Aspects on Diagnosis and Treatment of Gastrointestinal Neuroendocrine Tumours

Christina Swärd
2010

UNIVERSITY OF GOTHENBURG

Department of Surgery
Institute of Clinical Sciences at Sahlgrenska Academy
Lundberg Laboratory for Cancer Research
University of Gothenburg
Göteborg, Sweden
To Joakim and our beloved boys, John, Robin and Marcus

When the going gets tough, the tough get going

*Billy Ocean and others*
**ABSTRACT**

**Aspects on Diagnosis and Treatment of Gastrointestinal Neuroendocrine Tumours**

**Background.** Gastroenteropancreatic neuroendocrine tumours (GEP-NETs) originate from endocrine cells of the intestinal mucosa and pancreas. The tumour cells contain chromogranin A (CgA) and usually have a high expression of somatostatin receptors (SSTR), which can be used for diagnosis and treatment. The treatment alternatives in patients with disseminated GEP-NETs include debulking surgery, hepatic arterial embolization (HAE), in selected cases orthotopic liver transplantation (OLT), or somatostatin receptor (SSTR)-mediated radiation therapy besides medical treatment with somatostatin analogues, interferon, or combination of cytotoxic drugs.

**Material & Methods.** A xenograft model with a transplantable midgut carcinoid tumour (GOT 1) was used to study the correlation between tumour weight and the tumour marker chromogranin A (CgA) in plasma as well as the therapeutic effects of the somatostatin analogue octreotide. The uptake and biodistribution of the radiopharmaceuticals $^{177}$Lu-octreotide and $^{177}$Lu-octreotate in GOT 1-bearing nude mice were studied. A consecutive series of 107 patients treated with HAE was analyzed regarding biochemical response and survival. The effect of SSTR-mediated radiation therapy was analyzed in a consecutive series of 26 patients with disseminated GEP-NETs as well as in 6 patients with non-resectable recurrence of tumour after OLT. The absorbed dose to the kidney as a tool for optimization of radiotherapy was evaluated.

**Results.** In GOT 1-bearing nude mice there was a strong correlation between tumour weight and P-CgA ($P<0.00001$). The P-CgA/tumour weight ratio was significantly lowered by octreotide ($P<0.037$). The uptake of $^{177}$Lu-octreotide was twice that of $^{177}$Lu-octreotide ($P=0.00061$) and the reduction of tumour volume was significantly higher in animals given $^{177}$Lu-octreotate ($P=0.003$). The tumour volume correlated significantly with P-CgA, which served as a marker of treatment effect. A good biochemical response with decreased tumour markers correlated with prolonged survival in patients after HAE ($P=0.003$). There was also a significant correlation between the responses to primary and repeat HAE. SSTR-mediated radiation therapy resulted in 38% partial tumour reduction according to RECIST-criteria. By using the absorbed dose to the kidney as a limiting factor for therapy we found that 10 patients received fewer than 4 treatments but 4 patients were identified as candidates for additional treatment. There was a significant reduction of glomerular filtration rate (GFR) after radiotherapy ($P=0.0013$). Also in transplanted patients SSTR-mediated radiotherapy could be used to treat tumour recurrence.

**Conclusions.** 1. P-CgA was well suited for monitoring of the tumour burden and tumour response in GOT 1-bearing nude mice. 2. $^{177}$Lu-octreotate was a more suitable radiopharmaceutical than $^{177}$Lu-octreotide and the *in vivo* data allowed accurate determination of absorbed dose. 3. HAE provided prolonged survival in biochemically responsive patients. Repeat HAE can be considered in patients with favourable response to the first procedure. 4. Calculation of the accumulated dose to the kidneys during SSTR-mediated radiotherapy was valuable to optimize the treatment. 5. SSTR-mediated radiotherapy was a treatment option also for patients with non-resectable recurrence of SSTR-expressing tumours, previously treated with OLT.

**Key words:** Carcinoid, endocrine pancreatic tumour, neuroendocrine, octreotide, somatostatin receptor, peptide receptor radionuclide therapy, absorbed dose, chromogranin A, liver transplantation.
LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>b.w.</td>
<td>Body weight</td>
</tr>
<tr>
<td>CgA</td>
<td>Chromogranin A</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CR</td>
<td>Complete response</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CTC</td>
<td>Common toxicity criteria</td>
</tr>
<tr>
<td>EC cell</td>
<td>Enterochromaffin cell</td>
</tr>
<tr>
<td>ECL cell</td>
<td>Enterochromaffin-like cell</td>
</tr>
<tr>
<td>EPT</td>
<td>Endocrine pancreatic tumour</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescence in situ hybridization</td>
</tr>
<tr>
<td>GEP-NET</td>
<td>Gastroenteropancreatic neuroendocrine tumour</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>HAE</td>
<td>Hepatic artery embolization</td>
</tr>
<tr>
<td>5-FU</td>
<td>5-fluorouracil</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>5-hydroxyindole acetic acid</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine, serotonin</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>IFN-α</td>
<td>Interferon α</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mammalian target of rapamycin</td>
</tr>
<tr>
<td>MVT</td>
<td>Multivisceral transplantation</td>
</tr>
<tr>
<td>NAD+</td>
<td>Nicotine amide adenine dinucleotide</td>
</tr>
<tr>
<td>NE</td>
<td>Neuroendocrine</td>
</tr>
<tr>
<td>OLT</td>
<td>Orthotopic liver transplantation</td>
</tr>
<tr>
<td>PARP1</td>
<td>Poly (ADP-ribose) polymerase 1</td>
</tr>
<tr>
<td>P-CgA</td>
<td>Plasma chromogranin A</td>
</tr>
<tr>
<td>PD</td>
<td>Progressive disease</td>
</tr>
<tr>
<td>PDEC</td>
<td>Poorly differentiated endocrine carcinoma</td>
</tr>
<tr>
<td>PR</td>
<td>Partial response</td>
</tr>
<tr>
<td>PRRT</td>
<td>Peptide receptor-radionuclide therapy</td>
</tr>
<tr>
<td>RECIST</td>
<td>Response evaluation criteria in solid tumours</td>
</tr>
<tr>
<td>s.c.</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SD</td>
<td>Stable disease</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SIR</td>
<td>Selective internal radiotherapy</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
</tr>
<tr>
<td>SSTR</td>
<td>Somatostatin receptor</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>TKI</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>tU-5-HIAA</td>
<td>Total urinary 5-hydroxyindole acetic acid</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>WDEC</td>
<td>Well-differentiated endocrine carcinoma</td>
</tr>
<tr>
<td>WDET</td>
<td>Well-differentiated endocrine tumour</td>
</tr>
<tr>
<td>VMAT</td>
<td>Vesicular monoamine transporter</td>
</tr>
</tbody>
</table>
Determination of absorbed dose to tumours

Statistical analysis

Clinical studies (III-V)

Patients

Hepatic artery embolization procedure (III)

Peptide receptor radionuclide therapy (IV-V)

Somatostatin receptor scintigraphy

RESULTS

Tumour weight correlates with P-CgA in GOT 1-xenografted mice (I)

$^{177}$Lu-octreotate gives higher uptake, higher absorbed dose and better tumour reduction than $^{177}$Lu-octreotide in GOT 1-xenografted mice (II)

Successful HAE correlates with prolonged survival in patients with midgut carcinoid (III)

PRRT results in objective tumour reduction and impaired kidney function; it can be optimized by monitoring the absorbed dose to the kidney in patients with GEP-NETs (IV)

PRRT in transplanted patients with non-resectable recurrence (V)

DISCUSSION

Biochemical markers of tumour burden

Treatment of disseminated neuroendocrine tumours

Peptide receptor radionuclide therapy

Hepatic artery embolization

PRRT in patients liver transplanted for GEP-NETs

Optimization of current treatment alternatives

Future aspects

CONCLUSIONS

ACKNOWLEDGEMENTS

REFERENCES
INTRODUCTION

Pathology and clinical features of neuroendocrine tumours

Gastroenteropancreatic neuroendocrine tumours (GEP-NETs) originate from endocrine cells of the gastrointestinal mucosa and pancreas. There are at least 14 specialized cell types in the diffuse endocrine system of the gut. They are all part of a complex regulatory network, each cell type synthesizing and secreting specific peptide hormones and biogenic amines. The endocrine cells are characterized by neurosecretory granules containing both amines and peptide hormones. Examples of different subtypes of endocrine cells and their main secretory products are; A-cells/glucagon and B-cells/insulin in the pancreas, D-cells/somatostatin in the pancreas and intestine, EC-like (ECL) cells/ histamine and G-cells/gastrin in the stomach, enterochromaffin (EC) cells/serotonin (5-HT) and tachykinins in the intestine (Rindi et al., 2004).

