**DIFFUSE HORMONAL SYSTEMS**

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## Updated: December 10, 2017

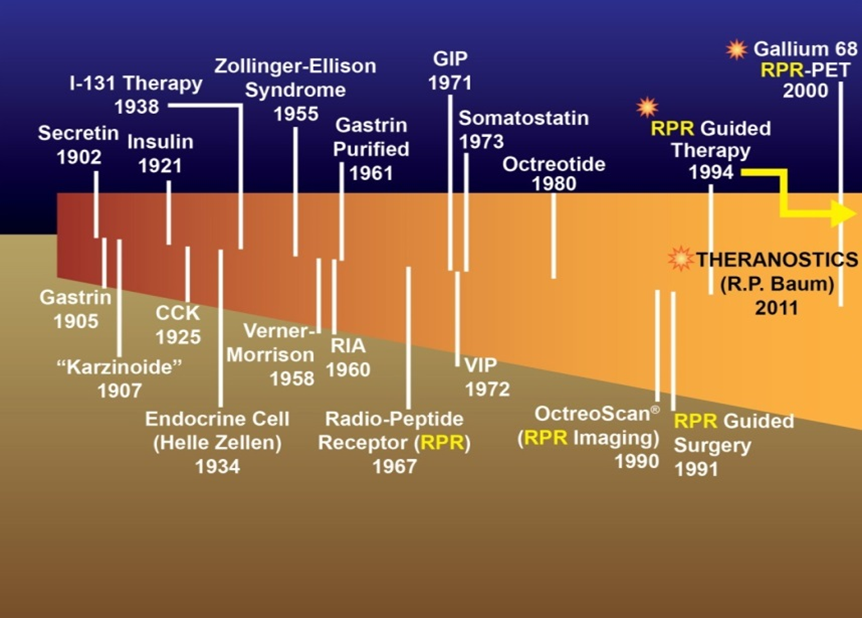
## ABSTRACT

A plethora of gut hormones have been discovered and are linked to clinical syndromes. The proliferation and totipotentiality of the enterochromaffin cell (EC) is responsible for many of the neuroendocrine tumors. Somatostatin has a myriad of physiologic actions in the human body ranging from control of secretions, hormones, gut motility and tumor growth and proliferation. Somatostatin has five receptors conferring specificity of function. Attaching the molecule to various tracers has fostered development of radiotracers to identify the sites of tumor formation and Gallium 68 RPR-PET has a 40-100 fold greater potency than Octreoscan. Combination with lutetium or yttrium creates powerful destructive tools and there has been born a period of “theranostics” in which the same molecule enhances detection and diagnosis but also the ability to treat. When Guillemin discovered somatostatin that inhibited growth hormone secretion from the pituitary he exclaimed that it had not escaped his attention that there may be multiple targets for this peptide. I am not sure that he anticipated the Theranostic explosion that lay ahead. And beyond somatostatin we have tyrosine kinase and MTOR inhibitors and peptide receptor radiotherapy (PRRT) for the treatment of NETS.

## INTRODUCTION

The birth of endocrinology was in the gastrointestinal tract! Figure 1 shows the progression from the discovery of the first hormone secretin when Bayliss and Starling instilled acid into the denervated duodenum of a dog and observed the flow of pancreatic secretions and said this must be due to a hormone which is a word derived from the Greek to “excite”. Subsequently gastrin was identified by Edkins as a potent gastric acid secretogogue in 1905 and Zollinger and Ellison in 1955 recognized it as the principal culprit for the Zollinger Ellison syndrome due to gastric acid overproduction. These discoveries led to a focus on pancreatic and gastric secretion. In 1925 Oberg and Ivy discovered CCK which contracted the gallbladder and it took eons to recognize a clinical syndrome associated with gastric hypersecretion by Jens Rehfeld. Meanwhile Verner and Morrison discovered VIP in 1972 which identified the culprit behind the watery diarrhea , hypokalemia acidosis syndrome (WDHHA) . A flurry of activity in the 60s and 70s led to the discovery of a host of peptides including gastric Inhibitory polypeptide (GIP) by John Brown who renamed it as glucose dependent insulin releasing peptide which has conferred upon it much greater notoriety. Advances in this field were catapulted with the development of radioimmunoassay by Berson and Yalow in 1959 led to the ability of quantification of hormones in the circulation in picomolar amounts and the application of the assay to glucagon by Roger Unger. The advances in immunological techniques and protein chemistry led to intensive progress with the purification and sequencing of a host of other peptides such as motilin, gastric inhibitory peptide somatostatin, leptin and ghrelin. Thus, grew the interest in physiologic mechanisms of action of these hormones digestive glandular secretion, gastrointestinal motility, visceral blood flow and tissue growth and proliferation as well as dedifferentiation of cells into their malignant counterparts. This era was recognized for the discovery of molecules without known biological function such as pancreatic polypeptide (PP), physiologic activity without known peptide regulators, hormones with dual function or as in the case of somatostatin discovered by Schally and Guillemin which was the second peptide captured for a role in peptide therapy. The ability to derive molecules from the parent molecule and its precursor enhanced the capacity to exploit its actions in controlling physiology as a virtual inhibitor of all secretions, to radiolabel the peptide in the development of imaging techniques such as the Octreoscan and the formation of long-acting analogs cable of restricting cell growth and proliferation. Further refinements have seen the evolution of peptide receptor radiotherapy (PRRT) and more potent Gallium DOTATOC and TATE scanning. This age has been referred to as Theranostics by Baum to reflect on the diagnostic capabilities as well as the therapeutic potential. The original recognition of Karzinoid as a tumor of the GI tract in 1907 led to the description by Feyrter of the cell type as Helle Zellen or transparent cells because of their characteristics gave birth to the recognition of the LC cell which is the grandfather of a far-reaching neuroendocrine tumor potential and expanded the role of the gut from pancreas and intestinal tract to a gut brain axis from which a number of disorders have blossomed. This theranostic era has witnessed the implications of tyrosine kinase inhibitors, MTOR involvement in tumor growth and therapy and these last few years have been party to a plethora of new discoveries of old hormones. New discoveries of new hormones and new discoveries of new functions for older peptides and new discoveries of actions outside of the GI tract for established GI hormones.

**Figure 1. History of GI Hormones**



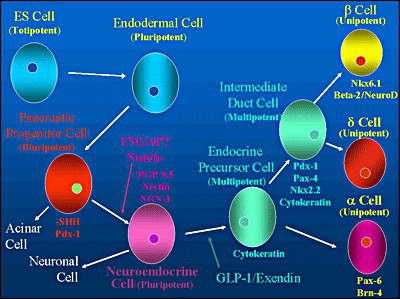
Endocrine tumors of the gastroenteropancreatic (GEP) axis consist of cells that are capable of amine precursor uptake and decarboxylation and therefore have been named APUDomas (1). The morphologic similarity of the APUD cells suggested a common embryologic origin, which was believed to be the neural crest but was later revised to include the neuroectoderm or, in the case of endocrine cells, the dorsal placoderm. However, most investigators agree that these tumors should be classified according to their secretory products (i.e., carcinoid, gastrinoma, insulinoma, somatostatinomas, glucagonoma, vasoactive intestinal peptide [VIPoma] and pancreatic polypeptide [PPoma] (2) (3) (4). Before presenting their clinical characteristics we will briefly review the currently held views on the embryologic origin of these cells and the factors regulating their growth, differentiation and apoptosis, that serve to maintain homeostatic balance. We will also indicate the derangements that result in tumor formation.

# DEVELOPMENTAL ORIGIN OF GEP CELLS DURING EMBRYOGENESIS

The pancreas is composed of exocrine tissue that produces enzymes for digestion, and an endocrine system designed to maintain glucose homeostasis within narrow confines. The adult endocrine pancreas contains four different cell types which produce insulin (β cells), glucagon (α cells) somatostatin (δ cells) and pancreatic polypeptide (PP cells). These are contained within a highly organized structure with beta cells in the interior and the remainder as a surrounding mantle. The vascular supply is elegantly organized to deliver blood to the central core of the islet and to perfuse the outer layers in a centrifugal manner form beta to alpha to δ (synonym B (b) A (a) D (δ). The adult pancreas also has a sophisticated ductal drainage system that ostensibly is present as a conduit for enzymes to reach the gastrointestinal tract but appears to retain cells capable of trans-differentiation into exocrine or endocrine components upon appropriate activation. A crucial question has arisen as to whether or not the adult pancreas retains these precursor cells and if they can be identified. In recent years a molecular fingerprint of embryonic islet precursors has begun to emerge (5) and precursor cells within embryos and postnatal mice are found in the ducts (4). These ductal precursor cells can be identified by their expression of Glut 2 (6). Recently cells expressing the neuronal antigen, an intermediary filament protein, were located in pancreatic ducts of adult rats and were found to differentiate into insulin expressing cells in vitro (7), further suggesting that the study of the characteristics of embryonic development might be able to assist in the capture of the elusive precursor in the adult pancreas.

