

Abstracts of presentations made at UK NETWORK/ENET Meeting on Neuroendocrine Tumours in London on 8–9 May 2003

1. Interferon and MIBG combinations in the treatment of metastatic carcinoid tumors

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Background: Metastatic carcinoid tumors are known for their production of vaso-active substances. Octreotide, but also interferon and MIBG have shown activity. In neuroblastoma cell lines improved uptake of ¹²⁵I-MIBG following interferon- γ has been described, based on a *de-novo* synthesis of MIBG receptor transporters.

In a phase II study we evaluated the effect of interferon-alpha upon ¹³¹I-MIBG uptake by carcinoid tumors, and whether the clinical response of the combination improved.

Methods: Patients with metastatic neuro-endocrine tumors in WHO performance scale 1-2, without prior interferon therapy, without right-sided heart failure and without concomitant life-threatening diseases were eligible.

Treatment was started with interferon-alpha 2a (Roferon*) 6 million IU three times a week subcutaneously. After 8 weeks, unlabeled MIBG was added in three cycles (10, 20 and 40 mg/m²) in a 4-hour intravenous infusion at 4 weeks interval. In addition, patients with a positive ¹³¹I-MIBG scan received two therapeutic doses of (200 μ Ci) ¹³¹I-MIBG administered in a 4 h iv infusion in week 21 and week 27. Interferon- α 2a was continued for 6 months. Prior to start, all patients underwent ¹³¹I-MIBG scintigraphy. This was repeated at week 8 to measure the effect of interferon.

Results: 26 patients (18 m, 8 f), median age of 63 years (range: 41–77) entered the study, with the primary tumor in the distal ileum ($n=6$), lungs ($n=5$), stomach ($n=1$) or remained unknown ($n=14$). Four patients received prior treatment (interval >2.5 years) with unlabeled and ¹³¹I-MIBG. After 8 weeks of interferon CT-evaluation showed stable disease (SD) in 85% ($n=22$); a biochemical response was found in 8 out of 23 (35%) patients with elevated urinary 5-HIAA. At 21 weeks, after the addition of unlabeled MIBG 20 patients (91%) had SD at CT-scan and overall biochemical PR response was 39%.

¹³¹I-MIBG tumor retention improved in 22% of the patients, who completed 8 weeks of interferon. In these patients with increased tumor retention at ¹³¹I-MIBG scintigraphy survival was not different ($p=0.89$) from the others (median 32 vs 38 months).

Conclusion: Interferon is active in carcinoid; improvement in tumor retention of ¹³¹I-MIBG occurred in only 22% and a synergistic effect on MIBG treatment was not seen.

2. Intravenous ⁹⁰Y-DOTA-lanreotide for the treatment of Neuroendocrine Tumours

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Background: The vast majority of neuroendocrine tumours express more than one of the five somatostatin receptors (sst 1–5), most frequently over-expressing sst 2. This high level of receptor expression has provided the molecular basis for the successful use of radiolabeled peptide analogues e.g. ¹¹¹In-Octreotide as a sensitive scintigraphic imaging modality for neuroendocrine tumours. ¹¹¹In-DOTA-lanreotide binds to sst 2,3,4, and 5 with high affinity, and to sst1 with lower affinity. The radio-peptide properties has led to the development of receptor-mediated radionuclide therapy with beta emitting particles. This provided the rationale for the development of ⁹⁰Y-DOTA-lanreotide.

Aim: To evaluate whether treatment of patients with progressive advanced neuroendocrine tumours with intravenous ⁹⁰Y-DOTA-lanreotide leads to a reduction in tumour size, extension of the time to tumour progression, and increase in patient survival.

Methods: Twenty-six patients (mean age, 55 years) with progressive neuroendocrine gastroenteropancreatic tumours and medullary thyroid carcinomas were included. These patients had disease that was not amenable to surgical resection. They had all been assessed for chemotherapy, and had either been treated with no effect, or their disease had been considered as untreatable with chemotherapy. Biotherapy with interferon and/or somatostatin analogues was not stopped during the treatment. The treatment consisted of intravenous injections of 1.2 GBq/m² ⁹⁰Y-DOTA-lanreotide administered at intervals of 4–6 weeks for 3 cycles. In some patients, a second set of 3 cycles was administered 6 months after the last previous treatment. Tumour growth and tumour response was evaluated every 3 months by either conventional CT or MRI.

Results: Tumour response was defined according to the World Health Organization (WHO) standard criteria. At the time of data analysis, regressive tumour disease was found in 27% (7 of 26) of patients, stabilization of tumour disease in 46% (12 of 26), and tumour progression in 27% (7 of 26) of patients. The total number of cycles given was 82 (median 3, range 1 to 6). The median duration of follow-up was 7.5 months (range 3–15 months). Side effects were grade 2 or 3 (NCI-CTC) toxicity in 15% (4 of 26). Of these 4 patients, 3 had been previously treated with chemotherapy, and 1 was also being treated with interferon. Fifty percent of the patients were alive at the time of analysis. The median survival time of those patients who had died was 6 months (range, 1 to 9). The median progression free survival time was 7 months (range, 1 to 15). The study is ongoing.

Conclusion: Intravenous ⁹⁰Y-DOTA-lanreotide is a well-tolerated treatment for progressive advanced neuroendocrine tumours, with a remarkable objective response rate.

3. The efficacy and safety of lanreotide Autogel® in relieving clinical symptoms associated with carcinoid tumours: a six-month, open, multicentre, dose-titration study

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Aim: Lanreotide Autogel® is a new aqueous gel formulation of this somatostatin analogue, which demonstrates sustained release over 28 days. The aim of this study was to investigate the efficacy and safety of lanreotide Autogel® in the control of diarrhoea and/or flushing associated with carcinoid tumours.

Patients: Seventy-one patients with symptomatic carcinoid tumours were recruited. Patients must have recorded ≥ 3 stools per day and/or ≥ 1 moderate or severe flushes per day over the week prior to the first treatment. The most troubling symptom for each patient at baseline was identified as the target symptom.

Methods: Patients received a deep subcutaneous injection of lanreotide Autogel®, 90 mg, every 28 days for 2 months. The dose was then titrated down to 60 mg if the patient was a responder, or up to 120 mg if the patient was a non-responder. Responders could have monthly dose titrations thereafter. A responder was defined as having a reduction of $\geq 50\%$ from baseline of the mean daily number of episodes of the target symptom.

Results: Diary card symptom assessments showed significant improvement from baseline (flushing, 3.0 ± 3.2 ; diarrhoea, 5.0 ± 2.7) throughout the study (Table 1). By the end of the study 25/31 (81%) flushing patients and 30/40 (75%) diarrhoea patients showed an improvement from baseline. Tumour marker levels also improved, so that by Month 6 the median 5-HIAA and Chromogranin A levels had decreased from baseline by 24% and 38%, respectively. The diarrhoea subscale of the EORTC-C30 questionnaire indicated a 33% improvement from baseline.

Abstract 3: Table 1

	Month						
	1	2	3	4	5	6	
Flushing (n=31)	Mean (SD) Episodes	2.2 (2.5)	1.9 (2.8)	1.8 (3.0)	1.7 (3.0)	1.7 (3.1)	1.7 (3.0)
	Δ from Baseline	-0.8	-1.1	-1.3	-1.3	-1.3	-1.3
		(-21%)	(-48%)	(-56%)	(-56%)	(-57%)	(-56%)
		$p=0.006$	$p<0.001$	$p=0.001$	$p=0.001$	$p<0.001$	$p=0.001$
Diarrhoea (n=40)	Responders (%)	39%	58%	61%	71%	65%	65%
	Mean (SD) Episodes	4.1 (2.3)	4.0 (2.2)	4.0 (2.2)	3.9 (2.2)	3.8 (2.3)	3.9 (2.2)
	Δ from Baseline	-0.9	-1.0	-1.0	-1.2	-1.2	-1.1
		(-15%)	(-18%)	(-16%)	(-20%)	(-21%)	(-19%)
	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	
	8%	13%	13%	15%	23%	18%	

Safety: The incidence of the most common drug-related adverse events were abdominal pain (20%), fatigue (13%), diarrhoea (11%) and cholelithiasis (10%). **Conclusions:** Lanreotide Autogel® was effective in reducing the flushing and diarrhoea associated with carcinoid tumours. The degree of improvement and the safety profile are consistent with previous studies with other formulations of lanreotide.