The most common neuroendocrine (NE) tumours of the gastrointestinal (GI) tract are the carcinoid tumours followed by endocrine pancreatic tumours (EPT), with an incidence in clinical series of 2.5/100.000 and 1/100.000, respectively (Crocetti et al., 1997; Hemminki & Li, 2001; Lepage et al., 2004; Levi et al., 2000; Modlin et al., 2003; Newton et al., 1994; Quaedvlieg et al., 2001; Westergaard et al., 1995). In a large Swedish autopsy study the annual incidence of GI carcinoids was 8.4 per 100.000 inhabitants (Berge & Linell, 1976). Many carcinoids are small and asymptomatic, which is one explanation for the higher incidence in autopsy series. Carcinoid tumours were initially classified into three groups, based on their embryological origin, i.e. foregut (thymus, lung, oesofagus, stomach and duodenum), midgut (jejunum, ileum, appendix) and hindgut (colon and rectum) tumours (Williams & Sandler, 1963). This classification has been replaced by the
WHO classification (Solcia et al., 2000) dividing NE tumours according to their degree of differentiation; well-differentiated endocrine tumours (benign or uncertain behaviour, WDET), well-differentiated endocrine carcinomas (low-grade malignant behaviour, WDEC), and poorly differentiated endocrine carcinomas (high-grade malignant carcinomas, PDEC). A TNM classification and grading system has been added by the European Neuroendocrine Tumour Society (ENETS) (Rindi et al., 2006).

GEP-NETs are rare, but still constitute the most common malignancy of the small bowel (Godwin, 1975; Modlin et al., 2003). Characteristic for these tumours is their hormonal secretory properties, causing clinical symptoms related to hormone overproduction. Carcinoid tumours in the small bowel (midgut) are composed of EC cells. Secretion of serotonin (5-HT) and tachykinins give rise to the midgut carcinoid syndrome characterized by facial flush, diarrhea (each 80%), right-sided cardiac valvular lesions (20-40%), and bronchoconstriction (10%, carcinoid asthma) (Grahame-Smith, 1972). The gastric carcinoid tumours arise from ECL cells of the corpus region and can result in a clinical syndrome characterized by lacrimation, bronchial obstruction, generalized flush, and oedema due to histamine release (the atypical carcinoid syndrome). The colorectal carcinoid tumours, originating from EC-, L- and P-cells, rarely result in a hormonal syndrome. Endocrine pancreatic tumours can be classified on clinical grounds as functioning, or non-functioning, depending on whether they are hormone-producing, or not (Jensen, 1999). Each specific cell type in pancreatic islets can give rise to a characteristic tumour and hormonal syndrome, e.g. A-cells give rise to glucagonomas, B-cells to insulinomas, and D-cells to somatostatinomas. Histopathologically, EPTs are also classified as WDET, WDEC and PDEC.

The prognosis of GEP-NETs is related to the grade and site of the primary and to the extent of disease at diagnosis (Godwin, 1975; Greenberg et al., 1987; McDermott et al., 1994; Rindi et al., 2007). Non-metastatic tumours with the primary site in the small intestine, or colon, have significantly worse prognosis than
those occurring in the lung, stomach, appendix or rectum (Modlin et al., 2003). WDEC have a relatively slow growth rate and are usually first clinically diagnosed with locoregional and distant spread. PDEC of the GI tract have sinister prognosis and are only rarely surgically curable, but temporary tumour responses can be achieved by chemotherapy (cisplatin and etoposide) (Brenner et al., 2004; Brenner et al., 2007).

**Biochemical markers of neuroendocrine tumours**

**Chromogranin A**

Chromogranin A (CgA) is a 75 kDa glycoprotein that was first isolated from chromaffin granules of bovine adrenal medulla (Smith & Winkler, 1967), and was found to be co-secreted with catecholamines. CgA is produced exclusively by endocrine and NE cells (Deftos, 1991). It is co-localized with amines and peptides in the dense core secretory granules. The function of CgA is not fully elucidated, but it plays a role in targeting peptide hormones and neurotransmitters to granules. It also serves as a precursor for several biologically active peptides such as vasostatin, β-granin, chromostatin, pancreastatin and parastatin. These peptides act predominantly as inhibitors of hormone and neurotransmitter release in an autocrine and paracrine fashion (Deftos, 1991; Helle et al., 2007; Hendy et al., 1995). The almost universal presence of CgA in NE cells provides a tool for immunohistochemical identification of NE tumours regardless of their hormonal activity, location, and degree of differentiation. For histopathological purposes, other NE markers are available, *i.e.* synaptic vesicle protein 2, neuron-specific enolase and synaptophysin (Jakobsen et al., 2002; Portela-Gomes et al., 1999; Vyberg et al., 1990). Since CgA is secreted into the circulation, it can also be used as a tumour marker in plasma (Stridsberg et al., 1995). P-CgA seems to be the most reliable marker for NE tumours, both in terms of specificity (86%) and sensitivity (68%) (Bajetta et al., 1999; Baudin et al., 1998).
5-Hydroxyindole acetic acid

The main secretory product of EC cells and ileal carcinoids is serotonin, 5-hydroxytryptamin (5-HT). After dehydrogenation and degradation of 5-HT by monoamine oxidase in the in liver and lung, 5-hydroxyindole acetic acid (5-HIAA) is excreted in the urine (Gillies, 1979; Grahame-Smith, 1972). The rapid turnover and the large 5-HT pool in platelets make measurements of 5-HT in serum less reliable for clinical use. Urinary 5-HIAA (24 hrs) is still one of the most frequently used diagnostic tools for midgut carcinoids. 5-HIAA levels have a high specificity (100%) but low sensitivity (35.1%) (Bajetta et al., 1999) in the detection of midgut carcinoid tumours; 5-HIAA is most valuable in patients with metastatic disease, in whom it serves as a tumour marker to follow the course of the disease and the effect of therapy.

The carcinoid valvular heart disease (usually tricuspid insufficiency sometimes combined with pulmonary stenosis) is believed to be caused by fibroblast-stimulating factors, i.e. 5-HT and tachykinins, secreted from liver metastases, or to local factors from the peritoneal cavity, e.g. transforming growth factor β1 and connective tissue growth factor (Kidd et al., 2007; Modlin et al., 2009). The presence of heart disease has been shown to correlate with high 5-HT turnover, manifested by high tU-5-HIAA (Bernheim et al., 2007; Himelman & Schiller, 1989; Pellikka et al., 1993; Westberg et al., 2001).

Somatostatin receptors

Somatostatin receptors (SSTR) are widely distributed in the body. They are involved in regulation of gut motility, fluid secretion, hormone release and growth. A variety of tumour types express SSTR, especially NE tumours which may over-express SSTR 100-fold compared to normal tissue resulting in high tumour-to-blood uptake ratios after administration of radiolabeled somatostatin analogue (Forssell-Aronsson et al., 2004; Forssell-Aronsson et al., 1995). There are 5 characterized subtypes of SSTR, SSTR1-5. They are all G-protein-coupled
receptors with 7 transmembrane domains. Using Northern blot, or real time quantitative PCR, the SSTR profile of individual tumours can be determined. Midgut carcinoid tumours express all subtypes, but with dominance of SSTR2&5 (Kölby et al., 1998). Native somatostatin has high affinity for all 5 subtypes, while the somatostatin analogue octreotide has high affinity only for SSTR2&5 (Susini & Buscail, 2006). The presence of SSTR on NE tumour cells provides a tool both for tumour visualization with scintigraphic techniques and peptide receptor radionuclide therapy (PRRT).

**Treatment modalities**

**Surgical treatment**

The only curative treatment for GEP-NETs is radical surgery at the time of localized disease. In the few patients who are diagnosed at an early stage, curative surgery should be attempted, including resection of the primary tumour, regional lymph node metastases and liver resection if limited hepatic spread is present. This can lead to total remission for prolonged periods (Wängberg et al., 1996). However, the tumour is often diagnosed first when the disease is widely disseminated. Patients with bilobar hepatic metastases should still undergo surgery aiming at reduction of extrahepatic tumour so that continued treatment can be focused on the hepatic tumour disease. Cholecystectomy is included in the surgical procedure to prevent somatostatin analogue-induced gallstones, and to avoid gall bladder gangrene during HAE (Simons et al., 1992; Takayasu et al., 1985). The slow progression of these tumours makes active tumour reduction highly motivated. Resection of lymph node metastases along the superior mesenteric artery (a common feature in ileal carcinoids) together with excision of mesenterial fibrosis, can be helpful to re-establish the circulation of the small bowel.

In the first report from our group (Wängberg et al., 1996), 64 patients with disseminated midgut carcinoids and hepatic metastases were treated according to a uniform clinical protocol aiming at aggressive tumour reduction by surgery alone,
or in combination with hepatic artery embolization (HAE). Fourteen patients (22%) reached remission by surgery alone, forty patients (63%) with bilobar liver metastases underwent HAE in combination with octreotide treatment. Ten patients (15%) with bilobar liver metastases were not embolized because of complicating diseases. The 5-year survival for the entire series was 58%, which compared most favourably with other patient series at that time; only 19-21% in the early series (Godwin, 1975; Moertel et al., 1961) and 38% in a simultaneous report (Jacobsen et al., 1995). In later series the 5-year survival of patients with liver metastases varied between 47-53% including data from single centres and large tertiary referral centres (Chamberlain et al., 2000; Nave et al., 2001; Sarmiento & Que, 2003; Sutcliffe et al., 2004).