Inductive signals important in the initiation of growth and development of the pancreas to a large extent have their signals enacted or amplified by genes targeted within the embryonic stem cells or cells committed to developing into a variety of pancreatic endocrine, exocrine or ductal cells.

Pancreatic islet-specific gene expression is mostly controlled at the transcriptional level by the binding of islet enriched transcription factors to sequences in islet genes (Figure 2).



**Figure 2.**

These transcription factors are involved in the temporal expression of genes that direct pancreatic development. Cell specific and extrinsic factors present in the endoderm act in a permissive or restrictive manner to direct the formation of the islets and the various cells and structures that comprise the adult islet (8). The PDX-1 encoded homeodomain protein in mammals (STF-1, IDF-1, IDX-1) was isolated as a transcriptional regulator of insulin and somatostatin (9) (10) (11). It binds and trans-activates the insulin promoter (12). PDX was first detected in embryonic pancreatic and duodenal endoderm. It is detected in all embryonic proto-differentiated epithelial cells during pancreatic development (13). In the pancreas it becomes progressively restricted to the islets, where it is produced in >90% of beta cells, 15% delta cells and 3% alpha cells. PDX-1 defines pancreatic gene expression pattern and cell lineage differentiation (14). Mice heterozygous for PDX-1 develop normally but in homozygous PDX mice the normal branching outgrowth of the pancreas is arrested at an early stage (12) (15). Maturity onset diabetes occurred in patients heterozygous for the gene (16) (17). Diabetes develops in aging transgenic mice following suppression of PDX-1 (18). The regulation of the PDX-1 gene appears to be central to the development of the pancreatic anlage during embryonic development as well as maintaining islet mass in the adult and contributing to the regulation of insulin secretion from the adult pancreas (19). The PDX-1 gene is initially expressed in exocrine and endocrine pancreatic precursors but later becomes restricted to the beta cells in the islets. Transgenic models leading to loss of PDX-expression, either via double knockouts, dominant negative control or elimination of the target binding protein leads to pancreatic agenesis in the case of the double knockout and with haplo-insufficiency, to defects in glucose-stimulated insulin secretion in mice and in humans (12) (17). Abolition of PDX-1 in differentiated β cells in mice results in loss of the β cell phenotype, and impaired expression of Glut 2, glucokinase required for β cell production of insulin in response to glucose (12). PDX-1 is also found where β cell neogenesis is occurring as in duct ligation model of neogenesis (20), partial pancreatectomy (21), overexpression of TGF alpha (22), or interferon gamma (23). In all instances the formation of new β cells is preceded by expression of PDX-1. It has been reported that stimulation of the initiation of trans-differentiation of adult stem cells with INGAP is associated with increased expression of PDX-1 in both ducts and subsequently newly formed islets (24) (25). This endorses the notion that cell differentiation in the adult can recapitulate normal fetal ontogeny. Further support for this notion derives from the observation that trans-differentiated cells stained positive for the neuronal antigen PGP 9.5. PGP 9.5 is an isoform of ubiquitin carboxy-terminal hydrolase (UCTH-LI) and is a marker for neurons and neuroendocrine cells in the skin (26-28) as well as the pancreas (29). It was also found in ductal cells during embryonic islet morphogenesis and in our studies on duct ligation of the hamster pancreas (30) (25). Thus, the combination of PDX-1 and PGP 9.5 suggest evolution from the ductal phenotype to a cell precursor en route to neoislet formation.

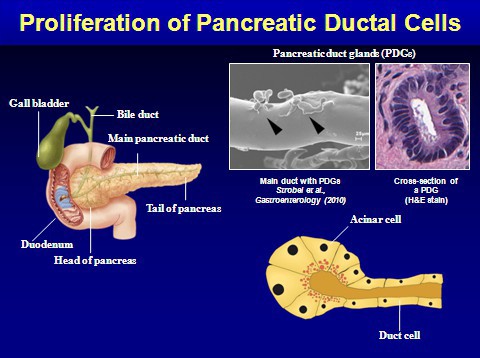
Various studies have shown that the hepatic nuclear transcription factors (HNFs) form a hierarchy of transcription factors that exert positive and negative influences on pancreatic islet growth and development (31) (32). Of particular relevance to islet development is the interpolation of HNF 3 b, a member of the forkhead/winged helix family of transcription factors, between inductive signals for **β** cell development and the expression of PDX-1 (33). Similarly, HNF1 a binds to a regulatory domain of PDX-1 and knockouts have reduced expression of PDX-1 (33). Cell specific and extrinsic factors are expressed during fetal development that determine the region of the endoderm destined to form the pancreatic bud (34-37). The initiation of the pancreatic program requires that signals specify the pancreatic region within the developing endoderm. Sonic hedgehog and Indian hedgehog genes dictate an intestinal differentiation, and for pancreatic development to occur these genes must be excluded. Candidate factors for excluding these genes are Activin–β and fibroblast growth factor (38). The dorsal and ventral buds may develop differently and the LIM homeodomain protein Isl-1 may be an important determinant of pancreatic development (39). Lateral specification of pancreatic development is mediated by Notch signaling by specifying a particular pathway in a field of initially equivalent cells. Notch signaling controls the choice between differentiated endocrine and progenitor cell fates in the developing pancreas and a block in activation of the Notch receptor resulting in high Neurogenin 3 expression and promotes an endocrine fate. These cells upon differentiation migrate into the adjacent mesenchyme where they cluster and upon receiving an inductive signal, for example INGAP; generate distinct endocrine cell types depending on the inductive milieu. This in turn activates PDX-1 which appears to act upstream of fibroblast growth factor (FGF) signaling (12) and induces the FGF1-5 ligands. This is necessary for full maturation of the glucose sensing mechanism of the β cell including expression of the low affinity glucose transporter, Glut 2, and the proinsulin processing machinery, the proinsulin convertase PCI/3 and PC2 responsible for converting proinsulin to insulin. This appears to be conserved between mice and men (34) (35) (37). HNF3 b is a candidate for initiating the positive response to the inductive signal and is expressed in the mouse fetus prior to the expression of PDX-1 at embryonic day 8 (E8.0) in the dorsal endoderm of the fore/midgut before the appearance of the insulin and /or the glucagon expressing cells (19). These primordial cells lack β cell specific markers. Around E13 the number of endocrine cells starts to increase and develop the characteristics of endocrine clusters destined to develop into the organ of Langerhans (4). The primordial cells not yet committed to develop into pancreatic islet cells express a number of neuronal markers including Neurogenin 3, (40), PGP 9.5 , and of Nestin (41). Cells destined to become islet cells appear to express the glut 2 transporter prior to development of hormone secretory capacity and this has been used as a marker for these committed cells in the pancreatic ductal system (6). The organization into individual α, β, δ and PP islet cells is dependent upon appropriately timed expression of a number of other genes including PAX 4, PAX 6 and PDX-1 (for a detailed review see (42) (25) and is complete by day 18, but further refinements and development of glucose sensing occurs in the 2 week postnatal period in mice (4). Although HNF-3 b may be necessary for the response to inductive factors in embryonic development, it appears that HNF-1α is necessary to maintain the islet specific expression pattern and is required throughout adult life (43).

Preliminary data suggest that embryonic stem cells can be differentiated into insulin secreting cells ex vivo, but these cells do not achieve terminal differentiation and have a low insulin content and poor response to glucose. Their growth is unbridled and despite production of β cells, they fail to cure diabetic mice (44). Israeli scientists found insulin-producing cells in embryoid bodies formed spontaneously from embryonic stem cells (ESCs) when they stop growing, but these too do not make sufficient insulin (45). Soria and colleagues used gene-trapping techniques to isolate insulin-producing cells and transfected an antibiotic resistant gene adjoined to the insulin promoter. When these cells formed three-dimensional structures the cells increased insulin to therapeutic levels but this of course remained unregulated (46). Others have created long-lived cell lines from β cells (47), while others have engineered beta cells from non-b cells (48). These have lacked the necessary ingredients of expandability, and physiologic regulation including glucose sensing and an off mechanism in the absence of glucose.

Rather than struggle with the propensity of non-pancreatic stem cells with their reversion to their former state and the difficulties of identifying the necessary control mechanisms for transitioning ESCs into pancreatic stem cells, some researchers have looked to the pancreas as a source of more mature stem cells. Peck and colleagues in a multi-step process, identified islet producing stem cells and transformed these into islet progenitor cells. These grew into islet like structures which increased pancreatic mass 10,000 fold but the cells never fully matured (7). Bonner–Weir and colleagues have applied growth promoting substances to ductal cells in culture and stimulated these to grow and express the IPF-1/PDX-1 protein, the transcription factor necessary for endocrine cell development. At this stage a switch to differentiation factors induces the cells to form cultivated human islet buds which produce a small amount of insulin in response to glucose. The single biggest limiting factor of course was the limited capacity for forming the number of cells required to reverse diabetes (49). Starting with adult human β cells, Levine and colleagues immortalize them by transfection with the SV40 T antigen and the K-ras oncogene to stimulate growth. Cells are induced to transdifferentiate by transfection with PDX-1 and formation of the three dimensional complexes with cell-to-cell contact conducive to insulin production. With the appropriate application of a growth- inducing stimulus these constructs are now capable of secreting insulin in response to glucose. However, these structures are not stable. When implanted they metastasize like tumors and lose their insulin secretory capacity (50) (51).