4. Expression of parathyroid hormone-related peptide and its receptor in neuroendocrine tumours

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Objectives: Certain pancreatic neuroendocrine tumours associated with hypercalcaemia are known to secrete PTH-related protein (PTHrP). PTHrP was identified in 1987 as the major factor responsible for humoral hypercalcaemia of malignancy (HHM). Since then, PTHrP has been found to be expressed in a paracrine/autocrine fashion in various normal tissues, and by tumours not typically associated with HHM, such as colon and prostate cancer. PTHrP has been shown to regulate cell growth and differentiation, independent of its hypercalcaemic effects. The purpose of this study is to evaluate the expression of the PTHrP and its corresponding PTH/PTHrP type 1 receptor (PTH1R) in human neuroendocrine tumours.

Methods: Immunohistochemical localization of PTHrP and PTH1R was performed on paraffin sections of resection specimens from 42 well-defined neuroendocrine tumours of differing types (19 carcinoid, 17 pancreatic endocrine tumours, 4 paragangliomas, 1 medullary thyroid, and 1 thymic carcinoma). All patients were pre-operatively eucalcaemic. Murine monoclonal antibodies to PTHrP(1-10) and PTH1R(146-169) were used, and the APAAP method of detection was employed. Specificity was demonstrated by pre-absorbance of antibodies with epitope. Immunohistochemistry, using the same antibodies, was carried out on the neuroendocrine cell lines, BON, CRI G1, H727, and RIN 57. Western Blot analysis using the anti PTHrP(1-10) antibody was used to assess the expression of PTHrP by the cell lines. PTHrP(1-10) peptide was labeled with Alexa Fluor 488 and incubated with the cell lines for 24 hours at 37°C. Cells were counterstained with fluorescent nuclear stain and examined under fluorescence microscope.

Results: Positive cytoplasmic staining for PTHrP and PTH1R was seen in tumour cells in the majority of the neuroendocrine tumour resection specimens (78.6% and 83% respectively). The intensity of positive staining for PTH1R in tumour cells was

significantly stronger in the carcinoid tumours compared to the GEP tumours ($p=0.04$), although there was no difference between the groups in the intensity of positive staining for PTHrP. Variable staining for PTHrP and receptor was seen in normal vascular endothelial cells, neurons, some normal epithelial cells, and in pancreatic islets. All four neuroendocrine cell lines showed cytoplasmic immunopositivity for PTHrP and PTH1R. CRI G1 also showed nuclear staining. Cellular PTHrP was detected by Western immunoblotting in all of the cell line extracts. Cytoplasmic and membranous uptake of labeled PTHrP(1–10) peptide was seen in up to 80% of the CRI G1 and RIN 57 cell lines.

Conclusion: PTHrP and its receptor PTH1R are expressed by neuroendocrine tumour cells. The expression of PTHrP peptide and receptor implies a possible autocrine/paracrine role for PTHrP, which may regulate tumour growth and differentiation. The identification of peptide and receptor expression may also enable the development of new therapeutic targets.

5. Somatostatin, vip and analo

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Background/Objective: The overexpression of peptide receptors has been utilized for several years in the diagnosis of neuroendocrine tumors. We here report the synthesis of peptide-dye conjugates consisting of near-infrared fluorescent dyes and somatostatin receptor ligands as contrast agents for optical imaging. Furthermore, we show binding and internalization characteristics of somatostatin analog conjugates as well as near-infrared detection of tumours using these conjugates.

Methods: Receptor binding was studied by inhibition assays. Internalization and subcellular localization of the dye conjugates were examined in BON, Rin38 and HT-29 cells, in cells expressing a receptor-EGFP fusion protein and in human primary neuroendocrine cells by confocal laser microscopy. Whole-body imaging of nude mouse xenografts was used to assess the conjugates *in vivo*. Binding and internalization studies were done by laser microscopy and biochemical assays.

Results: Fluorescence of the somatostatin analogues was rapidly eliminated from the cultured cells. Biodistribution analysis showed peptide-typic results with rather moderate values in kidney and high uptake in the liver. Whole-body imaging was done *in vivo* after intravenous injection into tumor-bearing nude mice by laser-induced fluorescence. In these animals, tumor fluorescence increased rapidly. Native peptides were removed from tumors within minutes while stable analogues accumulated over up to 24 hours. Experiments using several well known analogues (octreotide, octreotate) as well as some new analogs of somatostatin showed that internalization of ligand and receptor can differ greatly depending on the receptor subtype as well as the structure of the ligand. Structure-function relationships of these ligands with regard to endocytosis will be discussed.

Conclusion: Improving peptide ligands for tumor targeting can be done by rational design or combinatorial approaches. The effect of amino acid substitutions on the properties of the resulting analogues is still not predictable. Universal, "pan"-somatostatin analogs are valuable candidates for several diagnostic as well as therapeutic challenges.

6. CCK2 receptor as a therapeutic target for neuroendocrine tumours

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Aim: To investigate the role of gastrin and the CCK2 gastrin receptor in the proliferation of neuroendocrine tumour cell lines.

Methods: CRI-G1, NCI-H727, RIN 5F and SHP 77 neuroendocrine tumour cells were studied. CCK2 receptor was detected in cell lysates by immunoblotting using an antibody (anti-GRE1, Aphton Corp. CA.) raised against the C-terminal sequence of the receptor. For uptake studies, cells were cultured in chamber well slides. The cells were incubated with either gastrin 7 coupled to rhodol green dye or anti-GRE1 labelled with Alexa Fluor 546 dye for 1 hour at 37°C. After fixation cells were viewed under a fluorescence microscope. Cells exposed to the anti-GRE1 antibody were subsequently counterstained for apoptosis using the TUNEL method (ApopTag FITC kit). Proliferation studies were performed by incubating cells alone or with CCK2 antagonist PD 135 at 10^{-4} M for 96 hours followed by measurement of cell number using the MTT assay.

Results: Immunoreactivity to CCK2 was detected in all cell lines. A 122 kD band was seen in all 4 cell lines with an additional 186 kD band seen only in the CRI G1 and RIN 5F cells. Uptake of rhodol green labelled gastrin 7 and of Alexa Fluor 546 labelled anti-GRE1 antibody was seen in all 4 cell lines with the fluorescence being most prominent in the SHP 77 cells. ApopTag FITC staining revealed a coincidence of antibody uptake and apoptosis in the latter cell line. PD135 at a concentration of 10^{-4} M gave inhibition of proliferation in all 4 cell lines (27–92% reduction compared to controls).

Conclusions: We present evidence for the existence of CCK2 receptor in NET cell lines. We have demonstrated uptake of gastrin peptide and antibody by tumour cells and a role for CCK2 in proliferation. These results suggests that the gastrin pathway could be a potential target for treatment in neuroendocrine tumours.