**Somatostatin analogue treatment**

Somatostatin was first isolated from the hypothalamus (Krulich et al., 1968) and later found to be localized also in the D-cells of the GI tract (Reichlin, 1983a; Reichlin, 1983b; Shulkes, 1994). Somatostatin reduces smooth muscle contractility and glandular secretion by inhibiting cAMP. Physiologically somatostatin regulates hormone secretion from the anterior pituitary, pancreas and GI tract. Native somatostatin reduced symptoms in patients with GEP-NETs, but short half-life made its clinical use limited. In 1984, the first somatostatin analogue, octreotide, was tested in a phase II study. With a half-life of 90 minutes, octreotide can be administered every 6-12 hours. Octreotide has high affinity for SSTR2&5 (Bruns et al., 1994) and have direct inhibitory effects on the tumour cells, reducing the synthesis and release of 5-HT (Wängberg et al., 1991). With the introduction of somatostatin analogues, hormonal symptoms could be effectively treated and patients with the midgut carcinoid syndrome could undergo surgery without crisis reactions and unstable blood pressure (Ahlman et al., 1988; Ahlman et al., 1987; Eriksson & Öberg, 1999).
Common transient adverse effects during the first weeks of treatment with somatostatin analogues include abdominal discomfort, bloating and steatorrhea. Cholestasis with formation of cholecystolithiasis occur in up to 60% of the patients, due to inhibition of cholecystokinin and production of lithogenic bile (Redfern & Fortuner, 1995), which further supports prophylactic cholecystectomy at time for primary surgery in GEP-NET-patients. 

*In vitro* high doses of somatostatin analogues induce apoptosis in tumour cells leading to retarded growth. There are several clinical reports suggesting a tumour-stabilizing effect of somatostatin analogues (Arnold et al., 1996; Shojamanesh et al., 2002; Susini & Buscail, 2006; Verslype et al., 2009). In a recent randomized prospective study, long-lasting octreotide (LAR) prolonged the time to tumour progression from 6 to 14 months in patients with midgut carcinoids (Rinke et al., 2009).

**Peptide receptor radionuclide therapy**

The high expression of SSTR in NE tumours can be used for diagnostic and therapeutic purposes, since the receptor complex is internalized by the tumour cells after ligand binding (Andersson et al., 1996). Coupling of chelators may change the affinity of octreotide to SSTR. Using DOTA as a chelator decreases the SSTR2-affinity for octreotide 7-fold, while the somatostatin analogue octreotate retains very high SSTR2-affinity when chelated with DOTA (Reubi et al., 2000). The most common radiopharmaceutical for diagnostic procedures has been $[^{111}\text{In}]\text{-DTPA}\,^0\,\text{octreotide}$; $^{111}\text{In}$ emits primarily Auger electrons and photons. Treatment with $[^{111}\text{In}]\text{-DTPA}\,^0\,\text{octreotide}$ resulted in reduction of biochemical markers, but objective tumour regression was unusual (Fjälling et al., 1996; Krenning et al., 1999; Tiensuu Janson et al., 1999; Valkema et al., 2002). Other radionuclides (beta-emitters) were combined with new somatostatin analogues and chelators, which resulted in improved radiopharmaceuticals with enhanced binding to specific SSTR and more suitable radiation types emitted by the radionuclide aimed for therapy.
(Bernhardt et al., 2001). The use of $[^{90}\text{Y}-\text{DOTA}, \text{Tyr}^3]$-octreotide resulted in partial tumour regression in up to 24% of the first patients treated (Paganelli et al., 2001; Waldherr et al., 2001). Today the most frequently used radiopharmaceutical is $[^{177}\text{Lu}-\text{DOTA}^0, \text{Tyr}^3]$-octreotate. $^{177}\text{Lu}$ has a half-life of 6.7 days, and emits both beta- and gamma radiation, which allows imaging and dosimetry at the time of radiotherapy. $[^{177}\text{Lu}-\text{DOTA}^0, \text{Tyr}^3]$-octreotate has longer tumour residence time (by a factor of 2.1) than $[^{177}\text{Lu}-\text{DOTA}^0, \text{Tyr}^3]$-octreotide (Esser et al., 2006). This probably relates to higher affinity to SSTR2, which is the main SSTR subtype expressed by midgut carcinoids (Kwekkeboom et al., 2001; Kölby et al., 1998; Reubi et al., 2000).

$[^{177}\text{Lu}-\text{DOTA}^0, \text{Tyr}^3]$-octreotate treatment of patients with disseminated GEP-NETs resulted in 46% objective tumour responses (very few complete remissions and a majority of partial or minor tumour regressions) in a large series of 310 patients (Kwekkeboom et al., 2008). The dose-limiting factors are kidney and bone marrow toxicity. Careful dosimetry is essential to reach as high absorbed dose to the tumour tissue as possible without causing severe adverse effects, i.e. renal or bone marrow failure. Kidney doses can be calculated from planar scintigraphic images, or from single photon emission computed tomography (SPECT) images. Absorbed dose to the bone marrow is more difficult to assess, since it requires measurements of biopsy material.

PRRT-studies have so far reported that the procedure is well tolerated with few severe adverse effects. The tumour response rate is relatively high and the progression-free survival of patients is probably prolonged. The survival benefit has not yet been studied in randomized series. The most important factors for predicting objective tumour responses seem to be high radionuclide uptake on octreotide scintigraphy and a good performance status of the patient (Kwekkeboom et al., 2008).
**Hepatic artery embolization**

Interventions involving the hepatic artery for therapeutic purposes were first proposed by Markowitz in 1952 and various techniques have been employed to induce tumour ischaemia, initially hepatic artery ligation (Markowitz, 1952; Nilsson, 1966). This procedure rarely achieved adequate ischaemia due to a rich collateral blood supply. The interventions could not be repeated and the technique was associated with high mortality in the pre-somatostatin analogue era. The method most frequently used today is selective HAE. The hepatic artery is first catheterized followed by injection of the embolization material in peripheral branches related to vascular anatomy and tumour localization. This procedure causes a relatively selective ischaemia of the tumour metastases, since the normal liver parenchyma has a dual blood supply from the hepatic artery and the portal vein (Cho et al., 1976). Patency of the portal vein is therefore a prerequisite for HAE to be performed. HAE is a well established palliative treatment for bilobar liver metastases from NE tumours and has been shown to give good control of hormonal symptoms. It is effective in reducing hormone levels and symptoms in patients with objective radiological tumour regression in response to the procedure; a biochemical effect that can last for several years (Wängberg et al., 1996). In addition, a survival advantage after HAE has been suggested in previous studies (Gupta et al., 2005; Gupta et al., 2003; Jacobsen et al., 1995; Mitty et al., 1985).

HAE can be combined with intra-arterial injection of chemotherapeutic agents (HACE, or chemoembolization) to the liver. Combining HACE with octreotide treatment in patients with advanced hepatic carcinoid metastases resulted in shrinkage of the tumours in 85% of the patients; the mean reduction of tumour markers was 50-75%. The procedure was well-tolerated and without severe adverse effects (Drougas et al., 1998).
Radiofrequency ablation
Solitary liver metastases of moderate size (< 3 cm) can be successfully treated with ultrasound-guided percutaneous radiofrequency ablation (Elvin et al., 2005; Hellman et al., 2002). The method can also be applied during surgery (Berber et al., 2002). The method is limited not only by tumour size and number, but also by the location within the liver and the relation to adjacent vessels, e.g. the portal vein.

Radioembolization
Radioembolization with $^{90}$Y microspheres has been used to treat patients with non-resectable liver metastases and primary liver carcinoma since more than a decade with some success (Carr, 2004; Gray et al., 2001; Stubbs et al., 2001). The treatment is performed by injecting $^{90}$Y-labeled resin microspheres via the hepatic artery. Usually two thirds of the total dose is injected into the right hepatic lobe and one third into the left lobe (selective internal radiotherapy, SIR). Patients with liver metastases from NE tumours have recently been treated with objective tumour responses and few side-effects (Kalinowski et al., 2009; Kennedy et al., 2008; King et al., 2008; Rhee et al., 2008).

Chemotherapy
Chemotherapy can provide palliation for patients with disseminated GEP-NETs. The response rate to monotherapy is low, so combinations of chemotherapeutic agents are used (e.g. streptozotocin (STZ), fluorouracil (5-FU) and doxorubicin, or dacarbazine, etoposide and cisplatin). In one recent trial, patients with EPT were treated with STZ, 5-FU and doxorubicin (Kouvaraki et al., 2004) with a response rate of 39% and a median progression-free survival of 9.3 months in line with the early series (Moertel et al., 1994; Rivera & Ajani, 1998).