Others and we have elected to utilize the factors resident in the pancreas to stimulate islet cell growth and proliferation as an alternate to the above approaches (42) (52). It has been known for years that factors present in the pancreas mesenchyme may have an important role in islet integrity (4). More recently the close association between islets and their ducts of origin has been established by electron microscopy of pancreases using cytokeratin 20 markers of duct cells and islet hormone marker(53). The close contact between the islets and duct system has raised interesting possibilities. For example, the open nature of insulin and somatostatin cells allows secretion of hormones into the intestinal lumen, a feature we named “Lumone” many years ago (54) and indeed insulin, serotonin, gastrin, somatostatin and members of the Reg family of peptides have been found in the intestinal lumen and pancreatic juice (55-59). Receptors for insulin have also been found on the luminal surface of duct cells (60-62). The reciprocal relationship may have greater consequence for β cell function. Acinar cell proteins such as Reg are found in pancreatic juice (63-67) and their target may be the stimulation of growth and proliferation of duct cell proliferation and differentiation (63-67). Okamoto and colleagues established a model for islet regeneration in 90% depancreatectomized rats by the islets underwent considerable hypertrophy. They screened the islet derived cDNA library and found the novel regenerating gene and named it Reg. The rat Reg cDNA encoded a 165 amino acid protein with a 22 amino acid signal peptide. Subsequently they isolated the human counterpart which is 165 amino acids, with 68% homology to that of the rat Reg protein. The recombinant forms of Reg have been shown to expand β cell mass by inducing hypertrophy of existing islets and limited replication (68). They then isolated several Reg and Reg-related genes from human rat and mouse and grouped members of the family into three subclasses. Group I encodes a b cell growth factor and some of the type 111 (a, b,and g ) targets neuronal cells and cells of the epithelial alimentary tract where it is found extensively. In the process of ordering these genes (69) a novel form of Reg, Reg III δ, was found with 6 exons, spanning about 3Kb, encoding a 175 amino acid protein with 40-52% of homology to other Reg proteins. Unlike Reg I and Reg II which are expressed in hyperplastic islets, Reg III delta was expressed predominantly in the exocrine pancreas. This mouse form of Reg may be the counterpart of hamster and human INGAP gene that is found almost uniquely in the exocrine pancreas, appearing with islet neogenesis and responsible for stimulating proto-differentiated cells in the ductal system to proliferate, differentiate into islets and function physiologically to reverse diabetes (70). However, Sasahara and colleagues (71) cloned a novel cDNA from mouse pancreas having a 72% homology to hamster INGAP cDNA and 47-52% homology to other members of the Reg family including the different forms of Reg and pancreatitis associated protein (PAP) and pancreatic thread protein of rat, mouse and man. They refer to this protein as INGAP related protein (INGAPrP). In contrast to INGAP, which is expressed during neogenesis, INGAPrP was abundantly expressed in the normal mouse pancreas. Roman and colleagues (72) to determine whether islet angiogenesis and VEGFA production/release participate in the mechanism by which INGAP-PP enhances β-cell function and mass used two models: a) in vivo (normal rats injected with INGAP-PP for 10 days) and b) in vitro (normal islets cultured for 4 days with INGAP-PP, VEGFA, Rapamycin, and the specific VEGF-Receptor inhibitor, SU5416). INGAP-PP administration enhanced insulin secretion, b-cell mass, islet vascularization, and angiogenesis without affecting glucose homeostasis. Normal islets cultured with INGAP-PP and VEGFA increased insulin and VEGFA secretion while apoptosis decreased. INGAP-PP-induced effects were prevented by both Rapamycin and SU5416. INGAP-PP effects on b-cell mass and function were significantly associated with a positive effect on islet angiogenesis and VEGFA production/release. VEGF-A possibly potentiates INGAP-PP effect through mTORC pathway.

**Figure 3.**



The developing pancreas appears as a protrusion from the dorsal surface of the embryonic gut (4). Figure 3 shows the normal anatomy of the pancreas and duodenum in the adult. What is shown is the capability of proliferation duct glandular structures (PDGs) (73) with the capability of transformation to endocrine cells.

The different islet-cell types appear sequentially during development in vivo. Therefore, it seems reasonable to propose that coordinated growth depends on the specificity of growth factors.

Rosenberg and Vinik (74) used a model for new islet formation (i.e., nesidioblastosis) and showed that pancreatic ductal cells are capable of differentiating on stimulation into adult endocrine cells that are capable of secreting insulin in a fully regulated manner. This has led to the notion that endocrine tumors derive from a toti-potential stem cell in the gut that is capable of differentiating into any one of a variety of cells that may be responsible for the clinical syndromes. In HIP rats treated with Sitagliptin, a dipeptidyl peptidase 1V inhibitor prevents the catalytic breakdown of glucagon like peptide 1(GLP-1) thereby increasing endogenous GLP-1 inducing ductal metaplasia.

Human GLP-1 receptor is expressed in the ductal system in humans and upon stimulation with incretins like Exenatide or other GLP-1R agonists markedly increase the expression of GLP-1 receptor (Figure 3) leading to the formation of intraductal neoplasms called PanINs. Butler et al (75) showed expansion of exocrine and endocrine pancreas with Incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon producing tumors. Pancreases from Type 2 Diabetes organ donors on Incretin therapy (n=8), other therapies (n=12) and Diabetic Controls (n=14) were examined. In diabetic patients beta cell mass was reduced 60%. Incretin treatment increased islet mass by 40%. However, 3/8 developed glucagon microadenomas and 1 developed an alpha cell NET accompanied by exocrine cell proliferation and pancreatic intraepithelial neoplasia (PanIn) (Figure 3). Co-staining for insulin and glucagon increased in DM, and was even greater in Incretin treated patients. They concluded that Incretins expand exocrine and endocrine pancreas with proliferation, dysplasia and a cell hyperplasia with possible adenoma formation. While this data was found in postmortem specimens and there is little clinical evidence in thousands of patients treated with incretins, it raises an interesting possibility on the formation of adenomas and the role that GLP-1 may play (76). Figure 4 demonstrates the appearance of glucagon cells in the ducts of a patient who had been treated with an incretin (77). The suggestion is that, with the correct genetic predisposition, use of incretins may have the capacity to induce malignant transformation of cells with formation of neuroendocrine tumors.

A great deal of interest is now being focused on the factors responsible for the initiation of growth proliferation and differentiation into adult endocrine cells, and, in neuronal systems, for growth cessation and cell maintenance. Several models of pancreatic regeneration and tumor formation have been established (63) (78-87).

# GROWTH FACTORS AND THE DEVELOPMENT OF NEOPLASMS OF THE GASTROENTEROPANCREATIC AXIS (GEP-NETS)

Multiple growth factors and receptors are frequently expressed in GEP tumors. These growth factors may include insulin-like growth factor-1, platelet-derived growth factor, transforming growth factors (TGF) -α and β, basic fibroblast growth factors, nerve growth factor (88) (89) and GLP-1 (77). The frequent co-expression of TGF-β and its corresponding receptor, the epidermal growth factor receptor, suggests the presence of autocrine regulatory mechanisms in these tumors (89). TGF-β has been implicated in the desmoplastic reaction associated with carcinoid tumors (89) (90). Overall, the precise role of these growth factors and their importance in the growth and progression of GEP tumors is unknown.

Apoptosis (i.e., programmed cell death) has been shown to be an important process that may occur under normal physiologic conditions, including embryonic growth and development, the differentiation of β-cell populations, and the involution of cells deprived of necessary growth factors (91). Apoptosis may be induced by a variety of chemotherapeutic drugs and cytokines (92). Several growth factors and substances that are secreted by neuroendocrine tumors, including TGF-β (93), glucocorticoids, and somatostatin (94), have been shown in other model systems to induce apoptosis. The importance of apoptosis in the normal growth and differentiation of neuroendocrine tissues, however, and the importance of apoptosis in the response of GEP tumors to chemotherapy remain unknown.

# GENETIC FACTORS PREDISPOSING TO DEVELOPMENT OF NETs

The genetics of neuroendocrine tumorigenesis have yet to be elucidated. Although small familial clusters of midgut carcinoids have been described, there are no known genetic cancer syndromes associated with them. Tumors have clustered in several small families without MEN I, and multiplicity of tumors is a feature on one quarter of isolated cases. Among sporadic midgut carcinoids, several studies using comparative genomic hybridization or microsatellite markers have shown frequent allelic deletion of chromosome 18 (95) (96). On an epigenetic level, midgut NETs have been found to have global hypomethylation (97). There is little data about genetic aspects in NETs of the appendix or cecum. Tumor multiplicity is much less frequent in the appendix and cecum than the ileum.