7. Genetic abnormalities in carcinoid tumours

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Carcinoid tumours are neuroendocrine tumours that may arise as isolated non-familial cancers or in association with multiple

endocrine neoplasia type 1 (MEN1), which is an autosomal dominant disorder characterised by the combined occurrence of tumours of the parathyroids, the anterior pituitary and the pancreatic islets. The molecular mechanisms underlying carcinoid tumourogenesis have not been fully defined and in order to further characterise these, we undertook 2 approaches. Firstly, we performed comparative genomic hybridisation (CGH) studies, and secondly, we investigated the MEN1 gene for mutations in carcinoid tumours. We investigated 7 carcinoid tumours (6 non-familial and 1 from a familial MEN1 patient). Tumour DNA was extracted and used for: 1) CGH that utilized lymphocyte metaphase spreads from a healthy male donor; and 2) for mutational analysis of the MEN1 gene which is located on chromosome 11q13, consists of 10 exons and encodes a 610 amino acid protein. The CGH studies revealed losses of chromosomes 1p21-qter, 2q34-qter, 3, 9p21-pter, 11q12-qter, 20q, and 22q13-qter, and gains of chromosomes 5, 14, 17 and 20q in the carcinoid tumours. Mutational analysis of the MEN1 gene in the tumours revealed 3 mutations, which consisted of one nonsense mutation (Glu191Stop), one missense (Val162Phe) and one frame-shift insertion (nt234 ins CA). The Glu191Stop mutation was observed in the tumour and leukocyte DNA of the familial MEN1 patient, thereby indicating that this is a germline mutation. However, the frameshift insertion (nt234 ins CA) was only observed in the tumour DNA of the same familial MEN1 patient, thereby indicating that this is a somatic mutation. Similarly, the missense mutation (Val162Phe) was observed in tumour DNA from a non-familial carcinoid patient. The other chromosomal deletions and duplications were observed in tumours from the non-familial carcinoid patients. Thus, our studies have demonstrated 2 distinct molecular mechanisms that are implicated in carcinoid tumourogenesis, firstly, point mutations of the MEN1 gene and secondly, allelic deletions and amplifications at a number of different chromosomal loci.

8.

A glucagon-like peptide-1 secreting tumour causing reactive hypoglycaemia

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A 45 year old woman was referred with a 7 month history of dizzy spells, confusion, sweating and shaking. These episodes resolved with dextrose tablets. Clinical examination was unremarkable. Initial investigations included a random blood glucose of 21.0 mmol/l, diagnostic of diabetes mellitus. An OGTT showed a fasting glucose of 8.1 mmol/l and at 120 min of 1.9 mmol/l during which she was symptomatic. She was prescribed metformin and guar gum but her blood sugars continued to fluctuate between 14 mmol/L and 2.0 mmol/L. During these hypoglycaemic episodes she was symptomatic. She was referred to Hammersmith Hospital where 72 hour fast did not induce hypoglycaemia excluding an insulinoma. However, the patient suffered a profound hypoglycaemia of 1.8 mmol/l an hour after being given a meal, with high insulin and C-peptide values of 285 mU/l (NR<3) and >4000 pmol/l (NR<500) respectively. A sulphonylurea screen was negative. Plasma somatostatin and chromogranin B were markedly elevated at 4000 pmol/L (NR<15) and 181 pmol/L (NR<150) respectively. Somatostatin

(¹¹¹ Indium-pentetreotide) receptor scintigraphy revealed dense tracer uptake in the pelvis. Pelvic CT scan demonstrated a 12 cm×11 cm×12 cm encapsulated right-sided pelvic mass. The diagnosis of pelvic neuroendocrine tumour was made. A 5-hour OGTT was performed. The FBG was 10 mmol/L and increased to a delayed peak (17 mmol/L) at 90 minutes. Somatostatin levels were markedly elevated for the first 90 minutes at 3000-4000 pmol/L (NR<150) associated with hypoinulinaemia. Following the rise in plasma glucose, somatostatin levels fell and GLP-1 increased to six-times physiological levels to a peak of 270 pmol/L (normal postprandial peak 25–40 pmol/L) from a baseline 150 pmol/L (fasting NR 15–40 pmol/L). Consequently, insulin levels peaked at 340 mU/L (NR 30–100 mU/L) and hypoglycaemia ensued at 180 minutes reaching a nadir of 0.6 mmol/L. The glucagon levels were suppressed and failed to rise in response to the hypoglycaemia. An intravenous glucose tolerance test revealed similar results. The neuroendocrine tumour was therefore synthesizing GLP-1 and somatostatin. The patient underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy and the right ovary was replaced by a 15 cm mass. Histological examination revealed characteristic features of a strumal ovarian carcinoid tumour and immunohistochemistry confirmed the diagnosis with focal positivity for neuroendocrine marker chromogranin and there were strongly positive cells for GLP-1. The tumour cells were cultured. Elevated GLP-1 and somatostatin levels were demonstrated in the culture medium at 99 pmol/L and 50 pmol/L respectively. Insulin was not detected. Glucose (1–1000 nmol) increased GLP-1 secretion in a dose-dependent manner (control 68.3±19.4 fmol/ml vs glucose 1000 nmol, 172.8±8.2, *p*<0.05). Postoperatively, the patient was cured of both diabetes and reactive hypoglycaemia. A repeat OGTT, four weeks post-operatively, revealed normal hormonal profiles.

This is the first reported case of a neuroendocrine tumour secreting GLP-1 and causing reactive hypoglycaemia. GLP-1, an insulinotropic hormone, normally synthesized in the intestinal mucosa and released in response to a meal to enhance insulin secretion, is essential for normal glucose homeostasis. There is now increasing interest in the therapeutic use of GLP-1 in diabetes since GLP-1 treatment in patients with type 2 diabetes improves glycaemic control and the risk of hypoglycaemia is reported to be low. This highlights a potential problem with GLP-1 therapy in the treatment of diabetes.

9.

R-type Ca²⁺ channel activity is associated with chromogranin A secretion in human neuroendocrine tumor BON cells

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Background: Neuroendocrine tumor (NET) cells express voltage-operated Ca²⁺ channels (VOCCs) of the L-, N-, and P/Q-type. Ca²⁺ entry via these channels induces release of biogenic amines and hormones. Therefore, these channels could play a key role for secretion of peptide hormones, biogenic amines or growth factors.

Aim: This electrophysiological study was undertaken to investigate the relationship between distinct Ca²⁺ channel activities and CgA secretion in human NET BON cells.

Methods: Human NET BON cells were used as a representative model of NET disease. Patch-clamp techniques, measurements of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$), and secretion analysis were performed.

Results: Ba^{2+} inward currents through R-type channels (CaV2.3) were measured and identified by SNX-482 (10 nM), a novel voltage-sensitive R-type Ca^{2+} channel antagonist. In the presence of nifedipine (5 μM), α -Conotoxin GVIA (100 nM) and α -Agatoxin IVA (20 nM), R-type channel currents were also detectable. Release of Ca^{2+} from intracellular Ca^{2+} stores by intracellular application of inositol-1,4,5-trisphosphate (InsP3; 10 μM) via the patch-pipette during whole-cell configuration as well as induction of capacitative Ca^{2+} entry (CCE), a passive maneuver to release Ca^{2+} from intracellular Ca^{2+} stores led to an increase in $[\text{Ca}^{2+}]_i$. This effect could be reduced by SNX-482 (20 nM). In addition, SNX-482 (25 nM) also decreased chromogranin A (CgA) secretion whereas α -Conotoxin GVIA (500 nM) and nifedipine (5 μM) failed to reduce CgA secretion.

Conclusion: Neuronal R-type channel activity (CaV2.3) was detected in NET BON cells for the first time. Furthermore, a coupling of this channel subtype with CgA secretion was also found, suggesting association of Ca^{2+} channel activation with consecutive Ca^{2+} influx and stimulation of CgA secretion. In contrast to other Ca^{2+} channel subtypes, specific inhibition of R-type channel activity could be of clinical relevance for the control of hypersecretion in functional NET diseases.

10.

A case of false immunoassay results due to assay interference

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A 52 year old woman was referred to the Hammersmith Hospital in August 2001 for further investigation of her persistently elevated fasted gut hormones (GIH) [all previously assayed at the Hammersmith Hospital]. She had been initially diagnosed with irritable bowel syndrome 16 years ago. Following an exacerbation of her condition 9 years ago, fasting gut hormones were performed, which showed gross elevation of all peptides measured. The possibility of a neuroendocrine tumour was pursued. CT abdomen, MRI pancreas and an Octreotide scan performed at that time were all normal. The patient was monitored with bi-annual MRI scans (which were always normal) and annual fasting GIH, which remained both persistently and significantly elevated. Gastrin and neurotensin were the most affected peptides. The patient was not on any medication apart from oestrogen implants.