In patients with GI carcinoid tumours, chemotherapy seems to be less effective than in patients with EPT. A recent overview of 10 trials over 25 years (1979-2005) indicated a low median response rate of 25% and a median survival of 11 months
(Plöckinger, 2008). Etoposide and cisplatin are not effective in patients with WDEC tumours, but result in temporary tumour response in two thirds of the patients with PDEC tumours (Arnold et al., 2005; Brenner et al., 2004; Brenner et al., 2007).

**Interferon-α**

The cytokine interferon-α (IFN-α) has been shown to have anti-tumour effects mediated via several mechanisms. IFN-α became available for treatment of patients with the midgut carcinoid syndrome in the 1980s. The dose must be individually adjusted using haematological parameters such as leucocyte count. The adverse effects of IFN-α may include initial flu-like symptoms, but autoimmune reactions occur in 20% of patients (Öberg, 1994). Objective tumour reduction was observed in 10% of the patients, but stable disease in up to 70% (Dirix et al., 1996; Doberauer et al., 1991; Jacobsen et al., 1995; Janson et al., 1992; Moertel et al., 1989; Schober et al., 1992; Smith et al., 1987; Öberg & Eriksson, 1989; Öberg & Eriksson, 1991). However, in a national prospective randomized clinical trial of patients with midgut carcinoid tumours metastatic to the liver, who had previously undergone primary surgical treatment and HAE, no survival benefit of IFN-α was shown in addition to the use of a somatostatin analogue (Kölby et al., 2003).

**Liver transplantation**

Orthotopic liver (OLT), or multivisceral, transplantation (MVT), can be used for treatment of metastatic NE tumours, since metastases can be limited to the liver for long periods. It may offer control of hormonal symptoms, sometimes even cure, for selected patients with favourable biological features (WDEC tumours with limited proliferative activity). Resection of the primary tumour (intestinal or pancreatic body/tail resection) together with regional lymph nodes, should be performed prior to OLT. With an EPT located in the head of the pancreas, MVT may be a treatment
alternative. The experience of OLT/MVT is hitherto limited, but in a Gothenburg study on 15 patients over 8 years, good relief of hormonal symptoms, long disease-free survival and potential cure in individual patients were reported (Olausson et al., 2007).

**Treatment algorithm**

GEP-NETs can be managed by a multitude of treatment modalities. The mainstay in treatment of GEP-NETs is surgery and somatostatin analogues. For patients with localized disease surgery can lead to total remission. Primary surgery includes resection of the primary, lymph node metastases, sometimes liver resection and prophylactic cholecystectomy (in preparation for later HAE). As a majority of patients are diagnosed at an advanced tumour stage, only a minority can undergo curative surgery. Removal of the primary tumour and reduction of the metastases is still recommended to reduce local symptoms and hormonal effects. Somatostatin analogues alleviate hormonal symptoms and may also have a tumour-stabilizing effect (Rinke et al., 2009; Shojamanesh et al., 2002; Susini & Buscail, 2006; Verslype et al., 2009). Interferon can offer similar therapeutic effects as somatostatin analogues to certain patients (Öberg, 2000; Öberg, 2003). Bilobar hepatic metastases can be treated by applied ischemia (HAE), which leads to reduced hormone production and often tumour shrinkage. Liver transplantation can be a treatment option for selected patients with disease limited to the liver; the indications for transplantation have not yet been settled, e.g. the upper limit for the proliferative activity of the tumour (Ki67) today varies between 2.35-10% (Olausson et al., 2007). Progressive disease after attempts with medical and interventional therapy, may be treated systemically by PRRT or chemotherapy (Fig. 1).
Fig 1. Treatment algorithm. Flow chart representing the treatment algorithm for GEP-NETs employed at our unit. Bold arrows indicate our most common treatment pathways.
**RECIST**  
Response evaluation of anticancer treatment has not been uniform, which have made comparisons between studies difficult. Several organizations involved in clinical cancer research first met in 1994, which led to the definition of RECIST criteria (Response Evaluation Criteria in Solid Tumours) (Therasse et al., 2000). This consensus states that measurements of tumour lesions should be undertaken on CT or MRI images. The longest diameter of measurable (>10 mm on spiral CT or MRI, >20 mm on conventional CT) lesions up to a maximum of five per organ (a maximal total of 10) are measured in the axial plane, giving a sum of diameters at baseline. The measured lesions are designated target lesions. Lesions smaller than 10, or 20 mm, respectively, are considered non-measurable. Examples of non-measurable lesions are bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis in skin and lungs, abdominal and cystic lesions. At follow-up the target lesions are re-evaluated with the same examination modality as at baseline. The criteria for response are:  
*Complete response (CR)* – disappearance of all target lesions.  
*Partial response (PR)* – at least a 30% decrease of the sum of the longest diameter of target lesions, taking as reference the baseline sum of longest diameter.  
*Progressive disease (PD)* – at least a 20% increase of the sum of the longest diameter of target lesions, taking as reference the smallest sum of longest diameter, recorded since the treatment started, or at the appearance of new lesions.  
*Stable disease (SD)* – neither sufficient shrinkage to qualify for partial response, nor sufficient increase to qualify for progressive disease.  

**Tumour models**  
Few model systems are available for experimental studies of NE tumours. The BON cell line was derived from an endocrine pancreatic carcinoid (Evers et al., 1991), whereas KRJ-I, GOT 1 and CNDT2 cell lines were derived from ileal carcinoid tumours (Kölby et al., 2001; Pfragner et al., 1996; Van Buren et al.,
Primary cell cultures reflect the tumour biology of individual patients most accurately but are hampered by the limited availability of material. Studies of receptor mechanisms and secretory mechanisms have been performed in cell lines, BON being the most widely used. However, BON cells have been shown to have a mixed endocrine/exocrine phenotype. The GOT 1 model consists of a transplantable midgut carcinoid (EC cell type) derived from a liver metastasis of a human ileal carcinoid. Analysis of GOT 1 during several generations, have shown that the NE phenotype is well preserved, including expression of SSTR and vesicular monoamine transporters (VMAT1&2) (Kölby et al., 2001). This animal model with a human midgut carcinoid tumour transplanted s.c. to the back of the neck of nude mice, has been most useful for studies of SSTR-expression, secretory mechanisms, tumour markers and SSTR-targeted radiotherapy (Bernhardt et al., 2001; Bernhardt et al., 2003; Bernhardt et al., 2007; Kölby et al., 2005).
AIMS

1. To evaluate P-CgA as a tumour marker for NE tumours by studying the relationship between P-CgA and tumour burden in a xenograft model of a human midgut carcinoid (GOT 1) and to analyze the effect on P-CgA after medical treatment with a long-acting somatostatin analogue.

2. To evaluate the uptake and therapeutic effect of \([^{177}\text{Lu-DOTA}^0, \text{Tyr}^3] \)-octreotide and \([^{177}\text{Lu-DOTA}^0, \text{Tyr}^3] \)-octreotate in the GOT 1 model and to evaluate P-CgA as a marker of tumour response.

3. To study the effect of HAE in patients with metastatic ileal carcinoids by evaluating biochemical response and survival in a large consecutive series of patients treated with HAE.

4. To evaluate radiological and biochemical response to PRRT in patients with disseminated NE tumours and to analyze the clinical value of monitoring accumulated renal doses for optimization of such treatment.

5. To evaluate possible indications for PRRT and the clinical outcome after PRRT in a pilot series of patients with non-resectable recurrence of NE tumours, previously subjected to OLT.
MATERIAL AND METHODS

Experimental studies (I-II)

Tumour model
The xenograft model was established in our laboratory by injecting the human midgut carcinoid tumour cell line GOT 1 s.c. in the back of the neck of BALB/cABom-nude mice. This tumour has then been successfully propagated through several generations by transplanting small pieces of tumour tissue (Kölby et al., 2001). The phenotype has been preserved with identical expression of NE markers such as SSTR1-5 and VMAT1&2, as the original tumour. The human origin of the transplanted tumour was confirmed by cytogenetic and fluorescence in situ hybridization analyses (FISH) (Sjögren et al., 2000). When the tumours had grown to suitable size (2-20 mm in diameter), the animals were included in our studies I-II. Measurement of tumour volume was performed by measuring two perpendicular diameters with callipers. The volume was calculated by the formula $\frac{4}{3} \pi ab^2$, in which $a$ is the longitudinal radius, $b$ is the transverse radius (perpendicular radius).

