewski P, Scarpa A, Schmitt A, Solcia E, Wiedenmann B.** TNM staging of neoplasms of the endocrine pancreas: results from a large international cohort study. *Journal of the National Cancer Institute.* 2012;104:764-777.

Chapter 7

Prevalence and clinical features of the ectopic ACTH syndrome in patients with gastroenteropancreatic and thoracic neuroendocrine tumors

Kimberly Kamp[1], R. Ali Alwani[1], Esther Korpershoek[2], Gaston J. H. Franssen[3], Wouter W. de Herder[1], Richard A. Feelders[1]

[1]Department of Internal Medicine, Sector of Endocrinology, [2]Department of Pathology and [3]Department of Surgery, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands

***European Journal of Endocrinology* (2016) 174, 271-280.**

ABSTRACT

Objective: Several series report on the relative contribution of ectopic ACTH syndrome (EAS) in the spectrum of Cushing's syndrome. However, prevalence of EAS in patients with thoracic or gastroenteropancreatic neuroendocrine tumors (GEP-NETs) is currently unknown.

Design: We assessed, in a tertiary referral center, the prevalence of EAS in a large cohort of thoracic and GEP-NET patients including clinical, biochemical, and radiological features; management; and treatment outcome.

Methods: In total, 918 patients with thoracic or GEP-NETs were studied (1993–2012). Multiple endocrine neoplasia type 1 and small cell lung carcinoma patients were excluded. Differentiation between synchronous, metachronous, and cyclic occurrence of EAS was made.

Results: Out of the 918 patients with thoracic and GEP-NETs (469 males and 449 females; median age 58.7 years (range: 17.3–87.3)), 29 patients (3.2%) had EAS (ten males and 19 females; median age 48.1 years (range: 24.7–77.9)). EAS occurred synchronously in 23 patients (79%), metachronously in four patients (14%), and cyclical in two patients (7%) respectively. NETs causing EAS included lung/bronchus (n=9), pancreatic (n=9), and thymic (n=4). In four patients, the cause of EAS was unknown (n=4). Median overall survival (OS) of non-EAS thoracic and GEP-NET patients was 61.2 months (range: 0.6–249.4). Median OS of EAS patients was 41.4 months (range: 2.2–250.9). After comparison, only the first 5-year survival was significantly shorter (P=0.013) in EAS patients.

Conclusion: Prevalence of EAS in this large cohort of patients with thoracic and GEP-NETs was 3.2%. EAS was mostly caused by thoracic and pancreatic NETs. First 5-year survival of EAS patients was shorter compared with non-EAS patients.

INTRODUCTION

Cushing's syndrome (CS) is a rare and severe endocrine disorder characterized by a variety of typical signs and symptoms that occur due to chronic overproduction of cortisol. The estimated prevalence is two to three cases per million population per year[1].

Chronic hypercortisolism is associated with multiple systemic complications resulting in significant morbidity that severely impairs quality of life and an increased mortality when cortisol levels are not or suboptimally controlled[1].

CS is divided into adrenocorticotropin (ACTH)-dependent CS, and ACTH-independent CS. In ~80% of the cases, CS is ACTH-dependent, caused by either an ACTH-secreting pituitary adenoma (Cushing's disease) or, less often, by a non-pituitary ectopic ACTH-secreting tumor. ACTH-independent CS is caused by benign or malignant adrenal tumors or bilateral adrenal hyperplasia[1].

The ectopic ACTH syndrome (EAS) represents 20% of ACTH-dependent CS and about 10% of all types of CS[1-4]. EAS is associated with a variety of malignancies, predominantly of (neuro-)endocrine origin (bronchial, thymic, or pancreatic neuroendocrine tumors (NETs)). Other tumors associated with EAS are small cell lung carcinoma (SCLC), pheochromocytoma, medullary thyroid carcinoma (MTC), and prostate carcinoma[2, 5-8].

NETs originate from cells of the diffuse endocrine system and form a heterogeneous group of relatively rare neoplasms with various clinical manifestations and a distinct biological behavior[9]. Recent epidemiologic studies show that the age-adjusted incidence of all gastroenteropancreatic (GEP)-NETs is 3.65, for pancreatic NETs 0.43, for bronchial NETs between 0.2 and 2.0, and for thymic NETs, this is 0.4/100 000 population per year[10, 11]. Therefore, ectopic ACTH-secreting thoracic and GEP-NETs are extremely rare. Several large series report on the relative contribution of EAS in the spectrum of CS[2, 7, 12]. However, information on the incidence and prevalence of EAS in the setting of patients diagnosed with thoracic or GEP-NETs is currently unknown.

Therefore, the aim of this study was to assess the prevalence of EAS in a large cohort of patients with thoracic and GEP-NETs in a single tertiary academic referral center and to compare the prognosis in patients with and without EAS. Furthermore, clinical, biochemical, and radiological features; management; and treatment outcome of this patient cohort with EAS was evaluated.

PATIENTS AND METHODS

Patients

Patients with thoracic and GEP-NETs were identified from the Erasmus MC NET database. Patients diagnosed with the multiple endocrine neoplasia type 1 (MEN1) syndrome

were excluded. Thymic NETs are known to be associated with MEN1, and MEN1-related ACTH-secreting NETs can harbor germline menin or other somatic mutations[1].

Also patients with SCLC, prostate carcinoma, and MTC were excluded from the study. The medical records of 918 (non-MEN1) patients with thoracic and GEP-NETs, evaluated between 1993 and 2012 in the Erasmus MC, Rotterdam, the Netherlands, were reviewed. All thoracic and GEP-NET patients treated in the Erasmus MC, Rotterdam (as described in the present manuscript) gave written informed consent before inclusion in the studies, which were approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam.

Analysis of clinical and pathological data, laboratory parameters, and imaging findings was performed, and information on medical and surgical treatments was collected.

Diagnosis of thoracic NET and GEP-NET

Diagnosis of thoracic NET and GEP-NET was made on the basis of serological markers (chromogranin A and neuron-specific enolase), pathologic elevations of circulating, hypersecreted neuroendocrine hormones, or peptides[13], imaging according to international protocols and standards[14, 15] in combination with histological confirmation according to current guidelines[16, 17].

Diagnosis of EAS

The diagnosis of CS was based on a review of the patient's medical history, clinical features, and laboratory tests. The use of exogenous glucocorticoids was excluded before biochemical tests were conducted. Hypercortisolism was biochemically established by elevated excretion of 24-h urinary free cortisol (UFC) levels on at least two occasions, insufficient suppression of serum cortisol after 1 mg overnight dexamethasone (cutoff plasma cortisol (0800h): 50 nmol/l), loss of physiological cortisol diurnal rhythm (CDR) with assessment of midnight plasma and/or salivary cortisol levels, and non-suppressed plasma ACTH levels in the presence of normal or elevated plasma cortisol levels.

After the diagnosis of ACTH-dependent CS, the following tests were used to differentiate between pituitary and ectopic ACTH production: first, radiological imaging of the pituitary by magnetic resonance imaging (MRI); secondly, when no adenoma was present on radiological imaging or if the size of the adenoma was <6mm, bilateral inferior petrosal sinus sampling (BIPSS) was performed as the gold standard differentiation test. The iv 7 mg high-dose dexamethasone suppression test (HDDST) and the corticotropin-releasing hormone (CRH) stimulation test were not routinely used anymore from 2000 and onwards because of availability and better diagnostic performance of BIPSS[18].

Definition of synchronous, metachronous, and cyclic EAS

Further differentiation was made between synchronous (diagnosed within 3 months before or after thoracic or GEP-NET diagnosis), metachronous (diagnosed >3 months after

thoracic or GEP-NET diagnosis), and cyclical EAS (repeated episodes of hypercortisolism intermediated by phases of normal cortisol secretion) based on the time between the diagnosis of EAS and the diagnosis of the thoracic or GEP-NET.

Assays and investigations

Serum cortisol (reference range: 200–700 nmol/l), 24-h UFC excretion (reference range: 0–850 nmol/24-h), and plasma ACTH (reference range: 0–11 pmol/l) were measured using chemiluminescence-based immunoassays (Immulite 2000, Siemens, Los Angeles, CA, USA; inter- and intra-assay coefficients of variation respectively below 15 and 7% for cortisol and below 6.5 and 5.5% for ACTH). Urinary cortisol was measured without prior solvent extraction. Although this may be a less accurate method, the upper limit of normal of this in-house assay was carefully determined according to cortisol secretion rate in healthy controls[19]. Salivary cortisol was measured using a commercial kit (Salivary Cortisol ELISA SLV-2930, DRG Instruments GmbH, Marburg, Germany).

Statistical analysis

Thoracic and GEP-NET patients with EAS were compared to those without EAS, regarding various clinicopathological parameters. Comparisons between the two groups were performed with unpaired *t*-test for numerical data and chi-square test for categorical data. The Fisher's exact test was used when the chi-square test had one or more cells with an expected frequency of five or less. The level of statistical significance was set to 0.05. Overall survival (OS) and 5-year survival were analyzed using Kaplan–Meier methods. Log-rank testing was used to determine whether there was a statistically significant difference between the mortality in thoracic and GEP-NET patients with and without EAS. Calculations were performed using Statistical Package for Social Sciences (SPSS) Software V.21.0.

RESULTS

Patients

From 1993 to 2012, a total of 953 NET patients were treated in our department. After exclusion of 24 MEN1, four SCLCs, two prostate carcinoma, one MTC, and four other non-proven NET patients, 918 consecutive (non-MEN1) patients – 469 men and 449 women (female-to-male ratio, 1:1) – with thoracic and GEP-NETs were studied.

The median age of the patients at the time of the thoracic and GEP-NET diagnosis was 58.7 years (range: 17.3–87.3). The median follow-up of the study population was 61.2 months (range: 0.6–250.0). The majority of these tumors originated from the small intestine (267 patients – 29.1%), followed in order of frequency by the non-functioning

pancreas NETs (221 – 24.1%), lung/bronchus (51 – 5.6%), large intestine (47 – 5.1%), ileocecal (43 – 4.7%), and insulinoma (41 – 4.5%). In 131 (14.3%) patients, the primary tumor remained unknown until the completion of the study. The great majority of patients (81.8%) were diagnosed with ENETS Stage IV disease. Information on Tumor Grading was available in 523 patients: 240 (26.1%) were G1, 240 (26.1%) were G2, and 43 (4.7%) were G3 (Table 1).

Evaluation of the medical records of these 918 thoracic and GEP-NETs patients revealed EAS in 29 patients – 19 women and ten men (female-to-male ratio, 1.9:1), corresponding with a prevalence of 3.2%.

Patients were divided into two groups: patients with and without the EAS with respect to the patient's sex, age at diagnosis, localization of the primary thoracic or GEP-NET, localization of the metastases, Tumor Grade, and ENETS Stage. Highly significant differences were seen for the following parameters: a higher median age at diagnosis of the primary NET in the non-EAS group (58.9 years vs. 48.1 years, P<0.001), a higher prevalence of thoracic (lung/bronchus and thymic) NETs in the EAS group (31% vs. 4.7%, P<0.001), a higher prevalence of small intestine NETs in the non-EAS group (30% vs. 0%, P<0.001), a higher prevalence of liver metastases in the non-EAS group (78.7% vs. 55.2%, P 0.003), and a higher prevalence of lung metastases in the EAS group (27.6% vs. 7.8%, P 0.002); Grade 2 tumors were more frequently seen in patients in the EAS group (48.3% vs. 25.4%, P 0.006), and Stage IIIB was more frequently present in the EAS group (24.1% vs. 9.3%, P 0.018), whereas Stage IV was more frequently seen in the non-EAS group (82.5% vs. 62.1%, P 0.005) (Table 1).

Clinical presentation and complications of EAS

The majority of patients presented with the classic clinical signs and symptoms of CS were caused by known ectopic ACTH secretion: muscle weakness (79%), hypokalemia (72%), body weight changes (69%), truncal obesity (66%), full moon face (66%), hypertension (59%), and diabetes (59%) (Table 2).

Complications due to severe hypercortisolism included uncontrolled diabetes (59%) and severe or opportunistic infections (41%) involving the skin (n=2), urogenital tract (n=3), respiratory tract (n=5), sepsis (n=3), herpes zoster (n=1), and candida (n=1). Three patients had multiple recurrent infections. Other complications were severe hypertension (17%), pulmonary embolism (n=3) or thrombosis (n=1) (14%), and psychosis (14%) (Table 2).

Diagnosis of EAS

All patients but one (due to failure in urine collection) had elevated excretion of 24-h UFC levels above the normal reference range on at least two occasions: median baseline UFC – 5872 nmol/24-h (range: 1028–89392 nmol/24-h) and median highest UFC – 14405 nmol/24-h (range: 1127–30149 nmol/24-h). All patients had insufficient suppression of

Table 1. Clinicopathological characteristics of 918 patients with thoracic and GEP-NETs evaluated for the presence of EAS. Group analysis has been conducted between thoracic and GEP-NET patients with and without the EAS.

	All patients (n = 918)		Non-Cushing NET (n = 889)		Ect. ACTH Cushing NET (n = 29)		
	n	%	n	%	n	%	P-value
Gender							
Male	469	51.1	459	51.6	10	34.5	0.069
Female	449	48.9	430	48.4	19	65.5	
Age at diagnosis NET (years)	**58.7 (17.3-87.3)**		**58.9 (17.3-87.3)**		**48.1 (24.7-77.9)**		<0.001
< 50	205	22.3	189	21.3	16	55.2	<0.001
50-69	555	60.5	543	61.1	12	41.4	0.033
>70	158	17.2	157	17.7	1	3.4	0.045
Primary localization							
Lung/bronchus	51	5.6	42	4.7	9	31	<0.001
Thymus	6	0.7	2	0.2	4	13.8	<0.001
Stomach	18	2	17	1.9	1	3.4	0.442
Small intestine	267	29.1	267	30	0	0	<0.001
Appendix	16	1.7	15	1.7	1	3.4	0.404
Ileocecal	43	4.7	43	4.8	0	0	0.393
Large intestine	47	5.1	47	5.3	0	0	0.394
Rectum	32	3.5	32	3.6	0	0	0.62
Other *	12	1.3	12	1.3	0	0	1.000
Cancer of unknown primary	131	14.3	127	14.3	4	13.8	1.000
Pancreas							
Non-functioning	221	24.1	212	23.8	9	31	0.373
Insulinoma	41	4.5	41	4.6	0	0	0.636
Glucagonoma	6	0.7	6	0.7	0	0	1.000
Gastrinoma	16	1.7	15	1.7	1	3.4	0.404
VIPoma	10	1.1	10	1.1	0	0	1.000
Somatostatinoma	1	0.1	1	0.1	0	0	1.000
Metastases localization							
Lymph node	648	70.6	631	71	17	58.6	0.151
Liver	716	78	700	78.7	16	55.2	0.003
Bone	214	23.3	207	23.3	7	24.1	0.915
Lung	77	8.4	69	7.8	8	27.6	0.002
Other **	127	13.8	123	13.8	4	13.8	1.000
Tumor Grade							
G1	240	26.1	236	26.5	4	13.8	0.124
G2	240	26.1	226	25.4	14	48.3	0.006
G3	43	4.7	40	4.5	3	10.3	0.15
unknown	395	43	387	43.5	8	27.6	0.088
ENETS Stage							
I-IIIa	77	8.4	73	8.2	4	13.8	0.296
IIIb	90	9.8	83	9.3	7	24.1	0.018
IV	751	81.8	733	82.5	18	62.1	0.005

**Other primary tumors included: oesophagus, kidney and ovary NETs*

*** Other metastases included: adrenal, heart, brain, spleen, mammae, skin, thyroid, testis, eye and uterus*

serum cortisol after 1mg overnight dexamethasone (cutoff 50 nmol/l), loss of physiological CDR, and non-suppressed plasma ACTH levels (median 37.9 pmol/l, range: 1.8–169.0 pmol/l) in the presence of normal or elevated serum cortisol levels (median 1091 nmol/l, range: 324–3707 nmol/l).

For further differentiation between pituitary and ectopic ACTH production, 16 of 29 (55%) patients underwent MRI of the pituitary gland, but in no case did this show any clear evidence of an adenoma at presentation or follow-up. Overall, 20 patients underwent a HDDST, and 19 of 20 (95%) showed no serum cortisol suppression after HDSST. However, the only patient who showed cortisol suppression after HDSST (42.5% suppression of the baseline value) was diagnosed with a histologically proven EAS (lung

Table 2. Clinical symptoms and signs at presentation including complications in patients with EAS (n=29).

Clinical symptoms & signs	N	(%)
Muscle weakness	23	(79)
Hypokalemia	21	(72)
Body weight	20	(69)
Increase	17	(59)
Decrease	3	(10)
Truncal obesity	19	(66)
Full moon face	19	(66)
Hypertension	17	(59)
Diabetes	17	(59)
Edema	16	(55)
Bruising	15	(52)
Hirsutism	14	(48)
Buffalo hump	13	(45)
Psychiatric disorders	11	(38)
Osteopenia or osteoporosis	9	(31)
Acne	7	(24)
Hyperpigmentation	7	(24)
Insomnia	6	(21)
Impaired cognition or memory	5	(17)
Violaceous striae	4	(14)
Menstrual irregularities or amenorrhea	2	(7)
Libido	2	(7)
Fractures	2	(7)
Complications	**N**	**(%)**
Uncontrolled diabetes	17	(59)
Severe or opportunistic infections	12	(41)
Severe hypertension	5	(17)
Thrombosis or pulmonary embolism	4	(14)
Psychosis	4	(14)

NET). Data on the CRH stimulation test were available for 14 patients: 13 exhibited no cortisol or ACTH response after CRH administration; however, the same patient with a lung NET that showed cortisol suppression after HDSST also demonstrated after human CRH administration a 14 and 23% cortisol rise at 30 and 45 min respectively.

Eight patients underwent BIPSS, none of them showed a central-to-peripheral ACTH gradient.

In most patients, EAS was identified synchronously within 3 months before or after the thoracic or GEP-NET diagnosis (n=23). Metachronous EAS was only seen in few patients (n=4). In two patients, cortisol levels fluctuated markedly during follow-up (repeated episodes of hypercortisolism intermediated by phases of normal cortisol secretion), indicating cyclical EAS.

These two patients with cyclical EAS were diagnosed with a histologically proven thymic NET and pancreatic NET respectively.

Radiological investigation

A variety of imaging modalities was used over time to localize ACTH-producing thoracic or GEP-NET, as different imaging techniques became available. In total, 27 EAS patients underwent chest radiography, which revealed the presence of the primary thoracic tumor in six patients and metastases in only two out of eight patients with lung metastases. In total, 25 EAS patients underwent computed tomography (CT) imaging of the chest, which identified 13 with primary thoracic NETs, and 28 EAS patients had CT imaging of the abdomen, which identified eight of 12 GEP-NETs. Five EAS patients, who underwent CT imaging of the abdomen, additionally underwent MRI of the abdomen, and this revealed one with more primary pancreatic NET and other patient with metastases of the liver.

Octreotide scanning (^{111}In-pentetreotide scintigraphy) was performed in all 29 EAS patients, 20 had positive octreotide scintigraphy. In total, four of these 20 patients had an octreoscan that did not reveal the primary tumor but did reveal the presence of metastases (lymph nodes and liver). Two patients had an ^{123}I-MIBG scan that did not reveal the primary in either but did reveal the presence of metastases (liver) in one patient. Positron emission tomography (PET), a relatively new, non-invasive technique, was performed in six EAS patients. One of these six patients, with a G3 lung NET, underwent [^{18}F]-fluorodeoxyglucose PET which provided just limited information on lymphnode metastases. Additional ^{68}Ga-DOTA-TOC imaging in this patient revealed metastases of liver and bones. ^{18}F-DOPA scan performed in three patients revealed the presence of already known liver metastases in one patient. In another patient, both ^{18}F-DOPA and ^{11}C-5-HTP–PET scans were performed and were negative. ^{11}C-5-HTP–PET imaging was able to identify the primary thymic NET in one of our patients.

Management of EAS

Medical treatment

A total of 23 patients with EAS received medical treatment which consisted of the glucocorticoid receptor antagonist mifepristone, adrenolytic agents such as ketoconazole and etomidate, or ACTH inhibitory agents like somatostatin analogs (SSAs). Mifepristone, ketoconazole, and SSAs were used alone in five, three, and five patients respectively. Other patients received up to three drugs simultaneously or sequentially. Medication was discontinued or changed because of side effects (n=4) or inadequate inhibition (n=12). All patients (n=18), except two, had persistent hypercortisolism under treatment with ketoconazole or deteriorated under mifepristone treatment, and 15 of these patients underwent bilateral adrenalectomy. Of the two patients in whom clinical symptoms improved under treatment with mifepristone, one underwent surgery of the primary tumor, whereas the other patient ultimately underwent a bilateral adrenalectomy.

Surgical treatment

Curative resection or debulking of the primary tumor and its metastases was performed in 16 patients with EAS. Overall, seven patients had a curative resection and were cured of EAS; however, two of these patients previously underwent a bilateral adrenalectomy to control their hypercortisolism. In the other nine patients, surgery was not curative, and the EAS was controlled by bilateral adrenalectomy. In total, 22 out of 29 patients underwent a bilateral adrenalectomy to control the hypercortisolism caused by EAS.

Adjunctive therapy of the thoracic and GEP-NET

Seven patients were treated with peptide receptor radiotherapy, six patients (three pancreatic NET, two unknown primary NETs, and one thymic NET) were treated with a median dose of ^{177}Lu-octreotate of 29.0 GBq (range: 14.8–44.9 GBq) divided over two to six therapy cycles, and one patient with an appendix NET was treated with a total dose of ^{111}In-pentetreotide of 58.6 GBq divided over eight therapy cycles. In two patients, ^{177}Lu-octreotate was used as an adjuvant treatment to surgery, which resulted in two complete responses (23.4 and 34.4 months still ongoing at the end of this study). Other best responses of ^{177}Lu-octreotate were two partial responses (25.1 and 57.1 months) and two stable disease responses (32.5 and 6 months). Information regarding the response and duration of response of the patient treated with ^{111}In-pentetreotide therapy was not available.