At the Hammersmith, the fasting GIH were again repeated and were elevated as before. Imaging of the abdomen and Octreotide scan were also normal. The patient was initially scheduled for pancreatic angiography with calcium stimulation. However, the possibility that heterophilic [interfering] antibodies were present in this patient was considered. The radio-

immunoassays used for the GIH all employed rabbit antibodies. The patient and her husband had kept significant numbers of pet rabbits, with the patient having rabbit induced allergic rhinitis. The presence of heterophilic antibodies in the patient's serum was confirmed by the addition of small concentrations of non-immune rabbit serum (0.5% and 1% respectively) to assay buffer. This eliminated the interference found, by blocking the interfering antibodies, without otherwise affecting the assays. The presence of heterophilic antibodies must be considered when results generated by immunoassays do not correspond to the rest of the clinical and diagnostic pictures.

11.

Expression of molecular targets for tyrosine kinase receptor antagonists in malignant endocrine pancreatic tumors

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Purpose: Medical treatment of patients with metastatic endocrine pancreatic tumors (EPTs) is a challenge since both tumor growth and hormone related symptoms have to be controlled. Molecular targeting with monoclonal antibodies (Mab) and tyrosine kinase inhibitors (TKI) is a novel approach to cancer treatment. We have examined the expression of necessary molecular targets in patients with EPTs to justify further studies investigating their potential benefit from such treatment.

Experimental Design: Thirty-eight tumor tissues from patients with histopathologically verified malignant endocrine pancreatic tumors were examined with immunohistochemistry using specific rabbit polyclonal antibodies regarding the expression pattern of platelet-derived growth factor receptors (PDGFRs) α and β , c-kit and epidermal growth factor receptor (EGFR) (Santa Cruz Biotechnology, Ca, USA).

Results: All 38 tissue specimens expressed PDGFR α on tumor cells and 21 of 37 (57%) expressed PDGFR α in tumor stroma (1 non-evaluable). Twenty-eight (74%) samples stained positive for PDGFR β on tumor cells and 36 of 37 (97%) in the stroma (1 non-evaluable). Thirty-five (92%) tumor tissues stained positive for c-kit and 21 (55%) for EGFR on tumor cells. No differences were seen between syndromes or between poorly or well-differentiated tumors. Previous treatment did not influence expression pattern. Receptor expression pattern varied considerably between individuals.

Conclusions: We have found that tyrosine kinase receptors PDGFRs α and β , EGFR and c-kit are expressed in more than half of the patients with EPTs included in this study. Since these receptors represent molecular targets for STI571, ZD1839 (TKIs) and IMC-C225 (Mab), we propose that patients suffering from EPTs might benefit from this new treatment strategy and this possibility should be explored in forthcoming clinical trials. However, because of great variability in receptor expression pattern all patients' individual receptor expression should be examined.

12.

Expression of c-kit (cd117) in neuroendocrine tumours – a target for therapy?

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Introduction: Gleevec (imatinib mesylate) is currently being used in clinical trials for the treatment of gastrointestinal stromal tumours and is known to specifically inhibit the tyrosine coupled receptor encoded by the c-kit proto-oncogene. Gleevec is currently being assessed as a therapeutic possibility in other c-kit positive tumours.

Aim: To assess the expression of c-kit (CD117) in neuroendocrine tumours.

Methods: Immunohistochemistry was performed on paraffin embedded sections from 62 consecutive NET patients: 36 carcinoid, 16 pancreatic NET, 5 paraganglioma and 5 medullary carcinoma of thyroid. C-kit (CD117) polyclonal antibody (Dako) raised against synthesised c-kit peptide was used followed by a horseradish peroxidase detection step for immunohistochemistry. Appropriate negative controls were carried out simultaneously.

Results: 36% of carcinoids and 18% of pancreatic NET demonstrated expression of c-kit (CD117). No staining was observed on paragangliomas and medullary carcinoma of thyroid.

Conclusion: Immunohistochemical studies have demonstrated the presence of CD117 (c-kit) in carcinoid and pancreatic neuroendocrine tumours, with the most expression observed in carcinoid. With limited treatment availability, Gleevec may have therapeutic efficacy in the treatment of selected tumour patients.

13.

Neuroendocrine tumours of the pancreas: predictors of survival after surgical treatment

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Aims: Neuroendocrine tumours of pancreatic and duodenal origin (NETP) are rare and we present a significant experience from a single centre.

Methods: Data was collected on 44 patients who underwent surgery between 1988 and 2002. Prior to September 1997 data was collected retrospectively but since then data have been recorded prospectively on a dedicated database.

Results: There were 20 women and 24 men, with a median age of 48 years (range 17 to 75). Twenty-five patients had functioning tumours (16 insulinomas, 3 gastrinomas, 2 somatostatinoma, 1 pancreatic polypeptide tumour (PP), 1 vipoma, 1 glucagonoma and 1 carcinoid tumour). USS plus CT and MRI localized the tumours in 38 patients; however, 22 cases (50%) the precise diagnostic was made after surgery. The tumours were located in the head of pancreas in 28(63.6%), body/tail in

12 (27.3%) and duodenum in 3 patients (6.8%). Four insulinomas, 2 gastrinoma, 16 non-functioning, 1 somatostatinoma, 1 vipoma, 1 PP and 1 carcinoid had a malignant phenotype. Twenty pancreaticoduodenectomy, 9 local excisions, 7 distal and 2 total pancreatectomy, 5 bypasses and 1 exploratory laparotomy were performed. Two patients had hepatic resections and 2 had cryotherapy and embolization. Twelve patients had surgical complications (30%) with 1 peri-operative death (2.5%). The overall actuarial survival for resected cases was 74.4% and 42.5% at 5 and 10 years respectively (median = 81 months).

Correlations with survival: in univariate analysis included: gender, type of surgery (local vs extended resections), resection margin (R0 vs R1), lymph node invasion, metastases, location (head vs body and tail), pre-operative diagnostic (NEPT vs others), tumour size (>2cm vs <2cm) and function (functional vs non-functional). Size of tumour, referral diagnostic, lymph node invasion and metastases were significant predictors of survival ($p=0.012$, 0.0047 , 0.047 , and 0.007 respectively). By multivariate analysis, only the presence of metastases retained significance ($p=0.033$).

Conclusion: Surgical resection is the only curative treatment for NETP, can be safely carried out in a specialist centre and is associated with good long term survival. The presence of metastases is a significant factor to predict survival in NEPT in this series.

14.

Neuroendocrine tumours of gut, liver and pancreas: overall survival in a large cohort

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Introduction: Neuroendocrine tumours constitute a heterogeneous group of neoplasms, which originate from neuroendocrine cells in the gut, pancreatic islet cells, respiratory epithelium, thyroid and pituitary glands. They are rare tumours hence series tend to be small from individual centres. These are slow growing tumours for which many expensive therapies exist. It is important to assess background survival rates to compare to treated groups.

Aim: To determine the 1, 3, 5, and 10-yr survival in all the patient followed up in the carcinoid clinics at King's College and North Hampshire Hospitals.

Method: We carried out a retrospective analysis of the notes and computer database of the carcinoid and neuroendocrine tumour clinics at both hospitals.

Results: There were 212 patients on the database. 49 were excluded due to incomplete data. 163 were analysed with an average age of 54.61 yrs. There were 79 (48.5%) male and 84 (51.5%) female. There were 19(11.7%) pancreatic islet cell neuroendocrine tumours, 7(4.3%) fore gut (excluding pancreatic), 66(40.5%) midgut, 3(1.8%) hindgut, 9(5.5%) lung, 52(31.9%) unknown primary and 7(4.35) from various other sites. The peak age of diagnosis was 50-59 yrs. 92.64% of

patients had metastatic disease. 4.91% had regional lymph node metastases only and 86.5% had distant metastases. The 1, 3, 5, and 10-yr survival were 97.97%, 82.5%, 64.5% and 33.72% respectively. The mean survival was 5.46 yrs. The 5-yr survival for lung tumours was 100% and for midgut 84%. The worst prognosis was for tumours of unknown origin with 46%, 5-yr survival.