Determination of CgA
Blood was collected from the animals at sacrifice in heparinized syringes (40 µl heparin 5000 IE/ml + 1 ml blood). The samples were centrifuged for 20 min at 5000 x g and the plasma aliquot frozen at -70°C until assay. P-CgA was determined by using a competitive radioimmunoassay. Polyclonal antisera were raised against fragments of CgA, isolated from a patient with midgut carcinoid tumour. These fragments had molecular weights of about 30 kDa, covering the amino acid sequence 116-439 of human CgA. The CgA preparations were also used for standard and tracer preparations. The results were calculated with a logit-log transformation program (Pharmacia-LKB). The detection limit was <3 fmol/tube and the total coefficient of variation for the assay was <6.4%. No cross-reactions with other chromogranins, or fragments, were observed (Stridsberg et al., 1993; Stridsberg et al., 1995).
**Immunohistochemistry**

Tumour tissues from nude mice were characterized by immunohistochemistry (I). After formalin fixation and paraffin embedding the sections were incubated with primary monoclonal antibodies against CgA (Boehringer Mannheim, Germany). Bound antibodies were visualized by the indirect immunoperoxidase technique (Envision Plus, Dako, Denmark).

**Drugs**

The animals (I) were either given long-acting octreotide (Sandostatin-LAR) in low dose (0.286 mg/kg b.w.) or high dose (2.86 mg/kg b.w.). In study II, kits of \([\text{DOTA}^0, \text{Tyr}^3]\)-octreotate and \([\text{DOTA}^0, \text{Ty}^3]\)-octreotide were made by mixing 2.02 mg \([\text{DOTA}^0, \text{Tyr}^3]\)-octreotate and 2.02 mg \([\text{DOTA}^0, \text{Ty}^3]\)-octreotide (synthesized by Jörg Schmitt, Division of Radiological Chemistry, University Hospital, Basel, Switzerland) with 630 mg of sodium ascorbate, 125 mg of gentisic acid and 5 mL 0.05 M HCl, respectively. \(^{177}\text{LuCl}_3\) (Perkin Elmer, Life Sciences, Inc., Boston, MA, USA) was diluted in 0.05 M of HCl and mixed with \([\text{DOTA}^0, \text{Tyr}^3]\)-octreotate or \([\text{DOTA}^0, \text{Ty}^3]\)-octreotide to a specific activity of 30 MBq/µg, respectively. The peptide-bound fraction of \(^{177}\text{Lu}\) was assessed by instant thin layer chromatography with 0.1 M of sodium citrate (pH 5.0) as the mobile phase. The fraction of peptide-bound \(^{177}\text{Lu}\) was more than 99%. The exact amount of radiopharmaceutical administered was determined by measuring the activity in the syringes before and after administration, using a well-type ionization chamber. Two groups of tumour-bearing animals were established and injected with 15 MBq \([^{177}\text{Lu-DOTA}^0, \text{Tyr}^3]\)-octreotide (\(^{177}\text{Lu}\)-octreotide), or 15 MBq \([^{177}\text{Lu-DOTA}^0, \text{Ty}^3]\)-octreotate (\(^{177}\text{Lu}\)-octreotate), respectively.

**Determination of absorbed dose to tumours**

The absorbed dose to tumours was calculated (II). The cumulated activity concentration, \(i.e.\) the total number of radioactive decays per mass unit, was determined by estimating the area under the curve for the tumour activity concentration vs time. The electron energy emitted per decay was assumed to be 147 keV and absorbed within the tumour as previously described (Bernhardt et al., 2001). The contribution of photons was neglected. Also, the contribution from organs other than the tumour was neglected.

**Statistical analysis**

In study I, the dependency of P-CgA on weight was analyzed by covariance analysis using log (P-CgA) as the dependent variable, group as factor, and tumour weight as covariate. The
logarithmic transform of P-CgA and P-CgA/tumour weight was used to stabilize variances. The interaction between group and tumour weight was included in the model. The differences between the groups regarding P-CgA/tumour weight was analyzed by one-way analysis of variance followed by multiple comparisons of groups 2 & 3 vs group 1 using Dunnett’s method. As the dependent variable, log (P-CgA/tumour weight) was used.

In study II, the two-stage method (using the Statistical Analysis System (PROC MIXED)) was used for the uptake studies. The differences in tumour volume were analyzed by a two-tailed t test. For the correlation between tumour volume and P-CgA, Pearson’s correlation coefficient (R) was calculated.

**Clinical studies (III-V)**

**Patients**

In study III, 107 consecutive patients with the midgut carcinoid syndrome and non-resectable liver metastases were treated with HAE at the Sahlgrenska University Hospital (1987-2006) and analyzed. The patients had hormonal symptoms and tU-5-HIAA levels were increased at least twofold, or showed radiological progression. Contraindications for HAE were tumour burden exceeding 50% of the liver volume, portal vein occlusion, and hyperbilirubinaemia. Relative contraindications were contrast allergy, coagulopathy, persistently raised liver enzymes, extrahepatic tumour dominance, and poor general performance status.

In study IV, 26 patients with disseminated NE tumours (10 EPT, 10 midgut carcinoids, 3 rectal carcinoids, 1 pulmonary carcinoid, 1 duodenal gastrinoma, 1 NE pre-sacral tumour) treated with \(^{177}\)Lu-octreotate at our hospital (2006-2008), were analyzed regarding radiological (RECIST) and biochemical (P-CgA) response absorbed dose to the kidney and glomerular filtration rate (GFR). These patients had all been subjected to previous multimodal treatment with combinations of surgery (primary and repeated), chemotherapy, somatostatin analogues, interferon-\(\alpha\), HAE, RF ablation, external radiation of the skeleton and in 4 patients OLT.

In study V, 6 patients (three with carcinoids and three with EPT) previously treated by OLT for metastases confined to the liver, had developed recurrence. Four patients were treated with PRRT x 4 due to non-resectable recurrence, and 2 patients were treated in an adjuvant setting with PRRT x 2 after tumour excision. Routine haematology, liver and kidney function tests (GFR) and tumour markers were performed prior to each therapy and at follow-up. Radiological tumour responses of liver and lymph node metastases were evaluated according to RECIST-criteria.
**Hepatic artery embolization procedure (III)**

HAE was performed by a transfemoral approach using a Tracker®-18 infusion catheter (Target Therapeutics, Los Angeles, California, USA) (Chuang, 1988; Coldwell, 1990). Angiography was used to confirm the patency of the portal vein. Branches of the hepatic artery were embolized selectively, usually in two sessions, including the right and the left hepatic artery, respectively. In patients with large tumour burden, smaller branches were embolized selectively. The embolization material in the early part of the series was gelatin powder and later in the series polyvinyl alcohol particles (45-150 µm). The patients were provided with epidural anaesthesia during the procedure, and monitored haemodynamically. Broad-spectrum antibiotics and somatostatin analogues were administered. Repeat HAE was considered when progressive disease was detected by two consecutive CT scans with an interval of at least 6 months, and tU-5-HIAA concentration increased at least twofold relative to that recorded after the previous HAE; repeat HAE was performed in 19 out of 107 patients.

**Peptide receptor radionuclide therapy (IV-V)**

In study IV, each patient was scheduled for 4 treatment sessions, each with 8 GBq $^{177}$Lu-octreotate, 6 weeks apart. The indications for treatment were progressive disease despite conventional therapy, and a tumour uptake of the radiopharmaceutical of at least twice the liver-uptake on octreotide scintigraphy. Somatostatin analogue treatment was ccessated before PRRT to enhance the tumour uptake. During radiotherapy, a lysine/arginin-solution was administered i.v. for kidney protection (Rolleman et al., 2003). The absorbed dose to each kidney was calculated with the conjugate view method after each session. The accumulated kidney dose limit used was set at 27 Gy. The radiological response was evaluated by measuring tumour size using RECIST-criteria on CT-scans before and after treatment. Biochemical response was evaluated by comparing P-CgA before and after treatment. Haematological toxicity was evaluated according to the Common Toxicity Criteria (CTC) scale. Renal function was assessed by measuring GFR, either by Cr-EDTA-or iohexoleclearance.

In study V, two patients had their treatment at the Erasmus Medical Center, Rotterdam, The Netherlands. After 2005, all patients were treated at the Sahlgrenska University Hospital. The patients were treated up to a cumulative activity of 28-30 GBq, which corresponds to a bone marrow dose of 2 Gy. Patients with non-resectable recurrences received a cumulative renal dose less than 27.5 Gy (1-4 radiotherapies, 4-6 weeks apart). Two patients treated in an adjuvant setting only received PRRT x 2 each.
**Somatostatin receptor scintigraphy**

When examined with somatostatin receptor scintigraphy, the patients received 10-20 µg of \([^{111}\text{In DTPA-D-Phe}^1]\)-octreotide by i.v. injection. The administered activity was 190-300 MBq. A gamma camera (General Electric 400 AC/T) equipped with a medium-energy parallel-hole collimator connected to a GE STARCAM computer system was used. Static images were acquired for 10 min or until 500 kcounts were collected.