Three patients with thymic NETs received external radiotherapy to the mediastinum. In three GEP-NET patients, radiotherapy was directed to the bone metastases for pain

control. Other adjunctive treatments were cytotoxic chemotherapy (10.3%) and everolimus (6.9%).

Pathological findings

Of the 29 EAS thoracic and GEP-NET patients, 16 patients had a histopathological established diagnosis at Erasmus MC, Rotterdam, with the exception of seven patients who had a histopathological diagnosis before referral. Our pathology department reviewed all tumor tissues, obtained from the referring hospitals, in order to confirm the diagnosis. Furthermore, ACTH immunohistochemistry was positive in 13 out of 18 patients.

Prognosis and survival

At the last time point of follow-up, 15 of 29 (52%) EAS NET patients and 547 of 889 (62%) non-EAS thoracic and GEP-NET patients were still alive. In the EAS NET group, patients died of complications due to progression of the tumor itself (n=8) or as a consequence of previous excessive cortisol secretion (opportunistic infections (n=2), cardiac failure (n=2), and pulmonary embolism (n=1)); in one patient, the cause of death remained unknown. From this total of 14 deaths in the EAS NET group, two patients did not undergo bilateral adrenalectomy (one patient was in poor clinical condition and died within 2 months; the other patient refused and died after 12 months), nine patients had an early bilateral adrenalectomy (within 1 month after diagnosis of EAS), and three patients had a late bilateral adrenalectomy (after more than 4 months). Median survival of patients after early bilateral adrenalectomy was 6.2 months (range: 5.0–20.9 months), and median survival of patients after late bilateral adrenalectomy was 5.2 months (range: 2.2–20.9 months).

Median OS of the non-EAS patients was 61.2 months (range: 0.6–249.4), whereas median OS of the EAS patients was 41.4 months (range: 2.2–250.9). The OS in the patients with EAS was not significantly shorter (P=0.151) than in the group with non-EAS patients when compared for the complete duration of follow-up. However, the first 5-year survival of the EAS patients was significantly shorter (P=0.013) than in the group with non-EAS patients (Figure 1).

Survival according to Tumor Grade (Figure 2) and ENETS Tumor Stage (Figure 3) compared in patients with and without EAS showed no significant differences.

Figure 1. Overall survival and 5-year survival of EAS (n=29) vs. non-EAS thoracic and GEP-NET patients (n=889).

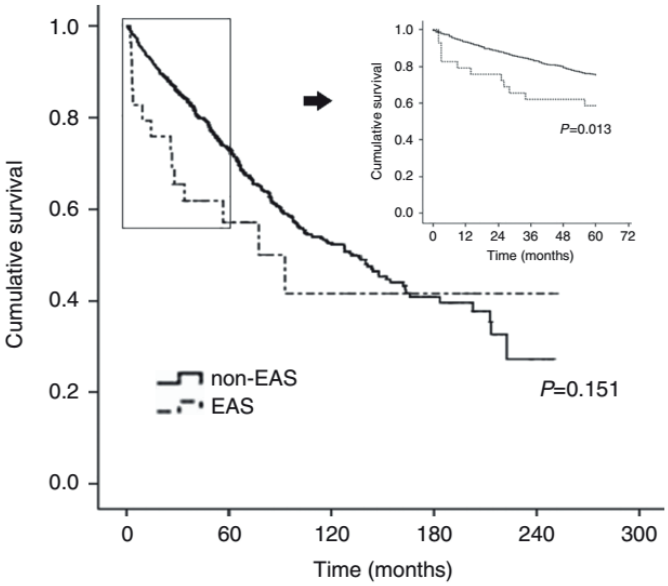
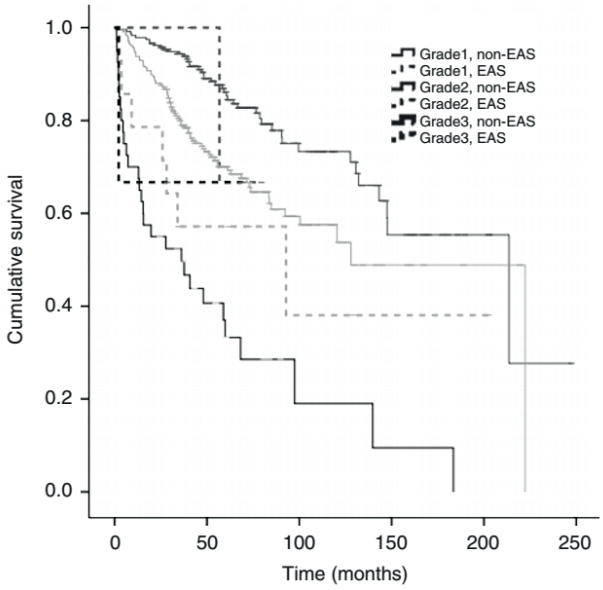
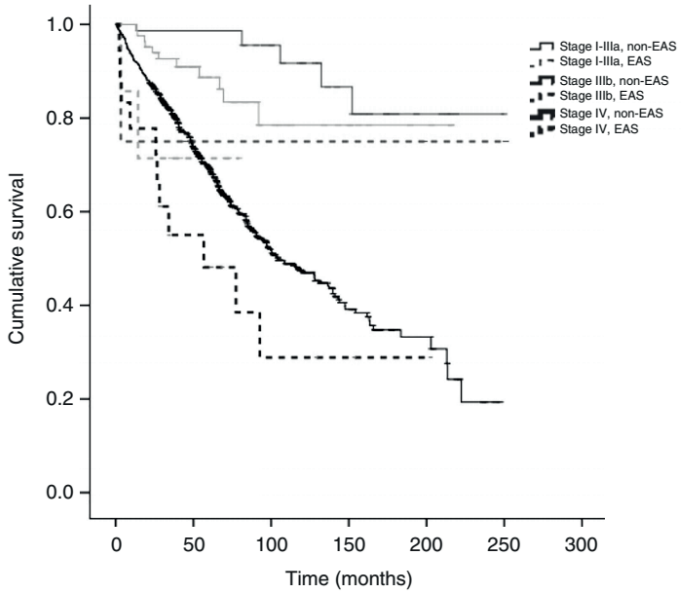


Figure 2. Survival curves by Tumor Grade (EAS vs. non-EAS patients).



Grade 1 EAS (n=4) vs. Grade 1 non-EAS (n=236), P=0.664; Grade 2 EAS (n=14) vs. Grade 2 non-EAS (n=226), P=0.147; and Grade 3 EAS (n=3) vs. non-EAS (n=40), P=0.502.

Figure 3. Survival curves by ENETS Tumor Stage Grade 3 (EAS vs. non-EAS patients).



Stage I–IIIA EAS (n=4) vs. Stage I-IIIA non-EAS (n=73), P=0.381; Stage IIIB EAS (n=7) vs. Stage IIIB non-EAS (n=83), P=0.182; and Stage IV EAS (n=18) vs. Stage IV non-EAS (n=733), P=0.064.

DISCUSSION

We have evaluated the prevalence of EAS in a large cohort of patients with thoracic and GEP-NETs, who were followed in a single, academic, and tertiary referral center. In our retrospective study, we found that, within a period of 20 years (1993–2012), 918 new patients with thoracic and GEP-NETs included 29 patients (3.2%) with proven EAS.

In addition, we present the clinical, endocrine, and radiological features; management; and treatment outcome of our cohort of 29 thoracic and GEP-NET patients with EAS evaluated and treated within a single center.

In line with literature, thoracic NETs (lung/bronchus and thymic) were the most common tumors with ectopic ACTH production (44.8%) in our study, followed by non-functioning pancreatic NETs (31%). Furthermore, EAS occurred only in one patient with an appendix NET in accordance with Ilias et al., which may explain why EAS is not a major concern in NETs originating from the midgut[1, 2, 7].

Comparisons were made between thoracic and GEP-NET patients with and without EAS which showed highly significant differences regarding various clinicopathological parameters. The lower median age at diagnosis of EAS patients is presumably explained by an earlier onset of symptoms due to the EAS. This probably also clarifies why Stage

IIIB tumors, without distant metastases, are more frequently seen in the EAS group. The differences in localization between the distant metastases, lung for the EAS group and liver for the non-EAS group, are in line with the primary tumor site.

This series of 29 patients illustrates the broad clinical spectrum of EAS that can present with a variable clinical phenotype as can be inferred from the prevalence of signs and symptoms[2, 7, 20].

In our cohort, all patients had disturbed first-line screenings tests for hypercortisolism. Although dynamic testing with CRH test and HDDST performed reasonably well, only BIPSS resulted in a sensitivity and specificity of 100% to differentiate between EAS and central ACTH overproduction.

CT or MRI of thorax and abdomen and octreoscan revealed the primary EAS lesion in most patients. Thoracic NETs were best detected by CT imaging of the chest (100%) and GEP-NETs by CT or MRI of the abdomen (75%). These results are in line with previously published studies that localized the primary tumor in 70–90% of the ectopic ACTH cases[2, 7]. The octreoscan was positive in 69% of patients and showed no superiority over CT or MRI in detecting more lesions. NETs express somatostatin receptors (SSTs), in particular subtype 2, and can be identified with SSTs scintigraphy with ^{111}In-pentetreotide octreoscan. In EAS patients, reported sensitivity of the diagnostic octreoscan varies between 25 and 80%, and this broad range may in part be explained by differences in imaging technique protocols[2, 7, 21, 22].

Furthermore, we found five patients with negative ACTH immunohistochemistry in our series (lung=2 and pancreatic NET=3). This is in line with Isidori et al.[7], they state that, in all patients, diagnosis was further confirmed by positive ACTH immunoreactivity (n=15) and/or complete/partial resolution of the hypercortisolemia after tumor removal/ debulking (n=19). They also found negative ACTH immunohistochemistry in several (lung) NET patients[7]. Another possible explanation could be bad fixation during immunohistochemistry in combination with the revision of relatively old tumor tissue samples.

Management of patients with ACTH-producing thoracic and GEP-NETs was aimed at control of cortisol excess, preferably also paralleled by tumor stabilization or reduction, resulting in prolongation of survival. Optimal primary treatment of EAS with surgical resection of the primary tumor and its metastases was possible in 24% of our EAS patients. Second-line treatment to control cortisol excess included bilateral adrenalectomy, medical therapy, and radiotherapy[1, 2, 6, 7, 23, 24]. Ultimately, most of our patients underwent bilateral adrenalectomy to control hypercortisolism.

Two large historical case record studies evaluated survival of EAS in NETs and other tumors. In the study by Ilias et al.[2], a cumulative survival of approximately 85% in pulmonary NET patients at 5 years was shown. In the study by Isidori et al.[7], cumulative survival of NET at 5 years without distant metastases was about 80% and with distant

metastases (Stage IV) 60%. OS in our cohort of 29 EAS patients (62% Stage IV) was 65% at 5 years and for non-EAS patients (82.5% Stage IV) 75% at 5 years. We conclude that, in our cohort, OS of patients with EAS was not significantly shorter than OS of non-EAS patients when compared for the complete duration of follow-up. However, the first 5-year survival of EAS patients was significantly shorter than the first 5-year survival of non-EAS patients. This difference could be explained by the proportion of 'cured' patients in the EAS group, in which survival is probably no longer largely determined by the EAS or the thoracic or GEP-NET. Literature shows that the overall prognosis of EAS patients is mainly determined by the Tumor Grade and Stage at the time of diagnosis[2]. In our study, eight EAS patients died of complications due to progression of the tumor itself, whereas five patients died due to complications of excessive cortisol secretion. Chronic hypercortisolism can induce multisystem morbidities and serious complications[25]. Cardiovascular risk factors (e.g. uncontrolled diabetes, severe hypertension, and obesity), immunosuppressive effects increasing the risk of opportunistic infections and sepsis, and venous thromboembolism occurred frequently in our patient cohort.

Although we report the prevalence of EAS in a very large cohort of patients with thoracic and GEP-NETs, our study has a minor limitation, since not all patients with thoracic and GEP-NETs were screened for hypercortisolism and EAS. Therefore, hypothetically patients with mild EAS might not have been diagnosed. However, EAS is usually symptomatic and associated with severe hypercortisolism.

In this retrospective study, we conclude that, within a period of 20 years (1993–2012), the prevalence of EAS in a large cohort of patients with sporadic thoracic and GEP-NETs was 3.2%. The first 5-year survival was shorter in patients with EAS compared with non-EAS patients. Our and other studies show that EAS in patients with thoracic and GEP-NETs is associated with serious morbidity and a high mortality risk. Therefore, aggressive treatment of hypercortisolism with (combination) medical therapy or rescue bilateral adrenalectomy is an essential part of patient management.

REFERENCES

1. **Lacroix A, Feelders RA, Stratakis CA, Nieman LK.** Cushing's syndrome. *Lancet.* 2015;386:913-927.
2. **Ilias I, Torpy DJ, Pacak K, Mullen N, Wesley RA, Nieman LK.** Cushing's syndrome due to ectopic corticotropin secretion: twenty years' experience at the National Institutes of Health. *The Journal of clinical endocrinology and metabolism.* 2005;90:4955-4962.
3. **Newell-Price J, Bertagna X, Grossman AB, Nieman LK.** Cushing's syndrome. *Lancet.* 2006;367:1605-1617.
4. **Tritos NA, Biller BM, Swearingen B.** Management of Cushing disease. *Nature reviews. Endocrinology.* 2011;7:279-289.
5. **Alwani RA, Neggers SJ, van der Klift M, Baggen MG, van Leenders GJ, van Aken MO, van der Lely AJ, de Herder WW, Feelders RA.** Cushing's syndrome due to ectopic ACTH production by (neuroendocrine) prostate carcinoma. *Pituitary.* 2009;12:280-283.
6. **Biller BM, Grossman AB, Stewart PM, Melmed S, Bertagna X, Bertherat J, Buchfelder M, Colao A, Hermus AR, Hofland LJ, Klibanski A, Lacroix A, Lindsay JR, Newell-Price J, Nieman LK, Petersenn S, Sonino N, Stalla GK, Swearingen B, Vance ML, Wass JA, Boscaro M.** Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement. *The Journal of clinical endocrinology and metabolism.* 2008;93:2454-2462.
7. **Isidori AM, Kaltsas GA, Pozza C, Frajese V, Newell-Price J, Reznek RH, Jenkins PJ, Monson JP, Grossman AB, Besser GM.** The ectopic adrenocorticotropin syndrome: clinical features, diagnosis, management, and long-term follow-up. *The Journal of clinical endocrinology and metabolism.* 2006;91:371-377.
8. **Phan AT, Yao JC.** Neuroendocrine tumors: novel approaches in the age of targeted therapy. *Oncology.* 2008;22:1617-1623; discussion 1623-1614, 1629.
9. **Modlin IM, Oberg K, Chung DC, Jensen RT, de Herder WW, Thakker RV, Caplin M, Delle Fave G, Kaltsas GA, Krenning EP, Moss SF, Nilsson O, Rindi G, Salazar R, Ruszniewski P, Sundin A.** Gastroenteropancreatic neuroendocrine tumours. *The Lancet. Oncology.* 2008;9:61-72.
10. **Fraenkel M, Kim M, Faggiano A, de Herder WW, Valk GD, Knowledge N.** Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocrine-related cancer.* 2014;21:R153-163.
11. **Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB.** One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *Journal of clinical oncology.* 2008;26:3063-3072.
12. **Aniszewski JP, Young WF, Jr., Thompson GB, Grant CS, van Heerden JA.** Cushing syndrome due to ectopic adrenocorticotropic hormone secretion. *World journal of surgery.* 2001;25:934-940.
13. **O'Toole D, Grossman A, Gross D, Delle Fave G, Barkmanova J, O'Connor J, Pape UF, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: biochemical markers. *Neuroendocrinology.* 2009;90:194-202.

14. **Kwekkeboom DJ, Krenning EP, Scheidhauer K, Lewington V, Lebtahi R, Grossman A, Vitek P, Sundin A, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: somatostatin receptor imaging with (111)In-pentetreotide. *Neuroendocrinology.* 2009;90:184-189.

15. **Sundin A, Vullierme MP, Kaltsas G, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: radiological examinations. *Neuroendocrinology.* 2009;90:167-183.

16. **Klimstra DS, Modlin IR, Coppola D, Lloyd RV, Suster S.** The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. *Pancreas.* 2010;39:707-712.

17. **Kloppel G, Couvelard A, Perren A, Komminoth P, McNicol AM, Nilsson O, Scarpa A, Scoazec JY, Wiedenmann B, Papotti M, Rindi G, Plockinger U, Mallorca Consensus Conference p, European Neuroendocrine Tumor S.** ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: towards a standardized approach to the diagnosis of gastroenteropancreatic neuroendocrine tumors and their prognostic stratification. *Neuroendocrinology.* 2009;90:162-166.

18. **van den Bogaert DP, de Herder WW, de Jong FH, Biemond P, van der Lely AJ, Lamberts SW.** The continuous 7-hour intravenous dexamethasone suppression test in the differential diagnosis of ACTH-dependent Cushing's syndrome. *Clinical endocrinology.* 1999;51:193-198.

19. **Croughs RJ, Docter R, de Jong FH.** Comparison of oral and intravenous dexamethasone suppression tests in the differential diagnosis of Cushing's syndrome. *Acta endocrinologica.* 1973;72:54-62.

20. **Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM.** The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *The Journal of clinical endocrinology and metabolism.* 2008;93:1526-1540.

21. **de Herder WW, Krenning EP, Malchoff CD, Hofland LJ, Reubi JC, Kwekkeboom DJ, Oei HY, Pols HA, Bruining HA, Nobels FR, et al.** Somatostatin receptor scintigraphy: its value in tumor localization in patients with Cushing's syndrome caused by ectopic corticotropin or corticotropin-releasing hormone secretion. *The American journal of medicine.* 1994;96:305-312.

22. **Tabarin A, Valli N, Chanson P, Bachelot Y, Rohmer V, Bex-Bachellerie V, Catargi B, Roger P, Laurent F.** Usefulness of somatostatin receptor scintigraphy in patients with occult ectopic adrenocorticotropin syndrome. *The Journal of clinical endocrinology and metabolism.* 1999;84:1193-1202.

23. **Reincke M, Ritzel K, Osswald A, Berr C, Stalla G, Hallfeldt K, Reisch N, Schopohl J, Beuschlein F.** A critical reappraisal of bilateral adrenalectomy for ACTH-dependent Cushing's syndrome. *European journal of endocrinology.* 2015;173:M23-32.

24. **van der Pas R, de Herder WW, Hofland LJ, Feelders RA.** New developments in the medical treatment of Cushing's syndrome. *Endocrine-related cancer.* 2012;19:R205-223.

25. **Feelders RA, Pulgar SJ, Kempel A, Pereira AM.** The burden of Cushing's disease: clinical and health-related quality of life aspects. *European journal of endocrinology.* 2012;167:311-326.

Chapter 8

Safety and efficacy of everolimus in gastrointestinal and pancreatic neuroendocrine tumors after ^{177}Lu-octreotate

Kimberly Kamp[1], Brenda Gumz[3], Richard A. Feelders[1], Dik J. Kwekkeboom[2], Gregory Kaltsas[4], Frederico P. Costa[3], Wouter W. de Herder[1]

[1]Department of Internal Medicine, Sector of Endocrinology, [2]Department of Pathology, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands
[3]Centro de Oncologia, Hospital Sírio Libanês, São Paulo, Brazil
[4]Department of Pathophysiology, National University of Athens, Athens, Greece

***Endocrine-Related Cancer* (2013) 20, 825-831.**

ABSTRACT

Although ^{177}Lu-octreotate is an effective treatment for patients with gastroenteropancreatic neuroendocrine tumors (GEP-NETs), some patients will fail or develop disease progression necessitating further treatment. We examined whether the safety and efficacy of everolimus after prior treatment with ^{177}Lu-octreotate is different from the published safety profile of everolimus in GEP-NETs. In this multicenter study, 24 GEP-NET patients were included. Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. Tumor response was measured according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0. Major clinical adverse events (grade 3 or 4) during treatment with everolimus were hyperglycemia (20.8%), fatigue (8.3%), thrombocytopenia (8.3%), and elevated alanine transaminase levels (8.3%). By radiological review, there were four partial responses (16.7%), fifteen patients (62.5%) with stable disease, and three patients (12.5%) with progressive disease. For two patients (8.3%), no data on tumor response were available. Median progression-free survival (PFS) was 13.1 months (95% CI, 11.5–21.2). Median PFS of the current study was longer when compared with the RADIANT-3 trial (13.1 vs. 11.4 months) and shorter when compared with the RADIANT-1 trial (13.1 vs. 16.7 months). In conclusion, the safety profile of everolimus is not influenced by previous treatment with peptide receptor radiotherapy.

INTRODUCTION

Neuroendocrine tumors (NETs) form a heterogeneous group of relatively rare neoplasms that originate from different types of neuroendocrine cells[1]. The incidence and prevalence of gastroenteropancreatic NETs (GEP-NETs) have shown a remarkable increase over the past three decades. The United States Surveillance Epidemiology and End Results (SEER) database and several other European databases currently estimate the GEP-NET incidence at between 2.5 and 6.2 cases/100,000 population[2]. Whether this is a true increase in incidence, the result of an increased use of imaging techniques, or a combination of the two has not been established yet.

Despite the constantly increasing incidence and prevalence of GEP-NETs, treatment options remain limited. Clinical symptomatology in patients with functioning well-differentiated GEP-NETs has already been successfully treated with somatostatin analogs for more than 25 years. Furthermore, in patients with metastatic NETs of the small intestinal tract, treatment with octreotide long-acting repeatable (LAR) im 30 mg/month resulted in an increase in time to progression from 6 to 16.3 months when compared with placebo[3].

However, upon progression, peptide receptor radionuclide therapy (PRRT) with radiolabeled somatostatin analogs has been shown to be effective regarding tumor control[4]. Although ^{177}Lu-octreotate is being increasingly used, there are limitations to its use and some patients who initially responded may became refractory after a certain number of treatment cycles necessitating further treatment.