The multiple endocrine neoplasia (MEN) characterized by the combined occurrence of tumors of the pituitary, pancreas, and parathyroid glands is associated with the loss of a tumor suppressor gene on chromosome 11q13 (98) (99). This is the same chromosome on which the insulin gene has been located (100). It has been linked to nesidioblastosis in certain families and parathyroid mitogenic activity can be identified in the plasma of patients with MEN-1 (101) (102). All of this suggests a genetic predisposition to tumor formation based on elaboration of a growth factor. Data from cell lineage analysis of pancreatic islet cells suggest that progenitor cells, which contain catecholamines, are present in pancreatic ducts and give rise to the glucagon and insulin cells of adult islets (103). These can be stimulated to grow by plasma from patients with MEN-1. Patients with MEN-1 also might elaborate into their plasma mitogenic circulating growth factor, involved in the initiation of GEP tumor growth (104). It has been suggested, but not proven, that allelic loses in the MEN-1 tumor suppressor gene located in the 11q13 region also might be responsible for sporadic parathyroid, pituitary, and neuroendocrine tumors of the stomach, pancreas, and intestine (105).The few cases of carcinoid tumors studied have not shown losses in this region.

In addition, MEN-2a (106) (107), MEN-2b, (108) (109) and familial medullary thyroid carcinoma are associated with mutations of the RET proto-oncogene, which is a conventional dominant oncogene located on 10q11.2. Although mutations in this region have been associated with sporadic medullary thyroid carcinoma, the role, if any, of this gene in sporadic GEP tumors is not known.

New molecular profiles of gastrointestinal (GI-NETs) and pancreatic neuroendocrine tumors (pNETs) have now been reported (110) (111). Frequent chromosomal gains occur on chromosomes 7 and 20, former also associated with metastases, together with losses on chromosomes 2, 6q, 21q, and Y in pNETs. Comparative genomic hybridization studies of GI-NETs show frequent gains on chromosomes 17 and 19, while frequent loss has been detected on chromosome 18. These findings indicate different molecular genetic background of these two tumors (112) (113). Therefore, molecular profiling of GEP-NETs demonstrates that pNET and GI-NET tumors display different genetic changes and should be considered different tumor entities; thereby, also differently managed clinically (114) (115).

Of great interest is the demonstration of the possible utility of the genomic information for treatment. In 68 pNETS (116) (117) reported mutations of genes involved in chromatin assembly were reported, as well as MEN-1 gene in > 44%, DAXX 25%, ATRX 17.6%, and MTOR pathway >14%, suggesting that these mutations may predict, for e.g., responses to the newly developed MTOR inhibitors and possibly others. More importantly these mutations have survival prediction. Mutations survive 10y while in patients without mutations > 60% died in 5 years.

# THE ROLE OF INFLAMMATORY CYTOKINES IN NETs

Several pro-inflammatory cytokines have been implicated in the development of carcinoid tumors (61-66) and may be prognostic in metastatic carcinoid (67) as well as host anti-tumor immunity (68) (69). TNF-α and IL-2 are associated with GEP-NET development (118) (119). Proinflammatory cytokines have been found in pNET tissue (120) (121) (122). IL-6 and IL-1ß may be involved in pNET development. According to SNP analyses, IL-6-174 CG and GG genotypes carriers and IL-1ß- 511/ + 3954 CTCC carriers were at risk of developing non-functional pNETs (118) (119), while IL-1-ß -511/ +3954 CTCT carriers were prone to development of functional pNETs (123) (84). Moreover, IL-6 GG genotype correlated with IL-6 serum levels that were significantly higher in patients with non-functioning pNETs. It now appears that cytokines may be important modifying factors in development, progression and prognosis of malignant tumors. In addition, many new antineoplastic drugs have been developed to target these specific genetic mutations.

# CHARACTERISTICS OF NEUROENDOCRINE CELLS

A number of peptides originally isolated from gut endocrine tissues have been shown to occur in nerves. These include gastrin, cholecystokinin, vasoactive intestinal polypeptide (VIP), and substance P (SP). As a corollary, peptides that have been found primarily in nervous tissues have now been identified in gut endocrine cells and include somatostatin, enkephalins, SP, neurotensin, and thyrotropin-releasing hormone (TRH) (124-126). Because many of these peptides occur both in endocrine cells and nerves, “endocrine” tumors of the gut may, in fact, be endocrine or neurocrine. Unique to the GEP axis is the ability of the endocrine cell to secrete a variety of peptides and amines. Hormonal peptides not only have been found within the same cell (e.g., motilin and serotonin in the enterochromaffin [EC] cell), but they have also been localized to the same secretory granule. Whether these act within the secretory granule in a paracrine manner or are co-regulated in some way is not clear. At any one point in time several hormones and amines are co-secreted and the symptom complex derives from one or more of the peptides and amines produced and cannot simply be ascribed to a single factor. Thus, a tumor may secrete one peptide, recur, and secrete yet another, and its metastases may secrete still other peptides. In the British National Supra-Regional Survey of National Health Service Hospitals, 58% of 353 patients with neuroendocrine tumors had increased serum levels of two or more hormones at diagnosis. Nine percent of patients had clinical symptoms related to different hormones, and four patients developed new symptoms from secretion of a second hormone after diagnosis (127).

Rick Lloyd has recently reviewed the use of various markers to identify neuroendocrine characteristics (128). Although there are many broad-spectrum neuroendocrine markers, chromogranin and synaptophysin are the principal ones used in diagnostic pathology. He has added to the armamentarium different keratins in the differential diagnosis and particularly the low molecular weight keratins such as CAM 5.2 to avoid false negative results in the workup of some neuroendocrine tumors. For low levels of expression he emphasizes the need for in situ hybridization to establish the presence of the message if not the protein.

The chromogranin/secreotogranin (Cg/Sg) family is composed of several acidic proteins present in secretory granules of neuroendocrine cells. The three major Cg/Sg proteins are currently designated as chromogranin (Cg) A and B and secretogranin 11 (Sg11). Others include Sg111, Sg1V and Sg V. The distribution of CgA has now been studied extensively. It is present in most neuroendocrine cells and neoplasms. A few neoplasms with only a few endocrine secretory granules such as the small cell carcinoma of the lung and Merkel cell carcinomas do not react strongly with CgA. The widespread distribution and high degree of specificity of Cg/Sg make these excellent markers for endocrine cells and their neoplasms. Cg A is endocrine specific but has limited sensitivity; for example, in hindgut carcinoids it is only positive for 25-50% of carcinoids, and adding CgB will increase the sensitivity for these tumors.

Synaptophysin, a 38kDa protein molecule is a component of the membrane presynaptic vesicles. It is widely distributed in neurons, neuroendocrine cells and their neoplasms and is a good broad spectrum neuroendocrine marker. Synaptophysin can be examined in formalin fixed tissues, which allows tumors to be revisited if initially not thought to be neuroendocrine. Although present in synaptic vesicles in tumors, it is found diffusely in the cytoplasm of the cell. It has however been found in adrenal cortical adenomas and carcinomas so, although sensitive, is not very specific. It therefore should always be used in conjunction with CgA. Synaptophysin belongs to a family of synaptic proteins that include synaptoganin (p65), SNAP-25, SNAP receptor (SNARE), Syntaxin and Rab3A. However, the utility of these proteins in routine diagnostic pathology has not been established to date.

The pro-convertases (PC) are enzymes that process pro-peptides into active peptides within cells. Some of these including PC1/PC3 and PC2 are highly specific for neuroendocrine cells and tumors and can be used as specific markers. Other such as PC4 is present in the tests whereas PC5/6 is more prevalent in the gastrointestinal tract.

Neuron Specific Enolase (NSE) is a very sensitive, but not very specific marker for neuroendocrine cells and tumors. It is commonly found in nerves, and neuroendocrine cells, but some non-neuroendocrine cells react with antisera to NSE. Therefore, NSE should only be used as a broad spectrum marker in the diagnosis of these tumors.

Bombesin, which is a tetrapeptide originally isolated from amphibian skin, is present in many endocrine cells as well as neurons. Gastrin releasing peptide (GRP), the mammalian analog of Bombesin, is found in many lung tumors and gastrointestinal endocrine tumors and can also be used as a broad-spectrum marker.

PGP-9.5 is a soluble protein that was originally isolated from the brain. It has now been shown to be a general marker for neuronal and neuroendocrine tissues. Interestingly about 50% of melanomas stain for PGP9.5 whereas these are negative for Cg/Sg.

Peptidylglycine Amidating Monooxygene (PAM): Amidation is an important step in the maturation of neuropeptides. PAM catalyzes the post-translational modification of many neuropeptides. The PAM proteins are usually released along with other peptides during exocytosis whereas membrane bound PAM remains in association with the cell. PAM expression is found in all neuroendocrine cell types (128-131). Scopsi found a close correlation between PAM expression and at least one of the three principal Cg/Sg proteins (CgA, CgB or Sg11). It is not clear that this protein provides additional information (132).