Conclusion: Neuroendocrine tumours have a good overall prognosis compared to other gastrointestinal malignancies. Those with lung and midgut primaries showed the best prognosis whereas those with an undiagnosed primary the worst. Trials of new therapies are rarely randomised but survival data needs to be compared with these figures to demonstrate efficacy.

15. Differences in the perception of quality of life issues between healthcare workers and patients with metastatic carcinoid or neuroendocrine tumours

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Introduction: Carcinoid and neuroendocrine tumours of gut origin give rise to symptoms not only from the presence of tumours in the liver (pain, ascites, pressure) but also from the output of hormones secreted by these tumours. The symptom complexes associated with the various syndromes and their treatments are unique, setting them apart from gastrointestinal malignancy in general. There is therefore the need to develop a disease-specific quality of life score questionnaire to supplement the already validated European Organisation for the Research and Treatment of Cancers core questionnaire (QLQ-C30)

Aims: Development of a disease-specific quality of life questionnaire module to supplement the QLQ-C30 in patients with carcinoid and neuroendocrine tumours.

Methods: Likely issues were raised from an in-depth literature search, followed by questionnaire interview of 15 healthcare workers (HCW) of different specialities involved with these patients and semi-structured interview of 23 patients with these tumours. In addition 60 patients were given the QLQ-C30 to assess general symptoms of malignancy.

Results: In general healthcare workers scored individual symptoms highest whereas patients scored emotional and anxiety issues highest. The highest average score for healthcare workers was for flushing—3.73, whereas the highest mean score from the patients was concern for family members—2.82. The greatest difference between the two groups was for wheezing where the healthcare workers scored 2.8 and the patients scored 1.3. From the QLQ-C30 values the patients scored 58.5 for global health status, 72.6 for physical function,

61.5 for role function, 67.25 for emotional function, 76 for cognitive function and 61 for social function.

Conclusion: There is a difference between the healthcare worker's perceptions of the importance of an issue in terms of quality of life as compared to the patient with carcinoid/neuroendocrine tumours:

- Healthcare workers are more pessimistic than the patients and therefore tend to score the issues higher than the patients.
- Healthcare workers attribute more importance to individual symptoms whereas patients attribute much more importance to issues of emotion and satisfaction with care than to symptoms.

Patients with carcinoid/neuroendocrine tumours maintain a good quality of life.

Further work is needed on developing a list of questions from these issues, which will need validating in multinational clinical trials.

16. Bowel surgery in metastatic carcinoid in a late phase: specific findings and clinical outcome

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Background: Carcinoid tumours are usually slowgrowing tumours arising from the small bowel. As treatment of metastatic disease has improved survival, in our referral hospital we are more often confronted with recurrent bowel obstruction and/or patients in a late phase of disease. To evaluate clinical outcome medical records were reviewed.

Patients: Among 12 m and 10 f (median age 63 yr, range 41–80; liver metastases in 15) prior surgery was performed in 13. Previous medical treatment: Octreotide in 15, MIBG in 9 and interferon in 5. Fifteen patients were still on octreotide treatment. Median urinary 5HIAA excretion was 265 U/24 hr in the 16 patients with preoperative elevated values. In 8 out of 15 patients with a cardiac ultrasound a tricuspid thickening and/or regurgitation was found. The perioperative approach was according to a standard protocol with continuous octreotide for at least 48 hours.

Results: Resection of the ileum was performed in 4, ileocecal region in 16 and a bypass in 2. The length of resection exceeded 100 cm in 11 patients. Mesenteric retraction was present in 15 patients; ischaemia in 8, necrosis in 2 and perforation in 2 patients. Peritonitis and/or ascites were present in 12 patients. Blood loss was 785 ml (range 0–2050); in 12 patients over 500 ml, while 200 ml is normal in abdominal surgery. Median duration of anaesthesia was 215 minutes (range 120–360). Hypotension occurred in 4 and hypertension in 1 patient. A dopamin infusion was needed in 3 cases. Stay at the intensive care unit was <24 hours in 15. Median duration of

hospitalization: 16 days (range 5–106). Mortality: one patient due to haemorrhage. Median survival in the additional 10 patients who died was 38 months (range 13–102); median follow-up in the patients alive ($n=11$) is 23 months (range 15–65). Clear improvement in symptoms was present in 19 cases.

Conclusion: In pretreated patients small bowel surgery lead in 50% to a large resection (>100cm) and heavy blood loss in 55%, but is still well tolerated and lead to clear improvement in 86%. Survival from surgery is favourable with a median of 23-38 months

17.

Cutaneous manifestations of the malignant carcinoid syndrome

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Carcinoid tumours are not uncommon, however the malignant carcinoid syndrome which occurs when neuroendocrine mediators produced by the tumours enter the systemic circulation is rare and occurs in less than 10% of patients.

Although cutaneous involvement is recognised in this rare syndrome, the small numbers of patients have led to a dermatological literature which is sparse and mainly limited to single case reports. Three main types of cutaneous involvement have been described. The most common is flushing, which is usually transient, although it may become permanent, evolving into an eruption indistinguishable from rosacea. Pellagra-like skin changes may occur and thirdly, a distinctive form of scleroderma has been described in association with the syndrome. Serotonin produced by the tumours has been linked to the pathogenesis of flushing although other mediators are likely to be involved. It is also thought to be involved in the fibrotic process which leads to scleroderma and carcinoid heart disease. Pellagra-like skin changes are due to an acquired deficiency of niacin as dietary tryptophan is utilised by the tumour in the production of serotonin.

We have studied a series of patients with the malignant carcinoid syndrome, performing a thorough cutaneous examination and recording positive findings by photography. Twenty-five patients were entered into the study. All had experienced flushing and in 80% of them it was a presenting feature of the syndrome. Three (12%) had progressed to rosacea. Six (24%) patients had clinical features of pellagra and two thirds of them had progressive disease suggesting that this may indicate an acceleration of disease activity with increased serotonin production. Two patients (8%) had developed scleroderma. Both had advanced disease and have subsequently died.

Thus, a simple cutaneous examination may provide useful information regarding disease activity in patients with carcinoid syndrome. In particular, development of scleroderma is associated with a poor prognosis and should prompt investigation for coexistent carcinoid heart disease.

18.

Treatment of metastatic lung carcinoids

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Introduction: The only curative treatment for patients with lung carcinoids is radical surgery. We report the results of medical treatment of 31 patients with metastatic lung carcinoids, all harbouring distant metastases. The treatment has consisted of alpha-interferon, with or without a somatostatin analogue, and various chemotherapy combinations. In addition, we discuss the circulating hormone markers in these patients.

Results: 5-year survival was 70% from initial diagnosis and 22% from treatment start. The combination of cisplatin and etoposide resulted in objective response in 2/8 patients and one additional patient had stable disease during 6–8 months. Streptozotocin combined with doxorubicin resulted in stable disease during 8–10 months in 2/2 patients, and paclitaxel combined with doxorubicin resulted in stable disease during 9 months in 2/2 patients. All 7 patients treated with streptozotocin + 5-fluorouracil showed progressive disease. Only 4/27 patients (15%) treated with alpha-interferon experienced stable disease during median 15 months. Four patients received a somatostatin analogue as single drug, all progressing in their tumour disease. Plasma chromogranin A was elevated in 28/30 of the patients (93%), plasma chromogranin B in 12/14 (86%) while urinary 5-HIAA was elevated in 21/31 patients (68%), 16 of whom had a carcinoid syndrome. Two patients had an ectopic Cushing's syndrome.

Conclusions: Treatment of lung carcinoids with distant metastases is generally discouraging. Chemotherapy with cisplatin + etoposide, or doxorubicin combined with streptozotocin or paclitaxel may be of value. Alpha-interferon and octreotide offer symptomatic relief, but stabilizes tumour growth in merely 15% of the patients. Plasma chromogranin A was the most frequently elevated tumour marker.

19.

Vascular enhancement of helical CT-scan is correlated with histological vessel density and tumour differentiation in pancreatic endocrine tumours

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Pancreatic endocrine tumours (PET) are frequently hypervascular. Contrast-enhancement at CT-scan is variable but often intense.