Everolimus, an mTOR inhibitor, has been shown to be a promising anti-tumor agent for patients with well-differentiated (WHO Grades 1 and 2) GEP-NETs with progressive disease (PD), with a well-established safety profile[5-8]. In the randomized phase III RADIANT-3 study, everolimus therapy was associated with a 2.4-fold improvement in median progression-free survival (PFS) in patients with progressive pancreatic NETs compared with placebo. Median PFS was 11 months in the everolimus arm compared with 4.6 months in the placebo arm (HR, 0.35; 95% CI, 0.27–0.45; log-rank test, P<0.0001)[8].

In another randomized phase III RADIANT-2 study in advanced mainly gastrointestinal (GI) NETs associated with carcinoid syndrome, it was demonstrated that everolimus plus octreotide LAR provided clinically meaningful 5.1-month improvement in PFS, which was not statistically significant, when compared to patients receiving placebo plus octreotide LAR[5].

However, there are currently no data regarding the toxicity and efficacy of everolimus after prior treatment with ^{177}Lu-octreotate radionuclide therapy. The aim of this study was to investigate whether the safety and efficacy profile of everolimus in patients who have progressed after ^{177}Lu-octreotate radionuclide therapy is different from the already known profile.

SUBJECTS AND METHODS

Patients

All patients with well-differentiated or moderately differentiated (G1-G2) GEP-NETs that received any dose (10 or 5 mg) of everolimus at least once immediately after documented progression following ^{177}Lu-octreotate therapy were included in this multicenter study. We retrospectively studied 24 patients with GEP-NETs, treated in three institutions in the Netherlands, Brazil, and Greece between 2010 and 2012. We retrospectively screened 30 patients for inclusion in this study. Patients were eligible to be included in the study if they had a G1-G2 GEP-NETs, a WHO performance status of 2 or less, hemoglobin levels of ≥8.9 g/dl, and a creatinine clearance above 50ml/min at baseline. Other prior antitumor therapies were not considered to be exclusion criteria. Six patients were excluded from this study: one patient had a primary lung NET, two patients had a WHO performance status of 3, two patients had a hemoglobin of ≥8.9 g/dl, and one patient whose follow-up data were missing.

According to the grading system proposed by the European Neuroendocrine Tumor Society (ENETS) – WHO, G1 (Ki-67 ≤2%) and G2 (Ki-67 2–20%) tumors are regarded as well- and moderately differentiated tumors[9, 10]. At baseline, all patients had radiological documentation of PD according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0[11].

All patients had given informed consent to participate in the ^{177}Lu-octreotate therapy series. Furthermore, the study was conducted in accordance with Good Clinical Practice principles and applicable local regulations.

Safety and efficacy assessments

The primary endpoint of this study was the retrospective evaluation of the safety profile of everolimus in patients previously treated with ^{177}Lu-octreotate radionuclide therapy. Patients were historically assessed for safety parameters at baseline, including previously reported adverse events during ^{177}Lu-octreotate radionuclide therapy and specific laboratory values. Baseline was defined as last documented parameters prior to initiation of everolimus (10 or 5 mg) therapy. All patients were seen in clinical settings at 40-day intervals with laboratory tests (including biomarkers), physical examination, and toxicity assessment. Tumor measurements (assessed by computed tomography (CT) and/or magnetic resonance imaging (MRI)) were performed at baseline and every 120 days unless there were any new symptoms. Chest imaging (chest X-ray or chest CT) was performed to document or exclude interstitial lung disease in all centers. These follow-up intervals (1–3 months) were taken into consideration retrospectively as documented by each individual institution. Follow-up intervals were similar to the intervals in the RADIANT-3 study[8].

Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0 (http://ctep. cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). Doses of everolimus were reduced and/or dose intervals were increased (5 mg daily or 5 mg every other day) when patients had clinically significant adverse events that were considered to be related to the treatment with everolimus. Treatment continued until the development of an unacceptable adverse event, PD, or death.

Secondary endpoints included PFS, objective response rate, duration of response, symptom control, and tumor marker control. All patients were assessed for efficacy according to RECIST, version 1.0[11]. PFS was documented according to RECIST, version 1.0 and defined as the time from initiation of everolimus therapy to the first radiological documentation of PD or death from any cause.

Statistical analyses

PFS was analyzed using Kaplan–Meier methods. Calculations were performed using Statistical Package for Social Sciences (SPSS) software, V.20.0.

RESULTS

Patients and treatment

Between September 2010 and July 2012, a total of 24 NET patients – 13 men and 11 women – from three centers in three countries were initiated on everolimus after tumor progression following ^{177}Lu-octreotate radionuclide therapy. The median interval between last ^{177}Lu-octreotate therapy and start of everolimus treatment was 18.7 months (range 2.9–47.6 months). The demographic and baseline clinical characteristics of the 24 patients are listed in Table 1. Patients had a median age of 60.0 years (range 32.2–65.9 years) at start of treatment with everolimus. The median interval between the initial diagnosis of the NET and the initiation of everolimus therapy was 64.2 months (range 9.9–273.5 months). The median follow-up of the study population was 11.0 months (range 1.9–28.9 months). Pancreatic NETs (75%) were the most common primary tumors when compared with NETs arising in the digestive tract (25%). Metastases were demonstrated in 23 patients (95.8%), main localizations were liver (91.7%) and lymph nodes (50%). The majority of patients (95.8%) included in our study were diagnosed with ENETS Stage IV disease[9, 10]. Information on Tumor Grading was available for 21 patients (87.5%): 7 (29.2%) were well-differentiated (G1) and 14 (58.3%) were moderately differentiated (G2).

Information regarding previous treatment regimens and interventions, responses and duration of responses is listed in Tables 2 and 3. Patients were treated with a median

Table 1. Demographic and baseline clinical characteristics of 24 patients with GEP-NETs treated with everolimus after ^{177}Lu-DOTA^{0}-Tyr^{3}-octreotate therapy failure.

Characteristics	N	Percentage (%)
Total	**24**	**100**
Gender		
Male	13	54.2
Female	11	45.8
Age (years)		
< 50	8	33.3
50-69	14	58.3
> 70	2	8.4
Race		
Caucasian	23	95.8
Asian	1	4.2
WHO performance status		
0	5	20.8
1	17	70.8
2	2	8.4
Primary localization		
Pancreas	18	75.0
Non-functioning	16	66.6
Glucagonoma	1	4.2
VIPoma	1	4.2
Duodenum	1	4.2
Small intestine	2	8.4
Colon	1	4.2
Rectum	1	4.2
CUP	1	4.2
Metastases localization		
Liver	22	91.7
Bone	7	29.2
Lymph node	12	50.0
Tumor Grade		
Well-differentiated	7	29.2
Moderately differentiated	14	58.3
Unknown	3	12.5
ENETS Stage		
IIB	1	4.2
IV	23	95.8

Table 2. Previous treatments: multicenter (n=24).

Previous Treatments	N	%
Total	**24**	**100**
^{177}Lu-octreotate (GBq)	**24**	**100**
0-10	1	4.2
10-20	3	12.5
20-30	4	16.7
30-40	2	8.3
40-50	6	25.0
50-60	2	8.3
60-70	1	4.2
not available	5	20.8
Median administered dose in GBq (range)	**37.0 (7.4-60.1)**	
Chemotherapy	**8**	**33.3**
Xelox	3	12.5
Interferon-α	1	4.2
5-FU-STZ	1	4.2
Cisplatin-Etoposide	1	4.2
Bevacizumab	1	4.2
Cisplatin-Etoposide + Cisplatin-Irinotecan + VAC	1	4.2
Surgery	**11**	**45.8**
Major (curative intent)	6	25.0
Palliative	5	20.8
Somatostatin analog use	**14**	**58.3**
Octreotide LAR 20mg/4wk	4	16.7
Octreotide LAR 30mg/4wk*	9	37.5
Short-acting octreotide	1	4.2

*Xelox, capecitabine plus oxaliplatin; 5-FU-STZ, 5-fluorouracil–streptozotocin; VAC, vincristine–adriamycin–cyclophosphamide; LAR, long-acting repeatable. *One patient used a combination of octreotide LAR 30 mg/4 weeks and short-acting octreotide.*

dose of ^{177}Lu-octreotate of 37.0 GBq (range 7.4–60.1 GBq) divided over one to eight therapy cycles. Other previous treatments and interventions were surgery (45.8%), cytotoxic chemotherapy (33.3%), and somatostatin analogs (58.3%). All 14 patients receiving somatostatin analogs (58.3%) continued treatment during treatment with everolimus in our study.

Twenty-two patients started with a daily dose of 10 mg everolimus and two patients started with a daily dose of 5 mg. Dose adjustments were required in 16.7% of the patients (two temporary reduction to 5 mg (8.3%) and two definite reductions to 5 mg (8.3%)).

Table 3. Response and duration of response in previous treatments (^{177}Lu-octreotate and chemotherapy): multicenter (n=24).

	Total		Best Response								Duration of response	
	Patients		PR		SD		PD		NA		Median	Range
	n	%	n	%	n	%	n	%	n	%	(months)	(months)
^{177}Lu-octreotate												
Cycles 1-4	24	100	8	33.3	14	58.3	1	4.2	1	4.2	25.9	0 – 98.8
Additional ^{177}Lu-octreotate												
Cycles 5, 5-6, 5-7*	11	45.8	0	0.0	10	41.7	1	4.2	0	0.0	26.5	0 – 45.4
Cycles 7-8	3	12.5	1	4.2	2	8.3	0	0.0	0	0.0	19.2	14.8 – 21.8
Chemotherapy												
1 regimen	7	29.2	1	4.2	3	12.5	2	8.3	1	4.2	3.5	0 – 56
>1 prior regimen **	1	4.2	0	0.0	1	4.2	0	0.0	0	0.0	NA	NA

NA, not available.

**Cycle 5 (n=1), cycles 5–6 (n=9), and cycles 5–7 (n=1).*

***Patients had already received one regimen of chemotherapy.*

Temporary interruptions were required in 29.2% of the patients. The median cumulative time of treatment with everolimus was 10.2 months (range 0.6–25.4 months). At the time the analysis was performed, treatment was ongoing for 45.8% of the patients. The primary reasons for discontinuation of treatment included radiological confirmed PD (29.2%), development of an unacceptable toxic adverse event (16.7%), and death (8.3%).

Safety

Most adverse events in the current study were grade 1 or 2. Table 4 shows the adverse events of everolimus and ^{177}Lu-octreotate therapies in patients with metastatic well-differentiated and moderately differentiated GEP-NETs compared with that reported in the RADIANT-3 trial[8] and the Rotterdam ^{177}Lu-octreotate trial[12]. The newly occurring adverse events after initiation of everolimus therapy in this study and those in patients treated with everolimus after failure of cytotoxic chemotherapy[6] are also listed in Table 4.

Most common new clinical adverse events occurring after initiation of everolimus therapy in this study were oromucosal sequelae (41.7%), pneumonitis (37.5%), fatigue (33.3%), peripheral edema (25%), and rash (25%). Drug-induced pneumonitis was mainly a radiological finding (1–9 months after initiation of everolimus therapy) without clinical consequences and did not lead to interruption or discontinuation of everolimus therapy.

The most common grade 3 or 4 drug-related adverse events that occurred after initiation of everolimus therapy were hyperglycemia (12.5%), fatigue (8.3%), thrombocytopenia (8.3%), and elevated alanine transaminase levels (8.3%).

Table 4. Adverse events for everolimus and ^{177}Lu-DOTA^{0}-Tyr^{3}-octreotate therapies in patients with metastatic well-differentiated or moderately differentiated GEP-NETs as reported in RADIANT-3 and ^{177}Lu-DOTA^{0}-Tyr^{3}-octreotate trials. This table includes the adverse events for everolimus after failure of cytotoxic chemotherapy and this study.

	Everolimus		^{177}Lu-octreotate		Everolimus post-Chemo		Everolimus post ^{177}Lu-octreotate	
Treatment	**N = 207**		**N = 504**		**N = 115**		**N = 24**	
	All Grades	**Grade 3/4**	**All Grades**	**Grade 3/4**	**All Grades**	**Grade 3/4**	**All Grades**	**Grade 3/4**
Adverse event	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Oral problems *	64	7	NR	NR	45.2	4.3	41.7	4.2
Rash	49	<1	NR	NR	40	0.9	25	4.2
Diarrhea	34	3	NR	NR	39.1	3.5	4.2	4.2
Fatigue	31	2	NR	NR	31.3	4.3	33.3	8.3
Infections **	23	2	NR	NR	NR	NR	8.3	0
Nausea	20	2	25	0	29.6	0.9	4.2	0
Peripheral edema	20	<1	NR	NR	14.8	0	25	0
Decreased appetite	20	0	NR	NR	NR	NR	4.2	0
Headache	19	0	NR	NR	21.7	0	4.2	0
Dysgeusia	17	0	NR	NR	10.4	0	16.7	0
Epistaxis	17	0	NR	NR	NR	NR	16.7	4.2
Pulmonic ***	17	2	NR	NR	6.1	0	37.5	0
Weight loss	16	0	NR	NR	14.8	0	ID	ID
Vomiting	15	0	10	0	17.4	0	4.2	0
Pruritus	15	0	NR	NR	12.2	0	8.3	4.2
Asthenia	13	1	NR	NR	14.8	5.2	4.2	0
Nail disorder	12	<1	NR	NR	NR	NR	8.3	0
Cough	11	0	NR	NR	NR	NR	12.5	0
Pyrexia	11	0	NR	NR	NR	NR	12.5	0
Dry skin	10	0	NR	NR	9.6	0	16.7	4.2
Abdominal pain	NR	NR	10	0	NR	NR	0	0
Hormone crisis	NR	NR	1	1	NR	NR	NR	NR
Hair loss	NR	NR	62	0	NR	NR	NR	NR

Table 4. Adverse events for everolimus and ^{177}Lu-DOTA^{0}-Tyr^{3}-octreotate therapies in patients with metastatic well-differentiated or moderately differentiated GEP-NETs as reported in RADIANT-3 and ^{177}Lu-DOTA^{0}-Tyr^{3}-octreotate trials. This table includes the adverse events for everolimus after failure of cytotoxic chemotherapy and this study. (continued)

	Everolimus		^{177}Lu-octreotate		Everolimus post-Chemo		Everolimus post ^{177}Lu-octreotate	
Treatment	**N = 207**		**N = 504**		**N = 115**		**N = 24**	
	All Grades	**Grade 3/4**	**All Grades**	**Grade 3/4**	**All Grades**	**Grade 3/4**	**All Grades Baseline/ New**	**Grade 3/4 Baseline/ New**
Laboratory event	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Anemia	17	6	0.4	0.3 / 0.1	13	4.3	(B:45.8) 20.9	(B:0) 0
Thrombocytopenia	13	4	2.7	1.9 / 0.8	7.8	2.6	(B:33.3) 16.7	(B:0) 8.3
Leukopenia	NR	NR	1.5	1.4 / 0.1	7	4.3	(B:20.8) 20.9	(B:0) 4.2
Myelodysplastic syndrome	NR	NR	0,8	0,8	NR	NR	NR	NR
Hyperglycemia	13	5	NR	NR	13	4.3	(B:25) 25.0	(B:8.3) 12.5
Hypercholesterolemia	NR	NR	NR	NR	NR	NR	(B:4.2) 16.6	(B:0) 0
ALT	NR	NR	1.0	0.6	NR	NR	(B:41.7) 12.5	(B:0) 8.3
AST	NR	NR	1.0	0.6	NR	NR	(B:45.8) 25.0	(B:0) 4.2
Kidney failure	NR	NR	NR	0.4	NR	NR	(B:29.2) 8.3	(B:0) 4.2

NR, Not Recorded; ID, Insufficient data, not routinely checked; (B:..) Baseline, before start everolimus; ALT alanine transaminase; AST aspartate transaminase.

** Included in this category are stomatitis, aphthous stomatitis, mouth ulceration and tongue ulceration.*

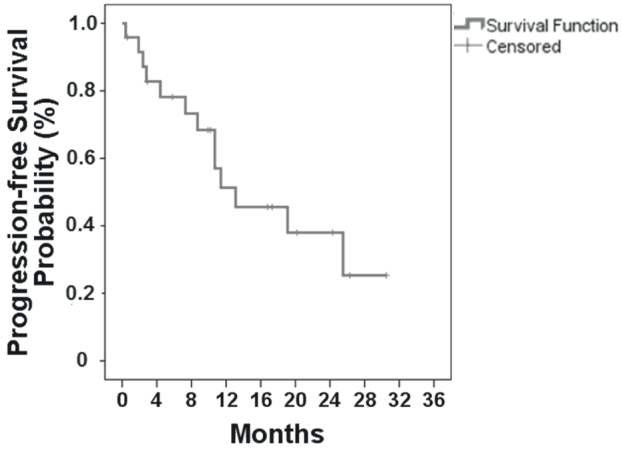
*** All types of infections are included.*

**** Included in this category are pneumonitis, interstitial lung disease, lung infiltrations and pulmonary fibrosis.*

Efficacy

The median PFS in our study was 13.1 months (95% CI 11.5–21.2; Figure 1). Best objective response rates were a partial response (PR) in 16.7% of the patients and stable disease (SD) in 62.5% of the patients whereas 12.5% of the patients continued having PD.

Figure 1. Progression-free survival: Kaplan-Meier curve, multicenter (n=24).



DISCUSSION

In this retrospective study, we evaluated the safety and efficacy profile of everolimus in well-differentiated or moderately differentiated (G1-G2) GEP-NET patients with documented disease progression after prior treatment with ^{177}Lu-octreotate. The median interval between the last ^{177}Lu-octreotate therapy and start of everolimus treatment was 18.7 months. Even 12 months after the last cycle of ^{177}Lu-octreotate, tumor responses can be observed (Kwekkeboom DJ, personal communication 2012). This international, multicenter study is, to our knowledge, the first study in which the occurrence of Grades 1–4 toxicities in patients treated with everolimus after peptide receptor radiotherapy was compared with the published safety profile of everolimus in GEP-NETs. Our findings with respect to safety were consistent with the known safety profile of everolimus established in earlier phase II and III studies, and most adverse events were either grade 1 or 2[5-8]. However, our results are not directly comparable with the results of prospective trials (RADIANT-trails) due to different study designs.

The most common drug-related adverse events in our study were oromucosal sequelae, which generally resolved during treatment. Other common and frequent adverse events were pulmonary (non-infectious pneumonitis and interstitial lung disease), fatigue, peripheral edema, and rash. Most adverse events were easily manageable, as evidenced by the low rate of discontinuation of treatment (16.7%).

We consider our patients to be a heavily pretreated group. The majority of our patients had received prior treatment with somatostatin analogs, chemotherapy, or a combination of those therapies. Somatostatin analog treatment was continued in all patients during treatment with everolimus in our study. All patients received treatment

with ^{177}Lu-octreotate and it is commonly known that with this therapy high cumulative dosages can cause serious side effects, including kidney failure, cytopenias, or myelodysplastic syndrome (MDS)[4]. With this knowledge, drug-related kidney failure was monitored in this study. However, data from the Rotterdam ^{177}Lu-octreotate trial show that serious hematological toxicity, MDS, and liver toxicity are relatively rare and occur in approximately 1% of the patients[12, 13]. Everolimus therapy can be associated with mild lymphopenia and neutropenia. In the current study, no MDS was reported, only two patients had a grades 1 and 2 infection and no grade 3 or 4 drug-related infections were reported. Grade 3 or 4 drug-related kidney failure and elevated transaminases occurred in 4.2% of the patients. The kidney failure can be most probably explained by the combined effect of the two therapies. Therefore, we can conclude that everolimus is well tolerated with regard to bone marrow function if PRRT has not lead to ongoing bone marrow suppression.

PFS results of this study are similar to the prolonged PFS that was reported in earlier phase II and III randomized trials, which included patients with advanced pancreatic NETs and gastrointestinal NETs[5-8]. In the initial open-label phase II study conducted at The MD Anderson Cancer Center (Houston, TX, USA), octreotide LAR (30 mg im) with everolimus (5 or 10 mg), a median PFS of 11.6 months (95% CI 7.1–16.1), was found[7]. In another open-label phase II study (RADIANT-1), patients were treated with everolimus in one arm vs. a combination of everolimus and octreotide LAR im in the other arm. Median PFS in the everolimus arm was 9.7 and 16.7 months in the everolimus plus octreotide LAR arm[6]. In another randomized phase III RADIANT-2 study in advanced NETs associated with carcinoid syndrome, it was demonstrated that everolimus plus octreotide LAR provided a clinically meaningful 5.1-month improvement in PFS in this patient population when compared with patients receiving placebo plus octreotide LAR[5]. The largest clinical trial, RADIANT-3, was a double-blind, placebo-controlled, randomized phase III study. In this study, everolimus therapy was associated with a 2.4-fold improvement in median PFS when compared with placebo (11.4 vs. 4.6 months)[8].

The median PFS of our current study was longer when compared with the RADIANT-3 trial (13.1 vs. 11.4 months) and shorter when compared with the RADIANT-1 trial (13.1 vs. 16.7 months). In the majority of patients (62.5%), SD was the best response, and PR was observed in 16.7% of patients. These results are similar to the response rates in the RADIANT-3 study. In this study, SD was achieved in 73% of patients[8]. Also in line with the RADIANT trials, a proportion of our patients (58%) was treated with somatostatin analogs. A potential bias with respect to the ongoing use of somatostatin analogs can, therefore, not be excluded. Together, the results support the clinical relevance and tolerability of everolimus in pancreatic NET and potentially in gastrointestinal NETs. Multicenter international trial with everolimus is still ongoing in patients with non-pancreatic NETs.

In conclusion, our results for pancreatic NET demonstrate that everolimus is an important and safe new drug option, including for those patients who show tumor progression after ^{177}Lu-octreotate radionuclide therapy.

REFERENCES

1. **Modlin IM, Oberg K, Chung DC, Jensen RT, de Herder WW, Thakker RV, Caplin M, Delle Fave G, Kaltsas GA, Krenning EP, Moss SF, Nilsson O, Rindi G, Salazar R, Ruszniewski P, Sundin A.** Gastroenteropancreatic neuroendocrine tumours. *The Lancet. Oncology.* 2008;9:61-72.

2. **Fraenkel M, Kim MK, Faggiano A, Valk GD.** Epidemiology of gastroenteropancreatic neuroendocrine tumours. *Best practice & research. Clinical gastroenterology.* 2012;26:691-703.