Pancreatic endocrine tumors are usually positive for cytokeratins in more than 90% of cases (133). The low molecular weight keratins such as CAM 5.2 are more sensitive for neuroendocrine tumors than the keratin cocktails such as AE1/AE3 and thus should be added to any regime that routinely examines pancreatic neoplasms to preclude misdiagnosis of an adenocarcinoma a for a neuroendocrine tumor. The latter has a much more benign prognosis.

Pulmonary tumors include typical and atypical carcinoids and large and small-cell carcinomas. About 85% are reactive with cytokeratins. Chromogranins are usually positive in carcinoids, atypical carcinoids and large-cell neuroendocrine carcinomas, while only about 50% of small cell carcinomas are positive. However other broad spectrum markers of neuroendocrine cells are positive in these tumors (134). While this may be helpful in delineating endocrine function of these tumors it is not clear that this alters treatment strategies or dictates a different prognostic value.

# CHARACTERISTICS OF NETS

Neuroendocrine tumors (NETs) are rare, slow growing neoplasms characterized by their ability to store and secrete different peptides and neuroamines (121). Some of these substances cause specific clinical syndromes (135) while others are not associated with specific syndromes or symptom complexes. There is no “ideal neuroendocrine tumor marker, (136)” but according to the presentation, the sensitivity and specificity of each marker varies and it is possible to choose those of greatest value for each clinical syndrome.

#### DEFINITION OF NETS:

* + Neuroendocrine tumors (NETs) are [neoplasms](http://en.wikipedia.org/wiki/Neoplasms) that arise from cells of the [endocrine](http://en.wikipedia.org/wiki/Endocrine) ([hormonal](http://en.wikipedia.org/wiki/Hormonal)) and [nervous systems](http://en.wikipedia.org/wiki/Nervous_system). Many are [benign](http://en.wikipedia.org/wiki/Benign), while some are [malignant](http://en.wikipedia.org/wiki/Malignant)
  + They share common features, such as looking similar, having special [secretory granules](http://en.wikipedia.org/wiki/Secretory_granule), and often producing biogenic [amines](http://en.wikipedia.org/wiki/Amines) and [polypeptide](http://en.wikipedia.org/wiki/Polypeptide) [hormones](http://en.wikipedia.org/wiki/Hormone) (121)
  + They arise from various [neuroendocrine cells](http://en.wikipedia.org/wiki/Neuroendocrine_cell) whose normal function is to serve at the  [neuroendocrine](http://en.wikipedia.org/wiki/Neuroendocrinology) interface. Neuroendocrine cells are present not only in [endocrine](http://en.wikipedia.org/wiki/Endocrine) glands throughout the body that produce [hormones](http://en.wikipedia.org/wiki/Hormone), but also diffusely in all body tissues (135).
  + [Enterochromaffin](http://en.wikipedia.org/wiki/Enterochromaffin) cells, give rise to carcinoid tumors, were identified in 1897 by [Kulchitsky](http://en.wikipedia.org/wiki/Kulchitsky) (136) and their secretion of [serotonin](http://en.wikipedia.org/wiki/Serotonin) established in 1953
  + NETs show [amine](http://en.wikipedia.org/wiki/Amine) precursor ([L-DOPA](http://en.wikipedia.org/wiki/L-DOPA) and [5-hydroxytryptophan](http://en.wikipedia.org/wiki/5-hydroxytryptophan)) uptake and decarboxylation to produce biogenic amines such as [catecholamines](http://en.wikipedia.org/wiki/Catecholamine) and [serotonin](http://en.wikipedia.org/wiki/Serotonin).

The current view of NETs has changed somewhat,

* Neuroendorine cells reside throughout the body in all tissues and can de-differentiate into tumor cells.
* NETs include:
  + - tumors of the gastrointestinal tract
    - the pancreatic [islet cells](http://en.wikipedia.org/wiki/Islet_cell) (121)
    - thymus and lung tumors
    - [medullary carcinoma](http://en.wikipedia.org/wiki/Medullary_thyroid_cancer) of the [parafollicular](http://en.wikipedia.org/wiki/Parafollicular) cells of the [thyroid](http://en.wikipedia.org/wiki/Thyroid) (121)
    - tumors in the [pituitary](http://en.wikipedia.org/wiki/Pituitary), [parathyroid](http://en.wikipedia.org/wiki/Parathyroid), and [adrenomedullary](http://en.wikipedia.org/wiki/Adrenal) glands and paraganglion cells (137)
    - NETs may be functioning or nonfunctioning but there is now a newly recognized entity of secretory tumors which are asymptomatic for a variety of reasons and may represent the largest category of NETs (138)

The annual incidence of neuroendocrine tumors (NETs) has risen to 40-50 cases per million; due to better diagnostic tools including the availability of highly specific and sensitive ways to measure tumor products and improved immunohistochemistry techniques for tumor detection. The perceived increase in incidence may be a false positive one. In a review of the SEERS database it has now been shown that the prevalence of NETS in the USA is about >100,000 cases which is twice the prevalence of gastric and pancreatic cancer combined. The great majority (56%) of these tumors are carcinoids and the remainder are pancreatic neuroendocrine (pNETS)

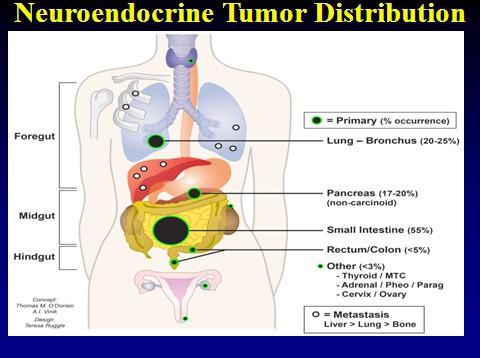
#### ANATOMIC DISTRIBUTION

More than 50% of neuroendocrine tumors in clinical practice are of the so-called carcinoid variety and are found incidentally at operation, after metastasis has occurred, in the small intestine (especially the appendix). The remaining fraction comprises approximately 50% gastrinomas, 30% insulinomas, 13% VIPomas, 5 to 10% glucagonomas, and, rarely, less than 5% neurotensinomas, somatostatinomas, and ectopic hormone-secreting tumors. Nonsecretory tumors were thought to make up the bulk of pancreatic tumors. However, with better immunohistochemical stains for endocrine cells, especially for neuron-specific enclose (NSE), chromogranin, synaptophysin, and receptors for somatostatin (139) there is increasing recognition that tumors masquerading as carcinomas of liver, small cell carcinoma of the lung, and others, are in reality neuroendocrine tumors. Most of these nonsecretory tumors actually store and secrete pancreatic polypeptide (PP), but because it has so little, if any, in the way of biologic activity, the tumor often remains silent until it is quite large.

Approximately 60% of pancreatic gastrinomas are concentrated in Pasarro’s Triangle, an area subtended by the head of pancreas, gastric antrum, and first portion of the duodenum. Other neuroendocrine tumors may be distributed more evenly across the pancreas or in ectopic sites such as the adrenal medulla, whereas carcinoid tumors most frequently occur in the appendix and small intestine.

The tumors are proliferative in nature and may take the form of hyperplasia or neoplasia (adenoma, adenomatous hyperplasia, microadenomatosis, nesidioblastosis, or carcinoma). Hyperplasia is relatively uncommon in benign sporadic tumors, but it is the rule in MEN-1 syndrome and often is present in the area of the pancreas surrounding a benign tumor.

Figure 4.



The tumors may be further subdivided into (a) orthoendocrine, when they secrete the normal product of the cell type (e.g., alpha cell glucagon), and (b) paraendocrine, when they secrete a peptide or amine that is foreign to the organ or cell of origin. Paraendocrine tumors are found in the adrenal medulla, kidney, lymph nodes, or liver and as a part of MEN-1 when a variety of peptides or amines are secreted. When tumors metastasize, they do so to local lymph nodes, liver, peritoneum, and, rarely, to bone, but this seems to be increasing in frequency as the natural history of these tumors changes with aggressive treatment. Metastases are notoriously highly vascular, which is a telltale sign of a GEP tumor. The occurrence of MEN-1 syndrome may be as frequent as one-third of the cases of GEP tumors, depending on the endemic area. In high-risk areas, measurements of ionized calcium, prolactin, and PP are important. Nonetheless these tumors are rare and slow growing. As Moertel once said, the study of neuroendocrine tumors of the gut is like an Odyssey in the land of slow growing tumors. Their characteristics are shown in Table 1.

#### Table 1. Characteristics of Neuroendocrine Tumors

* Rare
* Usually small, <1 cm
* Slow growing, months to years, “cancer in slow motion”
* Usually metastasize before becoming symptomatic, often when tumor is >2 cm
* Expression is episodic, may be silent for years
* Symptoms mimic commonplace conditions and often are misdiagnosed
* Complex diagnosis, rarely made clinically, requiring sophisticated laboratory and

scanning techniques

Table 2 lists the common clinical syndrome, the tumor types, the sites and the hormones or peptides/amines that are produced. The sections that follow focus on the specific syndromes that are ascribed to GEP hyperfunction.