Aims: (1) describe in detail the patterns of CT enhancement in a group of patients with PET; (2) compare CT-enhancement to vessel density analysed histologically; (3) correlate histological and CT data with survival.

Patients/methods: Accurate and sufficient helical CT with arterial (tumour enhancement) and portal (loco-regional invasion) acquisitions were available for 37 consecutive patients

with histologically proven PET (surgical specimen, $n=20$; biopsy, $n=17$). Contrast-enhancement was assessed using a 5-point scale: hypo-attenuating (0), iso-attenuating (1), and hyper-attenuating lesions as weak (2), moderate (3) or strong (4). Standard histology classification was performed for according to WHO criteria. In addition, tumour vessel quantification was also determined at light microscopy (5 fields at X25) with the aid of CD34 staining.

Results: PET were predominately well differentiated ($n=30$) and 7 were functional. Mean size of 44 detected lesions was 38 mm (5–100) which were largely solid and homogeneous (7 had a cystic component). Signs of vascular invasion, peritumoral lymph nodes and hepatic metastases were found in 43%, 30% and 43% of patients, respectively. Tumour contrast-enhancement was as follows: grade 0: 25%; grade 1: 14%, grades 2–4: 61%. Vascular density varied according to tumour differentiation (5.2 vs 40.4 vessels/mm² in poorly and well differentiated tumours, respectively: $p=0.007$) and CT-enhancement (grade 0: 5.9 vessels/mm²; grade 1: 15.3; grade 2: 36.9; grade 3–4: 56.3: $p<0.0001$). CT enhancement also correlated with tumour differentiation ($p=0.004$). Poorly-enhancing tumours at CT and low vessel density were poor survival factors at univariate analysis ($p=0.003$ and $p=0.04$, respectively).

Conclusion: CT enhancement of PET is correlated with microscopic vascular density and both of these factors are proportional to the degree of tumour differentiation. PET vascularity at assessed on CT and histology correlates inversely with survival and as such may be added to current arsenal of prognostic factors.

20.

A case of a metastatic insulinoma with refractory hypoglycaemia: response to embolization

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A 55 year old lady presented in 1996 with symptomatic hypoglycaemia. This was confirmed biochemically by hypoglycaemia and an elevated insulin and C-peptide. Abdominal CT scan revealed a 5 cm by 3 cm mass in the tail of the pancreas and a 1.5 cm mass in the head of the pancreas. She underwent surgical removal of these lesions and histological features were consistent with insulinomas. She made an uneventful recovery. A year later, she developed hypercalcaemia with plasma calcium 3.3 mmol/l, low phosphate 0.79 mmol/l and an inappropriately elevated parathyroid hormone 9.6 pmol/l (NR 0.95–5.7) consistent with primary hyperparathyroidism and the diagnosis of multiple endocrine neoplasia type 1 (MEN-1). She underwent exploration of her neck and one parathyroid adenoma was removed. She remained well until January 2000 when she again developed symptoms of hypoglycaemia. She had episodes of feeling vague, blurred vision, perioral tingling and these symptoms were relieved by eating. She had also gained 1½ stones in weight during the previous 3 months. Hypoglycaemia was confirmed within hours of fasting with a blood glucose of 1.8 mmol/l, with an inappropriately raised insulin level of 68.4 mU/l (NR<6 mU/l), and C-peptide level of 3324

umol/l (NR<300 pmol/l) consistent with a recurrence of an insulinoma.

Abdominal CT scan showed multiple poorly enhancing lesions within the right lobe of the liver suggestive of hepatic metastases and calcified areas within the pancreas consistent with previous surgery. Hepatic angiography revealed multiple blushes within the right lobe of the liver. Selective intra-arterial calcium injection into the hepatic artery resulted in a sharp rise in insulin levels sampled from the hepatic vein confirming hepatic insulinoma metastases. This lady required a continuous 10% dextrose infusion, diazoxide at a maximum dose of 200mg tds, guar gum and to eat every 2 hours in order to maintain her blood glucose above 3 mmol/l. As such she underwent embolization of the right hepatic artery in May 2000. She was discharged 10 days later, off all treatment and maintaining normal blood glucose levels. Post embolization abdominal CT scan showed good radiological evidence of resolution of her liver metastases. Initially, she remained off all treatment but at 6 months needed to restart low dose of diazoxide 50 mg tds to maintain her blood sugars. Since then she has remained well and has not required any further intervention and a recent CT scan confirms that her disease is stable.

In summary we present the case of a lady who had severe hypoglycaemia and was dependent on IV dextrose to maintain her blood glucose. However, following embolisation of her hepatic metastases she remains symptom-free on low dose diazoxide 32 months post-embolisation. This case proves that hepatic embolisation can cause sustained resolution of symptoms in patients with metastatic insulinoma and refractory hypoglycaemia.

21.

Localization of gastrinomas by selective intra-arterial calcium injection in patients on proton pump inhibitor or H₂-receptor antagonist therapy

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Preoperative localisation is important for the successful treatment of gastrinomas. Selective intra-arterial calcium injection and hepatic venous sampling has been used successfully to localise gastrinomas. Proton pump inhibitor (PPI) and histamine type 2 receptor (H₂-) antagonist therapy is routinely stopped prior to this test to allow fasting gastrin levels to return to baseline, although this can lead to gastrointestinal perforation. We examined whether selective intra-arterial calcium injection and hepatic venous sampling was able to localise gastrinomas in four patients who remained on PPI or H₂-antagonist therapy. Calcium gluconate was injected directly into the arteries supplying the pancreas and liver after standard selective angiography. Gastrin levels were then measured in samples taken from the right hepatic vein obtained before and 30, 60, 90, 120 and 180 seconds after each injection. Calcium gluconate produced a diagnostic rise (at least 2-fold) in serum gastrin and unequivocally localised the tumour to a specific vascular territory in each case. One patient did not undergo surgery and one patient is awaiting surgery. In the remaining

two patients, surgery confirmed the position and histology of the tumour. Our findings demonstrate that it is possible to localise gastrinomas by selective intra-arterial calcium injection in patients who remain on PPI or H2-antagonist therapy, reducing the potential complications of withdrawal of therapy without affecting the ability of the procedure to localise gastrinomas.

22.

Circadian rhythm and the effect of daily activity on the urinary excretion of 5-HIAA and their relationship with the carcinoid syndrome

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Background: Carcinoid tumours often secrete serotonin, which induce symptoms of the carcinoid syndrome in the presence of liver metastases. Symptoms seem to deteriorate by physical activity, food and alcohol intake and may be worst in the morning, but to what extent is not clear. Therefore, we investigated the effect of symptoms and daily activity on the 5-HIAA excretion (the degradation product of serotonin), searched for a possible circadian rhythm and evaluated whether the 24-hours collection could be replaced by a sample over a shorter period of time.

Methods: Urine was collected in four daily portions of 4 hours and an overnight portion of 8 hours during two days. Patients kept a diary for symptoms, consistency of stools, activities and food intake. Twenty-six consecutive patients entered the study between August 2001 and May 2002.

Results: Elevated levels of 5-HIAA in 24 hours urine were found in 22/26 patients on both collecting days (range 77–1529 $\mu\text{mol}/24\text{ hr}$). The overnight sample correlated best with the 24 hours 5-HIAA excretion (correlation coefficient 0.968) while the evening portion showed the poorest correlation (correlation coefficient 0.869). Median urinary 5-HIAA excretion was significant higher during collecting intervals with defecation (335 $\mu\text{mol}/24\text{ hr}$, $p=0.000$) compared to intervals without production of stools (158 $\mu\text{mol}/24\text{ hr}$). Moreover, a significant correlation was found with the consistency of the stools (solid/loose 332 $\mu\text{mol}/24\text{ hr}$ vs watery 790 $\mu\text{mol}/24\text{ hr}$). Contrary to expectations, 5-HIAA excretion was significantly higher in the collection periods without flushes (234 $\mu\text{mol}/24\text{ hr}$) compared to the periods with flushes (132 $\mu\text{mol}/24\text{ hr}$). No relation could be found for 5-HIAA excretion with activity or rest (220 and 174 $\mu\text{mol}/24\text{ hr}$ resp, $p=0.766$), nor was the degree of exercise related with the level of 5-HIAA excretion ($p=0.446$). No circadian rhythm in the 5-HIAA excretion could be confirmed during the two collecting days.