3. **Rinke A, Muller HH, Schade-Brittinger C, Klose KJ, Barth P, Wied M, Mayer C, Aminossadati B, Pape UF, Blaker M, Harder J, Arnold C, Gress T, Arnold R, Group PS.** Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2009;27:4656-4663.

4. **Bergsma H, van Vliet EI, Teunissen JJ, Kam BL, de Herder WW, Peeters RP, Krenning EP, Kwekkeboom DJ.** Peptide receptor radionuclide therapy (PRRT) for GEP-NETs. *Best practice & research. Clinical gastroenterology.* 2012;26:867-881.

5. **Pavel ME, Hainsworth JD, Baudin E, Peeters M, Horsch D, Winkler RE, Klimovsky J, Lebwohl D, Jehl V, Wolin EM, Oberg K, Van Cutsem E, Yao JC, Group R-S.** Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet.* 2011;378:2005-2012.

6. **Yao JC, Lombard-Bohas C, Baudin E, Kvols LK, Rougier P, Ruszniewski P, Hoosen S, St Peter J, Haas T, Lebwohl D, Van Cutsem E, Kulke MH, Hobday TJ, O'Dorisio TM, Shah MH, Cadiot G, Luppi G, Posey JA, Wiedenmann B.** Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumors after failure of cytotoxic chemotherapy: a phase II trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2010;28:69-76.

7. **Yao JC, Phan AT, Chang DZ, Wolff RA, Hess K, Gupta S, Jacobs C, Mares JE, Landgraf AN, Rashid A, Meric-Bernstam F.** Efficacy of RAD001 (everolimus) and octreotide LAR in advanced low- to intermediate-grade neuroendocrine tumors: results of a phase II study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2008;26:4311-4318.

8. **Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Oberg K, Rad001 in Advanced Neuroendocrine Tumors TTSG.** Everolimus for advanced pancreatic neuroendocrine tumors. *The New England journal of medicine.* 2011;364:514-523.

9. **Rindi G, Kloppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, Erikssson B, Falchetti A, Falconi M, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B, all other Frascati Consensus Conference p, European Neuroendocrine Tumor S.** TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Archiv : an international journal of pathology.* 2006;449:395-401.

10. **Rindi G, Kloppel G, Couvelard A, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B.** TNM staging of midgut and hindgut (neuro)

endocrine tumors: a consensus proposal including a grading system. *Virchows Archiv : an international journal of pathology.* 2007;451:757-762.

11. **Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG.** New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *Journal of the National Cancer Institute.* 2000;92:205-216.

12. **Kwekkeboom DJ, de Herder WW, Kam BL, van Eijck CH, van Essen M, Kooij PP, Feelders RA, van Aken MO, Krenning EP.** Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0,Tyr3]octreotate: toxicity, efficacy, and survival. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2008;26:2124-2130.

13. **van Essen M, Krenning EP, Kam BL, de Herder WW, Feelders RA, Kwekkeboom DJ.** Salvage therapy with (177)Lu-octreotate in patients with bronchial and gastroenteropancreatic neuroendocrine tumors. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine.* 2010;51:383-390.

Chapter 9

Sequential everolimus and sunitinib treatment in pancreatic metastatic well-differentiated neuroendocrine tumors resistant to prior treatments

Anna Angelousi[1]*, Kimberly Kamp[2]*, Maria Kaltsatou[1], Dermot O'Toole[3], Gregory Kaltsas[1]**, Wouter W. de Herder[2]**

[1]*Department of Pathophysiology, Sector of Endocrinology, National University of Athens, Greece.*
[2]*Department of Internal Medicine, Sector of Endocrinology, ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, the Netherlands*
[3]*Department of Gastroenterology, National NET Center at St. Vincent's University and St. James Hospital & Trinity College, Dublin, Ireland*

*joint first authors
**joint last authors

Submitted

ABSTRACT

Objective: Alternating treatment with sunitinib and everolimus has shown to be efficacious in renal cell carcinoma. However, no data exist for the role of alternate sequence administration of these molecules in well- and moderate-differentiated pancreatic neuroendocrine tumors (NETs).

Methods: Thirty-one patients were administered one compound and upon progression were changed to the other. All patients had Grade 1 or 2 tumors and Stage IV disease with similar metastatic pattern. The primary endpoint of the study was to calculate the progression-free survival (PFS), estimate overall survival (OS) and the development of adverse events (AEs).

Results: Median PFS after first-line everolimus was longer (16.3 months) compared to sunitinib (9 months) but not statistically significant (P=0.15). Upon progression, sequential second-line treatment with both agents showed no difference in the PFS (15.5 months for everolimus vs. 10.3 months for sunitinib, P=0.3). The order of the sequential treatment did not influence the overall median PFS HR=0.94 (95% CI: 0.45-1.97). No difference in OS between the two groups was observed. Discontinuation of treatment because of serious adverse events was less frequent with everolimus either as a first- or second-line treatment compared to sunitinib.

Conclusions: Treatment with sequential MT agents resulted in a clinical benefit regarding PFS although not statistical significant. Everolimus seemed to exert a longer PFS either as first- or second-line treatment compared to sunitinib, albeit without statistical significance. Larger prospective studies are required to investigate the efficacy of alternate sequence therapy with molecular targeting agents in metastatic pancreatic NETs.

INTRODUCTION

Digestive neuroendocrine tumors (NETs) arise from neuroendocrine cells of the pancreas (pancreatic NETs) and the gastrointestinal tract[1]. Although pancreatic NETs represent approximately 1.3% of all cases of pancreatic cancer their incidence and prevalence are increasing[1, 2]. Approximately 90% of pancreatic NETs are non-functional and are frequently diagnosed at a late stage. Indeed, patients presenting with unresectable or metastatic disease exhibit a worse prognosis with a median overall survival (OS) of 17 months compared to those diagnosed at earlier stages that exert a median OS of 100 months[3]. However, during the last years the median survival of patients with metastatic pancreatic NETs has improved substantially, reaching 41.7 months following the introduction of newer chemotherapeutic agents[4].

Recently the findings of a number of phase II and III studies have also documented the efficacy of molecular targeted (MT) therapies in pancreatic NETs[2, 5, 6]. Everolimus that inhibits the mammalian target of rapamycin (mTOR), a serine–threonine kinase that stimulates cell growth, proliferation, and angiogenesis[2, 5] has obtained a 65% reduction in the estimated risk for disease progression or death compared to placebo and a significant 6.4 month increase in the median progression-free survival (PFS) in advanced metastatic pancreatic NETs that had progressed despite previous treatment[6].

Vascular endothelial growth factor (VEGF) receptors inhibitors are also an alternative therapy option for patients with metastatic pancreatic NETs[7]. Sunitinib, a potent multi-targeted receptor of tyrosine kinase inhibitor with direct anti-tumor and anti-vascular effect, has been shown to selectively inhibit mutiple receptor tyrosine kinases (RTKs) that are implicated in tumour growth, neoangiogenesis and metastatic progression of cancer[8, 9]. A phase III trial evaluated sunitinib vs. placebo in patients with low- and intermediate-grade pancreatic NETs and found a statistically significant improvement in the PFS in the sunitinib arm (11.4 months vs. 5.5 months; $P<0.001$)[7].

Recent position statements and guidelines, propose that everolimus could be used as a treatment option after failure of chemotherapy in pancreatic NETs[10, 11]. Furthermore, it could also be considered as first-line therapy in selected cases as an alternative treatment to loco-regional therapies or chemotherapy[10]. Sunitinib should be considered as first-line therapy only in selected cases as an alternative treatment option if treatment with somatostatin analogues (SSAs), chemotherapy and/or loco-regional therapies is not feasible or promising[10, 11]. The efficacy of sunitinib appears to be similar regardless of the number of previous treatments or previous exposure to SSAs[12].

These two agents are standard therapy for other types of cancer such as metastatic renal carcinoma[13, 14]. Current treatment guidelines for patients with metastatic renal cell cancer recommend a first-line challenge with a vascular epithelial growth factor (VEGF) inhibitor, including sunitinib, followed by everolimus at progression[13, 14]. Recent trials

have shown the non-inferiority of everolimus compared to sunitinib as first-line treatment and support the standard treatment paradigm of first-line sunitinib followed by everolimus at progression[15].

In the present study we aim to investigate whether MT therapy with everolimus and sunitinib administrated sequentially in patients with advanced well-differentiated pancreatic NETs, that progressed following the administration of one of these agents, could be tolerated and affect disease progression and survival.

PATIENTS AND METHODS

Patients

We studied 31 patients with advanced pancreatic NETs with Stage IV/IIIB disease at initial diagnosis according to Response Evaluation Criteria in Solid tumors (RECIST) criteria who had documented disease progression besides different prior therapeutic modalities (surgery, peptide receptor radiotherapy treatment (PPRT), chemotherapy, SSAs and temozolomide). All patients had Stage IV disease at the time of initiation of MT therapies, similar tumor load, in respect of the different organs involved and the number of metastases found in each organ, and similar grading as shown by the Ki-67 proliferative index value. The study protocol was approved by the ethics committee of both participating centers, according to the 3rd edition of the guidelines on the practice of ethical committees in Medical Research. All patients gave informed consent according to the Declaration of Helsinki and Good Clinical Practice guidelines.

Study design and treatment

In this retrospective study, an analysis of institutional collected databases from two centers was performed. The study included consecutive patients with metastatic pancreatic NETs (Stage IV) resistant to prior therapeutic modalities who received a MT agent, everolimus (n=20) or sunitinib (n=11) as a first-line treatment followed by a second challenge by the other MT agent. Two patients had received no prior treatment, before the initiation of MT therapy. Patients received each drug until documented disease progression according to RECIST criteria or development of unacceptable toxicity. The everolimus full dosage was 10 mg daily, and the sunitinib 37.5 mg daily. However, dose modifications were permitted in the presence of adverse events (AEs), so that everolimus could be decreased to 5 mg and sunitinib to 25 mg respectively.

Primary and secondary endpoints

The primary endpoint was to assess median PFS in months and rate during different periods of the treatment with each agent. Median PFS period was assessed in both

groups after the first-line treatment (defined as the interval from the beginning to the discontinuation of the first-line treatment because of disease progression or toxicity) and the second-line treatment (defined as the interval from the beginning to the discontinuation of the second-line treatment because of disease progression, toxicity or death). All median PFS estimations were expressed in months. Secondary endpoints included the calculation of the OS defined as the time from the beginning of the MT therapy to death. Hazard ratios (HR) were also calculated for the PFS and OS as well as for the order of the sequencing of the treatment. Incidence of progressive disease (PD) and AEs were also studied. AEs were classified as grade 1 (mild and transient effect), grade 2 (moderate), grade 3 (severe necessitating medical intervention) and grade 4 (potentionally life theratening necessitating hospitalisation and medical intervention). Discontinuation of the treatment was decided when the AEs were grade III or IV and dosage modifications for grade I or II.

Statistical analysis

Statistical analysis was performed using Stata 10 (StataCorp. 2007. Stata Statistical Software: Release 10. College Station, TX: StataCorp LP). The survival analysis was performed using Kaplan-Meier methods and the results were compared using the log-rank test. Cox proportional hazard models (HR) with 95% confidence intervals (CI) were applied adapted for confounding factors such as age and gender. Results were considered significant at $P<0.05$ (95% CI). The distribution of continuous variabes is reported as median values or mean ± standard deviation (SD). Recurrent events analysis was performed using the Andersern and Gill Cox regression model[16].

RESULTS

Patient population

Thirty-one patients (8 female, mean age 52.3±12 years) were recruited from two European Centers (Department of Pathophysiology, Sector of Endocrinology, Laiko hospital, University of Athens, Greece and Department of Internal Medicine, Sector of Endocrinology, Erasmus MC, Rotterdam, the Netherlands). Twenty-three patients had Stage IV and four patients had Stage IIIB disease at initial diagnosis; however, at the initiation of MT therapy all patients had Stage IV disease. Nineteen (61%) patients had Grade 2 and 8 (26%) had Grade 1 tumors (in the remaining 4, the Grade was unknown). Twenty patients received first-line everolimus followed by sunitinib, and 11 were assigned to first-line sunitinib followed by everolimus. The most frequent previous therapeutic modalities included SSAs (in 58% cases), PPRT (in 58% cases), surgical ablation (in 55% cases) and chemotherapy (in 26% cases) **(**Table 1**).**

Table 1. Characteristics and comparison of the studied groups (sunitinib to everolimus group vs. everolimus to sunitinib group).

Characteristics	Sunitinib -> Everolimus	Everolimus ->Sunitinib	P
N	11	20	
Age (median, years)	51.5	57.9	
Sex (m)	9 (81%)	14 (80%)	
Stage	all Stage IV	all Stage IV	
Grade			
1	4 (36%)	4 (25%)	
2	6 (55%)	13 (65%)	
Unknown	1 (1%)	3 (15%)	
Ki-67, mean±SD (median)	5.0 ± 3.9 (5)	6.0 ± 3.5 (5)	0.33
Familiar	1 (MEN1)	3 (all MEN1)	
Functional status	3 (27%)	13 (65%)	
Previous treatment			
Surgery	7 (63%)	11 (55%)	
Chemotherapy	4 (36%)	4 (25%)	
PRRT	4 (36%)	14 (70%)	
SSAs	8 (72%)	13 (65%)	
TACE	1 (9%)	2 (10%)	
Radiofrequency	3 (27%)	1 (5%)	
Follow up, mean±SD (median)	11.7 ± 11.8 (7.4)	14.32 ± 12.85 (10.4)	0.6
Median PFS for pancreatic NETs*	9	16.3	0.15
Median PFS for pancreatic NETs **	15.5	10.4	0.3
Serious AEs (total)	5 (45%)	5 (25%)	0.6
Progression disease	7 (63%) after 1st line	18 (90%) after 1st line	0.3
	6 (55%) after 2nd line	12 (60%) after 2nd line	

** After first-line treatment with everolimus or sunitinib (before the switch)*

*** After second-line treatment with everolimus or sunitinib*

Abbreviations: PPRT= peptide receptor radionuclide therapy , SSAs= somatostatin analogs, TACE=transarterial chemo-embolization, SD=standard deviation , PFS= progression-free survival, AEs=adverse events.

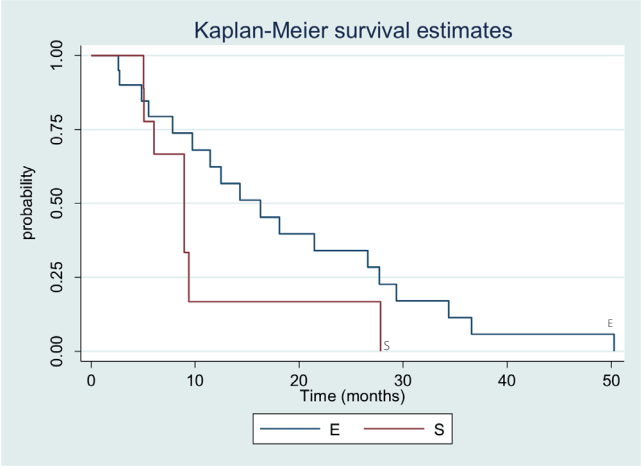
Sixteen patients (52%) had a functional pancreatic NET. Tumor load was similar in all patients. The median Ki-67 index was 5% in both groups (mean values = 6.0±3.5 for everolimus to sunitinib group vs. 5.0±3.9 for the sunitinib to everolimus group, P=0.33). All 20 patients from the group that received first-line everolimus had liver metastases, in 13 of whom (65%) were multiple (>5), 4 had also bone metastases and 2 pulmonary metastases. All 11 patients from the first-line sunitinib group had liver metastases, in 7 of whom (63%) were multiple (>5), one had also bone metastases, one peritoneal metastases and another one pulmonary metastases.

Primary endpoint

Median PFS was longer although not statistical significant (P=0.15), in patients with pancreatic NETs treated with first-line everolimus (n=20, median PFS=16.3 months) compared to sunitinib (n=11, median PFS=9 months) with a HR of 1.98 (95% CI: 0.77-5.14) for the sunitinib vs. the everolimus group (Figure 1). Median PFS for patients who received second-line treatment with everolimus (n=11, median PFS=15.5 months) was slightly longer but not statistical different (P=0.30) compared to that of patients with second-line sunitinib treatment (n=20, median PFS=10.3 months) with a HR of 1.66 (95% CI: 0.63-4.36) for the sunitinib vs. the everolimus group (Figure 2). The 2-year PFS rate during the first-line treatment with everolimus was 0.34 (95% CI: 0.14-0.55) and 0.17 (95% CI: 0.01-0.51) with sunitinib. The 2-year PFS rate during the second-line treatment with everolimus was 0.27 (95% CI: 0.04-0.58) and 0.11 (95% CI: 0.01-0.36) with sunitinib.

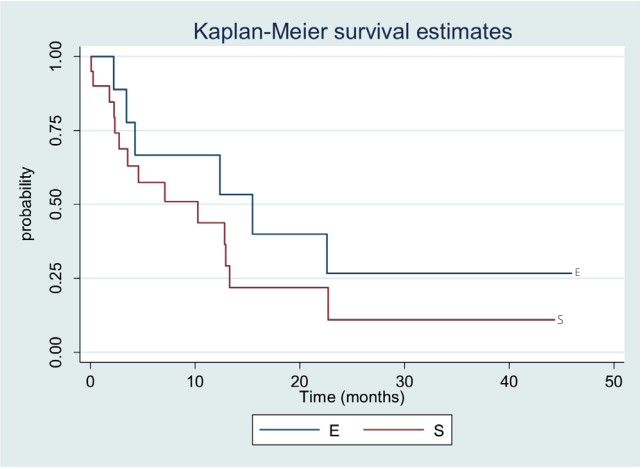
No significant differences were found for the overall median PFS from the beginning until the discontinuation of the MT therapies. In particular, the HR for the sunitinib to everolimus group vs. the everolimus to sunitinib group was 0.94 (95% CI: 0.45-1.97), implying that the order of the sequencing of the treatment did not exert a significant role to the median PFS time.

Figure 1. Kaplan-Meier plots for progression-free survival (PFS) in months after first-line treatment with everolimus or first-line with sunitinib.



E: Median PFS= 16.3 months
S: Median PFS= 9 monts

Figure 2. Kaplan-Meier plots for progression-free survival (PFS) in months after second-line treatment with everolimus or second-line with sunitinib.



E: Median PFS= 15.5 months
S: Median PFS= 10.3 months

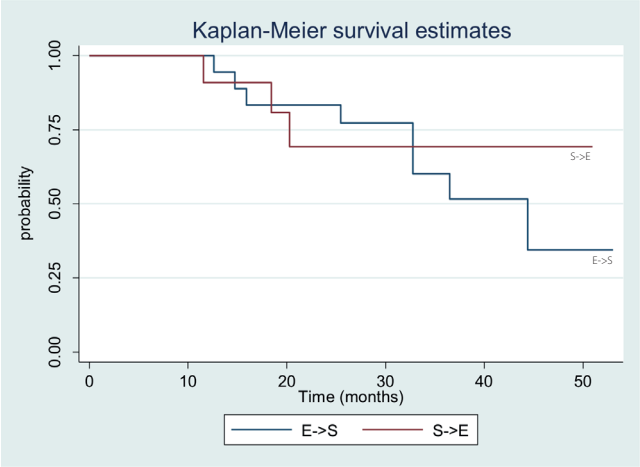
Secondary endpoint

No statistical significant differences were found in the median OS time in both groups (P=0.875) (Figure 3). The HR for the sunitinib to everolimus group vs. the everolimus to sunitinib group was 0.90 (95% CI: 0.23-3.45). The 2-year survival rate in the everolimus to sunitinib group was 0.83 (95% CI: 0.57-0.94) compared to 0.69 (95% CI: 0.31-0.89) in the sunitinib to everolimus group.

Disease progression after first-line treatment with everolimus was observed in 18 out of 20 patients with pancreatic NETs (90%) after a median duration of 13.6 months and in 7 out of 11 patients (63%) after first-line treatment with sunitinib after a median duration of 7.4 months. Disease progression after second-line treatment was observed in 12 out of 20 patients (60%) in the everolimus to sunitinib group after a median duration of 7.2 months, and in 6 out of 11 patients in the sunitinib to everolimus group (54.5%) after a median follow up of 7.0 months.

Overall mortality rate for both groups was 35% (11 patients out of the total 31). Eight of them (26%) were from the everolimus to sunitinib group and 3 of them (27%) from the sunitinib to everolimus group. All deaths were considered disease-related and were observed during the second-line treatment period with both agents.

Figure 3. Kaplan-Meier plots for the overall survival in the two groups.



HR = 0.90 (95% CI: 0.23-3.45)

Adverse events

During first-line treatment with everolimus, 2 out of 20 patients (10%) discontinued treatment because of serious AEs compared to 4 out of 11 patients who received first-line sunitinib (36%). During the second-line treatment, 3 out of 20 patients (15%) discontinued treatment due to serious AEs following sunitinib administration in the everolimus to sunitinib group and one out of 11 patients after everolimus (9%) in the sunitinib to everolimus group. Analysis for each group separately showed that 5 out of 20 patients (25%) discontinued treatment because of serious AEs in the everolimus to sunitinib group vs. 5 out of 11 (45%) in the sunitinib to everolimus group; however, these differences were not statistical significant different (P=0.6). Serious AEs after the first-line treatment with either single agent were less compared to those after the sequential treatment with the two agents (10% for everolimus as single agent treatment vs. 25% for the everolimus followed by sunitinib group, P=0.40, and 36% for sunitinib as single agent treatment vs. 45% for the sunitinib followed by the everolimus group, P=1.0). A higher percentage of patients required dose reduction during first-line (16%) and second-line (18%) treatment with everolimus compared to sunitinib (9% for first-line and 8% for second-line).

The overall frequency of AEs of all grades was 45% for the everolimus to sunitinib group and 80% for the sunitinib to everolimus group (P=0.13). Anemia was the most frequent for everolimus to sunitinib group treatment and fatigue/asthenia for sunitinib to everolimus group.