#### Table 2. The Clinical Presentations, Syndromes, Tumor Types, Sites and Hormones (139).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Clinical Presentation** | **Syndrome** | **Tumor Type** | **Sites** | **Hormones** |
| Flushing | Carcinoid  Medullary Carcinoma of Thyroid  Pheochromocytoma | Carcinoid C cell tumor  Tumor of Chromaffin cells | Mid/ foregut Adrenal medulla Gastric  Thyroid C cells  Adrenal and Sympathetic Nervous system | Serotonin GCRP  Calcitonin  Metanephrine and Normetanephrine |
| Diarrhea, abdominal pain and dyspepsia | Carcinoid, WDHHA, ZE, PP, MCT | Carcinoid, VIPoma, Gastrinoma, PPoma, Medullary carcinoma thyroid, mastocytoma | As above Pancreas, mast cells,  Thyroid | As above, VIP, gastrin, PP, calcitonin |
| Diarrhea/ Steatorrhea | Somatostatin Bleeding GI tract | Somatostatinoma, neurofibromatosis | Pancreas Duodenum | Somatostatin |
| Wheezing | Carcinoid | Carcinoid | Gut/pancreas/lung | SP, CGRP,  serotonin |
| Ulcer /dyspepsia | Zollinger Ellison, | Gastrinoma | Pancreas/ Duodenum | Gastrin |
| Hypoglycemia | Whipple’s triad | Insulinoma, sarcoma, hepatoma | Pancreas, retroperitoneum Liver | Insulin, IGF1, IGF2. |
| Dermatitis | Sweet Syndrome Pellagra | Glucagonoma Carcinoid | Pancreas Midgut | Glucagon Serotonin |
| Dementia | Sweet syndrome | Glucagonoma | Pancreas | Glucagon |
| Diabetes | Glucagonoma Somatostatin | Glucagonoma Somatostatinoma | Pancreas Pancreas | Glucagon Somatostatin |
| DVT, Steatorrhea, Cholelithiasis Neurofibromatosis | Somatostatin | Somatostatinoma | Pancreas Duodenum | Somatostatin |
| Silent, liver metastases | Silent | PPOMA | Pancreas | PP |

Table 2 summarizes the approach to diagnose a NET based upon the clinical presentation, the tumor type, their sites of origin and the possible means of diagnosis and the biochemical markers that should be measured. Abbreviations: CGRP: Calcitonin gene-related peptide.

**Table 3. Clinical presentation, syndrome, tumor type, sites and the hormones produced.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Clinical Presentation** | **Syndrome** | **Tumor Type** | **Sites** | **Hormones** |
| Acromegaly | Acromegal, Gigantism | NET, PNET,  Pheo | Pancreas islet | GHRH |
| Cushings | Cushings | NET, PNET,  Pheo | Pancreas islet, LUNG, PHEO, MTC | CRH, ACTH |
| Pigmentation | Pigmentation | NET | Pancreas islet | MSH |
| Anorexia, nausea, vomiting, abdominal pain | Hypercalcemia | NET, PNET,  Pheo | Pancreas islet, Pheo | PthRP, Pth,TGFb, IL, 25 0HD, 1,25 OHD – bone alk phos, NTx b = β |
| Hypoglycemia | Autonomic and CNS symptoms of hypoglycemia | NET, PNET | Pancreas Carcinoid | IGF, IGF2 and proIGF, GLP-1, GLP-2 |
| Weakness, lethargy, apathy | Hyponatremia, SIADH | NET, PNET,  Pheo | Lung, pancreas, pheo | ADH, ANP |
| Hyperandrogenism, gynecomastia, hyperthyroidism |  | PET | Pancreas | LH, FSH,  Prolactin, TSH |
| Hypertension | Malignant hypertension | PET, Pheo, Paraganglioma | Paraganglioma, NET | Renin, pro-renin, aldosterone |

Abbreviations:

PET = pancreatic NET

NET = neuroendocrine tumor

SIADH = syndrome of inappropriate secretion of antidiuretic hormone

Pheo = pheochromocytoma

MTC = medullary thyroid carcinoma

GHRH = growth hormone releasing hormone

CRH = corticotrophin releasing hormone

ACTH = adrenocorticotrophin

PthRP = parathyroid hormone related peptide

25 OHD = 25 hydroxy vitamin D

1,25 OHD = 1, 25 dihydroxy vitamin D

IGF = insulin like growth factor

ADH = antidiuretic hormone

ANP = atrial naturetic peptide

Alks phos = alkaline phosphatase

NTx = N telopeptide,

TSH = Thyrotropic stimulating hormone

LH – luteinizing hormone

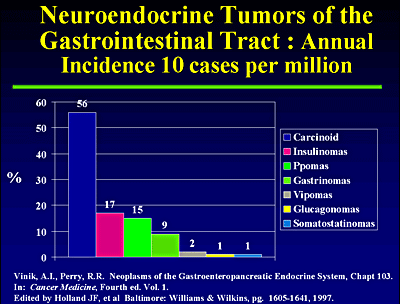
FSH = follicle stimulating hormone

**Table 4. Specific Biochemical Markers for each Tumor Type (140)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Site** | **Tumor Type** | **Marker** | **Specificity** |
| All |  | CgA and B  PP, NSE, Neurokinin, Neurotensin  HCG α and ß | High Intermediate Low |
| Thymus | Foregut Carcinoid | ACTH | Intermediate |
| Bronchus | Foregut Carcinoid, Small Cell Lung Carcinoma. | ACTH, ADH, Serotonin, 5- HIAA, Histamine, GRP, GHRH, VIP, PTHrp | Intermediate  Low |
| Stomach | Foregut Carcinoid, Gastrinoma, Ghrelinoma. | Histamine, Gastrin Ghrelin | Intermediate Low |
| Pancreas | Gastrinoma, Insulinoma, Glucagonoma, Somatostatinoma, PPoma, VIPoma. | Gastrin, Insulin, Proinsulin, Glucagon, Somatostatin  C-peptide, Neurotensin, VIP, PTHrp, Calcitonin | High Low |
| Duodenum | Gastrinoma, Somatostatinoma. | Somatostatin, Gastrin | High |
| Ileum | Midgut Carcinoid | Serotonin, 5-HIAA Neurokinin A, Neuropeptide K, Substance P | High Intermediate |
| Colon and Rectum | Hindgut Carcinoid | Peptide YY, Somatostatin | Intermediate |
| Bone | Metastasis | Bone Alkaline Phosphatase, N- Telopeptide  PTHrp | High (blastic lesions), Modest (lytic lesions)  Intermediate |
| Cardiac Involvement | Carcinoid | BNP | Intermediate |

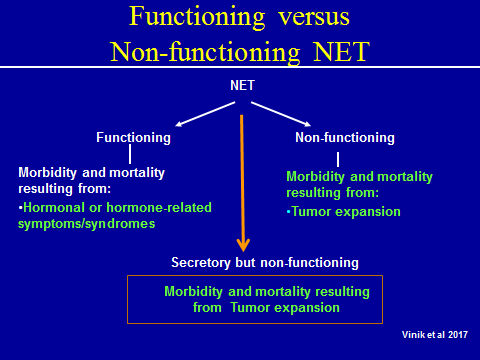
Table 4 shows the specific biochemical markers used for each tumor and their specificity. CgA and B: Chromagranin A and B; PP: pancreatic polypeptide; NSE: neuron-specific Enolase; HCG: human chorionic gonadotropin; ACTH: adrenocorticotropic hormone; ADH: anti diuretic hormone; 5-HIAA: 5 hydroxyindoleacetic acid; GRP: gastrin releasing peptide; GHRH: growth hormone releasing hormone; VIP: vasointestinal peptide; PTHrp: parathyroid hormone related peptide; BNP: brain natriuretic peptide

**Figure 5.**



The great majority of the symptomatic tumors are carcinoid tumors accounting for more than half of those presenting each year (Figure 5). Insulinomas, gastrinomas and PPomas account for 17,15 and 9%, while the remainder are around the 1% mark. These tumors are what is known in common parlance as “Zebras” because of their rarity, but physicians are fascinated by their complexity and the unusual nature of their presentations. For the most part the endocrinologist makes his living not by diagnosing one of these and treating it, but by excluding conditions that masquerade as a neuroendocrine tumor. For this reason it is probably more appropriate to consider the clinical presentations rather than the tumor types. By far the most frequent clinical manifestations found in practice are flushing and diarrhea (Table 3), which are the cardinal presentations of the most common tumor syndrome, carcinoid, and this will therefore be discussed first in the next Chapter. However, a new era is dawning wherein it has become increasingly recognized that tumors may be secretory but do not produce a clinical syndrome as indicated in Figure 6.