Conclusion(s): Levels of 5-HIAA are significantly correlated with consistency of stools, however, no correlation could be proved with flushes or activity. A circadian rhythm was not demonstrated in these two collecting days. Overnight urine sample correlates very well with 24-hours collected urine.

23.

Cause of death in carriers of a germ line mutation in the Multiple Endocrine Neoplasia type 1 gene

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Background: The benefit of periodic, clinical screening of carriers of a mutation in the Multiple Endocrine Neoplasia type 1 (MEN-1) gene remains controversial. Our study is aimed at cause of and age at death in MEN-1 patients. Our results are discussed in the light of literature data on MEN-1 regarding the benefit of screening.

Methods: Our study population consisted of all MEN-1 patients treated in a single institution (University Medical Center Utrecht, the Netherlands), during the period 1975–2001 and their affected relatives. Records of affected subjects who died were analysed for morbidity, cause of and age at death.

Results: During 25 years, we identified 87 MEN-1 affected individuals from 16 families. In 57% a mutation in the MEN-1 gene was demonstrated, 18% were obligate carriers and in 24% the diagnosis was only clinically confirmed. Twenty-nine patients died, 16 of who of MEN-1-related causes. MEN-1-related causes of death were malignancies ($n=11$), including pancreatic islet cell tumours ($n=6$) and carcinoid tumours ($n=5$), the Zollinger-Ellison syndrome ($n=4$) and Cushing's disease ($n=1$). The remaining patients died of causes probably related to MEN-1 ($n=3$), unrelated to MEN-1 ($n=7$), or of unknown causes ($n=3$).

Mean ages at death due to MEN-1 were 56.9 and 46.8 years for male, respectively female MEN-1 patients. In both cases this is significantly lower than the mean age at death in the average Dutch population ($p<0.05$).

Conclusions: Because of this increased risk of premature death in MEN-1 patients, it seems justified to recommend periodical clinical screening in MEN-1 gene mutation carriers. Early detection and treatment of abnormalities will probably reduce this risk.

24.

Implementation of programmatic prevention of complex hereditary tumour syndromes in national health care: Continuity in care has to be warranted

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The management of rare and complex hereditary tumour syndromes is facing a growing number of concerns. One of the problems of screening of families is continuity in attendance. At present, most screening programs are temporary, and not structured or incorporated in national health care programs. Mostly, medical care depends on incidental activities

i.e. knowledge, experience, and efforts of individual physicians. It is evident that interruption of periodical screening of patients delays intervention, affects quality of life, and impairs life expectancy. Always again, this induces commotion, frustration, and distress among families. In general, within the current reimbursement systems in health care insurance, preventive medicine such as periodic clinical monitoring of families at risk has a low priority, whereas evidence based medicine has demonstrated that continuity in attendance is cost effective.

In the Netherlands, a project of programmatic prevention is being initiated for 5 different tumour syndromes, involving about 8,500 patients and 15,000 close relatives. These syndromes include multiple endocrine neoplasias (MEN) type 1, MEN type 2, Von Hippel-Lindau (VHL) disease, tuberous sclerosis complex (TSC), and neurofibromatosis (NF). Individuals with a predisposition for an inherited tumour syndrome, such as MEN or VHL disease, are most likely to benefit from early identification of the disease (preferentially presymptomatic diagnosis by DNA analysis) followed by early treatment and periodic clinical monitoring. Potentially life-threatening tumours can be removed at an early stage and thus both prognosis and life expectancy are improved.

The main goal of this project is to investigate the requirements for a national service, which will guarantee periodic clinical monitoring, and secures optimal prevention in patients affected with these tumour syndromes as well as in their disease gene-carrying family members. In our opinion, this goal can be achieved by specially trained practical nurses, which will coordinate multidisciplinary guidance, and organize preventive and emergency cure for these patients. These nurses will cooperate with expert clinicians in the field, specialists for social and psychological issues, patient organizations and clinical genetic centres. In addition, they are responsible for providing patients with up to date clinical information (via newsletters, Internet, etc.).

In conclusion, we started a project to investigate how to achieve a national network supply, by which continuity of periodical examination and attendance is guaranteed and prevention promoted. In order to realize this plan, funding has to be warranted, preferably by health care insurers and supervised by patient interest groups.

25.

Radionuclide treatment of neuroendocrine tumours with the novel radiolabelled analogue ^{90}Y -SMT 487

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Neuroendocrine tumours (NT) are a diverse group of cancers with variable response rates to traditional therapies including radiation, chemotherapy and existing radionuclides. The majority of NT express somatostatin receptors (SMS), of which there are several subtypes. Octreotide is a synthetic somatostatin analogue which principally binds to the types 2 and 5 SMS receptors: ~80% of all NT display avidity for administered octreotide. Recognition of this mechanism has led to the development of targeted radionuclide therapy including ^{90}Y -SMT 487, an aggregate of the β -emitting ^{90}Y molecule, a complexing moiety (DOTA) and octreotide.

We report preliminary findings in 9 adult patients (7 males, 53 ± 4 yrs, mean \pm SE) with a variety of NT treated with a total of 360 mCi (13.2 GBq) ^{90}Y -SMT 487 administered in equal divided doses separated by a minimum of 6-weekly intervals. The majority of the patients had received previous treatment as listed below. In each case, response to ^{90}Y SMT 487 was followed using specific tumour markers in addition to radiological imaging. One patient with advanced hepatic involvement from GI carcinoid and pre-existing liver disease progressed rapidly early in the trial, and was withdrawn after receiving only two doses; therefore, data from 8 patients are listed below:

Transient GI upset was common during treatment and was related to concurrent administration of reno-protective amino acids. Mild thrombocytopenia occurred in 2 subjects but no major adverse events were recorded. Serum or urinary disease specific tumour markers were assessed just prior to commencement of treatment and 6 weeks following the 3rd dose of ^{90}Y SMT 487. A decrease in the disease specific tumour marker was

Abstract 25: Table 1

Diagnosis	Age (yrs)	Sex	Tumour marker	Previous treatment	Pre- ^{90}Y SMT	Post- ^{90}Y SMT
VIPoma	50	F	VIP (pmol/l)	S	89	56
IGF-IIoma	73	F	Pre-pro IGF-II	S, C, R	N/A	N/A
GI carcinoid	46	M	Urinary 5-HIAA ($\mu\text{mol/d}$)	C	264	239
GI carcinoid	61	M	Urinary 5-HIAA ($\mu\text{mol/d}$)	I	863	802
GI carcinoid	51	M	Urinary 5-HIAA ($\mu\text{mol/d}$)		2677	3243
MTC	30	M	Calcitonin ($\mu\text{g/l}$)	S, R	110	75
Insulinoma	51	M	Insulin (mU/l)	S	89	32
Gastrinoma	63	M	Gastrin (pmol/l)	S	>400	197

MTC, medullary thyroid carcinoma; S, surgery; C, chemotherapy; R, radiotherapy; I, interferon.

recorded in 6/7 individuals in whom data are available to date.

These preliminary results indicate that administration of ⁹⁰Y-SMT 487 in patients with a variety of advanced metastatic NT results in reduction of disease-specific tumour markers in the majority. These assessments were performed approximately 6 months after commencement of treatment, and longer-term follow up will be performed to determine the specific benefits and potential adverse effects in these patients.

26.

Computer aided diagnosis based on MRI imaging: Metering precision of repeated semi-automatic measurement of tumor volume and maximum cross-sectional area in carcinoid patients with liver metastases

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Background: Radiologic evaluation of tumor size is a surrogate marker of therapeutic response to anticancer therapy. According to WHO guidelines tumor measurement is standardized by determination of the maximum cross-sectional area. The value of volumetric tumor measurement is controversial. The aim of this study was to validate MRI-based metering precision of computer aided semi-automatic measurement of tumor volume and cross-sectional area determination.