DISCUSSION

This is the first study in pancreatic NETs showing that sequential treatment with currently available MT agents is feasible and associated with clinical meaningful additive PFS in patients with pancreatic NETs. Our findings show that a second challenge with a MT agent in patients with pancreatic NETs that have progressed following initial challenge with another MT agent, is associated with a clinical useful PFS although not statistically significant. These findings were obtained with relative moderate toxicity that necessitated treatment discontinuation in 25% of patients in the everolimus to sunitinib group and in 45% of patients in the sunitinib to everolimus group. In addition, the frequency of serious AEs was lower in patients treated with everolimus compared to sunitinib albeit without statistical significance.

Pancreatic NETs are in their great majority well-differentiated tumors and generally more indolent than adenocarcinomas; however, once these tumors progress beyond surgical resectability are essentially incurable[2]. Systemic treatment options have substantially been expanded in recent years with long acting SS analogues regarded as the first therapeutic option for well-differentiated pancreatic NETs with a Ki-67 index of <10%[17]. However, the majority of patients will develop progressive disease necessitating further systemic therapy. Following progression different therapeutic options are available including cytotoxic chemotherapy, PRRT and the administration of MT agents[18, 19]. Currently, there are no comparative studies evaluating the ideal sequence of these therapies upon progression although several algorithms have been proposed by relevant scientific societies taking into consideration parameters such as Ki-67 index, tumor growth rate, and disease burden[11, 19]. It has been suggested by the European Society of Neuroendocrine Tumors (ENETS) that everolimus could be used as first-line treatment whereas sunitinib should be considered in patients in whom other therapeutic modalities have failed[10]. However, the issue of sequential treatment with currently available MT agents has not been formally addressed up to now.

Alternate sequence of sunitinib and everolimus has been well studied in advanced renal cell carcinoma where sunitinib and everolimus are both approved as first-line and second-line therapies respectively[13, 14]. A prospective clinical trial in patients with advanced renal cancer (RECORD-3) comparing sunitinib until progression followed by everolimus vs. the reverse sequence, has shown the non-inferiority of everolimus compared to sunitinib in terms of PFS and OS, with however cross-over of patients (43-45%) from one group to the other[15]. Sunitinib exhibited a slightly longer PFS and OS without though reaching statistical significance[15]. In the RECORD-1 trial that compared everolimus to placebo after sunitinib or sorafenib failure in advanced renal cell carcinoma, median PFS was 4.9 months vs. 1.9 months compared to the placebo group[20]. These data suggest that a programmed sequential strategy administering sunitinib and everolimus

without waiting for disease progression may be considered as a potential option in patients with advanced renal cell carcinoma.

According to our data, the more beneficial effect of everolimus as first-line treatment in the median PFS of patients with pancreatic NETs compared to sunitinib, albeit not statistically significant, is in line with the standard treatment paradigm according to the ENETS guidelines[10]. Interestingly, second-line challenging treatment with everolimus in patients already treated with sunitinib seems to prolong more the median PFS time compared to the second-line treatment with sunitinib in patients already treated with everolimus, without though obtaining statistical significant difference. The order of the sequential treatment when comparing the 2 groups did not seem to have an effect in terms of the PFS.

Although there are no clinical data evaluating the effects of a second-line challenge treatment with a MT agent in pancreatic NETs, *in vitro* studies offer a better understanding of the mechanism underlying the role of the sequence of treatment of MT agents. The sequential combinations of everolimus and sunitinib in a renal cancer mouse model induced anti-angiogenic effects, leading to tumor necrosis[21]. In particular, everolimus addition after 5 weeks of sunitinib exposure of renal cancer cells slowed tumor growth[21]. Alternate sequence of sunitinib and everolimus mitigated the development of mesenchymal phenotype compared with sunitinib as single agent[22]. In particular mice, treated with sunitinib monotherapy, presented with controlled tumors characterized by lower vessel size compared to progressive tumors, while everolimus was more efficient on mature vessels than sunitinib that mainly affected unstable neoangiogenesis[22].

While the choice of targeted therapies in other malignancies is frequently driven by the findings of the precise molecular alterations present in the tumor, no such study has been done in NETs. This is particularly important given that the survival of patients with malignant NETs appears to be different based on the driver mutation(s) present in the tumor. An on-going study includes the analysis of the involved cell signaling pathways for the treatment of patients with advanced metastatic gastrointestinal and pancreatic NETs with currently approved MT agents according to the mutations present [sunitinib for *MEN1/PDGFR/KIT/FLT3* mutations, or everolimus for *NF1/PTEN/PI3K/AKT/mTOR/VHL/TP53* mutations][23].

Because of its design, the present study has its major inherent limitation in the retrospective nature of the analysis. This limitation, a common feature in most observational studies, may explain the allocation of different number of patients in each group and the different duration of treatment. Unfortunately, the small sample size didn't provide sufficient statistical power in any of our findings. Additionally, the lack of a separate group of patients receiving monotherapy with a MT agent did not permit a direct comparison between the single agent treatment and the combination of the two agents regard-

ing the PFS and the OS or mortality. However, this is the first clinical study comparing sequential challenge treatment in this rare category of neoplasms.

In summary, our results support current guidelines showing that in metastatic and resistant to prior therapeutic modalities pancreatic NETs, first-line treatment with everolimus increases the PFS. Although meaningful responses may be obtained following second challenge treatment, the small sample size and the retrospective nature of our study did not allow a robust conclusion to be made. However, these data provide evidence that programmed alternating sequential strategies studied in a prospective manner could document the therapeutic efficacy of this approach in patients with advanced metastatic pancreatic NETs.

REFERENCES

1. **Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB.** One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *Journal of clinical oncology.* 2008;26:3063-3072.

2. **Yao JC, Lombard-Bohas C, Baudin E, Kvols LK, Rougier P, Ruszniewski P, Hoosen S, St Peter J, Haas T, Lebwohl D, Van Cutsem E, Kulke MH, Hobday TJ, O'Dorisio TM, Shah MH, Cadiot G, Luppi G, Posey JA, Wiedenmann B.** Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumors after failure of cytotoxic chemotherapy: a phase II trial. *Journal of clinical oncology.* 2010;28:69-76.

3. **Halfdanarson TR, Rabe KG, Rubin J, Petersen GM.** Pancreatic neuroendocrine tumors (PNETs): incidence, prognosis and recent trend toward improved survival. *Annals of oncology.* 2008;19:1727-1733.

4. **Chan JA, Stuart K, Earle CC, Clark JW, Bhargava P, Miksad R, Blaszkowsky L, Enzinger PC, Meyerhardt JA, Zheng H, Fuchs CS, Kulke MH.** Prospective study of bevacizumab plus temozolomide in patients with advanced neuroendocrine tumors. *Journal of clinical oncology.* 2012;30:2963-2968.

5. **Yao JC, Phan AT, Chang DZ, Wolff RA, Hess K, Gupta S, Jacobs C, Mares JE, Landgraf AN, Rashid A, Meric-Bernstam F.** Efficacy of RAD001 (everolimus) and octreotide LAR in advanced low- to intermediate-grade neuroendocrine tumors: results of a phase II study. *Journal of clinical oncology.* 2008;26:4311-4318.

6. **Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Oberg K, Rad001 in Advanced Neuroendocrine Tumors TTSG.** Everolimus for advanced pancreatic neuroendocrine tumors. *The New England journal of medicine.* 2011;364:514-523.

7. **Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, Chen JS, Horsch D, Hammel P, Wiedenmann B, Van Cutsem E, Patyna S, Lu DR, Blanckmeister C, Chao R, Ruszniewski P.** Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *The New England journal of medicine.* 2011;364:501-513.

8. **Houk BE, Bello CL, Poland B, Rosen LS, Demetri GD, Motzer RJ.** Relationship between exposure to sunitinib and efficacy and tolerability endpoints in patients with cancer: results of a pharmacokinetic/pharmacodynamic meta-analysis. *Cancer chemotherapy and pharmacology.* 2010;66:357-371.

9. **Kulke MH, Lenz HJ, Meropol NJ, Posey J, Ryan DP, Picus J, Bergsland E, Stuart K, Tye L, Huang X, Li JZ, Baum CM, Fuchs CS.** Activity of sunitinib in patients with advanced neuroendocrine tumors. *Journal of clinical oncology.* 2008;26:3403-3410.

10. **Falconi M, Bartsch DK, Eriksson B, Kloppel G, Lopes JM, O'Connor JM, Salazar R, Taal BG, Vullierme MP, O'Toole D, Barcelona Consensus Conference p.** ENETS Consensus Guidelines for the management of patients with digestive neuroendocrine neoplasms of the digestive system: well-differentiated pancreatic non-functioning tumors. *Neuroendocrinology.* 2012;95:120-134.

11. **Kamp K, Damhuis RA, Feelders RA, de Herder WW.** Occurrence of second primary malignancies in patients with neuroendocrine tumors of the digestive tract and pancreas. *Endocrine-related cancer.* 2012;19:95-99.

12. **Pavel ME, Hainsworth JD, Baudin E, Peeters M, Horsch D, Winkler RE, Klimovsky J, Lebwohl D, Jehl V, Wolin EM, Oberg K, Van Cutsem E, Yao JC, Group R-S.** Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet.* 2011;378:2005-2012.

13. **Escudier B, Eisen T, Porta C, Patard JJ, Khoo V, Algaba F, Mulders P, Kataja V, Group EGW.** Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of oncology.* 2012;23 Suppl 7:vii65-71.

14. **Kanakis G, Kamp K, Tsiveriotis K, Feelders RA, Zormpala A, de Herder WW, Kaltsas G.** The prevalence and relevance of adrenal masses in patients with sporadic gastroenteropancreatic neuroendocrine tumours (GEP-NET). *Clinical endocrinology.* 2013;78:950-956.

15. **Motzer RJ, Barrios CH, Kim TM, Falcon S, Cosgriff T, Harker WG, Srimuninnimit V, Pittman K, Sabbatini R, Rha SY, Flaig TW, Page R, Bavbek S, Beck JT, Patel P, Cheung FY, Yadav S, Schiff EM, Wang X, Niolat J, Sellami D, Anak O, Knox JJ.** Phase II randomized trial comparing sequential first-line everolimus and second-line sunitinib versus first-line sunitinib and second-line everolimus in patients with metastatic renal cell carcinoma. *Journal of clinical oncology.* 2014;32:2765-2772.

16. **de Herder WW.** Biochemistry of neuroendocrine tumours. *Best practice & research. Clinical endocrinology & metabolism.* 2007;21:33-41.

17. **Caplin ME, Pavel M, Cwikla JB, Phan AT, Raderer M, Sedlackova E, Cadiot G, Wolin EM, Capdevila J, Wall L, Rindi G, Langley A, Martinez S, Blumberg J, Ruszniewski P, Investigators C.** Lanreotide in metastatic enteropancreatic neuroendocrine tumors. *The New England journal of medicine.* 2014;371:224-233.

18. **Fine RL, Gulati AP, Krantz BA, Moss RA, Schreibman S, Tsushima DA, Mowatt KB, Dinnen RD, Mao Y, Stevens PD, Schrope B, Allendorf J, Lee JA, Sherman WH, Chabot JA.** Capecitabine and temozolomide (CAPTEM) for metastatic, well-differentiated neuroendocrine cancers: The Pancreas Center at Columbia University experience. *Cancer chemotherapy and pharmacology.* 2013;71:663-670.

19. **Sorbye H, Strosberg J, Baudin E, Klimstra DS, Yao JC.** Gastroenteropancreatic high-grade neuroendocrine carcinoma. *Cancer.* 2014;120:2814-2823.

20. **Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grunwald V, Thompson JA, Figlin RA, Hollaender N, Kay A, Ravaud A, Group R-S.** Phase 3 trial of everolimus for metastatic renal cell carcinoma : final results and analysis of prognostic factors. *Cancer.* 2010;116:4256-4265.

21. **Rosa R, Damiano V, Nappi L, Formisano L, Massari F, Scarpa A, Martignoni G, Bianco R, Tortora G.** Angiogenic and signalling proteins correlate with sensitivity to sequential treatment in renal cell cancer. *British journal of cancer.* 2013;109:686-693.

22. **Lane HA, Wood JM, McSheehy PM, Allegrini PR, Boulay A, Brueggen J, Littlewood-Evans A, Maira SM, Martiny-Baron G, Schnell CR, Sini P, O'Reilly T.** mTOR inhibitor RAD001 (everolimus) has antiangiogenic/vascular properties distinct from a VEGFR tyrosine kinase inhibitor. *Clin Cancer Res.* 2009;15:1612-1622.

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

Chapter 10

General Discussion

GENERAL DISCUSSION

Progress has been made in the knowledge, diagnosis and treatment of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) over the past decades. In this chapter, the implications of the findings in the studies, as described in this thesis, are discussed in the light of the current and future diagnostics and treatments of GEP-NET patients.

EPIDEMIOLOGY

Over the course of the last thirty years, there has been an apparent increase in the prevalence and incidence of GEP-NETs[1, 2]. Whether this constitutes an actual increase in incidence and prevalence or is the result of better diagnostics or better detection or more awareness and more clearly defined nomenclature still remains unclear.

The Dutch National Cancer Registry has no complete data on GEP-NETs, due to the fact that some tumors (e.g. insulinomas and appendix NETs) were not considered to be malignant in the past. Furthermore, there was a lack of clear nomenclature and a wide variety of classification systems were used. As a result and as an example, we were required to analyze data from nationwide GEP-NET patient subpopulations in our second primary malignancy study.

The establishment of both the European Neuroendocrine Tumor Society (ENETS) and the North American Neuroendocrine Tumor Society (NANETS) has greatly improved collaboration between NET centers in various countries and across the ocean.

Although the Netherlands still lacks a national GEP-NET registry, ENETS has recently set up a multinational NET database, known as the ENETS European NET Registry. Consequently, international multicenter studies are able to source data from different national registries. It is possible that this will lead to the establishment of safe and effective NET treatment protocols and the initiation of international studies as well as to the determination of the actual epidemiology of NETs in Europe and its various geographical areas.

A further advantage of the ENETS European NET Registry is that uniformity in the registration of data becomes feasible and this in turn would benefit multicenter international studies.

Future Prospects in Epidemiology

Establishing a direct connection between the Dutch National Cancer Registry and hospital registrations in NET centers in the Netherlands could lead to a better and more complete registry of Dutch GEP-NET patients, eventually facilitating multicenter Dutch preclinical and clinical studies.

DIAGNOSIS

Clinical

A systematic clinical characterization of two rare humoral paraneoplastic syndromes was made: Humoral Hypercalcemia of Malignancy due to Parathyroid Hormone-related Protein (PTHrP) secretion by GEP-NETs and Cushing's syndrome caused by ectopic ACTH secretion (EAS) by thoracic and GEP-NETs. Improved awareness of the clinical signs and symptoms of these hormonal hypersecretory disorders will eventually lead to better identification, and, probably, earlier treatment of GEP-NET patients at risk.

The studies led to the identification of the most effective treatments for these very rare disorders; the realization of more tailored therapies in the near future have come a step closer as a consequence.

Biomarkers

At a recent consensus meeting, an international group of GEP-NET experts concluded that the currently available, circulating GEP-NET biomarkers have major limitations. Biomarkers for GEP-NETs should ideally comply with the multi-dimensional characteristics for accurate diagnosis (e.g. functional versus non-functional GEP-NETs), prediction of treatment effectiveness, quantification of tumor burden, indication of proliferative and metastatic capacity of the tumor, level of aggressiveness or benign behavior of the tumor[3].

New biomarkers for GEP-NETs are therefore needed to provide better diagnostic and prognostic information. We have shown that NSE is very useful as a predictive biomarker in GEP-NET patients, although its clinical use had been neglected to date. In addition, the performance of CgA as a marker for the diagnosis and follow-up of GEP-NET patients is under discussion. The study showed a favorable disease course in Stage IV GEP-NET patients without elevated CgA levels.

Imaging

Any progress in the field of radiology and molecular imaging can be brought into play in order to create so-called "Precision Medicine". This can involve the integration of (possible genomic/proteomic) biological information of the tumor, thus allowing for the development of so-called "Personalized Medicine", i.e. tailor-made individual treatments.

Future Prospects in Diagnostics

Circulating Tumor Cells (CTCs)

In recent years, interest in CTCs and their use as a potential new biomarker has gradually increased. As yet, the only FDA-approved test for the detection of CTCs is the CELL-

SEARCH® system, which uses the epithelial cell adhesion molecules (EpCAM) expression by NETs as an identification tool. This approach is similar to that used in other epithelial cancers such as breast, prostate, lung and colorectal cancer. Dynamic changes in CTC levels parallel responses to therapy and overall survival[4]. To further validate these findings, the role of CTCs in NETs is explored in two ongoing trials: the CALM-NET study in midgut NETs (NCT02075606) and the SEQTOR trial in pancreatic NETs (NCT02246127).

MicroRNA (miRNA) Profiling

Another possible new biomarker for GEP-NETs could be miRNA, known to be dysregulated in several malignancies. Overall, there are, as yet, still insufficient clinical data to support the use of miRNA expression measurement in GEP-NETs[3]. In addition, large differences in miRNA profiles have been reported between different subtypes of NETs[5,6].

NETest

The NETest (Wren Laboratories, Branford, CT, USA) represents a multianalyte qRT-PCR assay based on 51 marker genes. This test provides information on the biological nature of the GEP-NET and its extent of disease. The measurement of these values can furthermore be used to determine treatment effectiveness[7-10].

TREATMENT

Despite the increase in both the incidence and the prevalence of GEP-NETs over the past three decades, treatment options for patients with metastatic disease remain limited. Approved drugs for symptom control and inhibition of tumor growth include the somatostatin analogs (SSAs), octreotide and lanreotide, and the molecular targeted agents everolimus and sunitinib[11-14]. Due to the expanding knowledge on GEP-NET biology, several clinical trials with new biological and molecular targeted agents are expected in the near future. Currently available therapeutic agents for other cancer types will also be tested for their efficacy in the treatment of GEP-NETs in the near future. This will, ultimately, most certainly lead to an expansion of the current "armor" in the battle against GEP-NET disease.

Monotherapies

Peptide Receptor Radionuclide Therapy (PRRT)

PRRT with the radiolabeled SSA [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-octreotate, Lutathera®) is an important new treatment modality for GEP-NET patients with inoperable tumors or metastatic disease. The publication of the phase III, multicenter, stratified,

open, randomized, controlled trial on ^{177}Lu-octreotate in patients with midgut NETs (NETTER-1) may lead to formal approval and reimbursement of this therapy in Europe and the USA. It has yet to be determined whether prospective randomized trials with ^{177}Lu-octreotate are needed in pancreatic NETs in order to also obtain full approval for this GEP-NET subtype.

Molecular Targeted Agents

The molecular targeted agents everolimus and sunitinib have both been approved for the treatment of advanced pancreatic NETs[12, 14]. A recent study also demonstrated the efficacy of everolimus in advanced, non-functional lung or GI-NETs[15]. However, the issues of prioritization of one of these molecular targeted therapies and the role of sequential treatment with these therapies have not yet been formally addressed. We have shown that sequential therapy with these compounds is at least feasible, with acceptable side effects and toxicity profiles. This also corresponds to the standard treatment paradigm according to the ENETS guidelines[16]. We have also shown that the known safety profile of everolimus is not influenced by previous treatment with ^{177}Lu-octreotate.

Combination Therapies or Sequential Therapies?

While the treatment of GEP-NET patients in Centers of Excellence is increasingly successful, with a gradual increase in overall survival (OS), their treatment algorithms are becoming more and more complex. International organizations in the field, such as ENETS and NANETS, therefore recommend (or will require in the near future), that each GEP-NET patient must be discussed in a multidisciplinary tumor board consisting of specialists with experience in the diagnosis and treatment of GEP-NETs (http://www.soncos.org/).

Future Prospects in Treatment

Patient Selection and Sequential Treatment Strategies

It is necessary that we improve our skills in selecting patients who are likely to benefit from specific treatments at the time of diagnosis, or during the course of their disease. However, validated biomarkers, that guide the selection of the most appropriate treatment and/or follow-up for each individual patient, are still lacking.

Other important questions which still remain unanswered are: what is the recommended sequence of treatments and at which moment and at what stage of the disease should these be initiated? Therapeutic decisions are now mainly based on personal experience and expert consensus statements (expert, but generally not evidence based), but are also dependent on the local availability of these therapies as well as financial issues[17].

The feasibility of testing all possible sequential treatments, including SSAs, approved molecular targeted agents (everolimus, sunitinib), surgery, PRRT, embolization, chemotherapy and newly developed drugs with large prospective clinical trials is limited, since patient accrual will become problematic due to the relatively low prevalence and incidence figures. However, advances in molecular profiling could be used to address this issue at a different level. A better understanding of molecular tumor biology could lead to the development of new cancer medicines, prognostic and predictable biomarkers, and the increase of essential knowledge regarding the (baseline or acquired) tumor's resistance mechanisms[18]. These advances could also prove useful in the preselection of patients for a type of neo-adjuvant therapy by identifying those patients at risk for recurrence.

Serotonin Synthesis Inhibitors

In a phase III double-blind clinical trial (TELESTAR), telotristat etiprate (LX 1606), showed promising results by controlling diarrhea and/or flushing in patients with metastatic GEP-NETs and the carcinoid syndrome. It is to be expected that this drug will be approved for clinical use in 2016 (NCT01677910).

Immunotherapy

Recently, a study was initiated to test whether immune-based therapy in GEP-NETs provides the same promising results as it does in some other types of cancer[19, 20]. Ipilimumab is a monoclonal antibody that activates the immune system by targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), a receptor that downregulates the immune system. Ipilimumab stimulates cytotoxic T-lymphocytes to recognize tumor cells and destroy them[21]. A phase I/II trial, to examine the safety and efficacy of intratumoral injection of ipilimumab combined with antibodies to programmed cell death protein 1 (PD-L1 agent) in Grade 3 GEP-NETs, is in preparation (www.netrf.org/net-research-foundation-launches-major-immunotherapy-initiative). Further studies on the characterization of immune characteristics and genomes in GEP-NETs are also still needed to guide the testing of immunotherapy treatments.