**Figure 6.**



There have been major strides in the therapeutic options for patients with NETS. Studies that have come to fruition in the last decade include the CLARINET trial which evaluated lanreotide on tumor progression free survival in patients with non-functioning NETs; the ELECT trial (140) which showed that lanreotide was capable of controlling the major symptoms of flushing and diarrhea; The RADIANT-2 and RADIANT-4 studies which evaluated the MTOR inhibitor Everolimus in functioning and non-functioning =NETS of the gastrointestinal tract and lungs alone and in combination with somatostatin, the Telestar study which evaluated telotristat ethyl for control of symptoms of flushing and diarrhea by blocking serotonin synthesis and the tyrosine kinase inhibitor Sunitinib on secretory midgut NETS which demonstrated improvement in PFS (141) (142) in addition to improving quality of life (142) and most recently the 177 Lu-DOTOTATE in NETs of the small intestine and proximal colon (midgut) showing remarkable impact on PFS and to some extent overall survival (143). Perhaps as shown by Vinik and colleagues (144) quality of life was improved and the improvement was shown to be dependent on a change in the bulk of the tumor, progression free survival and the biomarkers secreted. In the earlier Vinik studies (144) the surprising findings were the intimate link of quality of life on the secretory product, the tumor bulk and the peptide or amine secreted. These remarkable advances amongst others have instigated NANETS to develop a Consensus statement on the current recommendations for the management of NETs (see the position statement and consensus guidelines) (145). In the ensuing pages we will entertain the reader to the many advances that have occurred in the neuroendocrine tumor world particularly the new syndromes, recognition of the importance an value of biomarkers in diagnosis, prediction of tumor behavior and the ramifications for patient survival and quality of life and mortality and welcome addition of new biologic agents, better and more powerful means of tumor identification and peptide targeted therapies including the scope for peptide radioactive receptor targeting (PRRT).

**REFERENCES**

1. Pearse AG. Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C cells and calcitonin. Proc R Soc Lond B Biol Sci 1968; 170(18):71-80.

2. Andrew A. An experimental investigation into the possible neural crest origin of pancreatic APUD (islet) cells. J Embryol Exp Morphol 1976; 35(3):577-593.

3. Le Douarin NM, Teillet MA. The migration of neural crest cells to the wall of the digestive tract in avian embryo. J Embryol Exp Morphol 30, 31. 1973.

4. Pictet RL, Rutter WJ. Development of the embryonic endocrine pancreas. In: Steiner DF, Freinkel N, editors. Handbook of Physiology, Section 7: endocrinology, vol. 1. Washington,D.C: American Physiological Society, 1972: 25-66.

5. Guz Y, Nasir I, Teitelman G. Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes. Endocrinology 2001; 142(11):4956-4968.

6. Pang K, Mukonoweshuro C, Wong GG. Beta cells arise from glucose transporter type 2 (Glut2)-expressing epithelial cells of the developing rat pancreas. Proc Natl Acad Sci U S A 1994; 91(20):9559-9563.

7. Ramiya VK, Maraist M, Arfors KE, Schatz DA, Peck AB, Cornelius JG. Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. Nat Med 2000; 6(3):278-282.

8. Grapin-Botton A, Melton DA. Endoderm development: from patterning to organogenesis. Trends Genet 2000; 16(3):124-130.

9. Peers B, Leonard J, Sharma S, Teitelman G, Montminy MR. Insulin expression in pancreatic islet cells relies on cooperative interactions between the helix loop helix factor E47 and the homeobox factor STF-1. Mol Endocrinol 1994; 8(12):1798-1806.

10. Peers B, Sharma S, Johnson T, Kamps M, Montminy M. The pancreatic islet factor STF-1 binds cooperatively with Pbx to a regulatory element in the somatostatin promoter: importance of the FPWMK motif and of the homeodomain. Mol Cell Biol 1995; 15(12):7091-7097.

11. Serup P, Petersen HV, Pedersen EE et al. The homeodomain protein IPF-1/STF-1 is expressed in a subset of islet cells and promotes rat insulin 1 gene expression dependent on an intact E1 helix-loop-helix factor binding site. Biochem J 1995; 310 ( Pt 3):997-1003.

12. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H. beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes Dev 1998; 12(12):1763-1768.

13. Guz Y, Montminy MR, Stein R et al. Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. Development 1995; 121(1):11-18.

14. Wang H, Maechler P, Ritz-Laser B et al. Pdx1 level defines pancreatic gene expression pattern and cell lineage differentiation. J Biol Chem 2001; 276(27):25279-25286.

15. Offield MF, Jetton TL, Labosky PA et al. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 1996; 122(3):983-995.

16. Stoffers HEJH, Kester ADM, Kaiser V, Rinkens PELM, Knottnerus JA. Diagnostic value of signs and symptoms associated with peripheral arterial occlusive disease seen in general practice: a multivariable approach. Med Decis Making 1997; 17(1):61-70.

17. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet 1997; 15(1):106-110.

18. Thomas MK, Devon ON, Lee JH et al. Development of diabetes mellitus in aging transgenic mice following suppression of pancreatic homeoprotein IDX-1. J Clin Invest 2001; 108(2):319-329.

19. Edlund H. Transcribing pancreas. Diabetes 1998; 47(12):1817-1823.

20. Jamal A, Lipsett M, Hazrati A, et al. INGAP peptide induces in vitro islet cell neogenesis from human duct-like progenitor cells. Endocrine Society 2002.

21. Sharma A, Zangen DH, Reitz P et al. The homeodomain protein IDX-1 increases after an early burst of proliferation during pancreatic regeneration. Diabetes 1999; 48(3):507-513.

22. Song SY, Gannon M, Washington MK et al. Expansion of Pdx1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor alpha. Gastroenterology 1999; 117(6):1416-1426.

23. Kritzik MR, Jones E, Chen Z et al. PDX-1 and Msx-2 expression in the regenerating and developing pancreas. J Endocrinol 1999; 163(3):523-530.

24. Pittenger GL, Malik RA, Burcus N, Boulton AJ, Vinik AI. Specific fiber deficits in sensorimotor diabetic polyneuropathy correspond to cytotoxicity against neuroblastoma cells of sera from patients with diabetes. Diabetes Care 1999; 22(11):1839-1844.

25. Taylor-Fishwick DA, Shi W, Pittenger GL, Vinik AI. PDX-1 can repress stimulus-induced activation of the INGAP promoter. J Endocrinol 2006; 188(3):611-621.

26. McCarthy BG, Hsieh ST, Stocks A et al. Cutaneous innervation in sensory neuropathies: evaluation by skin biopsy. Neurology 1995; 45:1848-1855.

27. McArthur JC, Stocks EA, Hauer P, Cornblath DR, Griffin JW. Epidermal nerve fiber density: normative reference range and diagnostic efficiency. Arch Neurol 1998; 55(12):1513-1520.

28. Basta B, Pittenger G, Ray M, Stansberry K, Vinik A. Intra-Epidermal Nerve Fiber Density (IENF) a New Marker for Small Fiber Neuropathy (SFNP) in Diabetes. Diabetes 2002; 51(Supplement 2).

29. Thompson NW, Pasieka J, Fukuuchi A. Duodenal gastrinomas, duodenotomy, and duodenal exploration in the surgical management of Zollinger-Ellison syndrome. World J Surg 1993; 17(4):455-462.

30. Pittenger GL, Fishwick-Taylor D, Kosuri S, Burcus N, Rosenberg L, Vinik A. INGAP peptide - an initiator of neuroendocrine cell (PGP9.5) development and islet transdifferentiation/transcription factor (Pdx-1) expression in normal dog pancreas. Endocrine Society 2002.

31. Shih DQ, Heimesaat M, Kuwajima S, Stein R, Wright CV, Stoffel M. Profound defects in pancreatic beta-cell function in mice with combined heterozygous mutations in Pdx-1, Hnf-1alpha, and Hnf-3beta. Proc Natl Acad Sci U S A 2002; 99(6):3818-3823.

32. Lee VM, Stoffel M. Bone marrow: an extra-pancreatic hideout for the elusive pancreatic stem cell? J Clin Invest 2003; 111(6):799-801.

33. Ben-Shushan E, Marshak S, Shoshkes M, Cerasi E, Melloul D. A pancreatic beta -cell-specific enhancer in the human PDX-1 gene is regulated by hepatocyte nuclear factor 3beta (HNF-3beta), HNF-1alpha, and SPs transcription factors. J Biol Chem 2001; 276(20):17533-17540.

34. Edlund H. Developmental biology of the pancreas. Diabetes 2001; 50 Suppl 1:S5-S9.

35. Sander M, German MS. The beta cell transcription factors and development of the pancreas. J Mol Med (Berl) 1997; 75(5):327-340.

36. St-Ogne L, Wehr R, Gruss P. Pancreas development and diabetes. Curr Opin Genet 1999; 9:295-300.

37. Kim SK, Hebrok M. Intercellular signals regulating pancreas development and function. Genes Dev 2001; 15(2):111-127.

38. Deutsch G, Jung J, Zheng M, Lora J, Zaret KS. A bipotential precursor population for pancreas and liver within the embryonic endoderm. Development 2001; 128(6):871-881.

39. Ahlgren U, Pfaff SL, Jessell TM, Edlund T, Edlund H. Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. Nature 1997; 385(6613):257-260.

