Current Medical Treatment of Endocrine Pancreatic Tumors and Future Aspects

by

Marie-Louise Fjällskog
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MARIE-LOUISE FJÄLLSKOG
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**ABSTRACT**


Endocrine pancreatic tumors (EPTs) are rare with an incidence of 4 per million inhabitants. In the majority of cases they grow slowly, but there are exceptions with very rapidly progressing malignant carcinomas. First-line medical treatment is streptozotocin combined with 5-fluorouracil.

We treated 16 patients with somatostatin analogs combined with $\alpha$-interferon and achieved a biochemical and/or radiological response in 56% (median duration 22 months). We consider this treatment a good alternative for patients who fail during chemotherapy or who do not want to/cannot receive cytotoxic drugs.

Thirty-six patients with neuroendocrine tumors were treated with cisplatin combined with etoposide. Of 14 patients with evaluable EPTs, 50% responded radiologically and/or biochemically (median duration 9 months). We consider this treatment useful as first-line medical treatment in aggressive EPTs or in patients failing prior chemotherapy.

Twenty-eight tumor tissues from EPTs were examined with immunohistochemistry regarding expression of somatostatin receptors (ssts) 1 to 5 on tumor cells and in intratumoral vessels. We found that sst$_1$ and sst$_5$ were highly expressed on tumor cells and in vessels. However, sst$_2$ and sst$_5$ were lacking in half of the tumor tissues and in most of the vessels. Because of the variability in sst expression, we recommend analysis of each individual's receptor expression before starting treatment.

We examined 38 tumor samples regarding expression of tyrosine kinase receptors platelet-derived growth factor receptors (PDGFRs), c-kit and epidermal growth factor receptor (EGFR). We found that the receptors were expressed in more than half of the tumor tissues. Further studies will reveal if tyrosine kinase antagonists can be part of the future treatment arsenal.

*Key words: endocrine pancreatic tumors, $\alpha$-interferon, somatostatin analogs, cisplatin, etoposide, somatostatin receptors, immunohistochemistry, tyrosine kinase receptors, platelet-derived growth factors receptors (PDGFRs), c-kit, epidermal growth factor receptor (EGFR)*

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To my precious family,
the true joy of life -
Gunnar, Lovisa and Tora
My thesis is based on the following papers, referred to in the text by their roman numerals:


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ABBREVIATIONS

5-FU  5-fluorouracil
5-HIAA  5-hydroxyindoleacetic acid
5-HT3  5-hydroxytryptamine
ACTH  adrenocorticotropic hormone
CEA  carcinoembryonic antigen
chrom  chromogranin
CML  chronic myeloid lymphoma
CPT-11  irinotecan
CT  computed tomography
DTIC  dacarbazine
EGFR  epidermal growth factor receptor
EPT  endocrine pancreatic tumor
GH  growth hormone
GHRH  growth hormone releasing hormone
GIST  gastrointestinal stromal tumor
HCG  human chorionic gonadotropin
HER2  human epidermal growth factor receptor 2
i.e.  that is
IFN  α-interferon
IFP  interstitial fluid pressure
IHC  immunohistochemistry
MU  million units
NCIC CTG  National Cancer Institute of Canada Clinical Trials Group
PDGF  platelet-derived growth factor
PDGFR  platelet-derived growth factor receptor
PP  pancreatic polypeptide
RT-PCR  reverse transcriptase – polymerase chain reaction
SPECT  single-photon-emission computed tomography
sst  somatostatin receptor
STZ  streptozotocin
VIP  vasoactive intestinal peptide
WDHA  watery diarrhea, hypokalemia and achlorhydria
WHO  World Health Organization
I. ENDOCRINE PANCREATIC TUMORS

Background

Neuroendocrine tumors in the gut and pancreas constitute 2% of all malignant gastrointestinal tumors. The two most common tumor types are carcinoids which can be divided into midgut, foregut and hindgut carcinoids, and endocrine pancreatic tumors (EPTs).

The incidence of EPTs is 4 per million inhabitants (Eriksson et al, 1990). They grow slowly in the majority of cases but there are exceptions with very rapidly progressing malignant carcinomas (Eriksson et al, 1990). Most EPTs, except for insulinomas, are malignant. They are highly vascularized tumors that equally often are localized in the head, tail and body of the pancreas (Grimelius et al, 1985). The plasma chromogranin A (O’Connor, 1983) is elevated in 80-100% of patients suffering from EPTs (Eriksson et al, 1989). The tumors are classified as functioning if they are associated with clinical syndromes due to hormone release and as non-functioning if they are not. The most common EPTs are non-functioning tumors followed by gastrinomas and insulinomas.

EPTs most often metastasize to the lymph nodes and to the liver. Bone metastases are rare occurring in about 5-10% of the patients (Eriksson et al, 1990). Most patients present with distant metastases at diagnosis and require both symptomatic and antitumor treatment. Few patients can be cured by surgery thus treatment aims to ameliorate clinical symptoms, stop tumor growth, improve quality of life and prolong survival (Oberg, 2000). Besides symptomatic treatment, such as proton inhibitors (ulcers in gastrinomas) or insulin (hyperglycemics in glucagonomas), chemotherapy and biotherapy (α-interferon and/or somatostatin analogs) are well-established therapies that effect both symptoms and tumor-growth (Eriksson et al, 1990). Since most tumor burden is located in...
the liver, hepatic artery embolization has proven effective as an alternative to systemic treatment (Eriksson et al, 1998).

**Clinical syndromes, symptoms and diagnosis**

See table 1, page 15.

**Insulinomas** In 1927, Wilder observed a correlation between hyperinsulinism and an unresectable pancreatic islet cell carcinoma. That same year, Graham surgically cured an insulinoma syndrome by removing a functioning islet beta cell adenoma. In 1935, Whipple and Frantz described a diagnostic triad as a preoperative diagnosis of the insulinoma syndrome:

1. biochemical hypoglycemia with  
2. symptoms that are  
3. relieved by elevation of the glucose level to normal

The clinical symptoms of insulinomas are due to hypoglycemia. The most common symptoms are visual disturbances, confusions, altered consciousness and weakness (Creutzfeld, 1995; Stefanini et al, 1974). Seizures occur in about 20% of the patients (Stefanini et al, 1974). Counter-regulatory catecholamine excess can also give rise to symptoms such as palpitations, sweating and pallor (Service et al, 1976). Symptoms often are precipitated by prolonged fasting, a delayed meal or exercise.

The insulin/glucose ratio derived from blood sampling during a fast (up to 72 hours) is the most reliable diagnostic test for insulinoma (Fajans et al, 1979). Insulin-producing tumors, especially malignant ones, can release large molecular forms of hormones and therefore determination of proinsulin and c-peptide may be of great value to establish the diagnosis (Turner and Heding, 1977).

**Gastrinomas (Zollinger-Ellison syndrome)** The Zollinger-Ellison syndrome was described in 1954 by Zollinger and Ellison, however it was not until 1964 that Gregory and Tracy purified gastrin from an islet cell tumor and showed it to be the cause of Zollinger-Ellison syndrome.

The Zollinger-Ellison syndrome is characterized by hypergastrinemia which causes persistent gastric acid hypersecretion. The clinical features can vary considerably and many patients present with only mild dyspeptic symptoms (Stadil
and Stage, 1983) which can easily be controlled medically. Many patients often complain about diarrhea at diagnosis (Jensen et al, 1983).

The diagnosis of a gastrinoma is based upon the demonstration of elevated basal gastric acid secretion and fasting hypergastrinemia (Mignon et al, 1995).

**Glucagonomas**

The hallmark in the diagnosis of a glucagonoma is a skin lesion called “necrolytic migratory erythema” (Wilkinson et al, 1977) which was already observed in 1942 by Becker and associates.

In 1974, Mallinson described the glucagonoma syndrome which is characterized by the above mentioned skin lesion, adult onset diabetes, weight loss, glossitis and thromboembolic complications. See fig 1.

The diagnosis is based upon elevated levels of serum glucagon and clinical symptoms.

**VIPomas**

In 1958, Verner and Morrison reported that two patients, dying from watery diarrhea and hypokalemia, were found to have benign islet cell tumors at autopsy. Vasoactive intestinal peptide (VIP) (Mutt and Said, 1970) was proposed in 1973 to be the diarrheogenic substance (Bloom et al) and a decade later, Kane and his group showed that infusion of VIP reproduced the symptoms of Verner-Morrison syndrome.

Overproduction of VIP causes profuse diarrheas and fluid loss, which leads to dehydration, and severe losses of potassium.

*Fig. 1 Photo of a patient suffering from a glucagonoma demonstrating necrolytic migratory erythema and severe weight loss.*
and bicarbonate thus causing hypokalemia and acidosis. Excess VIP production can also cause hypochlorhydria, diabetic glucose tolerance, hypercalcaemia and flushing. An elevated plasma level of VIP establishes the diagnosis.

**Somatostatinomas**  
In 1977, Ganda described a rare syndrome called the somatostatin syndrome. The symptomatology consists of diabetes mellitus, gall stones, steatorrhea or diarrhea, hypochlorhydria and weight loss. Determination of plasma somatostatin is the most important diagnostic tool.

**ACTHomas**  
Patients suffering from EPTs and Cushing’s syndrome (ACTHomas) have been reported by Maton et al in 1986 and by Amikura et al in 1995. Recent studies have shown that 4-16% of Cushing’s syndrome, due to ectopic ACTH (adrenocorticotropic hormone) production, is caused by a pancreatic tumor. The symptoms are those of Cushing’s syndrome i.e. weight gain, characteristic distribution of body fat, hypertension, hypokalemia, superficial bleedings in the skin and sometimes diabetes mellitus. In a subgroup of patients the symptoms progress within weeks and cause severe hypokalemia and pronounced proximal myopathy/muscular weakness (Öberg et al, 1997).

The diagnosis is based upon the findings of ectopic ACTH production and a pancreatic tumor.

**Miscellaneous syndromes**  
In rare instances EPTs can produce growth hormone (GH) or calcitonin. GH production causes acromegaly (Imura, 1980) but it is unclear if calcitonin production causes a distinct clinical syndrome (Jensen, 1999). EPTs can also produce multiple symptom-related hormones (Imura, 1980) in which case they are classified according to the dominant hormone. Quite often the tumor switches from one hormone production to another. In this case the tumor is classified according to the hormone initially produced.