**Statistical analysis**

In study III, a stepwise Cox regression was used to build a statistical model to select variables that correlated with survival; factors included were sex, age, previous octreotide treatment, previous treatment with cytotoxic agents, previous IFN-α treatment, presence of metastases to regional lymph nodes, peritoneum or skeleton, increased liver transaminase levels in response to HAE (within 3 days) and changes of tU-5-HIAA and P-CgA levels at follow-up. Cox regression was used to study the relationship between survival and the percentage change of tU-5-HIAA and P-CgA levels, and also to determine the influence on survival of liver transaminase levels after HAE. Pearson’s correlation coefficients were calculated to study the covariation of biochemical responses following the first and repeat HAE procedures. In study IV, the change in tumour size according to RECIST-criteria and the change in GFR were analyzed using Wilcoxon matched pairs signed rank sum test.
RESULTS

Tumour weight correlates with P-CgA in GOT 1-xenografted mice (I)

P-CgA levels correlated with tumour weight in all animals ($P<0.00001$) (Fig. 2). Octreotide decreased the P-CgA/tumour weight ratio and a significant reduction was observed in the high-dose octreotide group ($P<0.037$). We found no difference in mean tumour weight between the groups, and no significant difference in mean increase of tumour volume between control animals and octreotide treated animals. All tumours were positive for CgA immunohistochemically.

---

Fig 2. Correlation between P-CgA and tumour weight. **There was a significant correlation between tumour weight and P-CgA levels in GOT 1-xenografted mice. Long-acting somatostatin decreased the P-CgA/tumour weight ratio, without influencing tumour weight.**
\(^{177}\text{Lu}\)-octreotate gives higher uptake, higher absorbed dose and better tumour reduction than \(^{177}\text{Lu}\)-octreotide in GOT 1-xenografted mice (II)

We found that the tumour uptake of \(^{177}\text{Lu}\)-octreotate was significantly higher than the tumour uptake of \(^{177}\text{Lu}\)-octreotide \((P=0.00061)\). The difference in activity concentration remained during the observation period of 13 days. The mean absorbed dose in tumour was 3-fold higher in animals given of \(^{177}\text{Lu}\)-octreotate than in animals given of \(^{177}\text{Lu}\)-octreotide. The reduction of tumour volume was also much more prominent in the animals given \(^{177}\text{Lu}\)-octreotate \((P=0.003)\), with a reduction of tumour volume to 3% of its initial value after 37 days. There was a significant correlation between P-CgA levels and tumour volume \((P<0.001)\).

![Image of graphs showing activity concentration and relative tumour volume over time](image)

**Fig 3.** Uptake and effect of \(^{177}\text{Lu}\)-octreotate and \(^{177}\text{Lu}\)-octreotide in GOT 1-xenografts. The tumour uptake of \(^{177}\text{Lu}\)-octreotate was significantly higher than the uptake of \(^{177}\text{Lu}\)-octreotide (left panel). The tumour volume reduction was much more pronounced in animals given \(^{177}\text{Lu}\)-octreotate (right panel), note the logarithmic scale.
Successful HAE correlates with prolonged survival in patients with midgut carcinoid (III)

Twentysix out of 54 patients with complete data in their files showed >50% decrease of tU-5-HIAA and 19 out of 37 patients showed >50% decrease of P-CgA. The mean decrease of tU-5-HIAA was 30.4 ± 8.0 (SEM) % and the mean decrease of P-CgA was 28.0 ± 10.4 (SEM) %; 71% of the patients experienced symptomatic relief, i.e. less diarrhea, reduction of flush or improved general well being (reflected by weight gain).

Survival and complications. The median survival in the entire series (from the first HAE) was 56 months (range 1 – 204, 95% confidence interval (CI) 45 – 67) (Fig. 3).

![Fig 3. Median survival after HAE. The median survival (from the first HAE) in the entire series of patients subject to HAE was 56 months (range 1 –204, 95% CI 45–67).](image)

The multivariate stepwise Cox’ regression showed that male gender (Hazard Ratio (HR) 5.796), percentual decrease of tU-5-HIAA (HR 0.972), percentual decrease of P-CgA (HR 0.973) and post-embolization elevation of a liver transaminase (ASAT) (HR 1.104), were independent predictors of survival. There was a strong correlation by Cox’ regression between prolonged survival and reduction of tU-5-HIAA (HR 0.991, $P=0.003$), or reduction of P-CgA (HR 0.987, $P=0.001$). Based
on the reduction of tU-5-HIAA by HAE, predicted survival was estimated. There was a 6 months gain in survival if the reduction of tU-5-HIAA was $\geq 50\%$ vs no reduction of tU-5-HIAA; another 6 months gain if the reduction was $\geq 75\%$ (Fig. 4).

![Predicted survival based on decrease in 5-HIAA](image)

**Fig 4. Predicted survival based on decrease in tU-5-HIAA levels after HAE.** Compared to no reduction of tU-5-HIAA there was 6 months gain of survival if the tumour marker reduction was 50%; another 6 months gain of survival if the reduction was 75%.

There was also a strong correlation between reduced survival and elevated liver transaminase (ASAT) levels after HAE (HR 1.031, $P<0.001$).

Two patients died within one month after HAE; one due to hepatorenal syndrome and one due to rapidly progressive disease. The mortality rate was still low, 1.8%; at centres of expertise it should be <5% (Ajani et al., 1994). There was one patient with HAE-related sepsis with uneventful outcome. The minor complications that
occurred were 4 patients with liver abscess, one with mild pancreatitis and two with total occlusion of the common hepatic artery; all had uneventful outcomes.

Response to repeat HAE. For 6 out of 19 patients, who underwent repeat HAE on tumour progression, complete biochemical data were available to study changes of tU-5-HIAA in response to the first and repeat HAE. In these patients the biochemical response to repeat HAE correlated strongly with the biochemical response to the primary HAE ($P=0.002$) (Fig. 5).

![Fig. 5. Biochemical response to repeat HAE. The biochemical response (tU-5-HIAA) to repeat HAE correlated significantly ($P=0.002$) with the biochemical response to the primary HAE.](image)

PRRT results in objective tumour reduction and impaired kidney function; it can be optimized by monitoring absorbed dose to the kidney in patients with GEP-NETS (IV)

Twenty-six patients with disseminated GEP-NETs treated with PRRT using $^{177}$Lu-octreotate, were analyzed regarding absorbed dose to the kidneys, radiological and biochemical response, haematological toxicity and renal function.
Radiological response to PRRT. Sixteen out of 26 patients could be evaluated radiologically. Ten either lacked suitable lesions (not measurable on CT) \((n = 3)\), died of disease before follow-up \((n = 3)\) or are due to follow-up \((n = 4)\). The radiological tumour response varied from a 54% increase of tumour size (progression) to a 79% decrease (regression). Six out of 16 (38%) showed partial response (PR) according to RECIST-criteria. Eight out of 16 (50%) showed stable disease (SD) and two out of 16 (13%) showed progressive disease (PD). No complete remission (CR) was observed (Fig. 6).

![Radiological response to PRRT.](image)

**Fig. 6. Radiological response to PRRT.** A partial response (RECIST) was observed in 6 out of 16 patients with GEP-NETs treated with \(^{177}\)Lu-octreotate.

The radiological response according to RECIST was not significant, when analyzed by Wilcoxon rank sum test. Figure 7 illustrates a favourable radiological response in a 52 years old patient with hepatic metastases of EPT, accompanied by reduced uptake on octreotide scintigraphy (Fig. 7).
Fig. 7. Radiological and scintigraphic evaluation of PRRT. Marked radiological tumour response (upper panel) two years after PRRT and reduced scintigraphic tumour uptake one year after treatment (lower panel) in a patient with liver metastases of EPT. Comparable investigations before (left) and after (right) treatment (right). Tumours indicated by arrows.

Absorbed dose to the kidneys and glomerular filtration rate. The mean absorbed dose to the kidneys was 24 Gy ± 6 (SEM). Four PRRT were given 11 out of 26 patients. One patient had 5 PRRT, while 10 out of 26 had less than 4 PRRT, since they reached the kidney dose limit already after 2-3 treatments. Four patients did not reach the dose limit of the kidney; they thus have the possibility of additional PRRT. The mean GFR before treatment was 80 ml/1.73 m²/min ± 4 (SEM). At follow-up the mean GFR was 70 ml/1.73 m²/min ± 4 (SEM). The decrease of GFR was significant (P=0.0013).
**Biochemical response to PRRT.** Seventeen out of 26 patients could be evaluated biochemically by measuring P-CgA. Nine patients either had normal P-CgA-levels before treatment \((n = 4)\), died of disease before follow up \((n = 3)\), or are due to follow-up \((n = 2)\). Six out of 17 \((35\%)\) showed \(\geq 30\%\) decrease. Eight out of 17 \((47\%)\) showed \(\geq 20\%\) increase. Three out of 17 \((18\%)\) showed neither \(\geq 30\%\) decrease nor \(\geq 20\%\) increase.

**PRRT in transplanted patients with non-resectable recurrence (V)**

Two patients \((no\ 4\ &\ 6)\) with recurrence of GEP-NETs after OLT were treated with PRRT as an adjunct to surgical resection. Patient no 4 with an isolated skeletal metastasis has no evidence of disease after metastasectomy followed by PRRT x 2. Patient no 6 recurred a second time with abdominal lymph node metastases, and has stable disease after lymph node excision followed by PRRT x 2. Both patients have been observed 2.5 years after PRRT.