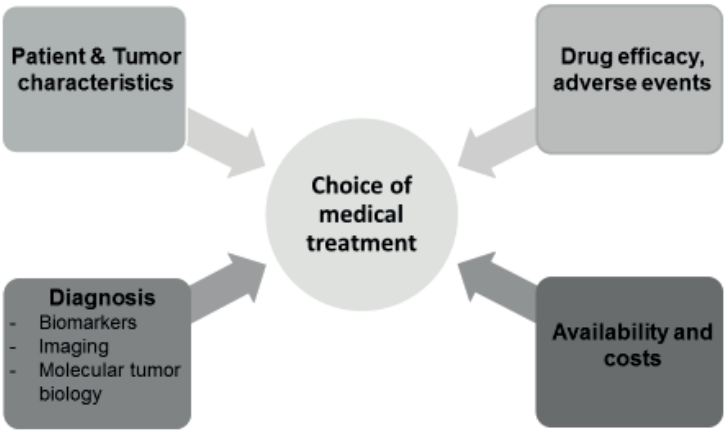
Oncolytic Viruses

Since 2008, an oncolytic adenovirus, modified with somatostatin motifs for selective infection of GEP-NET cells, has been developed and tested in mice[22, 23]. Oncolytic viruses ("cancer-eating viruses") are naturally occurring or genetically modified viruses that infect and destroy tumor cells by lysis, thereby releasing new virus particles which will infect neighboring tumor cells. The production and testing of a clinical batch of this virus named "Advince" is being executed at the time of writing. The final goal is to obtain

licenses from authorities and ethical committees to conduct phase I clinical trials (http://www.uu.se/en/support/oncolytic).

The combination of (new multi-dimensional) biomarkers and improved imaging techniques should help to diagnose the GEP-NET at an earlier stage and help to find the best tailored therapy for each individual patient.

Tailor-made medical therapy for GEP-NET patients.



REFERENCES

1. **Fraenkel M, Kim MK, Faggiano A, Valk GD.** Epidemiology of gastroenteropancreatic neuroendocrine tumours. *Best practice & research. Clinical gastroenterology.* 2012;26:691-703.

2. **Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB.** One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *Journal of clinical oncology.* 2008;26:3063-3072.

3. **Oberg K, Modlin IM, De Herder W, Pavel M, Klimstra D, Frilling A, Metz DC, Heaney A, Kwekkeboom D, Strosberg J, Meyer T, Moss SF, Washington K, Wolin E, Liu E, Goldenring J.** Consensus on biomarkers for neuroendocrine tumour disease. *The Lancet. Oncology.* 2015;16:e435-446.

4. **Khan MS, Kirkwood AA, Tsigani T, Lowe H, Goldstein R, Hartley JA, Caplin ME, Meyer T.** Early Changes in Circulating Tumor Cells Are Associated with Response and Survival Following Treatment of Metastatic Neuroendocrine Neoplasms. *Clin Cancer Res.* 2015;

5. **Li SC, Essaghir A, Martijn C, Lloyd RV, Demoulin JB, Oberg K, Giandomenico V.** Global microRNA profiling of well-differentiated small intestinal neuroendocrine tumors. *Mod Pathol.* 2013;26:685-696.

6. **Thorns C, Schurmann C, Gebauer N, Wallaschofski H, Kumpers C, Bernard V, Feller AC, Keck T, Habermann JK, Begum N, Lehnert H, Brabant G.** Global microRNA profiling of pancreatic neuroendocrine neoplasias. *Anticancer Res.* 2014;34:2249-2254.

7. **Modlin IM, Aslanian H, Bodei L, Drozdov I, Kidd M.** A PCR blood test outperforms chromogranin A in carcinoid detection and is unaffected by proton pump inhibitors. *Endocr Connect.* 2014;3:215-223.

8. **Modlin IM, Drozdov I, Alaimo D, Callahan S, Teixiera N, Bodei L, Kidd M.** A multianalyte PCR blood test outperforms single analyte ELISAs (chromogranin A, pancreastatin, neurokinin A) for neuroendocrine tumor detection. *Endocrine-related cancer.* 2014;21:615-628.

9. **Modlin IM, Drozdov I, Kidd M.** The identification of gut neuroendocrine tumor disease by multiple synchronous transcript analysis in blood. *PloS one.* 2013;8:e63364.

10. **Modlin IM, Drozdov I, Kidd M.** Gut neuroendocrine tumor blood qPCR fingerprint assay: characteristics and reproducibility. *Clin Chem Lab Med.* 2014;52:419-429.

11. **Caplin ME, Pavel M, Cwikla JB, Phan AT, Raderer M, Sedlackova E, Cadiot G, Wolin EM, Capdevila J, Wall L, Rindi G, Langley A, Martinez S, Blumberg J, Ruszniewski P, Investigators C.** Lanreotide in metastatic enteropancreatic neuroendocrine tumors. *The New England journal of medicine.* 2014;371:224-233.

12. **Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, Chen JS, Horsch D, Hammel P, Wiedenmann B, Van Cutsem E, Patyna S, Lu DR, Blanckmeister C, Chao R, Ruszniewski P.** Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *The New England journal of medicine.* 2011;364:501-513.

13. **Rinke A, Muller HH, Schade-Brittinger C, Klose KJ, Barth P, Wied M, Mayer C, Aminossadati B, Pape UF, Blaker M, Harder J, Arnold C, Gress T, Arnold R, Group PS.** Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. *Journal of clinical oncology.* 2009;27:4656-4663.

14. **Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Oberg K, Rad001 in Advanced Neuroendocrine Tumors TTSG.** Everolimus for advanced pancreatic neuroendocrine tumors. *The New England journal of medicine.* 2011;364:514-523.

15. **Yao JC, Fazio N, Singh S, Buzzoni R, Carnaghi C, Wolin E, Tomasek J, Raderer M, Lahner H, Voi M, Pacaud LB, Rouyrre N, Sachs C, Valle JW, Fave GD, Van Cutsem E, Tesselaar M, Shimada Y, Oh DY, Strosberg J, Kulke MH, Pavel ME, Rad001 in Advanced Neuroendocrine Tumours FTSG.** Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. *Lancet.* 2015;

16. **Falconi M, Bartsch DK, Eriksson B, Kloppel G, Lopes JM, O'Connor JM, Salazar R, Taal BG, Vullierme MP, O'Toole D, Barcelona Consensus Conference p.** ENETS Consensus Guidelines for the management of patients with digestive neuroendocrine neoplasms of the digestive system: well-differentiated pancreatic non-functioning tumors. *Neuroendocrinology.* 2012;95:120-134.

17. **Strosberg JR, Fisher GA, Benson AB, Anthony LB, Arslan B, Gibbs JF, Greeno E, Iyer RV, Kim MK, Maples WJ, Philip PA, Wolin EM, Cherepanov D, Broder MS.** Appropriateness of systemic treatments in unresectable metastatic well-differentiated pancreatic neuroendocrine tumors. *World J Gastroenterol.* 2015;21:2450-2459.

18. **Raymond E, Garcia-Carbonero R, Wiedenmann B, Grande E, Pavel M.** Systemic therapeutic strategies for GEP-NETS: what can we expect in the future? *Cancer metastases reviews.* 2014;33:367-372.

19. **Zhu Z, Liu W, Gotlieb V.** The rapidly evolving therapies for advanced melanoma-Towards immunotherapy, molecular targeted therapy, and beyond. *Crit Rev Oncol Hematol.* 2015;

20. **Champiat S, Lambotte O, Barreau E, Belkhir R, Berdelou A, Carbonnel F, Cauquil C, Chanson P, Collins M, Durrbach A, Ederhy S, Feuillet S, Francois H, Lazarovici J, Le Pavec J, De Martin E, Mateus C, Michot JM, Samuel D, Soria JC, Robert C, Eggermont A, Marabelle A.** Management of Immune Checkpoint Blockade Dysimmune Toxicities: a collaborative position paper. *Annals of oncology.* 2015;

21. **Ribas A.** Tumor immunotherapy directed at PD-1. *The New England journal of medicine.* 2012;366:2517-2519.

22. **Essand M, Leja J, Giandomenico V, Oberg KE.** Oncolytic viruses for the treatment of neuroendocrine tumors. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme.* 2011;43:877-883.

23. **Leja J, Yu D, Nilsson B, Gedda L, Zieba A, Hakkarainen T, Akerstrom G, Oberg K, Giandomenico V, Essand M.** Oncolytic adenovirus modified with somatostatin motifs for selective infection of neuroendocrine tumor cells. *Gene Ther.* 2011;18:1052-1062.

Chapter 11

Summary – Samenvatting

SUMMARY

NETs form a heterogeneous group of relatively rare tumors with a fair degree of variability in clinical manifestations and biological behavior. The primary tumor site localizations of the majority of metastatic NETs are the gastrointestinal (GI) and bronchopulmonary tracts, and the pancreas. GEP-NETs originating from cells of the diffuse neuroendocrine system of the GI tract and pancreas have shown an increasing incidence and prevalence over the past 30 years.

This thesis evaluates the clinical and biochemical characteristics of GEP-NETs, and includes data on the safety and efficacy of different sequential treatments. **Chapter 1** provides a general introduction to GEP-NETs with a short history of their nomenclature and a general overview of the scientists who first described these rare tumors. This chapter also contains a summary of the current literature with regard to the epidemiological, clinical and biochemical aspects of GEP-NETs as well as diagnostic strategies and different treatment modalities for patients with these malignancies.

The first section of this thesis analyzes the prognostic value of two general serum biomarkers: chromogranin A (CgA) and neuron-specific enolase (NSE). To date, this has been poorly studied in patients with ENETS TNM Stage IV GEP-NETs.

Chapter 2 describes a retrospective study of 616 and 524 patients with ENETS TNM Stage IV GEP-NETs for the analysis of baseline and follow-up levels of serum CgA, respectively. At the time of diagnosis and follow-up, there were those patients with elevated CgA levels and those with CgA levels within the reference range (so-called 'true non-secretors'). The hypothesis was put forward that these non-secretors would have a less favorable prognosis on the basis that lack of secretion can be considered a sign of further tumor dedifferentiation. The results of this single-center study demonstrate that, contrary to our hypotheses, true non-secretion of CgA does not constitute an unfavorable prognostic factor in patients with Stage IV well-moderately differentiated GEP-NETs and is, therefore, an independent biomarker for OS, both at baseline and at follow-up.

Chapter 3 discusses a retrospective study on serum NSE at first consultation in 592 patients with sporadic (non-familial) ENETS TNM Stage IV GEP-NETs. Elevated levels of NSE were found in over 40% of patients, confirming already published data. The results of this study demonstrate that NSE is a biomarker for overall survival in ENETS TNM Stage IV GEP-NET patients. Elevated levels of serum NSE indicate a more aggressive disease course and the determination of NSE at the first consultation could, therefore, have prognostic implications.

The second part of this thesis focuses on the association between GEP-NETs and the presence of other neoplastic lesions. In the literature, several small case series and autopsy studies reported an increased incidence of second primary malignancies (SPMs) in patients with GEP-NETs. **Chapter 4** examines whether there is indeed an actual, increased risk for SPMs in GEP-NET patients. The occurrence of SPMs was evaluated in a large cohort of 459 GEP-NET patients and compared with an age- and sex-matched control group of patients with identical malignancies, using linkage to the Dutch National Cancer Registry. In total, 63 (13.7%) GEP-NET patients were diagnosed with 67 SPMs: 25 previous, 13 synchronous and 29 metachronous cancers. Despite the fact that these results are in line with findings from historical series, a statistical quantification of risk using a population-based reference group led to different conclusions. Contrary to what other studies found, this study concludes that GEP-NET patients show a higher frequency only in the occurrence of synchronous SPMs, mainly colorectal cancers, as compared to the general population.

The widespread application of modern imaging techniques has revealed an increased prevalence of incidentally discovered adrenal masses ("adrenal incidentalomas"). In **Chapter 5** an estimation of the prevalence of adrenal incidentalomas in 438 GEP-NET patients has been made retrospectively. The results show that the prevalence of adrenal incidentalomas in GEP-NET patients (8.4%) is higher than in the general population (0.98-4%), but significantly lower than in patients with other malignancies (27%). In addition, it was found that most incidentalomas in our study were benign adrenal adenomas. However, adrenal incidentalomas were more frequently observed in patients with Grade 3 tumors, suggesting that the aggressive biological behavior of the GEP-NET may predict the possibility that a concurrent adrenal lesion is metastatic and, therefore, in these cases should not be considered as an incidentaloma.

The third part of this thesis examines two humoral paraneoplastic syndromes in a large cohort of GEP-NET patients. These two syndromes are Humoral Hypercalcemia of Malignancy due to Parathyroid Hormone-related Protein (PTHrP) secretion and Cushing's syndrome caused by ectopic ACTH secretion (EAS).

Only a few case reports of PTHrP hypersecreting GEP-NETs had been reported in literature, showing that investigational protocols and treatment management varied considerably from patient to patient and from institution to institution. **Chapter 6** describes a large single-centre case series of 10 successive PTHrP hypersecreting GEP-NET patients in a total cohort of 895 GEP-NET patients. PTHrP hypersecretion seemed to be exclusively associated with metastatic pancreatic NETs. As shown in our series, paraneoplastic PTHrP hypersecretion had a major clinical impact as, apart from the poorly controllable hypercalcemia, it was associated with an increased morbidity and mortality. Various therapeutic regimens and combination therapies were investigated

and reported, including information on treatment response and duration of response. The results demonstrated that somatostatin analogs (SSAs) and peptide receptor radionuclide therapy (PRRT) using radiolabeled SSAs are most successful in terms of long-term control and achieving normalization of serum calcium, paralleled by tumor stabilization or reduction, and prolongation of progression-free survival (PFS). Isotonic saline and bisphosphonates are considered to be the best supportive therapies.

Due to the fact that several large series solely reported on the relative contribution of EAS in the spectrum of Cushing's syndrome, an assessment of the prevalence of EAS in a large cohort of 918 patients with thoracic and GEP-NETs has been made in **Chapter 7**. This retrospective, single-center study revealed that, within a time frame of 20 years (1993–2012), 29 patients (3.2%) were diagnosed with EAS: 23 patients synchronously, 4 patients metachronously, and 2 patients with cyclical EAS. In addition, clinical, biochemical, and radiological features as well as management and outcome of treatment of this EAS patient cohort are detailed. In accordance with the literature, thoracic NETs (lung/bronchus and thymic) were shown to be the most common tumors causing EAS, followed by pancreatic NETs. Comparisons between thoracic and GEP-NET patients with and without EAS showed highly significant differences in various clinicopathological parameters: a lower median age at diagnosis and more Stage IIIB tumors in the EAS group, presumably explained by an earlier onset of symptoms due to the EAS. Overall survival analysis showed that only the first 5-year survival rate was lower in patients with EAS as compared with non-EAS patients. We concluded that EAS in patients with thoracic and GEP-NETs is associated with serious morbidity and a high mortality risk. Aggressive treatment of hypercortisolism with medical therapy or rescue endoscopic bilateral adrenalectomy are therefore of the essence for the management of these patients.

In the last part of this thesis, the safety and efficacy of different sequential treatments are evaluated in order to assess new possible treatment algorithms.

Although PRRT with ^{177}Lu-octreotate has proven to be an effective treatment for GEP-NET patients, there are limitations to its use as some patients become refractory to this therapy or develop progressive disease, necessitating further treatments.

Chapter 8 compares the safety and efficacy profile of everolimus in 24 GEP-NET patients, who have previously been treated with ^{177}Lu-octreotate. In this multicenter study, the safety profile of everolimus was assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) and efficacy in all patients was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST). Safety issues were similar to the known safety profile of everolimus and most adverse events were either grade 1 or 2. The most common major clinical adverse events (grade 3 or 4) during treatment with everolimus were hyperglycemia (20.8%), fatigue (8.3%), thrombocytopenia (8.3%), and elevated levels of alanine transaminase levels (8.3%).

Median PFS of our study was longer as compared to the RADIANT-3 trial (13.1 versus 11.4 months). We concluded that the known safety profile of everolimus is not influenced by previous treatment with ^{177}Lu-octreotate and is, therefore, a safe and feasible treatment option for GEP-NET patients with progressive disease.

The molecular targeted agents everolimus and sunitinib have both been approved for the treatment of advanced pancreatic NETs. However, the issue of sequential treatment with these molecular targeted therapies has not been formally addressed to date. In other types of cancer, such as metastatic renal carcinoma, alternating treatment with sunitinib and everolimus has been shown to be efficacious.

In **Chapter 9,** sequential molecular targeted therapy with everolimus and sunitinib is evaluated in terms of safety and its effect on disease progression and survival in 31 patients with advanced well-differentiated pancreatic NETs. This multicenter study analyzed efficacy according to RECIST criteria and classified adverse events into 4 grades. With regards to safety issues, discontinuation of treatment because of serious adverse events occurred less frequently with everolimus either as a first- or second-line treatment as compared to sunitinib. Sequential treatment with MT agents everolimus and sunitinib showed a clinical benefit on PFS, although not statistically significant. Everolimus treatment seemed to result in a longer PFS, either as a first- or second-line treatment as compared to sunitinib, albeit without statistical significance. The order of the sequential treatment did not influence the overall median PFS. More extensive prospective studies are required to further investigate the efficacy of alternate sequence therapy with molecular targeting agents in metastatic pancreatic NETs.

Finally, in **Chapter 10,** the results and consequences of the aforementioned studies, as described in this thesis, are discussed in relation to current and future diagnostic modalities and new treatment options for GEP-NET patients.

SAMENVATTING

Neuroendocriene tumoren (NETs) vormen een heterogene groep van relatief zeldzame tumoren, die diverse klinische verschijningsvormen hebben en zich biologisch uiteenlopend kunnen gedragen. De voornaamste locaties in het lichaam waar uitgezaaide NETs voorkomen zijn het maagdarmstelsel, de luchtwegen en de pancreas. De gastroenteropancreatische NETs (GEP-NETs), welke afkomstig zijn uit het diffuse neuroendocriene systeem van het maagdarmstelsel en de pancreas laten in de periode van de afgelopen dertig jaar een stijgende incidentie en prevalentie zien.

In dit proefschrift worden de klinische en biochemische eigenschappen van GEP-NETs besproken, inclusief de veiligheid en effectiviteit van verschillende behandelmethodes. **Hoofdstuk 1** is een algemene introductie over GEP-NETs, met een korte historische achtergrond van hun naamgeving en een vermelding van de artsen die deze zeldzame tumoren voor het eerst hebben beschreven. Ook wordt in dit hoofdstuk de huidige literatuur met betrekking tot de epidemiologische, klinische en biochemische aspecten van GEP-NETs samengevat en worden de diagnostische strategieën en behandelingstechnieken genoemd.

Na dit inleidende hoofdstuk volgt het eerste deel van het proefschrift, waarin de prognostische waarde van twee algemene serum biomarkers – chromogranine-A (CgA) en neuron-specifieke enolase (NSE) – wordt onderzocht. Tot op heden is dat weinig bestudeerd bij patiënten met ENETS TNM Stadium IV GEP-NETs.

In **Hoofdstuk 2** wordt een retrospectief onderzoek beschreven van een meting van serum CgA tijdens het eerste consult en het verdere ziekteverloop van 616 patiënten met ENETS TNM Stadium IV GEP-NETs, onder wie 524 patiënten die geen protonpompremmers voorgeschreven kregen.

Op het moment van eerste consult (diagnose) en bij het vervolgonderzoek waren er zowel patiënten met verhoogde CgA-spiegels, maar ook patiënten met CgA-spiegels binnen het referentiegebied (de zogenaamde 'echte non-secretors'). De hypothese was dat deze laatste groep een minder gunstige prognose zou hebben, omdat minder CgA-productie immers een teken zou kunnen zijn van dedifferentiatie van de tumor. De bevindingen uit het onderzoek lieten verrassend genoeg juist het tegenovergestelde zien: non-secretie van CgA is voor patiënten met Stadium IV goedgedifferentieerde (Graad 1-2) GEP-NETs juist niet een ongunstige prognostische factor en kan daarom fungeren als een onafhankelijke biomarker voor totale overleving, zowel ten tijde van diagnose stelling als tijdens het vervolgonderzoek.

Hoofdstuk 3 behandelt een retrospectief onderzoek naar de serum NSE-bepalingen tijdens het eerste consult van 592 patiënten met sporadische ENETS TNM Stadium IV GEP-NETs. Bij ruim 40% van de patiënten werd een verhoogde NSE-spiegel gevonden, wat overeen komt met in de literatuur gepubliceerde data. De resultaten van het onderzoek onderstrepen dat NSE als biomarker kan dienen voor de totale overleving van patiënten met ENETS TNM Stadium IV GEP-NETs. Verhoogde spiegels van het serum NSE kunnen wijzen op een agressiever ziekteverloop en de bloedbepaling van NSE tijdens het eerste consult kan daarom prognostische implicaties hebben.

Het tweede deel van dit proefschrift richt zich op het verband tussen GEP-NETs en de aanwezigheid van andere neoplastische laesies. In de bestaande literatuur worden een aantal kleinschalige patiënten series en autopsiestudies beschreven, waarin bij patiënten met GEP-NETs een verhoogde incidentie van een tweede primaire tumor (SPMs) wordt gerapporteerd. In **Hoofdstuk 4** werd onderzocht of GEP-NET-patiënten daadwerkelijk een verhoogd risico hebben voor SPMs. Het voor komen van SPMs werd getoetst binnen een groot cohort van 459 patiënten met GEP-NETs en vergeleken met een controlegroep bestaande uit leeftijds- en geslachtsgelijke patiënten met vergelijkbare tumoren. Voor deze vergelijking werd gebruik gemaakt van gegevens van het Nederlands Kanker Instituut (NKI).

Bij 63 GEP-NET-patiënten (13,7%) werden in totaal 67 SPMs geconstateerd: 25 eerder gediagnostiseerde tumoren, en 13 synchrone en 29 metachrone maligniteiten. Deze resultaten kwamen overeen met die uit gepubliceerde series. Na statistische risicoanalyse, gebruik makend van een populatie-gerelateerde referentiegroep, kwamen we echter tot een andere conclusie. Het bleek dat, anders dan wordt beschreven in eerdere gepubliceerde onderzoeken, patiënten met GEP-NETs in vergelijking met de algemene bevolking alleen frequenter synchrone colorectale tumoren als SPM hebben.