**Non-functioning tumors**  
Symptoms from these tumors arise from mechanical effects of the carcinoma and therefore often present late when they are large and locally advanced. At diagnosis these patients often have jaundice or pain because of the large tumor mass (Kent et al, 1981).

Besides elevation of chromogranin A (chrom A), these
patients often have elevated levels of peptides that do not produce specific clinical symptoms such as pancreatic polypeptide (PP) and human chorionic gonadotropin (HCG) α and β.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Syndrome</th>
<th>Hormone producing symptoms</th>
<th>Malignant</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>gastrinoma</td>
<td>Zollinger-Ellison</td>
<td>gastrin</td>
<td>60-90%</td>
<td>pancreas (30-60%) duodenum (30-43%)</td>
</tr>
<tr>
<td>insulinoma</td>
<td>Insulinoma</td>
<td>insulin</td>
<td>10-15%</td>
<td>pancreas (&gt;99%)</td>
</tr>
<tr>
<td>VIPoma</td>
<td>Verner-Morrison</td>
<td>VIP</td>
<td>80%</td>
<td>pancreas (90%) adrenal (10%)</td>
</tr>
<tr>
<td>glucagonoma</td>
<td>glucagonoma</td>
<td>glucagon</td>
<td>60%</td>
<td>pancreas (&gt;99%)</td>
</tr>
<tr>
<td>somatostatinoma</td>
<td>somatostatinoma</td>
<td>somatostatin</td>
<td>70-90%</td>
<td>pancreas (56%), upper small intestine (44%)</td>
</tr>
<tr>
<td>ACTHoma</td>
<td>ectopic Cushing’s</td>
<td>ACTH</td>
<td>&gt;95% (pa)</td>
<td>pancreas (4-16%)</td>
</tr>
<tr>
<td>nonfunctioning</td>
<td>nonfunctioning</td>
<td>non</td>
<td>&gt;60%</td>
<td>pancreas</td>
</tr>
</tbody>
</table>

*VIP=vasoactive intestinal peptide, ACTH=adrenocorticotropic hormone, pa=pancreas*
Clinicopathological classification

The endocrine part of pancreas is formed by four islet cell types, the glucagon (A) cells, insulin (B) cells, somatostatin (D) cells and pancreatic polypeptide (PP) cells. These cells can also be found scattered in the exocrine part of the pancreas. EPTs can contain each of these cell types and related hormones (Larsson LI, 1978). The tumors can also contain cells not present in normal adult human pancreas, i.e. cells producing inappropriate hormones such as gastrin (G cells), VIP, growth hormone releasing hormone (GHRH), ACTH and calcitonin (Capella et al, 1995; LaRosa et al, 1996).

EPTs can be divided into well-differentiated endocrine tumors (mild atypia) with mostly benign behavior, well-differentiated endocrine carcinomas (moderate atypia) which are low grade malignant and poorly differentiated carcinomas (high atypia) which are high-grade malignant (Rindi et al, 1998). See table 2.


<table>
<thead>
<tr>
<th>Well-differentiated endocrine pancreatic tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign behavior: confined to the pancreas, nonangioinvasive, &lt;2cm in size*, Ki67≤2%</td>
</tr>
<tr>
<td>Functioning</td>
</tr>
<tr>
<td>– Insulinoma</td>
</tr>
<tr>
<td>Nonfunctioning</td>
</tr>
<tr>
<td>Uncertain behavior: confined to the pancreas, &gt;2cm in size or angioinvasive, Ki67≥2%</td>
</tr>
<tr>
<td>Functioning</td>
</tr>
<tr>
<td>– Gastrinoma, insulinoma, VIPoma, glucagonoma, somatostatinoma, or inappropriate syndromesb</td>
</tr>
<tr>
<td>Non-functioning</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Well-differentiated endocrine carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade malignant with gross local invasion and/or metastases, Ki67 2-10%</td>
</tr>
<tr>
<td>Functioning</td>
</tr>
<tr>
<td>– Gastrinoma, insulinoma, VIPoma, glucagonoma, somatostatinoma, or inappropriate syndromesb</td>
</tr>
<tr>
<td>Non-functioning</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Poorly differentiated endocrine carcinoma</th>
</tr>
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<tbody>
<tr>
<td>High grade malignant (small to intermediate cell) carcinoma, Ki67&gt;10%</td>
</tr>
</tbody>
</table>

* <2cm and <3cm in site implies near to 100% and 90% probability of benign behavior, respectively.

b Inappropriate hormone syndromes: Cushing (ACTH), acromegaly or gigantism, hypercalcemia, etc.
Treatment

Surgery Complete surgical removal of an endocrine pancreatic tumor is the treatment of choice which is potentially curative (Welbourn et al, 1981) but rarely possible due to metastatic disease. However, despite the often advanced stage at diagnosis, surgery should always be considered. Even if the patient has liver metastases, surgical resection combining partial hepatectomy and removal of the primary tumor can be life-extending and, in some cases, curative (Carty et al, 1992; Que et al, 1995). However, since most patients have multiple or diffuse liver metastases, this is seldom possible. The proportion of patients with resectable liver metastases is only around 10% (Carty et al, 1992). EPTs have a relatively high rate of resectability compared to adenocarcinomas of the pancreas. Surgical debulking of primary or metastatic tumors can provide effective palliation (Montenegro et al, 1980; Nagorney et al, 1983) by decreasing hormonal overproduction and local mechanical pressure. Debulking also delays and maybe reduces the need for medical treatment (McEntee et al, 1990). Radiofrequency ablation of liver metastases can be used instead of, or as a complement, to surgery, although data are limited (Hellman et al, 2002).

During the last few years, liver transplantation has evolved as a potentially curative therapeutic option for patients without metastases outside the liver. Whereas most patients develop recurrent tumors within the first years after liver transplantation, some survive long-term and a few even remain free of tumor (Lang et al, 1997 and 1999). Because of the shortage of donor organs, research on selection of patients with favorable long-term prognosis is of uttermost importance.

Liver embolization The majority of patients suffering from malignant EPTs have most of their tumor burden in the liver. The hepatic metastases derive almost all of their blood supply from the hepatic artery. The normal liver parenchyma receives about 20-25% of its blood supply from the hepatic artery and the rest from the portal vein (Ackerman et al, 1972). Because of its dual blood supply, the normal liver is protected against infarction caused by occlusion of hepatic arterial branches, while hepatic metastases undergo varying degrees of necrosis. Several reports have demonstrated that selective embolization of a hepatic artery branch can provide reduction of hormonal
symptoms and tumor burden in patients with neuroendocrine gastrointestinal tumors (Ajani et al. 1988; Carrasco et al., 1983; Eriksson et al., 1998). Peripheral arterial embolization allows repeated embolizations since the vessels undergo recanalization.

Overall response rates from liver embolization varies between 50-90% and median duration of response is 10-15 months. Symptomatic and biochemical responses are more frequent than radiological responses (40-90% versus 15-40%) (Ajani et al., 1988; Carrasco et al., 1983; Eriksson et al., 1998).

Complications such as postembolic syndrome with pain, fever, nausea, leucocytosis and liver enzyme derangements occur in 50-90% of the patients. Severe complications such as renal failure, liver necrosis, intestinal ischemia or cholecystitis, is noted in 10% of patients and the mortality rate is 3-7% (Ajani et al., 1988; Carrasco et al., 1983; Eriksson et al., 1998).

Chemoembolization has been performed in some studies whereby embolization is combined with chemotherapy, for example 5-fluorouracil (5-FU), adriamycin and/or streptozotocin. Long-lasting responses have been seen (Carrasco et al. 1983), but the place for chemoembolization in the treatment arsenal is not yet clear.

**Chemotherapy**

Streptozotocin (STZ) is a broad-spectrum antibiotic which is composed of a nitrosurea moiety with a methyl group on one end and a glucosamine on the other (Herr et al., 1967).

![Fig 2. Structure of streptozotocin.](image)
INTRODUCTION

STZ’s primary effect is inhibition of DNA synthesis (Reusser, 1971) which affects all stages of the cell cycle (Bhuyan et al, 1970). It was chosen for treatment of islet cell carcinomas because of its activity against mouse leukemia (Carter et al, 1971) and its preclinical diabetogenic effect in monkey and beagle (Gans et al, 1971; Pitkin et al, 1970).

In 1973, Broder and Carter reported a 42% response rate with STZ in patients with EPTs. One decade later, Moertel reported a 20% response rate with adriamycin and Altimari reported a 50% response rate with dacarbazine (DTIC). See table 3.

Polychemotherapy has produced better results. The combination of STZ and 5-FU has proven superior to STZ alone (63% response rate versus 36%) (Moertel et al, 1980). In 1992, Moertel et al published data from a randomized trial comparing STZ combined with 5-FU or adriamycin or single treatment with chlorozotocin. Combined treatment with STZ and adriamycin showed superior response rates compared to the two other treatments (69% versus 45% and 30%, respectively). However, the duration of responses were generally low (14-17 months) and median survival was only 1.4-2.2 years. In a previous study, comparing STZ combined with 5-FU or adriamycin, Eriksson et al (1990) reached response rates of 58% and 36%, respectively. Median duration of response was 36 months and 22 months, respectively, and median survival was 4.9 years. See table 3. Because of these contradictory results and known cardiotoxicity for adriamycin, STZ combined with 5-FU is first-line medical treatment at our center.

STZ is known to be a nauseating drug and before the introduction of 5-hydroxytryptamine (5-HT3) blockers almost all patients had nausea and vomiting. With 5-HT3 blockers this problem is much less pronounced even though approximately 10 % still vomit despite adequate premedication (Eriksson et al, 1993). Dose-related renal dysfunction including protein-urea and decrease in creatinine clearance is the dose-limiting factor which occurs in 20-70% of the patients. However, blood counts are rarely affected (Weiss, 1982).
However, some EPTs are poorly differentiated and behave aggressively from the initial diagnosis and others start out as indolent tumors that suddenly change their behavior into rapidly progressing tumors. For these, the previously mentioned treatments are not enough. The most common malignancy derived from neuroendocrine cells, small cell lung cancer, responds well to cisplatin combined with etoposide (Krook et al, 1989). Therefore Moertel et al (1991) decided to use the same regimen in patients with neuroendocrine gastroenteropancreatic tumors. Among patients with anaplastic neuroendocrine tumors, 67% responded partially or completely whereas only 7% of patients with well-differentiated tumors responded partially. Mitry et al (1999) have later published data using a similar regimen confirming the therapy’s efficacy in patients with poorly differentiated endocrine pancreatic tumors.
Nephrotoxicity is the dose-limiting factor in treatment with cisplatin and etoposide occurring in two-thirds of the patients. Hematological toxicity is universal, even though septic fevers and/or hospitalizations due to this are uncommon (Moertel et al, 1991).