Methods: We analyzed patients with liver metastases in neuroendocrine tumors. Based on MRI imaging (1-T system, Magnetom Expert, Siemens, Germany), tumor volume of reference metastases was repeatedly evaluated in T2-weighted turbo spin-echo sequences (TSE) and in T1-weighted fast low-angle shot sequences (FLASH 2D) using a semi-automatic image segmentation algorithm with manual marker placement (GradientWatershed, ILAB4, Mevis). Furthermore repeated computer aided bidimensional measurement was performed in each sequence using an electronic caliper (SimpleImage-Stat).

Results: Subjective metastases were better visible and defined in TSE sequences. The mean difference of the repeated cross-sectional product was 2.8% (range 1.6% to 5%) in TSE sequences and 3.8% (range 1.3% to 7.2%) in FLASH 2D sequences. The variation was greater in small metastases. The mean difference in repeated semi-automatic tumor volumetry was 5.2% (range 0.8% to 15.7%) in TSE sequences and 24.1% (range 6.3% to 62.2%) in FLASH 2D sequences. Here, too, the variation was greater in small metastases compared to large metastases. For tumor volumetry a mean of 240 markers (range 70 to 814) had to be placed.

Conclusions: Based on MRI imaging bidimensional and volumetric measurement of liver metastases in carcinoid patients should be performed on TSE sequences. For determination of computed aided maximum cross-sectional products reproducible results were obtained with a variability of 5%. Inferior image quality due to long acquisition times and breath artefacts induced a variability in semi-automatic tumor

volumetry up to 15.7% in TSE sequences. The time consuming manual placement of markers for semi-automatic tumor volumetry has to be rationalized.

27.

Primary endocrine tumors of the liver: features and prognosis

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Despite extensive work-up, a primary tumor is not always found in patients with liver endocrine tumors.

Patients and Methods: All patients with histologically confirmed liver endocrine tumors and without primary tumor seen on thoraco-abdomino-pelvic CT, upper GI endoscopy, ileocolonoscopy, pancreatic endoscopic ultrasonography and somatostatin receptor scintigraphy (SRS) were included in this study. Clinical and morphological characteristics, tumor biology, therapeutic strategy and survival were assessed.

Results: Among 393 patients with digestive endocrine tumors seen since 1993, 17 (4.3%) had liver endocrine tumors without a primitive at diagnosis and after a median follow-up of 30 (4–99) months. There were 7 men and 10 women, median age 55 (26–69) years. Liver endocrine tumors were non-functioning in 71%. Serum chromogranin A and NSE were elevated in 67 and 75% of the patients, respectively. Liver endocrine tumors presented as multiple (>5) nodules in 10 patients or as a single large tumor (median diameter 16 cm) in 7 patients. SRS was positive in 65% of cases. Liver endocrine tumors were well-differentiated in 82% and Ki-67 proliferation index was > 10% in 36% of patients. Resection with curative intent was performed in 7 patients (all single tumor), 3 of whom were tumor-free after a median of 16 months, while an extrahepatic tumor occurred in the 4 other patients after a median of 5 months. In patients with unresectable liver endocrine tumors ($n=10$: diffuse liver endocrine tumors, undifferentiated liver endocrine tumors, synchronous bone metastases), chemotherapy ($n=9$) and/or chemoembolization ($n=5$) was administered. Four patients died, 3 from tumor progression after 18, 30 and 63 months. Actuarial survival was 100 and 93% after 1 and 3 years, respectively. It was 100% at 3 years in patients who underwent curative surgery.

Conclusions: (a) endocrine tumors of the liver may be of primary origin in 5% of patients; (b) most primary liver endocrine tumors are non-functioning and well-differentiated; (c) almost half are amenable to liver resection; (d) the prognosis in patients with primary liver endocrine tumors seems better than in those with endocrine liver metastases.

28.

Twenty year experience of pancreatic neuroendocrine tumours (PNTs) at Royal Liverpool and Broadgreen University Hospitals Trust (RLBUHT)

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Background: We report a 20-year single centre experience of PNTs.

Methods: Patients presenting with PNTs to the RLBUHT from 1981-2001 identified from hospital and cancer registry databases were validated by case note review.

Results: Twenty-seven males and 25 females (median age 60 years, range 15–89) presented with PNTs during the study period; multidisciplinary management was evident during the latter half. Symptoms were hormonal in 24 (46.2%); 3 had MEN-1. Fifty patients underwent CT scanning, with tumour visible in 42; 19 had angiography, with tumour evident in 15. Fourteen patients (26.9%) had metastases at presentation. Surgery included Whipple's (11), left pancreatectomy (10), total pancreatectomy (5), enucleation (4), Beger's procedure (1), debulking (1), bypass (3) or no procedure (3); 2 went on to completion pancreatectomy. Chemotherapy was used on presentation (8), for residual disease (2) or recurrence (1). Pathological diagnoses were insulinoma (10), gastrinoma (8), carcinoid (2), neuroendocrine tumour (11), neuroendocrine carcinoma (18) or small cell carcinoma (3). Median follow-up was 23 months (range 6 days to 14 years). Resection was associated with improved survival ($p < 0.0002$).

Conclusion: Management of PNT is distinct from that of other types of pancreatic neoplasia and is optimised by specialist multidisciplinary teams.

29.

Clinical epidemiology of pancreatic neuroendocrine tumours (PNTs): comparison of data from three UK cancer registries (CRs)

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Background: We compared data from three UK CRs to determine the feasibility of a national approach to assess PNT clinical epidemiology.

Methods: All PNT cases (including age, sex, treatment and survival) were identified from three CRs (West Midlands, North Western and Mersey) for 1996–2000.

Results: Forty-three cases (median age 63 years, range 34–77; 23 male: 20 female) were identified from West Midlands CR (population 5.3 m; 1.6 per m per year) with a median survival of 760 days (range 77–1834) in those undergoing resection and 834 days (range 1–2055) overall. Forty-five (median age 63 years, range 34–77; 25 male: 20 female) were identified from North Western CR (4.2 m; 2.1 per m per year); median survival 1602 days (977–1910) after resection and 458 days (range 4–2050) overall. Twenty-seven (median age 60 years, range 25–72; 17 male: 10 female) were identified from Merseyside and Cheshire CR (2.4 m; 2.1 per m per year); median survival 1180 days (72–1827) after resection and 668 days (4–2374) overall.

Conclusion: These results from CRs alone confirm the feasibility of a national approach to PNT clinical epidemiology and estimate incidence at ~2.0 per m per year.

30.

Enhancement of cancer registry (CR) pancreatic neuroendocrine tumour (PNT) data using Royal Liverpool University Hospital (RLUH) databases

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Background: We have assessed the completeness of CR case ascertainment by comparison of contemporaneous CR and hospital records.

Methods: All registered PNT cases were identified from Merseyside and Cheshire CR (MCCR, 1974–2000), and from databases in the Pathology (1986–2000), Clinical Chemistry (1995–2000), and Surgery (1996–2000) Departments at RLUH.

Results: Sixty-five PNT cases of uncertain or malignant status were registered by MCCR between 1974 and 2000 (24 between 1996 and 2000, incidence 2.0 per m per year; overall incidence 1.0 per m per year). Thirty-three cases of benign, uncertain or malignant status were recorded in the Pathology database, 13 (40%) of which were registered by MCCR. Three further unregistered cases were identified from amongst those in Clinical Chemistry and Surgical databases. Addition of unregistered cases (including benign cases not registered by CRs) to those held by MCCR gave an enhanced incidence of ~3.0 per m per year (~6.0 per m per year for 1996–2000).

Conclusion: Calculation of PNT incidence rates using CR data underestimates rates by at least 50%, partly because benign cases are not registered; hospital databases should be used to check estimates derived from CR data. The number of PNT cases registered may be rising because of increasing resection rates.