Door de brede toepassing van moderne radiologische technieken is er sprake van een verhoogde prevalentie van het aantal bij toeval ontdekte bijniermassa's ("adrenale incidentalomen"). **Hoofdstuk 5** behelst een retrospectief onderzoek waarin de prevalentie van adrenale incidentalomen bij 438 patiënten met GEP-NETs werd gecontroleerd. Het bleek dat deze bijniermassa's bij deze patiënten categorie vaker voorkomen dan in de algemene bevolking (8.4% ten opzichte van 0.98-4%), maar wel significant minder dan bij patiënten met andersoortige tumoren (27%). Bovendien werd vastgesteld dat de meeste incidentalomen in onze studie benigne adrenale adenomen waren. Echter, adrenale incidentalomen werden vaker gevonden bij patiënten met Graad 3 tumoren, wat suggereert dat het agressieve biologische gedrag van de GEP-NET de mogelijkheid kan voorspellen of een tegelijk ontdekte bijniermassa een metastase is, en daarom niet meer onder de noemer 'incidentalomen' kan vallen.

In het derde deel van dit proefschrift worden twee humorale paraneoplastische syndromen in een groot cohort GEP-NET-patiënten beschreven. Het gaat hier om zogenaamde Humoral Hypercalcaemia of Malignancy (hypercalciëmie) door Parathyreoïdhormoongerelateerde Proteïnesecretie (PTHrP) en het syndroom van Cushing veroorzaakt door ectopische secretie van het Adrenocorticotrophine (ACTH) (EAS).

In de literatuur worden maar enkele PTHrP-producerende GEP-NETs gerapporteerd, en uit deze publicaties blijkt dat er per patiënt en per instelling aanzienlijke verschillen zijn met betrekking tot onderzoeks- en behandelingsprotocollen. In **Hoofdstuk 6** staat een in één medisch centrum uitgevoerd grootschalig onderzoek centraal bij 895 GEP-NET patiënten, waarvan er 10 met een PTHrP-producerende GEP-NET werden gediagnostiseerd. Er werd gevonden dat PTHrP-hypersecretie alleen voor lijkt te komen als sprake is van metastatische neuroendocriene pancreastumoren. Paraneoplastische PTHrP-hypersecretie heeft een sterk negatief effect op het klinische beloop bij de patient, omdat de slecht controleerbare hypercalciëmie leidt tot een verhoogde morbiditeit en mortaliteit. Meerdere behandelregimes en -combinaties werden toegepast. Informatie over de behandelrespons en -duur werden vastgelegd. De resultaten laten zien dat somatostatine analogen (SSAs) en peptide receptor radionuclide therapie (PRRT) met radioactief gelabelde somatostatine analogen het meest geschikt waren om langdurige controle, het bereiken van normalisatie van het serum calcium en stabilisatie dan wel reductie van de tumor en de prolongatie van progressie-vrije overleving (PFS) te bewerkstelligen. NaCl en bisfosfaten blijken het meest geschikt te zijn als ondersteunende therapie.

Aangezien meerdere grote onderzoeken zich enkel hebben gericht op de relatieve bijdrage van EAS in het spectrum van het syndroom van Cushing, behandelt **Hoofdstuk 7** de prevalentie van EAS in een groot cohort van 918 thorax- en GEP-NET patiënten. In deze retrospectieve analyse werden over een tijdsspanne van 20 jaar (1993-2012), 29 patiënten (3.2%) gediagnosticeerd met EAS, waarvan 23 patiënten met synchrone, 4 patiënten met metachrone en 2 patiënten met cyclische EAS. Hiernaast werden ook klinische, biochemische en radiologische eigenschappen van het gehele EAS-cohort vastgelegd, en tevens de behandelmethodes en de behandelingsuitkomsten. In overeenstemming met de medische literatuur veroorzaken neuroendocriene thoraxtumoren (in long/bronchus en thymus) het vaakst EAS, gevolgd door pancreas NETs. Vergelijkingen tussen thorax- en GEP-NET patiënten met en zonder EAS laten significante verschillen zien met betrekking tot diverse clinicopathologische parameters. Een lagere mediane leeftijd bij diagnose en meer Stadium IIIb-tumoren in de EAS-groep, vermoedelijk te verklaren door een eerder optreden van symptomen van EAS, zijn hiervan de belangrijkste parameters. Het overlevingspercentage was alleen in de eerste vijf jaar lager bij patiënten met EAS in vergelijking met patiënten zonder EAS. Verder werd geconcludeerd dat patiënten met thorax- en GEP-NETs een belangrijk morbiteits- en mortaliteitsrisico lopen. Een agres-

sieve benadering van het hypercortisolisme met ofwel medicamenteuze therapie of endoscopische bilaterale adrenalectomie is daarom essentieel.

In het laatste deel van dit proefschrift worden de veiligheid en effectiviteit van verschillende sequentiële behandelingen onderzocht om potentieel nieuwe behandelingsalgoritmes te introduceren.

Hoewel PRRT met ^{177}Lu-octreotaat een bewezen effectieve behandeling voor GEP-NET patiënten is, zijn er ook beperkingen. Sommige patiënten reageren niet meer op deze therapie, of ontwikkelen progressieve ziekte waardoor aanvullende behandeling nodig is.

Hoofdstuk 8 beschrijft de vergelijking tussen het veiligheids- en effectiviteitsprofiel van everolimus in 24 GEP-NET patiënten, nadat ze eerder zijn behandeld met ^{177}Lu-octreotaat (Lutathera) Het veiligheidsprofiel van everolimus werd in een retrospectieve studie vastgelegd door middel van de *National Cancer Institute Common Terminology Criteria for Adverse Events* (CTCAE). De effectiviteit van het middel op de tumoren van alle GEP-NET patiënten werd geëvalueerd met behulp van de *Response Evaluation Criteria in Solid Tumors* (RECIST). De geconstateerde veiligheidsproblemen waren gelijk aan het al bekende veiligheidsprofiel van everolimus, en de meeste bijwerkingen waren ofwel graad 1 of 2. De meest veelvoorkomende klinische bijwerkingen (graad 3 of 4) bij de behandeling met everolimus waren hyperglycemie (20.8%), vermoeidheid (8.3%), thrombocytopenie (8.3%), en verhoogde alanine-transaminaseniveaus (8.3%). De mediane PFS van ons onderzoek was in vergelijking met de RADIANT-3 trial langer (13.1 versus 11.4 maanden). We concluderen dat een eerdere behandeling met ^{177}Lu-octreotaat geen invloed heeft op het bekende veiligheidsprofiel van everolimus en dat de combinatie daarom een veilige en effectieve behandelingsoptie is voor GEP-NET patiënten met progressieve ziekte.

De moleculaire kankertherapiemiddelen everolimus en sunitinib zijn beide goedgekeurd als behandelopties voor uitgezaaide pancreas NETs. Tot op heden is sequentiële behandeling met deze middelen echter nog niet verder uitgezocht. Bij andere kankervarianten, zoals gemetastaseerd niercelcarcinoom, is een alternerende behandeling met sunitinib en everolimus effectief gebleken.

In **Hoofdstuk 9** worden de resultaten van een sequentiële moleculaire therapiebehandeling met everolimus en sunitinib beschreven en met name met betrekking op veiligheid, effectiviteit, progressie en overleving in een groep van 31 patiënten met gemetastaseerde en goed-gedifferentieerde pancreas NETs. Dit onderzoek analyseerde de effectiviteit middels de RECIST-criteria en rangschikte de bijwerkingen in vier gradaties. Wat betreft de veiligheidscriteria werd de behandeling met everolimus minder vaak afgebroken, zowel in het val van eerste- of tweedelijnsbehandeling, als die met sunitinib.

Een belangrijke conclusie was dat everolimus en sunitinib als sequentiële MT-middelen een positief doch niet statistisch significant verschillend klinisch effect hebben op het gebied van PFS. Everolimus lijkt als eerste-, of tweedelijnsbehandeling een langere

progressievrije overleving te geven dan sunitinib, maar ook hier was het verschil niet statistisch significant. De totale mediane PFS werd niet beïnvloed door de volgorde waarin beide middelen werden ingezet in de sequentiële behandeling. Uitgebreidere prospectieve onderzoeken zijn verder nodig om de effectiviteit van alternerende sequentietherapieën met moleculaire kankertherapieën op pancreas NETs te bepalen.

Tot slot worden in **Hoofdstuk 10** de resultaten van bovengenoemde onderzoeken in dit proefschrift in het kader van de huidige en toekomstige diagnostische en therapeutische opties voor GEP-NET-patiënten geplaatst.

Chapter 12

List of Publications

Curriculum Vitae

PhD Portfolio

List of Abbreviations

Dankwoord

LIST OF PUBLICATIONS

1. van Schaik E, van Vliet EI, Feelders RA, Krenning EP, Khan S, **Kamp K**, Valkema R, van Nederveen FH, Teunissen JJM, Kwekkeboom DJ, de Herder WW. Improved Control of Severe Hypoglycemia in Patients with Malignant Insulinomas by Peptide Receptor Radionuclide Therapy. *Journal of Clinical Endocrinology and Metabolism* (2011) **96**, 3381-3389.

2. **Kamp K**, Damhuis RAM, Feelders RA, Herder WW. Occurrence of second primary malignancies in patients with neuroendocrine tumors of the digestive tract and pancreas. *Endocrine-Related Cancer* (2012) **19**, 95–99.

3. Kanakis G*, **Kamp K***, Tsiveriotis K, Feelders RA, Zormpala A, de Herder WW**, Kaltsas G**. The prevalence and relevance of adrenal masses in patients with sporadic gastroenteropancreatic neuroendocrine tumours (GEP-NET). *Clinical Endocrinology* (2013) **78**, 950-956.

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

5. **Kamp K**, Feelders RA, van Adrichem RC, de Rijke YB, van Nederveen FH, Kwekkeboom DJ, de Herder WW. Parathyroid hormone-related peptide (PTHrP) secretion by gastroenteropancreatic neuroendocrine tumors (GEP-NETs): clinical features, diagnosis, management, and follow-up. *Journal of Clinical Endocrinology and Metabolism* (2014) **99**, 3060-3069.

6. van Adrichem RC, **Kamp K**, van Deurzen CH, Biermann K, Feelders RA, Franssen GJ, Kwekkeboom DJ, Hofland LJ, de Herder WW. Is there an Additional Value of Somatostatin Receptor Subtype 2A Immunohistochemistry over Somatostatin Receptor Scintigraphy Uptake in Predicting Gastroenteropancreatic Neuroendocrine Tumor Response? *Neuroendocrinology* (2015) Epub Nov 5.

7. van Adrichem RC*, **Kamp K***, Vandamme T, Peeters M, Feelders RA, de Herder WW. Serum neuron-specific enolase level is an independent predictor of overall survival in patients with gastroenteropancreatic neuroendocrine tumors. *Annals of Oncology* (2015) Epub Dec 27.

8. **Kamp K**, Alwani RA, Korpershoek E, Franssen GJ, de Herder WW, Feelders RA. Prevalence and clinical features of the ectopic ACTH syndrome in patients with gastroenteropancreatic and thoracic neuroendocrine tumors. *European Journal of Endocrinology* (2016) **174**, 271-280.

9. van Adrichem RC, de Herder WW, **Kamp K**, Brugts MP, de Krijger RR, Sprij-Mooij DM, Lamberts SW, van Koetsveld PM, Janssen JA, Hofland LJ. Effects of Somatostatin Analogs and Dopamine Agonists on Insulin-Like Growth Factor 2-Induced Insulin Receptor Isoform-A Activation by Gastroenteropancreatic Neuroendocrine Tumor Cells. *Neuroendocrinology* (2016) Epub Feb 2.

10. **Kamp K***, van Adrichem RC*, Vandamme T, de Rijke YB, Peeters M, Feelders RA, de Herder WW. Is true non-secretion of Chromogranin A an unfavorable prognostic factor in patients with ENETS TNM Stage IV gastroenteropancreatic neuroendocrine tumors? *Submitted.*

11. Angelousi A*, **Kamp K***, Kaltsatou M, O'Toole D, Kaltsas G**, de Herder WW**. Sequential everolimus and sunitinib treatment in pancreatic metastatic well-differentiated neuroendocrine tumors resistant to prior treatments. *In revision.*

12. Zandee WT, **Kamp K**, van Adrichem RC, Feelders RA, de Herder WW. Limited value for urinary 5-HIAA excretion as prognostic marker in gastrointestinal neuroendocrine tumors. *Manuscript in preparation.*

13. **Kamp K**, van Adrichem RC, Feelders RA, de Herder WW. Clinical Overview of Gastroenteropancreatic Neuroendocrine Tumors (GEP-NETs). *Invited review: Netherlands Journal of Medicine: Manuscript in preparation.*

14. **Kamp K**, Feelders RA, Kwekkeboom DJ, de Herder WW. A retrospective Analysis of Safety and Efficacy of 177Lu-octreotate in Gastroenteropancreatic Neuroendocrine Tumors after prior treatment with-Everolimus. *Manuscript in preparation.*

* joint first authors

** joint last authors

CURRICULUM VITAE

Kimberly Kamp was born on June 3rd 1988 in Oosterhout, the Netherlands. After primary school, she moved to Belgium and attended the first four years of secondary school at the College van het Eucharistisch Hart in Essen and the last two years at the Kindsheid Jesu College in Hasselt, where she graduated cum laude in 2006. In the same year, she started her medical studies at the Medical Faculty of the Erasmus University in Rotterdam. From 2007 to 2011 she worked in the nursing staff at the Geriatrics-Oncology ward of the Erasmus MC.

In 2010 she executed her graduation research project, entitled "Occurrence of Second Primary Malignancies in Patients with Neuroendocrine Tumors of the Digestive Tract and Pancreas" at the section of Endocrinology of the Department of Internal Medicine, Erasmus MC under the supervision of Prof.dr. W.W. de Herder and Dr. R.A. Feelders. For this research she received an ENETS young investigator award in the category of clinical research. During her internships in 2011 she continued further research on neuroendocrine tumors.

She interrupted her internships in October 2011 to focus on her PhD project "Clinical & Biochemical Characterization of Gastroenteropancreatic Neuroendocrine Tumors" at the section of Endocrinology of the Department of Internal Medicine, Erasmus MC, under the supervision of Prof.dr. W.W. de Herder and Dr. R.A. Feelders. During this project the author received two travel grants, one abstract award and was involved as a sub-investigator in various clinical trials.

In March 2015 she resumed her internships to obtain her medical degree. Parallel to her medical studies and PhD project, she was quite a successful coxswain for student rowing club "A.R.S.R. Skadi" in Rotterdam. She won various (inter)national regattas such as the Head of the Charles in Boston, USA, and the esteemed Varsity Boat Race in the Netherlands, as well as being invited to try out as coxswain for the Dutch national women's eight.

PHD PORTFOLIO

Name PhD student:	Kimberly Kamp
Erasmus MC Department:	Internal Medicine – Section of Endocrinology
Research School:	MolMed
PhD period:	November 2011 – February 2015
Promotor:	Prof. dr. W.W. de Herder
Supervisor:	Dr. R.A. Feelders

General academic courses – research skills	**Year**	**Workload (ECTS)**
BROK (Basiscursus Regelgeving Klinisch Onderzoek) (Clinical Research Course)	2014	2
Course Molecular Diagnostics IX	2014	1
Research Integrity	2014	0.5
Biomedical English Writing Course	2012-2013	2
Basic Introduction Course on SPSS	2012	1
Basic Introductory Course on Statistics & Survival Analysis	2012	0.5
Good Clinical Practice Training	2012	1

Clinical Courses & Meetings/projects/participation	**Year**	**Workload (ECTS)**
Werkgroep NET, the Netherlands	2013-2015	2
ENETS Center of Excellence	2012-2015	3
Erasmus MC Zorgpad NET	2012-2015	4
Weekly Grand Round Endocrinology Department	2011-2015	6
Bi-weekly Multidisciplinary NET Tumorboard	2011-2015	3
Erasmus MC Course Neuro-Endocrinology	2012-2014	0.5
Weekly Scientific Meeting/Workdiscussion Endocrinology Department	2011-2015	1
NET Outpatient Clinics	2012-2013	2
Database NET Registration, Erasmus Medical Center	2010-2016	10

(International) conferences – oral presentations	**Year**	**Workload (ECTS)**
Biochemical Markers in GEP-NETs: Chromogranin A *NeuroEndocrine Tumour Masterclass on site, Rotterdam, the Netherlands*	2015	0.3
Neuroendocriene Tumoren van Database naar Resultaten *Investigator Meeting Ipsen, Rotterdam, the Netherlands*	2015	0.3
Prevalence and Clinical Features of the Ectopic ACTH Syndrome in Patients with GastroEnteroPancreatic and Thoracic Neuroendocrine Tumors. *Dutch Endocrine Meeting, Noordwijkerhout, the Netherlands*	2015	0.8

NETs from a Rotterdam Perspective - Share of Experience *Latin American Knowledge Network Expert Meeting, Rotterdam, the Netherlands*	2014	0.3
NET Diagnosis *Latin American Knowledge Network Expert Meeting, Rotterdam, the Netherlands*	2014	0.3
Parathyroid Hormone-related Peptide (PTHrP) Secretion by GastroEnteroPancreatic Neuroendocrine Tumors (GEP-NETs): Clinical Features, Diagnosis, Management, and Follow-up. *Dutch Endocrine Meeting, Noordwijkerhout, the Netherlands*	2014	0.8
Safety and Efficacy of Everolimus in GastroIntestinal and Pancreatic Neuroendocrine Tumors after (177)Lu-octreotate. *Network Europe 2013 meeting, Munich, Germany*	2013	0.5
Safety and Efficacy of Everolimus in Gastrointestinal and Pancreatic Neuroendocrine Tumors after (177)Lu-octreotate. *Dutch Endocrine Meeting, Noordwijkerhout, the Netherlands*	2013	0.8

(International) conferences – poster presentations	**Year**	**Workload (ECTS)**
Is True Non-Secretion of Chromogranin A an Unfavorable Prognostic Factor in Patients with ENETS TNM Stage IV GastroEnteroPancreatic Neuroendocrine Tumors? *13th Annual ENETS Conference, Barcelona, Spain*	2016	0.3
Little Additional Value for Urinary 5-Hydroxyindoleacetic Acid as Prognostic Marker in Midgut Neuroendocrine Tumors. *13th Annual ENETS Conference, Barcelona, Spain*	2016	0.3
GastroEnteroPancreatic and Thoracic Neuroendocrine Tumors and the Ectopic Adrenocorticotropin Syndrome. *12th Annual ENETS Conference, Barcelona, Spain*	2015	1
Parathyroid Hormone-related Peptide (PTHrP) Secretion by Metastatic Gastroenteropancreatic Neuroendocrine Tumors (GEP-NET): Clinical Features, Diagnosis, Management, and Long-Term Follow-Up. *11th Annual ENETS Conference, Barcelona, Spain*	2014	1
Parathyroid Hormone-related Peptide (PTHrP) Secretion by Metastatic Gastroenteropancreatic Neuroendocrine Tumors (GEP-NET): Clinical Features, Diagnosis, Management, and Long-Term Follow-Up. *Internal Medicine Science Days, Antwerp, Belgium*	2014	0.7
A Retrospective Analysis of Safety and Efficacy of Everolimus in GastroEnteroPancreatic Neuroendocrine Tumor (GEP-NET) Patients that Showed Progression after PRRT. *10th Annual ENETS Conference, Barcelona, Spain*	2013	1
A Retrospective Analysis of Safety and Efficacy of Everolimus in GastroEnteroPancreatic Neuroendocrine Tumor (GEP-NET) Patients that Showed Progression after PRRT. *Internal Medicine Science Days, Antwerp, Belgium*	2013	0.7
Thoracic and GastroEnteroPancreatic (GEP) Neuroendocrine Tumors (NETs) and the Ectopic Adrenocorticotropin (ACTH) Syndrome (EAS). *9th Annual ENETS Conference, Copenhagen, Denmark*	2012	1

Thoracic and GastroEnteroPancreatic (GEP) Neuroendocrine Tumors (NETs) and the Ectopic Adrenocorticotropin (ACTH) Syndrome (EAS). *European Congress of Endocrinology, Rotterdam, the Netherlands*	2011	0.3
Occurrence of Other Primary Malignancies in Patients with Endocrine Tumors of the Digestive Tract and Pancreas. *8th Annual ENETS Conference, Lisbon, Portugal*	2011	1

Symposia, seminars & workshops	**Year**	**Workload (ECTS)**
Bi-annual Regional Endocrinology Meeting, Rotterdam, the Netherlands	2011-2015	0.3
Interactieve Nascholingscursus Pancreas NET, Rotterdam, the Netherlands	2012	0.3
NET Minisymposium, Oratie Prof.dr. W.W. de Herder, Rotterdam, the Netherlands	2011	0.3

Teaching activities - supervising internships	**Year**	**Workload (ECTS)**
Supervising Master's thesis: Mw. M. van den Dool	2014	0.3
NET Database: diversity of projects	2010-2016	10

Clinical Research Trials	**Year**	**Workload (ECTS)**
Sub-investigator Telotristat, Lexicon Pharmaceuticals: A Phase 3, Randomized, Placebo-controlled, Multicenter, Double blind Study to Evaluate the Safety and Efficacy of Telotristat Etiprate (LX1606) in Patients with Carcinoid Syndrome.	2013-2015	1
Sub-investigator RADIANT-4, Novartis Pharmaceuticals: A Randomized, Double-blind, Multicenter, Phase III Study of Everolimus (RAD001) plus Best Supportive Care vs. Placebo plus Best Supportive Care in the Treatment of Patients With Advanced NET of GI or Lung Origin.	2012-2013	1
Investigator Meetings (London, Madrid, Sitges)	2012-2014	1

Awards and prices – travel grants	**Year**
Travel Grant Award *9th Annual ENETS Conference, Copenhagen, Denmark*	2012
Young Investigator Award, Clinical Science category, 3rd prize Occurrence of Other Primary Malignancies in Patients with Endocrine Tumors of the Digestive Tract and Pancreas. *8th Annual ENETS Conference, Lisbon, Portugal*	2011
Travel Grant Award *8th Annual ENETS Conference, Lisbon, Portugal*	2011