**α-interferon**

In 1957, Isaacs and Lindenmann discovered an inducible cell factor that could transfer antiviral resistance to other virus-susceptible cells. They called it interferon, the bearer of interference. The interferon family consists of α-interferon, β-interferon and γ-interferon which bind to two types of receptors, one common for α- and β-interferon and one separate for γ-interferon. α-interferon stimulates natural killer cell function and controls hormone secretion, clinical symptoms and tumor growth in carcinoid tumors (Öberg et al, 1983). See table 4.

**Table 4. α-interferon’s mechanism of action. From Grandé et al, 1990; Andersson T et al, 1990.**

<table>
<thead>
<tr>
<th>Mechanism of action</th>
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<tbody>
<tr>
<td>1. Cell blocking in Go and G1.</td>
</tr>
<tr>
<td>2. Induction of 2'-5'-A-synthetase and thereby reduction of mRNA for hormones and growth factors.</td>
</tr>
<tr>
<td>3. Induction of class I antigens on the cell surface.</td>
</tr>
<tr>
<td>4. General stimulation of the immune system.</td>
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</tbody>
</table>

Natural human leukocyte interferon contains more than 15 subtypes of α-interferon, whereas recombinant interferon, for example α-interferon2b (Intronα®), contains only one subtype of interferon.

The first data on treatment of EPTs with human leukocyte α-interferon (IFN) were reported by Eriksson et al in 1986. Since the drug had produced promising results in midgut carcinoids (Öberg et al, 1983), the authors wanted to try it in advanced cases of the closely related EPTs. Eriksson et al treated 22 patients with advanced EPTs, of which 18 were refractory to chemotherapy, with 3-6 MU of IFN daily. Objective (radiological and/or biochemical) response was seen in 77% of the patients (median duration 8.5 months) and 35% had a radiological response. In 1993 the same authors reported an
objective response rate of 51% (median duration 20 months) and a radiological response rate of 12% in a study where half of the patients received IFN as first-line and half as second-line after chemotherapy.

Previously reported side-effects are influenza-like symptoms (77%), weight loss (59%), chronic fatigue syndrome (50%), bone-marrow depression (anemia, leucopenia, trombocytopenia) (38%-66%) and transient liver dysfunction (13%) (Eriksson et al, 1986; Oberg, 1999). Autoimmune phenomena, such as systemic lupus erythematosus, vasculitis, polymyositis or thyroiditis, are experienced by about 10-15 % of the patients (Rönnblom et al, 1991). About 5% of the patients develop neutralizing antibodies with loss of therapeutic effect which can be restored after switching to natural IFN (Öberg et al, 1989). Another side effect is depression (Merimsky et al, 1992) which can be successfully treated with antidepressive therapy.

**Somatostatin analogs**

Somatostatin is a naturally occurring peptide hormone which was discovered almost 30 years ago (Brazeau et al, 1973) and is mainly present in two biologically active molecular forms, somatostatin-14 and somatostatin-28. See fig 3. Somatostatin is found within the CNS and in many organs including the gastrointestinal and genitourinary system, pancreas (D-cells), thyroid, heart, eye and skin (Reichlin, 1983; Brazeau P, 1986; Polak et al, 1986; Arimura et al, 1975).

The two most common and commercially available somatostatin analogs are octreotide (SMS 201-995) and lanreotide (BIM-23014C). See fig 3. Octreotide was the first long-acting somatostatin analog to be synthesized in 1982 by Bauer et al and lanreotide was introduced by Heiman et al in 1987.

---

**Fig 3. Native somatostatin and somatostatin analogs.**


### Effects of somatostatin and its analogs

- 1. Inhibits the release of growth hormones and almost all gut hormones
- 2. Inhibitory effect on gut exocrine secretion (gastric acid, bile, colonic fluid)
- 3. Inhibition of gastric emptying, gallbladder contraction, intestinal motility
- 4. Suppresses ACTH in Addison’s disease and in ACTH-producing tumors
- 5. Reduces levels of chromogranin A in neuroendocrine tumors
- 6. Inhibition of angiogenesis
- 7. Direct antimitotic effect via somatostatin receptors on the tumor cell

ACTH = adrenocorticotropic hormone

It is well known that neuroendocrine tumors express somatostatin receptors (Reubi et al, 1987) and this is the rationale for treatment with somatostatin analogs in EPTs. Somatostatin analogs are mainly used for symptomatic treatment because of their ability to decrease the level of circulating hormones (Long et al, 1979). The antiproliferative effect seen in vitro (Buscail et al, 1995) is rarely seen in patients.

The short half-life of native somatostatin, 3 minutes (Sheppard et al, 1979), makes it impractical for more frequent clinical use since it has to be administered as a continuous intravenous infusion. The half-life of octreotide has been reported to be 113 minutes after a subcutaneous injection (del Pozo et al, 1986). Slow release formulations have recently been developed for octreotide, Sandostatin-LAR®, and lanreotide, Somatuline-PR®. Both substances are given as intramuscular injections every fourth and second weeks, respectively.
Arnold et al (1996) treated progressing patients with octreotide and achieved tumor stabilization as best response in 36.5% of them (median duration 18 months). However, symptomatic or subjective responses as well as biochemical responses are achieved in 30-90% of the patients (Scarpignato et al, 1995; Ruszniewski P et al, 1993; Arnold et al, 1996).

Treatment with high-dose (up to 12000 μg/day) lanreotide showed biochemical response in 58% and tumor response rate in 11% of patients with neuroendocrine gastrointestinal tumors failing on treatment with standard doses of octreotide (Eriksson et al, 1997). Also, induction of apoptosis, thought to be one of the regulating processes of tumor cell turn-over, was seen. The importance of this is not yet known.

Adverse effects from treatment with somatostatin analogs are mild compared to those seen with chemotherapy or treatment with IFN. Most side-effects such as nausea, transient abdominal pains, flatulence, diarrhea and local reaction at the injection site dissolve with time. In 20-50% of the patients, gall-stones are formed, but these virtually always remain asymptomatic (Trendle et al, 1997).

New high-affinity and subtype selective somatostatin analogs, for each of the five somatostatin receptors, were identified by Rohrer et al in 1998. These analogs are not yet commercially available but hopefully they can contribute to the treatment of EPTs in the future.

In patients suffering from carcinoid tumors, the combination of IFN and somatostatin analogs has been shown to produce a higher biochemical response rate than either drug alone (Janson et al, 1992). Whether this is also true for patients suffering from EPTs is not yet established even though there is one publication from Frank et al, 1999, suggesting that addition of IFN to octreotide has antiproliferative efficacy in a subgroup of patients with advanced metastatic disease unresponsive to octreotide monotherapy. However, in Frank’s study, patients with carcinoid tumors, non-functioning tumors and gastrinomas were mixed and radiological response was defined as >25% reduction of tumor mass instead of >50% reduction, making it difficult to compare the results.

The combination treatment is not accompanied by additional adverse effects, apart from those previously described for each drug.
The scintigraphic technique with radiolabeled somatostatin analogs, of which the most common is radiolabeled octreotide (Octreoscan), is frequently used to visualize and stage patients with somatostatin receptor expressing tumors (Krenning et al, 1993). Studies of biodistribution show a high uptake of octreotide in these tumors.

During the recent years, much attention has been drawn to the possibility of using radiolabeled somatostatin analogs for tumor targeted therapy. There have been several reports (Krenning et al, 1996; Anthony et al, 2002; Janson et al, 1999) concerning the use of such therapy with positive effects on hormone levels, symptoms and tumor size (in the 2 studies first mentioned). Side-effects usually have been transient and include nephrotoxicity and bone-marrow depression.

### Survival

The survival of patients with malignant EPTs depends on the stage and resectability at diagnosis. In patients with unresectable tumors, the primary determinant of survival is the development of liver metastases (Weber et al, 1996). It has been shown that curative surgery of non-metastatic gastrinomas significantly decreases the incidence of hepatic metastases (Fraker et al, 1994). However, only few patients have non-metastatic disease (10-15%) or resectable liver metastases (10%) making them eligible for curative surgery. In a non-randomized study, aggressive resection of metastatic EPTs resulted in a 5-year survival of almost 80% compared to 30% in inoperable patients (Carty et al, 1992).

Most patients have unresectable tumors and need medical treatment which can prolong median survival from diagnosis to 72 months (Eriksson et al, 1993). This can be compared to median survival of insulinomas which was 9.9 months from onset of symptoms in 1950 (Howard et al) when medical treatment was not available.
II. SOMATOSTATIN RECEPTORS

Background

Somatostatin acts via somatostatin receptors (ssts) 1 to 5 which belong to a G-coupled receptor family having seven transmembrane spanning segments (Bell et al, 1993).

The first report on cloning of ssts (sst₁ and sst₂) was published in 1992 by Yamada et al and one year later three additional subtypes (sst₃, sst₄ and sst₅) were identified by the same group.

The ssts are widely distributed in the brain and periphery in a tissue and subtype specific manner. The complete tissue distribution is not yet known, but it has been shown that all five receptors can be found in the pancreas, brain and stomach. Additionally, sst₁ can be found in the liver, pituitary and kidneys, sst₂ in the pituitary and kidneys, sst₃ in the pituitary, sst₄ in the lungs and placenta and sst₅ in the pituitary (Lamberts et al, 1996; Patel, 1997).

Somatostatin (and its analogs) have many different modes of actions which are mediated through different receptors. Inhibition of hormone secretion is mediated through sst₁ and sst₂ (Chen et al, 1992), growth-inhibition is mediated through sst₃, sst₄ and sst₅ (Buscail et al, 1994 and 1995) and somatostatin-induced apoptosis is mediated through sst₃ (Sharma et al, 1996 and 1998). The physiological function of sst₅ is not yet well studied.