Four patients \((no\ 1,\ 2,\ 3&5)\) with non-resectable recurrence of GEP-NETs after OLT, received PRRT for palliation. Patient no 1 was accepted for OLT despite tumour regions with high proliferation rate \((\text{hot spots with Ki67} > 10\%)\). She developed a s.c. metastasis in the neck, which was excised. After diagnosed continued dissemination she received PRRT x 1, but died of rapid tumour progression. Patient no 2 developed lymph node and skeletal metastases and severe skeletal pain. After metastasectomy and external radiation he received PRRT x 4 due to rapid tumour progression. There was an initial stabilization, but the patient died from progressive liver tumours 4.5 years after OLT. Patient no 3 developed multiple lymph node metastases and was treated with PRRT x 4, which stabilized the disease for 5 years. When graft metastases were detected rapid tumour progression followed; he died 10 years after OLT. Patient no 5 developed liver metastases and a pelvic recurrence. After resection of the pelvic tumour, PRRT x 4 led to marked radiological response of the liver metastases. After 4 years he
recurred with bilobar liver metastases and abdominal para-aortic lymph node metastases. To avoid further renal toxicity, the liver metastases were treated with SIR, and subsequent excision of metastatic lymph nodes. He had an objective tumour response also to this treatment; he has now been observed 10 years after OLT (Fig. 8).

**Fig 8. Clinical outcome and time course of therapy in patients with GEP-NETs at tumour recurrence after OLT.** Six patients were treated with PRRT for non-resectable recurrence of NE tumours. A survival of 100-120 months was observed in 4 patients.

Renal function. Glomerular filtration rate (GFR) was retrieved in 5 out of 6 patients prior to OLT and varied between 67-129 ml/1.73 m$^2$/min. There was a moderate reduction of GFR in 4 patients and a pronounced reduction in one patient
after OLT/immunosuppression. Five patients (no 2-6) could be evaluated before and after PRRT. Two patients with adjuvant treatment (no 4 & 6) each received PRRT x 2 with no impairment of GFR. Three patients with palliative treatment (PRRT x 4) showed a reduction of GFR by 29% (pat no 3) and 15% (pat no 5), and stable function in one (pat no 2) (Fig. 9).

Fig. 9. Influence of immunosuppression and subsequent PRRT on renal function in patients with metastastic GEP-NETs, subjected to OLT. *GFR remained stable after PRRT in 5 evaluable patients with recurrence of GEP-NETs after OLT; pat no 4 & 6 received PRRT x 2, pat no 2, 3 & 5 received PRRT x 4.*
DISCUSSION

Biochemical markers of tumour burden
To evaluate the therapeutic effect of different treatments, reliable and readily available markers of tumour burden are needed. P-CgA is used both for diagnostic purposes and to follow the clinical course of NE tumour disease. P-CgA has been claimed to be the most sensitive marker for GEP-NETs (Bajetta et al., 1999; Baudin et al., 1998; Nobels et al., 1998). Sampling of P-CgA is a simple test compared to urinary collections for 5-HIAA analyses. It can be used both for functioning and non-functioning tumours. Earlier studies have shown a correlation between tumour size and P-CgA levels in hereditary (small) pheocromocytoma (Hsiao et al., 1990b) and in nude mice bearing human neuroblastoma (Wassberg et al., 1996). These correlations cannot directly be translated to the clinical situation of NE tumours with spread to several organs. Assessing total tumour volume from a secretory product released into the circulation called for another approach, and our nude mice model with xenografted human carcinoid (GOT 1) offered a possibility to study the relationship between P-CgA and carcinoid tumour volume. One must bear in mind that P-CgA levels may be influenced by several host factors. Impaired renal function prevents the excretion of peptides and elevates P-CgA (Hsiao et al., 1990a). Atrophic gastritis, or proton pump inhibition, causes hypergastrinemia and ECL-cell hyperplasia associated with elevated P-CgA (Borch et al., 1997; Sanduleanu et al., 1999). Serotonin release in response to hyperemesis in patients after treatment with cytotoxic agents, may also increase P-CgA (Cubeddu et al., 1995). We found that tumour weight strongly correlated with P-CgA levels in GOT 1-xenografted mice (I). Octreotide treatment decreased P-CgA in a dose-dependent manner, but tumour size was not reduced in response to such
Treatment of disseminated neuroendocrine tumours

Peptide receptor radionuclide therapy

PRRT is a new modality to treat disseminated GEP-NETs, taking advantage of the high SSTR-expression in these tumours. We compared the tumour uptake and tumour response in our xenograft model of the human midgut carcinoid GOT 1, when treated with either $^{177}\text{Lu}$-octreotide or $^{177}\text{Lu}$-octreotate (II). Treatment with $^{177}\text{Lu}$-octreotate resulted in 2-fold higher tumour uptake. The activity concentration level remained significantly higher for a prolonged period, which contributed to a 3-fold higher absorbed dose vs treatment with $^{177}\text{Lu}$-octreotide. These results provided in vivo data of the uptake and also enabled detailed estimation of absorbed doses.

PRRT to patients with GEP-NETs was initiated at the Sahlgrenska University Hospital 2006. The radiological (RECIST) and biochemical (P-CgA) evaluation of the first 26 treated patients (IV) revealed a 38% partial response rate radiologically, in line with previous reports (Kwekkeboom et al., 2008; Kwekkeboom et al., 2005). There was no evident correlation between the radiological and biochemical responses, which might partly be explained by the relatively short follow-up. Furthermore, P-CgA levels are influenced by renal function (Hsiao et al., 1990a), which can be impaired by PRRT. Other centres have reported mild renal effects of PRRT, but we found a 12% reduction of GFR ($P = 0.0013$). This must be considered when changes in P-CgA levels by PRRT are evaluated. It also
emphasizes that the kidney is a dose-limiting organ. Individualization of PRRT can be achieved, if the accumulated absorbed kidney dose is monitored. In our series (IV), four out of 26 patients were identified as candidates for additional PRRT without exceeding the kidney dose limit (27 Gy). More precise methods for calculating the kidney dose, e.g. using SPECT images for quantification of the activity concentration together with better knowledge of the kidney dose limit, will contribute to an optimized individual dose planning aiming at delivering a high dose to the tumour without serious impairment of kidney function.

**Hepatic artery embolization**

HAE is an established palliative treatment for liver metastases of GEP-NETs resulting in good control of hormonal symptoms (Chamberlain et al., 2000; Coupe et al., 1989; Geterud et al., 1990; Gupta et al., 2003; Schell et al., 2002; Wängberg et al., 1996). It appears to be especially favourable for patients with midgut carcinoid tumours (Eriksson et al., 1998). A survival advantage has been suggested in previous small series (Jacobsen et al., 1995; Mitty et al., 1985). In our series of 107 consecutive patients with midgut carcinoid (III), we found a correlation between tumour marker reduction (tU-5-HIAA and P-CgA) and prolonged survival. It was possible to estimate the gain in survival; the relative reduction in tumour markers corresponded to a graded survival advantage. Also immediate biochemical changes, *i.e.* the increase of liver transaminase levels, correlated with reduced survival. This finding supports a more selective HAE-procedure, *i.e.* limited embolization of more peripheral branches to avoid damage to normal liver parenchyma. Induction of tumour ischemia is most likely not the only mechanism of action of HAE. Natural killer cells with increased tumouricidal activity rapidly accumulates in central venous blood of patients with radiological responses to HAE. Bilobar tumour regression was shown in individual patients after unilateral HAE, suggesting involvement of systemic mechanisms. It was suggested that 5-HT released from tumour cells by ischemia decreased the monocyte inhibition of
natural killer cells (Wängberg et al., 1995). Criteria to identify responsive patients will lead to more precise indications for HAE. Our finding that the response to the first HAE correlated with the response to repeat HAE, indicates that it will be possible to identify responsive patients in advance. A randomized, prospective study of HAE in patients with hepatic metastases of carcinoids has not been performed, since no effective pharmacological treatment arm has been available.