LIST OF ABBREVIATIONS

^{11}C-5-HTP	β-[(11)C]-5-Hydroxy-L-Tryptophan
^{18}F-DOPA	6-(18)F-L-3,4-Dihydroxyphenylalanine
25(OH)D	25-Hydroxyvitamin D3, Cholecalciferol
1,25(OH)$_2$D	1,25-Dihydroxyvitamin D3, Calcitriol, 1,25-Dihydroxycholecalciferol
5-FU	5-Fluorouacil
5-FU-STZ	5-Fluorouracil–Streptozotocin
5-HIAA	5-Hydroxy Indole Acetic Acid
5-HT	5-Hydroxytryptamine, Serotonin
5-HTP	5-Hydroxytryptophan
^{68}Ga-DOTA-TATE	Gallium-68 labelled Octreotate
ACTH	Adrenocorticotropic Hormone
ADH	Vasopressin
AEs	Adverse Events
AI	Adrenal Incidentaloma
AJCC	American Joint Committee on Cancer
ALT	Alanine Transaminase = SGPT = Serum Glutamic Pyruvic Transaminase
ANP	Atrial Natriuretic Peptide
APC	Adenomatous Polyposis Coli
AST	Aspartate Transaminase = SGOT = Serum Glutamic Oxaloacetic Transaminase
BAI	Bilateral Adrenal Involvement
BIPSS	Bilateral Inferior Petrosal Sinus Sampling
B rep	Bisphosphonate repetitive
CDR	Cortisol Diurnal Rhythm
CgA	Chromogranin A
CgB	Chromogranin B
CgC	Chromogranin C
CI	Confidence Interval
CRH	Corticotropin-releasing Hormone
CS	Cushing's Syndrome
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTCs	Circulating Tumor Cells
CTGF	Connective Tissue Growth Factor

CTLA-4	Cytotoxic T-lymphocyte-associated Protein 4
DHEA-S	Dehydroepiandrosterone-Sulphate
DST	Dexamethasone Suppression Test
EAS	Ectopic ACTH Secretion
ECL-cells	Enterochromaffin-like Cells
ELISA	Enzyme-linked Immunosorbent Assay
ENETS	European Neuroendocrine Tumor Society
EpCAM	Epithelial Cell Adhesion Molecule
EUS	Endoscopic Ultrasound
FDA	Food and Drug Administration (USA)
FDG	[^{18}F]-Fluorodeoxyglucose
FLT3	Fms related Tyrosine Kinase 3
FSG	Fasting Serum Gastrin
G1	Grade 1
G2	Grade 2
G3	Grade 3
GBq	Gigabecquerel
G-cell	Gastrin-producing Cell
GEP-NET	Gastroenteropancreatic Neuroendocrine Tumor
GH	Growth Hormone
GHRH	Growth Hormone-releasing Hormone
GI	Gastrointestinal
G(H)RF	Growth Hormone–releasing Factor
H2-blockers	Histamine 2-blockers
HDSST	High-Dose Dexamethasone Suppression Test
HE	Hematoxylin and Eosin
HHM	Humoral Hypercalcemia of Malignancy
HPF	High Power Field
HR	Hazard Ratio
IFN-α	Interferon-alfa
IFN-β	Interferon-beta
IGF1	Insulin-like Growth Factor 1
IGF2	Insulin-like Growth Factor 2
IL	Interleukin
im	intramuscular
IR	Immediate Release
iv	intravenous
Ki-67	Ki-67 labeling index (using MIB-1 antibody)
KIT	KIT Proto-Oncogene Receptor Tyrosine Kinase

LAR	Long-Acting Release/Repeatable
LH	Luteinizing Hormone
luthatera, ^{177}Lu-octreotate	[^{177}Lu-DOTA^{0},Tyr^{3}]octreotate
MANEC	Mixed Adeno-Neuroendocrine Carcinoma
MDS	Myelodysplastic Syndrome
MEN1	Multiple Endocrine Neoplasia Syndrome type 1
MIB-1 antibody	Monoclonal Antibody Ki-67 index
miRNA	microRNA
mo	months
mOS	median Overall Survival
MRI	Magnetic Resonance Imaging
MT	Molecular Targeted
MTC	Medullary Thyroid Carcinoma
mTOR	mammalian Target of Rapamycin
NA	not available
NANETS	North American Neuroendocrine Tumor Society
NEC	Neuroendocrine Carcinoma
NET	Neuroendocrine Tumor
NF	Non-Functioning
NF1	Neurofibromatosis type I/ Neurofibromin 1 gene
NICTH	Non-islet Cell Tumor Hypoglycemia
NSE	Neuron-specific Enolase
Octreoscan	^{111}In-pentetreotide scintigraphy
OS	Overall Survival
PA	Pathology Assessment
PAC	Plasma Aldosterone Concentration
PD	Progressive Disease
PDGFR	Platelet-derived Growth Factor Receptors
PD-L1	Programmed cell death protein 1
PET	Positron Emission Tomography
PET-CT	Positron Emission Tomography-Computed Tomography
PFS	Progression-Free Survival
PHM	Peptide Histidine-methionine
PI3K	Phosphatidylinositol 3-kinase
PPI	Proton Pump Inhibitor
PR	Partial Response
PRA	Plasma Renin Activity
PRRT	Peptide Receptor Radio(nuclide)therapy
PTEN	Phosphatase and Tensin Homolog

PTH	Parathyroid Hormone
PTHrP	Parathyroid Hormone-related Peptide/Protein
QOL	Quality of Life
qRT-PCR	quantitative Reverse Transcriptase Polymerase Chain Reaction
RANK	Receptor Activator of Nuclear factor-κB
RECIST	Response Evaluation Criteria in Solid Tumors
RFA	Radiofrequency Ablation
RR	Reference Range
RTK	Receptor Tyrosine Kinase
sc	subcutaneous
SCLC	Small Cell Lung Carcinoma
SCS	Subclinical Cushing's Syndrome
SD	Standard Deviation
SD	Stable Disease
SEER	Surveillance, Epidemiology, and End Results
SGH	Subclinical Glucocorticoid Hypersecretion
SIADH	Syndrome of Inappropriate Antidiuretic Hormone Secretion
siNET	small intestine Neuroendocrine Tumor
SIR	Standardized Incidence Ratio
SPECT	Single Positron Emission Computed Tomography
SPM	Second Primary Malignancy
SPSS	Statistical Package for Social Sciences
SRS	Somatostatin Receptor Scintigraphy, *OctreoScan®*
SSA	Somatostatin Analog
sst	somatostatin receptor subtype
STZ-5FU	Streptozotocin-5-Fluorouracil
TACE	Transcatheter Arterial Chemoembolization
TGF	Transforming Growth Factor
TI	Therapeutic Intervention
TNF	Tumor Necrosis Factor
TNM	Tumor, Lymph Node, Metastasies
TP53	Tumor Protein p53
TTP	Time To Progression
UFC	Urinary Free Cortisol excretion
ULN	Upper Limit of Normal
VAC	Vincristine–Adriamycin–Cyclophosphamide
VEGF	Vascular Endothelial Growth Factor
VHL	Von Hippel-Lindau
VIP	Vasoactive Intestinal Peptide

vs.	versus
WHO	World Health Organization
Xelox	Capecitabine plus Oxaliplatin
ZES	Zollinger-Ellison Syndrome

DANKWOORD

Als stuurvrouw in het roeien ben ik het gewend om na een gewonnen race ceremonieel in het water gegooid te worden. Nu dit proefschrift ook de eindstreep heeft gehaald, wens ik een aantal mensen datzelfde eervolle natte pak toe, als dank voor de (jarenlange) steun.

Allereerst mijn promotor, **Prof.dr. W.W. de Herder. Wouter**, je hebt me met meer dan alleen raad en daad bijgestaan. Bij jou komt klinisch- en basaal onderzoek samen, ik heb veel waardering voor je betrokkenheid bij patiënten en je toewijding tijdens het opleiden van jonge artsen. Je bent de spin in het web van neuroendocriene tumoren. Door jou ben ik medisch onderzoek gaan waarderen en je bent er ook buiten het ziekenhuis om voor me geweest tijdens de vermissing van mijn hond Berber. De memorabele mails – de 'nachttrein' die ook om 03:00 uur stukken retour stuurde – zullen me misschien nog wel het meeste bijblijven, met afgelopen Kerst als hoogtepunt. Er zijn weinig mensen met wie ik tijdens de feestdagen liever tot diep in de nacht digitaal spar. Dankzij jou hoef ik niet aan de boterhammen met pindakaas – dankjewel.

Mijn co-promotor, **dr. R.A. Feelders. Richard**, jij en Wouter waren de gangmakers van de afdeling endocrinologie in het Erasmus MC, waardoor menig grote visite wel eens uitliep. Bloed prikken ging nog nooit zó duidelijk volgens de MacGyver-methode. Jouw scherpe blik – tot de laatste spelfouten vlak voor de deadline aan toe! – en je inzichten hebben mijn proefschrift beter gemaakt. Bij jou was nooit een afspraak nodig, en je droge gevoel voor humor zorgde voor de nodige ontspanning in het promotieproces.

Prof.dr. L.J. Hofland, **Prof.dr. D.J. Kwekkeboom** en **Prof.dr. G.D. Valk**, hartelijk dank dat u allen mijn proefschrift heeft willen toetsen en positief heeft willen beoordelen als leden van de leescommissie. **Leo** en **Dick**, ik ben jullie daarnaast veel dank verschuldigd voor het meedenken over en het bediscussiëren van mijn onderzoeksresultaten de afgelopen jaren.

De overige leden van de promotiecommissie. **Prof.dr. J.C. Kluin-Nelemans**, **Prof.dr. Y.B. de Rijke** en **Dr. E.J.M. Nieveen-van Dijkum**, bedankt voor het plaatsnemen in de commissie. **Hanneke**, op driejarige leeftijd was je de eerste aan wie ik vertelde dat ik dokter wilde worden, vervolgens heb ik vele jaren later mijn eerste geneeskundestage in het UMCG gelopen, wat het heel speciaal maakt dat je in mijn grote commissie wilde plaatsnemen. **Yolanda**, dank voor je enthousiasme, hulp en inzichten bij het klinisch-biochemische aspect van mijn proefschrift. **Els**, dank dat je als chirurg wilde deelnemen

aan dit vrij "internistische" gebeuren en voor het delen van alle mooie roeiverhalen op congressen.

Dank aan alle mede-auteurs, de volgende personen in het bijzonder. **Timon**, jij weet met al je enthousiasme als geen ander statistiek begrijpelijk te maken. Dank voor alle hulp bij de analyses van de biomarker studies. **Ali**, bedankt voor alle tijd en energie om mee te denken over het ectopische ACTH stuk. Ik kijk nu al uit naar mijn oudste coschap in het IJsselland Ziekenhuis. Heel veel succes in de nabije toekomst met je eigen promotie. PS: mocht ik ooit zonneschermen nodig hebben, weet ik bij wie ik aan moet kloppen. **Esther Korpershoek**, bedankt voor je hulp bij de immunohistochemische ACTH-bepalingen voor de ectopische ACTH studie.

Prof.dr. G. Kaltsas, **Prof.dr. F.P. Costa**, **Prof.dr. M. Peeters**, **George Kanakis**, **Anna Angelousi** and **Brenda Gumz**: thank you for your contributions and assistance with regards to the studies described in this thesis. Your knowledge and expertise in the field of NETs was invaluable.

Mijn paranimfen. **Ro(derick)**, jij bent al vanaf de eerste colleges geneeskunde mijn maatje. Jij bent de hoofdschuldige van mijn Skadi-verslaving, want door jou ben ik daar lid geworden. Ik ben ervan overtuigd dat je een fantastische chirurg zal worden, je promotie tot een goed einde zal brengen, en dat je gelukkig wordt met Claire. Jij bent een goedzak, met een onmetelijk relativeringsvermogen en de gave om altijd rustig te blijven.

Neelke. Mijn beste vriendinnetje, tante van Pluis, partner in crime van menig vrijdag-middagborrel. Of überhaupt gewoon een borrel. Of koffie. Ik ben er zo trots op dat jij in Amsterdam promotieonderzoek gaat doen bij de kindergeneeskunde. Op de een of andere manier kunnen wij elkaar weken niet spreken en toch zo verdergaan waar het vorige gesprek eindigde. Zelfs jouw jaar in Houston was niet genoeg om elkaar uit het oog te verliezen. Sinds jullie hachelijke auto-avontuur in de rimboe van Zuid-Amerika, weet ik één ding zeker: jij en Tim kunnen samen alles aan.

Collega PhD's van kamer Z-626: **Karin**, dank voor al je wijze raad en adviezen tijdens mijn promotie en coschappen! Ik was zo groen als gras toen ik startte met mijn onderzoek. Samen met Stijn reis je langzaamaan de hele wereld rond, ik wens jullie alle geluk toe. **Sanne**, ik waardeer je gedrevenheid voor onderzoek, logistieke talenten tijdens reizen, gezelligheid tijdens eten en borrels, het denken in oplossingen (hoe neem ik mijn degen mee in de trein?) en de passie voor de schermsport. Wat waren de interne skiweekenden mooi: denk aan die steile zwarte piste, leek initieel een goed idee(?) en de outfits van de Disney's Peter Pan vertolking. Succes met de laatste fase van je proefschrift, je weet me te vinden. **Roxanne**, wat hebben we veel dagen, avonden en weekenden samen aan de

NET database gewerkt, zonder jou was het niet gelukt! Ik heb diep respect voor al jouw uurtjes en geduld in het lab. Naast je onderzoek heb je een bewogen jaar achter de rug, het mag gezegd worden, je bent een enorme doorzetter! Succes met de laatste loodjes van je proefschrift! Ik zie je snel weer in het IJsselland Ziekenhuis. Dames, dank dat jullie naast collega's ook vriendinnetjes zijn geworden!

Mark en **Ammar**, jullie kwamen als twee serieuze heren in een kippenhok terecht. Excuses (nogmaals) voor de af en toe vrij slechte muziekkeuzes op vrijdagmiddag. Veel succes met de verdere "rustige" voortzetting van jullie onderzoek. **Wouter** (Zandee niet te verwarren), ik heb de NET database met een gerust hart aan je overgedragen. Heel veel succes met je promotieonderzoek en sterkte met het databasen, maar dat zit wel goed.

Collega's van de interne geneeskunde-endocrinologie, **Aart Jan**, **Robin**, **Liesbeth**, **Sebastian**, **Carola** en **Joop**, **Ellen**, **Elske**, **Michel**, **Kees**, **Marlies**, **Carolien**, **Laura**, **Bruno**, **Swasti, Nicolaas, Roos, Corina**, **Edward**, **Hans, Marianne, Tineke** en **Judith**, dankzij jullie heb ik enorm veel geleerd over het mooiste vak, de endocrinologie. Bedankt voor de ontspannen en prettige werksfeer in het Erasmus MC en op menig congres, en niet te vergeten de altijd gezellige lunches.

Wanda, in mijn ogen ben je onmisbaar voor de endocrinologie. Je regelt alles voor de patiënten, van A tot Z, en nu je zwanger bent merkt iedereen hoeveel bergen werk je verzet. Ook voor mij was je van onschatbare waarde, al was het alleen al vanwege de congressen waar we samen naar toe gingen en de talloze databaselijstjes die we samen hebben opgesteld en doorgeworsteld. **Sjaan**, jou wil ik graag bedanken voor de fijne samenwerking en al je kennis en hulp bij de Clinical trials.

Karin en **Anneke**, jullie dansten jullie een weg door de onmetelijke wirwar van administraties, declaraties, afspraken en het organiseren van congressen. Jullie zijn de stille krachten achter mijn promotie.

Jelmer en **Michel**, grote broers met doorlopend gratis advies. Het was een eer om met jullie de legendarische 'pluizig en blauw'-skireis te organiseren voor de afdeling interne geneeskunde van het Erasmus MC. Ik ga ervan uit dat jullie kersverse kroost ook de komende jaren pluizig en blauw door het leven zullen gaan.

Leden en aanwezigen van de Tumorwerkgroep (Neuro)Endocriene Tumoren: dank voor het delen van jullie multidisciplinaire kennis en de altijd boeiende discussies. Vanuit de nucleaire geneeskunde **Jaap**, **Boen**, **Esther**, **Hendrik** en **Wouter**, vanuit de pathologie **Prof.dr. F.J. van Kemenade** en **Francien Nederveen**, bij oncologie **Ferry Eskens**, en tot slot binnen de chirurgie **Prof.dr. C.H.J. van Eijck**, **Gaston Franssen** en **Aleida.**

Andere collega PhD's: **Gerard**, **Vincent**, **Thomas**, **Sabine**, **Marije**, **Sara**, **Mesut, Anneke**, **Martijn**, **Layal**, en **Tim**, bedankt voor de altijd gevarieerde besprekingen op donderdagmiddag, het sparren over endocrinologische problemen, de gezellige congresbezoeken, borrels en hardloopperikelen.

Dames van de polikliniek, dank voor de ondersteuning bij het zoeken en vinden van alle oude statussen voor de opbouw van de NET database. **Verpleging en secretaresses van 5-midden**, dank dat ik altijd welkom was om op de afdeling te werken.

Cogroep 14.33, dankzij jullie is de herstart van mijn coschappen een stuk leuker en soepeler verlopen dan ik had durven denken. Dank voor alle briljante humor tijdens de ICK weken en de *tips and tricks* omtrent alle administratieve rompslomp. Ik ga de spam van de Psy-Co-groep 14.33 app enorm missen. Op het afstuderen!

Marli(eke), maffe, lieve en vooral gekke huisgenote van Huize Honingerdijk. Wat hebben we veel lol gehad op menig doordeweekse avond met slechte tv-programma's, oreo's, chips, cola-light en wijn! Met daarna steevast het voornemen om weer te gaan sporten of hardlopen. Wat mis ik die avonden sinds we allebei verhuisd zijn!

Skadi eerstejaars dames 2011 (Fleur, Avalon, Carola, Anne-Jet, Sianne, Anouk, Jitte, Carline & Melody): van slag tot boeg totaal verschillende types, maar toch de meest hechte groep dames die ik ken. Ik hoop dat we nog heel lang vriendinnen blijven. Al onze EJD avonturen, de prettige chaos tijdens het ploegeten en de feestjes (met prosecco): het zijn stuk voor stuk dierbare herinneringen. Oh ja, het roeien was natuurlijk ook extreem belangrijk. Vanaf nu ben ik er weer volop bij om gezellig te doen.

Heren van de Oude Vier: Spillie, Jappie, Fox & Floris. Bedankt dat ik voorin jullie boot mocht liggen toen we de mooiste overwinning ooit behaalden op de Varsity van 2015. Ik weet dat ik de laatste kilometer vooral hysterisch heb gegild, maar misschien wilden jullie daarom zo snel mogelijk over de streep? Hugo en Thijs, ook jullie waren belangrijk voor het succes, al roeiden jullie niet mee tijdens die race. Als ik aan het goud van Houten denk, denk ik ook aan jullie.

Oud-Skadi bestuur, **Remco**, **Robbert**, **Corneel**, **Gijs** en **Mattis**. Dank voor jullie altijd (h) eerlijke roei-relativeringsvermogen en het tijdelijk waarnemen van mijn taken in deze afgelopen drukke periode.

Vriendinnetjes, vrienden, collega's en kennissen, van binnen en buiten de roeiwereld, ook jullie wil ik niet ongenoemd laten. Dank voor jullie steun, interesse en luisterend oor tijdens vele etentjes en borrels, ook al hadden jullie vaak geen idee waar ik het over had.

Dan nu het belangrijkste deel van mijn dankwoord. **Mijn familie**.

Oma Kamp, helaas maak je de afronding van mijn proefschrift en afstuderen niet meer mee, ook al had je dit dolgraag gewild. Je was voor mij de allerliefste oma die er bestaat!

Tante Nelly en **oom Rien**: jullie zijn mijn tweede thuis, staan altijd klaar en niks is voor jullie te gek. Ik heb bijna een kwart van mijn coschappen bij jullie in Etten-Leur doorgebracht, en ik durf met mijn hand op mijn hart te beweren dat ik het zonder jullie warmte en zorg niet tot een goed einde had kunnen brengen. Jullie omschrijven jullie huis als de zoete inval, en als jullie het niet erg vinden wil ik nog heel lang van jullie gastvrijheid blijven snoepen.

Lieve paps, jij bent een doorzetter. Koppig (ik heb het af en toe niet van een vreemde), maar niet iemand die bij de pakken neerzit. Toen ik nog heel jong was werd je ernstig ziek, maar hoewel ik als driejarig meisje riep dat ik het was die je beter ging maken, was dat gelukkig niet nodig. Je helpt altijd met klussen en als ik je hulp nodig heb kan ik je bellen. Zullen we afspreken dat je tijdens mijn promotieplechtigheid geen foto's maakt zoals tijdens mijn doctoraal uitreiking?

Lieve mama, ook al woon je ongeveer aan de andere kant van de wereld, jij bent altijd dichtbij. Je bent er altijd voor me geweest, dag en nacht, en ik weet dat je er ook altijd voor me zult zijn. Je hebt je ontfermd over Berber en Pluis toen het nodig was, was er op álle roeiwedstrijden om me aan te moedigen en steunt me door dik en dun (ook als ik niet de ideale dochter ben of je midden in de nacht bel als ik alleen op de fiets naar huis moet). Ik heb ontzettend veel respect voor wat jij in het leven voor elkaar hebt gebokst. Ik hoop dat ik later net zo'n moeder kan worden als jij.

Michiel, je kwam letterlijk uit de lucht vallen. Je weet me altijd te verrassen en aan het lachen te maken. Op de een of ander manier houd je me rustig, dring je tot me door, en ook al gaat alles in een sneltreinvaart: het voelt alsof het zo hoort. Ik denk dat je mijn promotie inmiddels van binnen en buiten kent – tegen wil en dank – aangezien je aan een half woord van mij genoeg hebt om een alinea over neuroendocriene tumoren te herschrijven. Wil je alsjeblieft nooit je maffe tulpenpak weggooien? Ben stapelgek op je!