The two most common somatostatin analogs, octreotide and lanreotide, bind with high affinity to sst₂ and sst₅, and with moderate affinity to sst₁ (Patel, 1999).
Expression of somatostatin receptors 1 to 5

In 2000, Portela-Gomes et al reported on the expression of sst 1-5 in endocrine cells of the pancreas. They found a high expression of sst subtypes 1, 3 and 4 in all endocrine pancreatic cells, applying immunohistochemistry (IHC) with subtype specific antibodies. Sst₁ was frequently expressed in alpha and beta cells, whereas expression was low in pancreatic polypeptide cells and intermediate in delta cells. Sst₅ was expressed in most beta and delta cells but almost absent in alpha and pancreatic polypeptide cells. See table 6.

Earlier this year, Kulacsiz et al described the expression pattern for sst subtypes 1, 2, 3 and 5 in neuroendocrine gastroenteropancreatic tumors with subtype specific antibodies (IHC). They found a low expression of sst₁ (30%) in both insulinomas and gastrinomas. Sst subtypes 2, 3 and 5 were expressed in 60-80% of the insulinomas and in 75-100% of the gastrinomas. See table 6. A distinction between benign and malignant tumors was not made in this papers.

Recently, a paper was published by Papotti et al where the authors found a correlation in somatostatin receptor expression between pancreatic islet cells and related endocrine tumors. Papotti et al examined sst subtypes 2, 3 and 5 with

<table>
<thead>
<tr>
<th>Sst subtype</th>
<th>Alpha-cells</th>
<th>Beta-cells</th>
<th>Delta-cells</th>
<th>PP-cells</th>
<th>Gastrinomas ns</th>
<th>Insulinomas ns</th>
<th>Benign ETP</th>
<th>Malignant ETP</th>
</tr>
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<tr>
<td>Sst₁</td>
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<td>100</td>
<td>70</td>
<td>83</td>
<td>30</td>
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<td>82</td>
</tr>
<tr>
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<td>90</td>
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<td>53</td>
</tr>
<tr>
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<td>96</td>
<td>99</td>
<td>0</td>
<td>76</td>
<td>78</td>
<td>58</td>
<td>65</td>
</tr>
</tbody>
</table>

*RT-PCR=reverse transcriptase – polymerase chain reaction,
IHC=immunohistochemistry, ns=not specified benign or malignant, nd=not done
both IHC and reversed transcriptase polymerase chain reaction (RT-PCR) whereas sst subtypes 1 and 4 were examined with RT-PCR only. The receptor expression in tumors was generally high (50-90%) except for sst₄ which was expressed in 35% of the benign tumors but none of the malignant tumors. See table 6.

The expression pattern did not differ between benign or malignant EPTs. In lymph node or liver metastases the sst expression was overlapping when studied in parallel with the corresponding primary tumor. Papotti et al also found a possible relation to tumor grade with high-grade malignant endocrine carcinomas having a reduced somatostatin receptor content.

In peritumoral vessels

The vascular system of the tumor bed and the intratumoral neogenesis are of great importance for the development of a tumor (Vaupel et al, 1989; Folkman et al, 1995). In 1994, Reubi et al (1994) identified ssts in the vessels in the tumor bed. They found a several-fold overexpression of ssts in veins located within 2 cm around colorectal tumors compared to veins located at distances 5 to 10 cm from the tumors. Dentzler and Reubi (1999) confirmed their observation a few years later examining tumors and metastases of various origin but also found that overexpression of ssts in peritumoral veins is highly variable between different tumors. Recently, Watson et al showed that sst₂ is present on proliferating endothelial cells but not on non-proliferating endothelial cells. Furthermore, Abini et al (1999) have demonstrated that somatostatin potently inhibits angiogenesis in an animal model.

The expression of somatostatin receptors in per- or intratumoral vessels in EPTs is not clearly established. Since there now are subtype selective somatostatin analogs for each of the five receptor subtypes, it would be interesting to gain further knowledge of the expression of sst₂ and sst₄ in vessels and also to begin studying the expression of sst₁, sst₃ and sst₄.
III. MOLECULAR TARGETING

Tyrosine kinase receptors

Molecular targeting is becoming increasingly important in modern cancer treatment. Among the most interesting and promising targets today are platelet-derived growth factor receptors (PDGFRs), c-kit and epidermal growth factor receptor (EGFR) which all belong to the tyrosine kinase receptor family.

PDGFRs  PDGFRs are normally found in connective tissue and glia but lacking in most epithelia. PDGF paracrine or autocrine loops seem to be involved in growth deregulation of gliomas and sarcomas as well as various human epithelial tumors (Buchdunger et al, 2000). Expression of PDGFRs has been observed in the stroma of a broad range of solid tumors (Lindmark et al and Sundberg et al, 1993). Tumor stroma has a higher interstitial fluid pressure (IFP) than normal stroma (Jain, 1987) which aggravates drug uptake into the tumor. Signaling through PDGFRβ is thought to have a role in the control of IFP (Rodt et al, 1996; Pietras, 2001).

c-kit  C-kit is the cellular homolog of the v-kit retroviral oncogene. The c-kit gene product is expressed in melanocytes, spermatagonia, breast epithelium and mast cells (Natali et al, 1992; Lammie et al, 1994). C-kit is essential for maintenance of normal hematopoiesis, gametogenesis and growth/differentiation of mast cells in mice (Nocka et al, 1989). Deregulation of c-kit is thought to play a role in certain human tumors, including germ cell tumors, mast cell tumors, gastrointestinal stromal tumors (GISTs), small cell lung cancer and breast cancer (Hirota et al, 1998; Tian et al, 1999; Tsuura et al, 1994; Palmu et al, 2002). It has been shown that the deregulation of c-kit in mast cell tumors, germ cell tumors
and GISTs are caused by gain-of-function mutations (Hirota et al, 1998; Tian et al, 1999).

**EGFR**

EGFR is expressed in a variety of different cell types (Salomon et al, 1995). It is important for cell proliferation, cell motility, cell adhesion, invasion, cell survival and angiogenesis (Woodburn, 1999). Coexpression of high levels of EGFR and its ligands leads to a transformed cellular phenotype (DiMarco et al, 1989). Epithelial tumors often overexpress EGFR including cancers of the head and neck, breast, lung, colon, kidney and bladder. Overexpression of EGFR often correlates to a poor clinical prognosis (Salomon et al, 1995) and therefore regulation of EGFR has become an attractive target for developing new strategies in cancer therapeutics (Baselga, 2000).

**Expression of tyrosine kinase receptors in endocrine pancreatic tumors**

In 1992, Chaudhry et al examined five patients with EPTs regarding the expression of PDGFRβ, using immunohistochemistry (IHC). He found that the tumors expressed PDGFRβ in the stroma, but not on the tumor cells. Wulbrand et al (1998) examined (IHC) the expression of EGFR in 10 insulinomas, 9 gastrinomas and 9 non-functioning tumors and found that the receptor was expressed almost exclusively in gastrinomas. Tsuura et al (1994) did not find any expression of c-kit in 8 EPTs examined with IHC. In summary, the expression of tyrosine kinase receptors in EPTs has only been briefly examined.

**Tyrosine kinase receptor antagonists**

STI571 (Glivec®) is a protein tyrosine kinase inhibitor that selectively inhibits the bcr-abl, PDGFRs and c-kit tyrosine kinases (Druker et al, 1996; Buchdunger et al, 2000). Clinically, STI571 has shown good response rates in chronic myeloid leukemia (CML) where the nearly patognomone Philadelphia gene results in a continuously activated bcr-abl fusion protein with tyrosine activity (Druker et al, 2001). STI571 has also induced dramatic tumor responses in GISTs which frequently have mutations in the c-kit tyrosine kinase (Joensuu et al, 2001; Blanke et al, 2001). Additionally, recent experimental work by Pietras et al (2001) has shown that treatment with STI571 inhibits PDGFRβ in tumor stroma which reduces interstitial hypertension and increases transcapillary transport in subcutaneously growing rat colonic

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Accordingly, co-treatment with ST1571 has been demonstrated to enhance the effect by taxol and 5-FU and also increase the tumor uptake of taxol (Pietras et al, 2002).

ZD1839 (Iressa®) is a selective inhibitor of EGFR tyrosine kinase and preliminary data from phase I and II trials show encouraging antitumor activity (Ferry et al, 2000; Baselga et al, 2001). See figure 4. Furthermore, ZD1839 has been shown to potentiate the effect of cytotoxic drugs, such as taxanes, gemcitabine and platinum salts in human cancer cells (Giardello et al, 2000).

**Monoclonal antibodies** IMC-C225 (cetuximab) is a chimeric antibody which binds to the EGFR with very high affinity and prevents ligand binding, thereby blocking ligand-induced tyrosine kinase activity (Baselga, 2000). See figure 4. IMC-C225 is capable of inducing complete regression of well-established tumor xenografts overexpressing the EGF receptor in nude mice (Goldstein, 1995). Preclinical studies have shown that IMC-C225 markedly enhances the antitumor activity of cytotoxic

*Fig 4. Clinical anti-EGFR receptor therapies. From Baselga, 2001.*
agents, such as cisplatin, doxorubicin, paclitaxel, among others (Baselga et al, 1993 and 1994; Fan et al, 1993). Promising results have been reported from a phase I study where IMC-C225 has been used as single drug or in combination with cisplatin (Baselga, 2000). Furthermore, IMC-C225 is a potent radio sensitizer (Saleh et al, 1999). This can be exemplified by a phase I study where patients with unresectable and locally advanced squamous cell cancer of the head and neck were treated with standard irradiation therapy in combination with IMC-C225 and 92% achieved a complete remission (Ezekiel et al, 1998). A randomized comparison between this strategy and irradiation alone is on the way.

Rationale for treatment with tyrosine kinase receptor antagonists

Tyrosine kinase inhibitors are effective if they inhibit targets whose functions are essential for maintenance of the cancer phenotype (Sawyers, 2002). This is shown in GISTs where patients expressing a mutated c-kit respond better to treatment with STI571 than those not carrying the mutation (Blanke et al, 2001) and in CML where the target for STI571 is the bcr-abl fusion protein which is a product of the patognomone Philadelphia gene (Druker et al, 2001). Furthermore, breast cancer patients respond to treatment with trastuzumab, a monoclonal antibody against human epidermal growth factor receptor 2 (HER2), only if HER2 is overexpressed (3+) (Vogel et al, 2002).