**PRRT in patients liver transplanted for GEP-NETs**

In patients, who have been treated with OLT due to metastatic GEP-NETs limited to the liver, recurrence is early detected due to a strict follow-up protocol. Surgical treatment is preferred if the recurrent lesion is resectable, but in case of multilocular recurrences systemic therapy is required, especially since there is no effective chemotherapy for many of these tumours (Rougier & Mitry, 2000); chemotherapy can also be hazardous for immunosuppressed patients. PRRT therefore offers an attractive therapeutic alternative for transplanted patients, even though they have reduced GFR (Herlénius et al., 2008) and PRRT can cause radiation-related nephrotoxicity (IV). In our pilot series of 6 OLT patients, with monitored absorbed doses to the kidney, we found a moderate reduction of GFR by PRRT only in two patients, suggesting that PRRT can be safely used also under these conditions. Multilocular recurrences in OLT patients may be well suited for PRRT, since recurrent disease is early detected, *i.e.* the total tumour volume is relatively small, which is favourable for the therapeutic outcome (Valkema et al., 2002). In our study, two patients with minimal residual disease received PRRT x 2 in an adjuvant setting: One has no sign of recurrence and the other has SD after 2.5 years. These results support that PRRT can be a valuable treatment option to prevent, or delay, tumour recurrence in OLT patients.
Optimization of current treatment alternatives

The palliative treatment for disseminated GEP-NETs needs to be further optimized, e.g. methods for early identification of responders to HAE and superselective embolization procedures are required. PRRT via highly expressed SSTR has a proven effect in clinical studies. To date most treated patients had advanced tumours and were beyond curative therapy. Experimental studies indicate that the treatment protocols can be optimized, e.g. low amounts of $^{177}$Lu-octreotide prior to PRRT can upregulate SSTR2 in the tumour tissue leading to increased uptake of radionuclide and enhanced therapeutic effects (Bernhardt et al., 2007; Oddstig et al., 2006). Another promising option is the radiosensitizing action of the pyridyl cyanoguanidine CHS 828, which is a poly(ADP-ribose)polymerase 1 (PARP1) inhibitor (Olesen et al., 2008; Watson et al., 2009). Nicotinic amide adenine dinucleotide (NAD+) is essential for enzymatic redox reactions, including adenosine triphosphate (ATP) production, but is also a substrate for poly (ADP) ribosylation (catalyzed by PARP1), which is activated by DNA strand breaks, e.g. induced by radiotherapy. If the re-synthesis of NAD+ is inhibited by PARP1 inhibition, the energy metabolism of the irradiated tumour is severely impaired and radiation-induced tumour cell damage is enhanced (Watson et al., 2009). Interference with the apoptotic pathway and death receptors can probably also potentiate the radiotherapeutic effects (Schulze-Bergkamen et al., 2009).

Our series of patients (IV) was characterized by multimodal therapy prior to PRRT. The patients all had advanced disease with large tumour burden and our results were similar to the Rotterdam series (Kwekkeboom et al., 2008). One way to better select patients for PRRT is to analyze the profile of expressed SSTR subtypes in tumour biopsies (Kubota et al., 1994; Kölby et al., 1998), so that a suitable radiopharmaceutical with a high affinity for the particular subtypes expressed, or a pan-analogue (SOM 230), can be selected in order to obtain an optimal tumour-specific uptake. Radionuclides with different ranges can be chosen in relation to tumour size (Bernhardt et al., 2001). Combinations of several radionuclides can
also be used when the size of different lesions vary (de Jong et al., 2005). Finally monitoring of the total absorbed dose to the kidneys is a valuable tool to optimize the dose to each patient. The use of PRRT in an adjuvant setting to eradicate minimal residual disease, or as a precaution in patients with high-risk tumours after intentionally curative surgery, has not yet been established. The two pilot cases in study V may indicate curative treatment, or long periods of stable disease, when minimal tumour volumes are treated.

Future aspects

The increasing knowledge of cancer cell biology has revealed a number of signalling pathways in cancer cells that may be selectively targeted for anti-cancer therapy. Our knowledge of cellular signalling in GEP-NETs is limited. However, interference with some of the signalling pathways has been attempted in experimental studies as well as in phase 1 & 2 studies. Targeting growth factors (e.g. epidermal growth factor or platelet derived growth factor) and their receptors [using tyrosine kinase inhibitors (TKI) (Hobday et al., 2006; Hobday et al., 2007) or monoclonal antibodies (ongoing study NIH:NCT 00397384)], angiogenic factors (e.g. vascular endothelial growth factor) [using TKI (Raymond et al., 2009), monoclonals (Yao et al., 2008), endostatin (Kulke et al., 2006a), thalidomide (Kulke et al., 2006b)] and hormone receptors (SSTR and insulin-like growth factor 1 receptor) [using inhibitory analogues (Rinke et al., 2009; Schally, 2008) or small molecules] expressed on NE tumours has been attempted and may provide new therapeutic options. Monotherapy with the multitarget TKI sunitinib significantly increased the progression-free survival of patients with advanced EPT in a recent randomized study (Raymond et al., 2009). In carcinoids interference with some signalling pathways (mTOR, PI3K, RAS/RAF and Notch) seems effective in experimental models (Greenblatt et al., 2007; Pitt et al., 2009; Van Gompel et al., 2005; Zitzmann et al., 2007) besides more general mechanisms as inhibition of the proteasome or histone deacetylation (Bolden et al.,
2006; Karin et al., 2004; Shah et al., 2004). Several new agents to treat the rare NE tumour diseases will evidently appear in the near future. To gain rapid information about their usefulness randomized multicentre studies are required.
CONCLUSIONS

1. Chromogranin A levels in plasma were closely related with tumour volume and were significantly reduced by a somatostatin analogue, i.e. P-CgA is well suited for monitoring of the tumour volume in our xenograft model, but is influenced by somatostatin analogue treatment.

2. $^{177}$Lu-octreotide is a more suitable radiopharmaceutical than $^{177}$Lu-octreotide for treatment of SSTR-expressing NE tumours. P-CgA was an accurate marker of volume and response to treatment in an experimental setting.

3. Hepatic artery embolization was a safe and well tolerated treatment modality. It provided good hormonal control in patients with the midgut carcinoid syndrome and prolonged survival of biochemically responsive patients. Furthermore, repeat HAE can be considered in patients with favourable response to the first procedure.

4. Peptide receptor radionuclide therapy can be optimized by using the accumulated dose to the kidneys as a limiting factor, so that the highest dose possible can be used without causing nephrotoxicity.

5. Peptide receptor radionuclide therapy provided a safe treatment option for patients with non-resectable recurrence of SSTR-expressing NE tumours, previously treated with liver transplantation; in individual patients objective tumour regression, or disease-stabilization, was seen.
ACKNOWLEDGEMENTS

First of all, I want to express my deepest gratitude to everyone who in different ways has contributed to this work. I especially want to thank:

Lars Kölby, my tutor, for your endless energy, stubbornness and patience. I am so grateful that you took me on as your first postgraduate student.

Ola Nilsson, for your support and brilliant mind. You have a way of clarifying complicated issues, so that even I get a glimpse of understanding.

Håkan Ahlman, for accepting me in the team and letting me grow while generously guided by your enormous knowledge in the field of endocrine science.

Bo Wängberg, for appreciated advice in many areas, and enormous support through good and bad.

Svante Jansson, for taking me on at the unit for endocrine surgery in the first place. An excellent clinician and forever a role-model in many aspects.

Bengt Nilsson, Viktor Johanson, Per Bümming and Anna-Karin Elf for friendly collaboration and contribution to the scientific spirit of our team.

Christer Andersson, my first clinical tutor, who taught me the fundamental skills of surgery and more. Thanks to you, endocrine surgery opened as an opportunity for me.

Bengt Lindberg, who led me in to the field of surgery. Without you, I would probably not have become a surgeon.

Eva Forssell-Aronsson for inspiring support and collaboration.

Peter Bernhardt, Rauni Rossi-Norrlund, Johanna Svensson and Yvonne Arvidsson for generous help and friendly, constructive collaboration.

Agneta Särén and Ingela Stave for friendly and excellent secretarial assistance.

Barbro Krüger for joyful collaboration and help in many ways.

Ann Wikström, Gülay Altiparmark, Ellinor Andersson, Ann-Christine Illerskog-Lindström, Siw Tuneberg, Malin Berntsson and Linda Arvidsson for
their skilful technical assistance and friendly collaboration in the laboratory and with the mice.

**Ulric Pedersen** for expert assistance with computers.

**Mats Stridsberg** for generous help with CgA analyses.

**Helene Thörne** for always listening and cheering me up with your positive attitude.

And above all, **my family**, my everything.

This study was supported by grants from The Swedish MRC (5220 and 6834), The Swedish Cancer Society (654, 2690, 3427, 3911 and 4956), I.B. and A. Lundberg Research Foundation, Assar Gabrielsson Foundation, The Sedish Society of Medicine, The Swedish Society for Medical Research, The Göteborg Medical Society, The King Gustav V Jubilee Clinic Cancer Fund, Göteborg, Sahlgrenska University Hospital Research Funds, Gunvor and Josef Anérs Stiftelse, Axel Linders Stiftelse, Gunvor, Arvid och Elisabet Nilssons Stiftelse, B. Uhlanders fond, C. Landgrens Minnesfond, Wilhelm och Martina Lundgrens Vetenskapsfond, Serena Ehrenströms Foundation, The Selanders Foundation. [DOTA$^0$-Tyr$^3$]-octreotate was provided through a collaboration within COST B12/D18 action.
REFERENCES


(177)Lu-DOTA (0), Tyr (3) octreotide: which peptide is preferable for PRRT? Eur J Nucl Med Mol Imaging, 33, 1346-51.


53