However, tyrosine kinase antagonists have been effective both as single drugs and as potentiators of chemotherapy and radiotherapy in many studies where receptor expression is not analyzed or correlated to treatment results. In this situation, the need for pathological or overexpressed receptors is not clear. Seemingly, overexpression of the EGFR is not needed for growth inhibition by monoclonal antibodies (Mendelsohn and Baselga, 2000). This has been shown in a phase II study evaluating IMC-C225 in combination with irinotecan (CPT-11) in CPT-11 refractory colorectal carcinomas where the response rates were similar regardless of EGFR expression (1+ to 3+) (Saltz et al, 2001). The level of EGFR required in the tumor to obtain clinical benefit from ZD1839 is not yet established and needs further investigation (Baselga, 2000). Co-treatment with STI571 has been found to increase the antitumor effects by 5-FU and paclitaxel (Pietras et al, 2002). Whether receptor overexpression is needed or not for increased drug uptake (Pietras et al, 2002) is not yet known.
- To evaluate the efficacy and the tolerability of the combined treatment with IFN and somatostatin analogs in patients with EPTs.

- To confirm previously reported good results from treatment with cisplatin and etoposide in patients with aggressive neuroendocrine tumors using a slightly modified chemotherapy schedule.

- To examine the expression pattern of somatostatin receptors 1 to 5 in tumor cells and intratumoral vessels in tumor samples from patients with EPTs.

- To map the expression of tyrosine kinase receptors PDGFRs, c-kit and EGFR in EPTs to gain knowledge if they express necessary molecular targets for currently available tyrosine kinase antagonists.
**Patients**

**Paper I**  
Sixteen patients, 7 men and 9 women, with EPTs were treated with somatostatin analogs combined with IFN. Median age at diagnosis was 58 years and median duration of disease since diagnosis was 32 months. Eight patients had non-functioning tumors, 3 patients gastrinomas, 3 glucagonomas, 1 an insulinoma and 1 a VIPoma. All patients had metastatic disease. All patients except one were progressing when starting the new treatment. Most patients (15/16) had received previous systemic treatment of which 8 had received IFN, 6 somatostatin analogs, and 11 chemotherapy. Three patients had undergone liver embolization. Thirteen patients were examined with somatostatin receptor scintigraphy and 12 (92%) had pathological uptake.

**Paper II**  
Thirty-six patients, 23 men and 13 women, with neuroendocrine tumors were treated with cisplatin combined with etoposide. The median age at diagnosis was 47.5 years and the median duration of disease from diagnosis was 12 months. All tumors were either poorly differentiated or rapidly progressing. Eighteen patients had carcinoid tumors of foregut origin, of which 5 were atypical. Fifteen patients had EPTs (11 non-functioning and 4 functioning) of which 4 were poorly differentiated carcinomas. Three patients had rapidly progressing midgut carcinoids. All patients had metastatic disease. Thirty of the patients had received prior therapy of which 17 had received chemotherapy, 13 somatostatin analogs combined with IFN, 9 IFN as single drug, 10 surgery, 4 liver embolization and 8 radiotherapy. Thirty-four patients were examined with somatostatin receptor scintigraphy and 30 of them (88%) had pathological uptake.
**Tumor tissue**

**Paper III**  Tumor tissue was collected from 28 patients with histopathologically verified EPTs. Twenty-three patients had well-differentiated tumors of which 10 were non-functioning tumors, 6 gastrinomas, 3 glucagonomas, 3 insulinomas and 1 VIPoma. Five patients had poorly differentiated non-functioning tumors. All patients had malignant disease and half of them had received prior medical treatment. Twenty-six patients were examined with somatostatin receptor scintigraphy and 24 (92%) of them had pathological uptake.

Ten tumor specimens came from primary tumors and 18 from metastases (14 liver metastases and 4 lymph node metastases).

**Paper IV**  Tumor tissue was obtained from 38 patients with malignant EPT. Thirty patients had well-differentiated tumors of which 18 were non-functioning, 4 insulinomas, 3 gastrinomas, 2 glucagonomas, 2 VIPomas and 1 ACTHoma. Eight patients had poorly differentiated non-functioning tumors. All had metastatic disease and 20 patients had received prior chemotherapy and biotherapy. Thirty-five patients were examined with somatostatin receptor scintigraphy and 31 (89%) of them had pathological uptake.

Eight tumor samples came from primary tumors and 30 from metastases (26 liver metastases and 4 lymph node metastases).

Tissue samples (paper III-IV) were obtained by surgery or ultrasound-guided biopsy. The material obtained was frozen in liquid nitrogen and kept at -86°C until sectioned. Five μm thick sections were cut, dried for 5 minutes and fixed in ice-cold acetone for ten minutes. The sections were then dried for 15 minutes and stored in -20°C.

**Biochemistry**

**Paper I-II**  Prior to the start of therapy patients with EPTs were screened for the following serum levels: HCG α and β, gastrin, insulin, c-peptide, pro-insulin, somatostatin, PP and the following plasma levels: chrom A and B, glucagon and VIP. Patients with foregut carcinoids were screened for the following serum levels: HCG α and β, gastrin, PP and the following plasma levels: chrom A and B, glucagon, calcitonin and...
ACTH. Urinary cortisol and histamine metabolite levels were also measured. Patients with poorly differentiated EPTs and foregut carcinoids were screened for tumor markers CA19-9, CA50 and carcinoembryonic antigen (CEA). Patients with midgut carcinoids were screened for plasma chrom A and B and urinary 5-hydroxyindoleacetic acid (5-HIAA) levels. During treatment, analyses of routine biochemistry and individual tumor-marker profiles were performed every third month/treatment.

Patients receiving chemotherapy were examined regarding kidney function using creatinine clearance before each treatment and bone marrow function was monitored weekly between treatments.

**Radiology**

**Papers I-II** Radiologic evaluation was performed every third month/treatment including alternating contrast-enhanced computed tomography (CT) and ultrasonography of the abdomen/thorax. In paper I, CT examinations were re-evaluated by a board-certified specialist in diagnostic radiology.

**Somatostatin receptor scintigraphy**

**Papers I-IV** Somatostatin receptor scintigraphy was performed as described in a previous article (Westlin et al, 1992). Briefly, [111In-DTPA-D-Phe1]-octreotide was injected. Twenty-four hours after injection, static whole-body images were collected and single-photon-emission computed tomography (SPECT) was performed across the body using γ-scintillation SPECT camera equipped with a medium-energy general-purpose collimator (Nuclear Diagnostics, Hägersten, Sweden and London, UK). The collection of original data for SPECT images was performed using a 64-step rotation of 360° in a 64x64-word matrix. Energy windows of 173 and 247 keV ± 10% were used. The collection time for each angle was 40 seconds, amounting to approximately 20,000-35,000 counts per angle. For the reconstruction of SPECT images, a Wiener filter was applied to the original data.
**Definition of response**

**Papers I-II** Radiological tumor response was defined as >50% reduction of the tumor size which was defined as the sum of the products of perpendicular diameters of measurable lesions. Stable disease was defined as <50% reduction or <25% increase of tumor size. Progressive disease was defined as >25% increase of tumor size.

Biochemical response was defined as >50% reduction of at least one tumor marker and remaining tumor markers reduced or stable. Stable disease was defined as <50% reduction or <25% increase of one or more tumor markers. Progressive biochemistry was defined as >25% increase of any tumor marker.

In paper II the definition “overall response” was used meaning a biochemical response and/or radiological response.

**Therapy**

**Paper I** Nine patients were treated with subcutaneous injections of recombinant IFN-α2b (Intron®), 4 with lymphoblastoid IFN (Wellferon®) and 3 with human leucocyte IFN (Finnferon®). Doses were individually titrated and ranged from 3-5 million units (MU) of IFN 3-7 days/week (0-25 MU/wk). Median dose was 9 MU/week. Fourteen patients were treated with 50-500μg of octreotide (Sandostatin®) 2-3 times daily (median dose 450 μg daily) and 2 with 3000 μg lanreotide (Somatuline®) twice daily. All patients received replacement of pancreatic enzymes.

**Paper II** Patients were treated with etoposide at a dose of 100 mg/m² given daily for three days and cisplatin at a dose of 45 mg/m² given daily on the second and third day by continuous infusion in a solution of 5% dextrose and 0.45% saline. As antiemetics, 5-HT3 blockers and cortisone were given. Courses were repeated every four weeks. Because of known nephrotoxicity, diuresis was monitored and furosemid administered, if required. Patients suffering from severe neutropenia received filgastrim.
Development of antibodies

Paper III  The production of somatostatin receptor subtype specific antibodies has been described earlier (Portela-Gomes et al, 2000). Deduced from the amino acid sequence of human sst subtypes 1-5, 5 polypeptides were synthesized by a solid-phase system using Fmoc chemistry (Model 430 A; Applied Biosystems, Foster City, CA, USA). The peptides were purified by reversed phase chromatography and analyzed by plasma resorption mass spectrometry (P DMS biotin 20; Bioion Nordic AB, Uppsala, Sweden). The sequences were selected to be specific for each sst subtype. The homology was <48% to any other protein sequence in the protein database SWISS-PROT, release 37.0, Dec 1998, except for the respective sequences of ssts from other species. The selected sequences were amino acids 363-380 for sst₁, amino acids 330-334 for sst₁, amino acids 366-381 for sst₂, amino acids 332-345 for sst₃, and amino acids 327-341 for sst₄. All had additional tyrosine residues at the N-terminals (tyr₀) and amidated C-terminals. Before immunization, the peptides were coupled to a carrier protein. Two milligrams peptide and 20 mg bovine serum albumin or keyhole limpet hemocyanogen were dissolved in a 50 mmol/L sodium phosphate buffer at pH 7.4, containing 150 mmol/L sodium chloride. Coupling was then induced by addition of 90 μL glutaraldehyde (O’Shaugnessy, 1982). The resulting complexes were injected into New Zealand white rabbits using intradermal injection technique to produce antibodies (Vaitukaitis et al, 1971).

Immunohistochemistry

Papers III-IV  Endogenous peroxidase was blocked with 1% hydrogen peroxidase in phosphate buffered saline (PBS). Avidin-binding protein was blocked by incubating the sections sequentially with avidin and biotin in Blocking Kit (Vector Laboritories, Burlingame, CA, USA). Polyclonal antibodies against chromogranin A and sst subtypes 1-5 were provided by M. Stridsberg, M.D., Ph.D., University Hospital, Uppsala, Sweden, whereas polyclonal antibodies directed towards PDGFR α and β, c-kit and EGFR were purchased from Santa Cruz Biotechnology, CA, USA. The dilutions of antibodies used were 1:1200 for sst₁, 1:5000 for sst subtypes 2, 3 and 5, 1:1000 for sst₃, 1:400 for PDGFRα, 1:100 for PDGFRβ,
MATERIALS AND METHODS

1:250 for c-kit and 1:150 for EGFR. Incubation was performed overnight at 4°C. The immunoreaction was visualized with an Elite Kit (Vector Laboratories), 3-amino-9-ethylcarbazol was used as a chromogen and 0.02% hydrogen peroxidase was used as a catalyst. The sections were counterstained by Mayer’s hematoxylin (Apoteksbolaget, Sweden).

Statistics

For comparison of proportions Chi-square test and Students t-test (two-sided) were used. Survival analysis was performed according to the Kaplan-Meier method.
TREATMENT WITH IFN AND SOMATOSTATIN ANALOGS (I)

Biochemical and radiological responses

Radiological response was seen in 3 out of 16 (19%) patients (median duration 23 months) and stable disease in 11 patients (69%). Of patients previously progressing on treatment with IFN, 1 out of 8 (12%) achieved partial response (median duration 23 months) and 5 (62%) stable disease. All patients previously progressing on treatment with somatostatin analogs had stable disease. See table 7.

Biochemical response was noted in 10 out of 16 (62%) patients (median duration 22 months) and stable disease in 5 patients (31%). Three of 8 (38%) patients previously progressing on IFN treatment showed partial response (median duration 23 months) and 4 had stable disease. Two of 6 (33%) patients progressing on somatostatin analogs achieved partial response (median duration 27 months) and 4 (67%) had stable disease.

Three patients who had progressed on prior treatment with both IFN and somatostatin analogs as single drugs achieved a stabilization of the disease biochemically and radiologically for a median of 10 months.

Relation to somatostatin scintigraphy

All patients with a positive somatostatin scintigraphy obtained a biochemical partial response (9 patients) or stable disease (3 patients). Two patients achieved a radiological partial response and 9 stable disease whereas 1 progressed.
Table 7. Patients’ characteristics and results from treatment with IFN and somatostatin analogs.

<table>
<thead>
<tr>
<th>Case</th>
<th>Syndrome</th>
<th>Previous treatment</th>
<th>Response</th>
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<td>SD 6</td>
<td>SD 6</td>
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<td>10</td>
<td>non-func</td>
<td>x      x</td>
<td>SD 21</td>
<td>PR 21</td>
</tr>
<tr>
<td>11</td>
<td>non-func</td>
<td>x      SD 19</td>
<td>PR 22</td>
<td>pos</td>
</tr>
<tr>
<td>12</td>
<td>non-func</td>
<td>x      x</td>
<td>SD 4</td>
<td>SD 4</td>
</tr>
<tr>
<td>13</td>
<td>non-func</td>
<td>x      x</td>
<td>PD 0</td>
<td>PD 0</td>
</tr>
<tr>
<td>14</td>
<td>non-func</td>
<td>x      x</td>
<td>SD 10</td>
<td>PR 10</td>
</tr>
<tr>
<td>15</td>
<td>non-func</td>
<td>x      x</td>
<td>SD 13</td>
<td>SD 20</td>
</tr>
<tr>
<td>16</td>
<td>non-func</td>
<td>PR 19</td>
<td>PR 19</td>
<td>pos</td>
</tr>
</tbody>
</table>

op=operated on, Som=somatostatin analog, IFN=interferon, chemo=chemotherapy, Emb=liver embolization, rad=radiological, dur=duration of response, biochem=biochemical, non-func=non-functioning, PR=partial response, SD=stable disease, PD=progressive disease, NE=not evaluable, ND=not done
Toxicity

The most common toxicities were fatigue (cortical neurotoxicity grade 1–2) and mild transitory bone marrow toxicity. Four patients developed mild to moderate depression, requiring antidepressant medications. Gastrointestinal adverse effects were also common with nausea and diarrhea. See table 8.

Diarrhea and nausea can most likely be attributed to treatment with somatostatin analogs, the rest to IFN treatment. All side-effects were mild to moderate except for 2 patients experiencing confusion (cortical neurotoxicity grade 3). Both these patients and one additional patient experiencing cortical neurological toxicity grade 2 ended the treatment because of side effects.

Table 8. Toxicity – IFN and somatostatin analogs.

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>No of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diarrhea</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nausea</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anorexia</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gastrointestinal pain</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mouth dryness</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>flatulence</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>white blood cell count</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>platelets</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurologic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sensory</td>
<td>1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>personal change</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortical</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flu-like symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myalgia</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>alopecia</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dry skin</td>
<td>1</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypothyreosis</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Toxicity according to National Cancer Institute of Canada Clinical Trials Groups (NCIC CTG) expanded common toxicity criteria.
grade 1=mild, grade 2=moderate, grade 3=severe, grade 4=life threatening
RESULTS

TREATMENT WITH CISPLATIN AND ETOPOSIDE (II)

Biochemical and radiological responses

EPTs  Seven of 14 (50%) evaluable patients responded overall i.e., biochemically and/or radiologically (median duration 9 months) and 5 (36%) had stable disease. Five patients responded radiologically and 5 biochemically. About half of the patients improved symptomatically. See table 9.

Four patients had poorly differentiated tumors and, of these, 2 responded radiologically, but none biochemically. Eleven patients had well-differentiated tumors and 5 (45%) of them responded overall. Biochemical response was seen in 5 (45%) patients and radiological response in 3 (27%). No difference in response rates was noted between poorly and well-differentiated tumors.

Foregut carcinoids  Ten of 18 (56%) patients responded overall (median duration 9 months). Seven (39%) patients responded radiologically and 4 (22%) biochemically. More than half of the patients improved symptomatically. See table 9.

Five patients had atypical foregut carcinoids and, of these, 2 patients responded radiologically and overall (median duration 11 months). None responded biochemically. Eight (62%) of thirteen patients with typical foregut carcinoids responded overall (median duration 9 months) with 5 (38%) patients responding radiologically and 4 (31%) biochemically. No difference in response rates was noted between atypical and typical tumors.

Midgut carcinoids  Two patients were evaluable biochemically but none responded. The only patient that was radiologically evaluable responded. One patient improved symptomatically. See table 9.
Table 9. Results of treatment with cisplatin and etoposide.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Total No.</th>
<th>Objective response</th>
<th>SD</th>
<th>PD</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biochem.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiol.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foregut carcinoids</td>
<td>18</td>
<td>10 (56%)</td>
<td>4</td>
<td>7</td>
<td>6 (33%)</td>
</tr>
<tr>
<td>atypical carcinoid</td>
<td>5</td>
<td>2 (40%)</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>typical carcinoid</td>
<td>13</td>
<td>8 (62%)</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>EPTs</td>
<td>15 (14)*</td>
<td>7 (50%)</td>
<td>5</td>
<td>5</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>poorly differentiated</td>
<td>4 (3)*</td>
<td>2 (67%)</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>well-differentiated</td>
<td>11</td>
<td>5 (45%)</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Midgut carcinoids</td>
<td>3 (1)**</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>36 (33)***</td>
<td>18 (55%)</td>
<td>9</td>
<td>13</td>
<td>11 (33%)</td>
</tr>
</tbody>
</table>

SD=stable disease, PD=progressive disease, Symptoms=symptomatic response, Biochem.=biochemical, Radiol.=Radiological
* = One patient not evaluable biochemically or overall. ** = One patient not evaluable biochemically, two patients not evaluable radiologically or overall. *** = Two patients not evaluable biochemically, two not radiologically and three not evaluable overall.

Relation to previous treatment

Seven of 17 (41%) patients previously treated with chemotherapy achieved overall response as did 11 of 19 (58%) patients previously chemotherapy naïve. The difference was not significant.

Toxicity

The median number of courses delivered in chemotherapy naïve patients was 5 and, in patients previously treated with chemotherapy, it was 6. However, the median number of non-reduced courses was 2 for chemotherapy naïve patients and 0.5 for patients previously treated with chemotherapy. Dose reductions were frequent, most commonly caused by kidney toxicity. Median creatinine clearance before and after treatment was 83 and 53 mL per 1.73 m², respectively. The reduction was highly significant.

Other adverse reactions included neutropenia and alopecia, see table 10.
Table 10. Toxicity – cisplatin and etoposide.

<table>
<thead>
<tr>
<th>Adverse effect</th>
<th>No.</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of patients</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Nephrotoxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO grade 1</td>
<td>17</td>
<td>47</td>
</tr>
<tr>
<td>WHO grade 2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Neutropenia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO grade 3-4</td>
<td>23</td>
<td>64</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO grade 1-2</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Ototoxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Alopecia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO grade 3</td>
<td>36</td>
<td>100</td>
</tr>
</tbody>
</table>

WHO=World Health Organisation

Survival

Median survival from diagnosis was 38 months for EPTs and foregut carcinoids and 93 months for midgut carcinoids whereas median survival from start of treatment was 13 months for EPTs, 26 months for foregut carcinoids and 1 month for midgut carcinoids. See fig 6.

Fig 6. Median cumulative survival from time of diagnosis (left) and from start of treatment with cisplatin and etoposide in patients with foregut carcinoids (foregut) and EPTs.
### SOMATOSTATIN RECEPTORS 1-5 IN TUMORS AND VESSELS (III)

Expression of ssts in tumor tissue

Nineteen of 28 (68%) tumor specimens stained positive for sst₁, 24 (86 %) for sst₂, 13 (46%) for sst₃, 26 (93%) for sst₄, and 16 (57%) tumor specimens for sst₅. See table 10 and fig 7.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Syndrome</th>
<th>Tissue</th>
<th>Octreoscan</th>
<th>Somatostatin receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46/F</td>
<td>insulinoma</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>2</td>
<td>69/M</td>
<td>insulinoma</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>3</td>
<td>76/M</td>
<td>insulinoma</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>4</td>
<td>52/M</td>
<td>gastrinoma</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>5</td>
<td>52/F</td>
<td>gastrinoma</td>
<td>lymph</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>6</td>
<td>53/M</td>
<td>gastrinoma</td>
<td>lymph</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>7</td>
<td>71/F</td>
<td>gastrinoma</td>
<td>primary</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>8</td>
<td>36/M</td>
<td>gastrinoma</td>
<td>primary</td>
<td>ND</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>9</td>
<td>46/M</td>
<td>gastrinoma</td>
<td>primary</td>
<td>ND</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>10</td>
<td>43/M</td>
<td>glucagonoma</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>11</td>
<td>58/M</td>
<td>glucagonoma</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>12</td>
<td>53/M</td>
<td>glucagonoma</td>
<td>lymph</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>13</td>
<td>58/M</td>
<td>VIPoma</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>14</td>
<td>71/F</td>
<td>nf, well diff</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>15</td>
<td>22/F</td>
<td>nf, well diff</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>16</td>
<td>57/F</td>
<td>nf, well diff</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>17</td>
<td>70/F</td>
<td>nf, well diff</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>18</td>
<td>49/M</td>
<td>nf, well diff</td>
<td>lymph</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>19</td>
<td>51/M</td>
<td>nf, well diff</td>
<td>primary</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>20</td>
<td>53/M</td>
<td>nf, well diff</td>
<td>primary</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>21</td>
<td>51/M</td>
<td>nf, well diff</td>
<td>primary</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>22</td>
<td>33/F</td>
<td>nf, well diff</td>
<td>primary</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>23</td>
<td>64/F</td>
<td>nf, well diff</td>
<td>primary</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>24</td>
<td>43/F</td>
<td>nf, poorly diff</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>25</td>
<td>49/M</td>
<td>nf, poorly diff</td>
<td>liver</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>26</td>
<td>43/F</td>
<td>nf, poorly diff</td>
<td>liver</td>
<td>neg</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>27</td>
<td>25/M</td>
<td>nf, poorly diff</td>
<td>primary</td>
<td>pos</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
<tr>
<td>28</td>
<td>64/F</td>
<td>nf, poorly diff</td>
<td>primary</td>
<td>neg</td>
<td>sst₁, sst₂, sst₃, sst₄, sst₅</td>
</tr>
</tbody>
</table>

RESULTS

Fig 7. Immunohistochemical stainings in two non-functioning tumors (a-f and g-l, respectively). The left tumor stains positive for chrom A (a), sst1, sst3, and sst5 (b, c, e), and negative for sst3, and sst4 (d,f). The right tumor stains positive for chrom A (g), sst1, sst3, and sst1 (i, k, l), and negative for sst3, and sst4 (h, j), x400.
The receptors were evenly distributed among the different tumor subtypes. The individual expression pattern for the sst subtypes varied considerably. One gastrinoma stained negative for all receptors. We could not find any differences in expression pattern between primary tumors and metastases or between well- and poorly differentiated tumors. Nor could we find any differences between previously medically treated tumors or previously non-treated tumors. See table 11.

Table 11. Expression of ssts on tumor cells.

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Positive staining in tumors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sst₁</td>
</tr>
<tr>
<td>All tumors</td>
<td>68</td>
</tr>
<tr>
<td>insulinomas</td>
<td>33</td>
</tr>
<tr>
<td>gastrinomas</td>
<td>33</td>
</tr>
<tr>
<td>glucagonomas</td>
<td>67</td>
</tr>
<tr>
<td>VIPoma</td>
<td>100</td>
</tr>
<tr>
<td>non-func, well diff</td>
<td>80</td>
</tr>
<tr>
<td>non-func, poorly</td>
<td>100</td>
</tr>
<tr>
<td>Well-diff tumors</td>
<td>61</td>
</tr>
<tr>
<td>Poorly diff tumors</td>
<td>100</td>
</tr>
<tr>
<td>Primary tumors</td>
<td>70</td>
</tr>
<tr>
<td>Metastases</td>
<td>67</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>71</td>
</tr>
<tr>
<td>Non-treated</td>
<td>64</td>
</tr>
</tbody>
</table>

diff=differentiated
RESULTS

Expression of sst in intratumoral vessels

Twenty-four specimens were assessable regarding expression of sst\_1, sst\_2, sst\_3, and sst\_4 and 23 regarding expression of sst\_5 in intratumoral vessels. Sst\_1 was found in 9 (38\%) of the vessels, sst\_2 in 19 (79\%), sst\_3 in 3 (13\%), sst\_4 in 19 (79\%) and sst\_5 in 1 (4\%) of the vessels. See table 12 and fig 8.

The receptors were evenly distributed among the different tumor subtypes. The individual expression pattern for the sst subtypes varied considerably. We could not find any differences in expression pattern between primary tumors and metastases or between well- and poorly differentiated tumors. Nor could we find any differences between previously medically treated tumors or previously non-treated tumors.

Table 12. Expression of ssts in intratumoral vessels.

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Pos stainings in intratumoral vessels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sst_1</td>
</tr>
<tr>
<td>All tumors</td>
<td>38</td>
</tr>
<tr>
<td>insulinomas</td>
<td>33</td>
</tr>
<tr>
<td>gastrinomas</td>
<td>25</td>
</tr>
<tr>
<td>glucagonomas</td>
<td>50</td>
</tr>
<tr>
<td>VIPoma</td>
<td>0</td>
</tr>
<tr>
<td>non-func, well diff</td>
<td>33</td>
</tr>
<tr>
<td>non-func, poorly</td>
<td>60</td>
</tr>
<tr>
<td>Well-diff tumors</td>
<td>32</td>
</tr>
<tr>
<td>Poorly diff tumors</td>
<td>60</td>
</tr>
<tr>
<td>Primary tumors</td>
<td>38</td>
</tr>
<tr>
<td>Metastases</td>
<td>38</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>50</td>
</tr>
<tr>
<td>Non-treated</td>
<td>25</td>
</tr>
</tbody>
</table>

Pos=positive
Fig 8. Immunohistochemical stainings of intratumoral vessels in a glucagonoma. The vessels stain positive for sst₂, sst₃, and sst₅ (b, d, e), and negative for sst₁ and sst₆, x140.
Results

Tyrosine Kinase Receptors in Tumors (IV)

Expression of tyrosine kinase receptors

All 38 tumor tissues expressed PDGFRα on tumor cells and 21 of 37 (57%) expressed PDGFRα in tumor stroma. Twenty-eight of 38 (74%) tissues stained positive for PDGFRβ on tumor cells whereas almost all, 36 of 37 (97%), stained positive for PDGFRβ in tumor stroma. C-kit was expressed on tumor cells by 35 of 38 (92%) tumor tissues and EGFR by 21 of 38 (55%) tissues. See table 13 and fig 9.

No differences in expression pattern could be seen between syndromes nor between poorly or well-differentiated tumors. Prior treatment did not influence the expression of receptors. The individual receptor expression pattern varied considerably.

Table 13. Expression of tyrosine kinase receptors in EPTs.

<table>
<thead>
<tr>
<th></th>
<th>PDGFRα tumor</th>
<th>PDGFRα stroma</th>
<th>PDGFRβ tumor</th>
<th>PDGFRβ stroma</th>
<th>c-kit</th>
<th>EGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tumors</td>
<td>38/38</td>
<td>21/37* (57%)</td>
<td>36/37 (97%)</td>
<td>35/38 (92%)</td>
<td>22/39 (56%)</td>
<td></td>
</tr>
<tr>
<td>insulinomas</td>
<td>4/4</td>
<td>3/4</td>
<td>0/4</td>
<td>4/4</td>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td>gastrinomas</td>
<td>3/3</td>
<td>0/3</td>
<td>3/3</td>
<td>2/3</td>
<td>3/3</td>
<td>1/3</td>
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<td>glucagonomas</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>VIPoma</td>
<td>2/2</td>
<td>1/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>1/2</td>
</tr>
<tr>
<td>ACTHoma</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>non-functioning</td>
<td>26/26</td>
<td>16/25* (64%)</td>
<td>20/26 (77%)</td>
<td>25/25</td>
<td>23/26 (88%)</td>
<td>15/26 (58%)</td>
</tr>
<tr>
<td>Well-diff tumors</td>
<td>30/30</td>
<td>15/29* (52%)</td>
<td>24/30 (80%)</td>
<td>28/29* (97%)</td>
<td>27/30 (90%)</td>
<td>17/30 (57%)</td>
</tr>
<tr>
<td>Poorly diff tumors</td>
<td>8/8</td>
<td>6/8</td>
<td>4/6</td>
<td>8/8</td>
<td>8/8</td>
<td>4/8</td>
</tr>
<tr>
<td>Primary tumors</td>
<td>8/8</td>
<td>5/8 (63%)</td>
<td>4/8 (50%)</td>
<td>8/8</td>
<td>7/8</td>
<td>3/8 (38%)</td>
</tr>
<tr>
<td>Metastases</td>
<td>30/30</td>
<td>16/29* (55%)</td>
<td>24/30 (80%)</td>
<td>28/29* (97%)</td>
<td>28/30 (93%)</td>
<td>18/30 (60%)</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>20/20</td>
<td>9/19* (47%)</td>
<td>17/20 (85%)</td>
<td>18/19* (95%)</td>
<td>19/20 (95%)</td>
<td>12/20 (60%)</td>
</tr>
<tr>
<td>Non-treated</td>
<td>18/18</td>
<td>12/18 (67%)</td>
<td>11/18 (61%)</td>
<td>18/18</td>
<td>16/18 (89%)</td>
<td>9/18 (50%)</td>
</tr>
</tbody>
</table>

a) 1 not evaluable
Fig 9. Immunohistochemical stainings in two patients with non-functioning tumors (a-e and f-j, respectively). Both tumors stain positive for chrom A (a+f), PDGFRα (b+g) on tumor cells and in stroma, PDGFRβ (c+h) on tumor cells and in stroma, c-kit (d+i) and EGFR (e+j), x400.
Surgery is the treatment of choice and should always be considered in a patient with a newly diagnosed EPT. However, in most patients, this is not possible because of advanced disease with non-resectable liver metastases (Carty et al, 1992). First-line medical treatment is STZ combined with 5-FU or Adriamycin which has a response rate of 36-69% with a median response duration of 14-59 months (Moertel et al, 1980 and 1992; Eriksson et al, 1990).

In paper I, we treated patients with a combination of IFN and somatostatin analogs and we achieved an objective (biochemical and/or radiological) response rate of 56%, with a median response duration of 22 months. Both radiological and biochemical responses were about the same as, or slightly higher than, that achieved with either drug alone (19% versus 12% and 0%; 65% versus 48% and 31.5%) (Eriksson et al, 1993). Furthermore, patients treated with IFN as single drug received 15MU of IFN weekly compared to 9MU in the combination treatment and half of them were previously untreated whereas the patients in the present study were heavily pretreated. Today, we use comparable IFN doses ranging from 9-25MU (mean 15MU), but considerably higher doses of octreotide than those in the previous studies, 500-1500 daily (mean 1000 μg). We cannot claim that combination treatment is better than either drug alone, even though it seems reasonable. Probably, first-line treatment with the combination in adequate doses would achieve higher response rates. Frank et al (1999) reported a higher radiological response rate from treatment with combined IFN and somatostatin analogs in a mixed material on neuroendocrine tumors than we did. However, they used response criteria different from ours, with radiological PR defined as > 25% reduction in tumor mass (instead of >50%), making comparison difficult.
The treatment was well tolerated. It seems adding somatostatin analogs to IFN makes it possible to lower the IFN dose without losing any efficacy, but instead reducing toxicity.

We consider the treatment a good alternative for patients who fail during chemotherapy as well as for those who cannot or do not want to receive cytotoxic drugs.

In paper II, we treated patients with poorly differentiated or rapidly progressing neuroendocrine tumors, for whom above mentioned treatments are not sufficient, with cisplatin combined with etoposide. Radiological and/or biochemical response was achieved in 50% of EPTs with a median response duration of 9 months comparable to that achieved by Moertel et al (1991). Consequently, we have confirmed their results using the same regimen as they did except for a slightly lower etoposide dose.

The toxicity was considerable with more than half of the patients experiencing nephrotoxicity and most patients experiencing some degree of bone marrow toxicity.

Even though this treatment is tough, it induces a partial response or stabilization of disease in 86% of the patients which is impressive considering the advanced stage of these patients. Survival from diagnosis was only 38 months which is 34 months less than that previously reported for EPTs (Eriksson et al, 1993), confirming that this patient group indeed has an aggressive disease.

Mitry et al (1999) have reported equally good response rates with considerably less nephrotoxicity using a similar chemotherapy regimen with 2-hour infusions. Because of the high incidence of nephrotoxicity using continuous intravenous infusion of cisplatin, we now have adopted the 2 hour infusions and use this as standard delivery of the drugs.

We consider combined treatment with cisplatin and etoposide useful in patients with aggressive tumors or in patients failing on treatment with a STZ-containing regimen.

**Future aspects**

Today available somatostatin analogs bind to sst₂ and sst₃ (Patel, 1999) and are well established in the treatment of EPTs because of their ability to decrease the level of circulating hormones (Scarpignato et al, 1995). The inhibition of tumor growth seen in vitro (Buscail et al, 1995) is rarely seen in patients.
It is suggested that somatostatin analogs act not only directly on tumor cells but also on peritumoral vessels (Reubi et al, 1994). It has been shown that proliferating angiogenic vessels express sst₁₂, whereas non-proliferating vessels do not, (Watson, 2001) and that angiogenesis can be inhibited by somatostatin (Albini et al, 1999). New high-affinity, subtype selective somatostatin analogs for each of the 5 ssts have recently been identified (Rohrer et al, 1998) and will hopefully soon be available.

In paper III, we have examined the expression of somatostatin receptors on tumor cells and in intratumoral vessels. We found that sst₁ and sst₅ were expressed in most tumor tissues and sst₅ in almost 70% of the samples. However, sst₁ and sst₅ were missing in about half of the tumor tissues. Intratumoral vessels stained positive to a high extent for sst₂ and sst₃ (almost 80%), to a lesser extent for sst₁ (40%) and poorly for sst₇ and sst₅ (<15%). No differences in expression pattern were seen between poorly and well-differentiated tumors.

Our findings of sst expression in tumors are in coherence with those observed by Papotti et al (2002) regarding sst₅, sst₁, and sst₅ in malignant tumors whereas we found a slightly lower expression of sst₁ than they did. However, there is a big difference in our findings regarding sst₅ expression: 0% in malignant tumors reported by Papotti et al compared to 93% in our study. Note that different methods were used examining the expression of sst₁ and sst₅, Papotti’s group using RT PCR and our group using IHC. Kulacsiz et al (2002) have examined sst₁, sst₅, sst₃, and sst₅ in insulinomas and gastrinomas, using IHC, and found a lower expression of sst₁ and a slightly higher expression of sst₃ and sst₅ than both our and Papotti’s group did.

Because of the great variability in receptor expression, we suggest that each patient’s individual expression pattern be examined before starting treatment. It has been shown that expression of sst₅ can predict the outcome of treatment with octreotide in carcinoids (Janson et al, 1996 176). It seems reasonable that the same should be true for the other sst subtypes and their respective somatostatin analogs in EPTs. By tailoring treatments according to each individual’s receptor expression, unnecessary expenses and side-effects may be avoided.

Growth-inhibition is mediated through sst₁, sst₂, and sst₅ (Buscail et al, 1994 and 1955) and apoptosis through sst₅ (Sharma et al, 1996 and 1998). Maybe we can achieve a higher
anti-proliferative effect by tailoring treatment with these subtype specific analogs for patients expressing these receptors. It has already been shown that high doses of lanreotide, which binds with moderate affinity to sst$_2$, can induce apoptosis (Eriksson et al, 1997). Hopefully, a somatostatin analog with high affinity for sst$_3$ can do the same to an even higher degree.

We found that ssts were equally often expressed in tumors and intratumoral vessels in poorly differentiated EPTs as in well-differentiated tumors. Today well-differentiated EPTs are more often subject to treatment with somatostatin analogs than poorly differentiated tumors. Maybe the addition of somatostatin analogs to chemotherapy treatment could potentiate the treatment.

Several new antiangiogenetic drugs will soon be on the market. Combination treatment with these products and somatostatin analogs might possibly achieve stronger inhibitory effects on angiogenesis than either drug alone.

We look forward to investigating the impact treatment with new subtype specific somatostatin analogs may have on tumor growth and angiogenesis.

Tyrosin kinase receptors and antagonists

Molecular targeting is a new and exciting approach to cancer treatment. Today, STI571, which inhibits bcr-abl, PDGFRs and c-kit, is registered for treatment of CML (Druker et al, 2001) and GISTs (Blanke et al, 2001). Furthermore, animal tumor models have shown increased drug uptake of taxol and increased effect by 5-FU and taxol with concomitant STI571 treatment (Pietras et al, 2002). The EGFR antagonists, ZD1839 and IMC-C225, which have shown promising antitumor activity in phase I-II studies, potentiation of cytotoxic drugs (Baselga et al, 1993, 1994, 2000; Saltz et al, 2001) and radiotherapy (Ezekiel et al, 1998) will probably be on the market within 1-2 years.

In paper IV, we have examined the expression of PDGFR $\alpha$ and $\beta$, EGFR and c-kit in malignant EPTs. We found that all tumor cells and 50% of the stroma cells expressed PDGFR$\alpha$ whereas almost all stroma cells and 75% of the tumor tissues expressed PDGFR$\beta$. C-kit and EGFR were expressed on tumor cells in more than 90% and 50% of the tumor tissues, respectively.

Our results differ considerably from those previously published by Chaudry et al (1992) who did not find any expres-

However, we now believe that tyrosine kinase receptors are expressed in EPTs, but we do not yet know if they are important for maintenance of the cancer phenotype. We need to further investigate if there exists any important mutations or amplifications in the receptors. However, combination treatment with IMC-C225, ZD1839 or STI571 could be considered in order to enhance the effects of chemotherapy or radiotherapy.

Carefully monitored studies, including examination of and correlation to, receptor expression are needed. We hope that patients with EPTs will benefit from treatment with tyrosine kinase receptor antagonists in the future.
CONCLUSIONS

• Combined treatment with IFN and somatostatin analogs is a good alternative for patients with EPT who are progressing on chemotherapy as well as for those that cannot or do not want to receive cytotoxic drugs. It is well tolerated.

• Cisplatin in combination with etoposide is an efficacious treatment and should be considered as first-line medical treatment in patients with poorly differentiated or rapidly progressing neuroendocrine tumors. Toxicity is high but can be altered by optimizing drug delivery.

• Sst₁ and sst₃ are highly (90%) expressed and sst₅ is moderately (70%) expressed in EPTs whereas almost 50% of the tumors lack sst₁ and sst₅. Analysis of exact receptor expression in each tumor before initiation of treatment with current (and future) receptor subtype specific analogs might increase response rates and avoid unnecessary treatments.

• Sst₁ and sst₃ are highly (80%) expressed and sst₅ is moderately (40%) expressed in intratumoral vessels whereas most (90%) vessels lack sst₁ and sst₃. The relevance of sst expression in intratumoral vessels remains to be elucidated.

• EPTs express tyrosine kinase receptors PDGFRs, c-kit and EGFR in more than half of the tumors. Further studies will reveal if tyrosine kinase antagonists will be part of the future treatment arsenal for EPTs, either as single drugs or as potentiators of chemo- and radiotherapy.
I am grateful to lots of co-workers and friends who have contributed to this work. In particular I would like to express my gratitude to:

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Fredrik and Andrea,
Barbro and Erik,
Peter and Annakarin,
Bengt and Lamjuan,
Tommy and Karin

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REFERENCES


REFERENCES


Buscail L, Estève JP, Saint-Laurent N, Bertrand V, Reisine T, O’Carroll AM, Bell GI, Schally AV, Susini C. Inhibition of cell proliferation by the somatostatin analogue RC-160 is mediated by somatostatin receptors subtypes sSTR2 and sSTR through different mechanisms. Proc Natl Acad Sci USA 1995;92:1580-1584.


CURRENT MEDICAL TREATMENT OF ENDOCRINE PANCREATIC TUMORS AND FUTURE ASPECTS


REFERENCES


Isaac A, Lindemann J. Virus interference. I. The interferon. By A Isaacs and J Lindemann,
Lindmark G, Sundberg C, Glimelius B, Pålman L, Rubin K, Gerdin B. Stromal expression of platelet-derived growth factor beta-


Palmu S, Soderstrom KO, Quazi K, Isola J, Salminen E. Expression of C-KIT and HER-2 tyrosine kinase receptors in poor-prognosis


Saleh MN, Raisch KP, Stackhouse MA, Grizzle

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REFERENCES


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