40. Smith SB, Gasa R, Watada H, Wang J, Griffen SC, German MS. Neurogenin3 and hepatic nuclear factor 1 cooperate in activating pancreatic expression of Pax4. J Biol Chem 2003; 278(40):38254-38259.

41. Rooman I, Heremans Y, Heimberg H, Bouwens L. Modulation of rat pancreatic acinoductal transdifferentiation and expression of PDX-1 in vitro. Diabetologia 2000; 43(7):907-914.

42. Vinik A, Rafaeloff R, Pittenger G, Rosenberg L, Duguid W. Induction of pancreatic islet neogenesis. Horm Metab Res 1997; 29(6):278-293.

43. Shih DQ, Screenan S, Munoz KN et al. Loss of HNF-1alpha function in mice leads to abnormal expression of genes involved in pancreatic islet development and metabolism. Diabetes 2001; 50(11):2472-2480.

44. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 2001; 292(5520):1389-1394.

45. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. Diabetes 2001; 50(8):1691-1697.

46. Soria B, Roche E, Berna G, Leon-Quinto T, Reig JA, Martin F. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes 2000; 49(2):157-162.

47. Beattie GM, Itkin-Ansari P, Cirulli V et al. Sustained proliferation of PDX-1+ cells derived from human islets. Diabetes 1999; 48(5):1013-1019.

48. Cheung AT, Dayanandan B, Lewis JT et al. Glucose-dependent insulin release from genetically engineered K cells. Science 2000; 290(5498):1959-1962.

49. Bonner-Weir S, Taneja M, Weir GC et al. In vitro cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci U S A 2000; 97(14):7999-8004.

50. Itkin-Ansari P, Demeterco C, Bossie S et al. PDX-1 and cell-cell contact act in synergy to promote delta-cell development in a human pancreatic endocrine precursor cell line. Mol Endocrinol 2000; 14(6):814-822.

51. de la Tour D, Halvorsen T, Demeterco C et al. Beta-cell differentiation from a human pancreatic cell line in vitro and in vivo. Mol Endocrinol 2001; 15(3):476-483.

52. Vinik A, Pittenger G, Rafaeloff R, Rosenberg L. Factors controlling pancreatic islet neogenesis. Tumour Biol 1993; 14(3):184-200.

53. Bertelli E, Regoli M, Orazioli D, Bendayan M. Association between islets of Langerhans and pancreatic ductal system in adult rat. Where endocrine and exocrine meet together? Diabetologia 2001; 44(5):575-584.

54. Fiddian-Green RG, Pittenger G, Kothary P, Vinik AI. Role of calcium in the stimulus-secretion coupling of antral gastrin release. Endocrinology 1983; 112:753-760.

55. Bendayan M. Anatomic basis oc islet-acinar interaction in the pancreas. Regulatory Peptide Letter 1990; 2:8.

56. Creutzfeldt W, Folsch UR, Stockmann F. Clinical implications of islet-acinar interactions. Regulatory Peptide Letter 1990; 2:8.

57. Conlon JM, Rouiller D, Boden G, Unger RH. Characterization of immunoreactive components of insulin and somatostatin in canine pancreatic juice. FEBS Lett 1979; 105(1):23-26.

58. Ertan A, Taminato T, Akdamar K et al. Immunoreactive somatostatin in human pancreatic secretion. J Clin Endocrinol Metab 1981; 52(3):589-591.

59. Sarfati PD, Green GM, Brazeau P, Morisset J. Presence of somatostatin-like immunoreactivity in rat pancreatic juice: a physiological phenomenon. Can J Physiol Pharmacol 1986; 64(5):539-544.

60. Buts JP, De KN, Marandi S et al. Expression of insulin receptors and of 60-kDa receptor substrate in rat mature and immature enterocytes. Am J Physiol 1997; 273(1 Pt 1):G217-G226.

61. Gingerich RL, Gilbert WR, Comens PG, Gavin JR, III. Identification and characterization of insulin receptors in basolateral membranes of dog intestinal mucosa. Diabetes 1987; 36(10):1124-1129.

62. Pillion DJ, Ganapathy V, Leibach FH. Identification of insulin receptors on the mucosal surface of colon epithelial cells. J Biol Chem 1985; 260(9):5244-5247.

63. Miyaura C, Chen L, Appel M et al. Expression of reg/PSP, a pancreatic exocrine gene: relationship to changes in islet beta-cell mass. Mol Endocrinol 1991; 5:226-234.

64. Ohno T, Ishii C, Kato N et al. Increased expression of a regenerating (reg) gene protein in neonatal rat pancreas treated with streptozotocin. Endocr J 1995; 42(5):649-653.

65. Rafaeloff R, Barlow SW, Rosenberg L, Vinik AI. Expression of Reg gene in the Syrian golden hamster pancreatic islet regeneration model. Diabetologia 1995; 38(8):906-913.

66. Zenilman ME, Chen J, Danesh B, Zheng QH. Characteristics of rat pancreatic regenerating protein. Surgery 1998; 124(5):855-863.

67. Sanchez D, Baeza N, Blouin R et al. Overexpression of the reg gene in non-obese diabetic mouse pancreas during active diabetogenesis is restricted to exocrine tissue. J Histochem Cytochem 2000; 48(10):1401-1410.

68. Anastasi E, Ponte E, Gradini R et al. Expression of Reg and cytokeratin 20 during ductal cell differentiation and proliferation in a mouse model of autoimmune diabetes. Eur J Endocrinol 1999; 141(6):644-652.

69. Abe M, Nata K, Akiyama T et al. Identification of a novel Reg family gene, Reg IIIdelta, and mapping of all three types of Reg family gene in a 75 kilobase mouse genomic region. Gene 2000; 246(1-2):111-122.

70. Rafaeloff R, Pittenger GL, Barlow SW et al. Cloning and sequencing of the pancreatic islet neogenesis associated protein (INGAP) gene and its expression in islet neogenesis in hamsters. J Clin Invest 1997; 99(9):2100-2109.

71. Sasahara K, Yamaoka T, Moritani M, Yoshimoto K, Kuroda Y, Itakura M. Molecular cloning and tissue-specific expression of a new member of the regenerating protein family, islet neogenesis-associated protein-related protein. Biochim Biophys Acta 2000; 1500(1):142-146.

72. Roman CL, Maiztegui B, Del ZH, Gagliardino JJ, Flores LE. INGAP-PP effects on beta-cell mass and function are related to its positive effect on islet angiogenesis and VEGFA production. Mol Cell Endocrinol 2017.

73. Strobel O, Rosow DE, Rakhlin EY et al. Pancreatic duct glands are distinct ductal compartments that react to chronic injury and mediate Shh-induced metaplasia. Gastroenterology 2010; 138(3):1166-1177.

74. Rosenberg L, Vinik AI. Regulation of pancreatic islet growth and differentiation - Evidence for paracrine and/or autocrine growth factor(s). Clin Res 1990; 38:271A.

75. Butler AE, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC. Marked Expansion of Exocrine and Endocrine Pancreas with Incretin Therapy in Humans with increased Exocrine Pancreas Dysplasia and the potential for Glucagon-producing Neuroendocrine Tumors. Diabetes 2013; 62(7):2595-2604.

76. Butler PC, Elashoff M, Elashoff R, Gale EA. A Critical Analysis of the Clinical Use of Incretin-Based Therapies: Are the GLP-1 therapies safe? Diabetes Care 2013; 36(7):2118-2125.

77. Butler PC, Dry S, Elashoff R. GLP-1-based therapy for diabetes: what you do not know can hurt you. Diabetes Care 2010; 33(2):453-455.

78. Brockenbrough JS, Weir GC, Bonner-Weir S. Discordance of exocrine and endocrine growth after 90% pancreatectomy in rats. Diabetes 1988; 37:232-236.

79. Pour P, Mohr U, Cardesa A, Althoff J, Kruger FW. Pancreatic neoplasms in an animal model: morphological, biological, and comparative studies. Cancer 1975; 36:379-389.

80. Rosenberg L, Duguid WP, Brown RA, Vinik AI. Induction of nesidioblastosis will reverse diabetes in Syrian golden hamster. Diabetes 1988; 37(3):334-341.

81. Rosenberg L, Duguid WP, Vinik AI. Cell proliferation in the pancreas of the Syrian golden hamster. Dig Dis Sci 1987; 32:1185.

82. Sarvetnick N. Islet cell destruction and regeneration in IFN-gamma transgenic mice. J Cell Biochem 1991; CB019:49.

83. Smith DB, Scarffe JH, Wagstaff J, Johnston RJ. Phase II trial of rDNA alfa 2b interferon in patients with malignant carcinoid tumor. Cancer Treat Rep 1987; 71(12):1265-1266.

84. Takasawa S, Yamamoto H, Terazono K, Okamoto H. Novel gene activated in rat insulinomas. Diabetes 1986; 35:1178-1180.

85. Terazono K, Uchiyama Y, Ide M et al. Expression of reg protein in rat regenerating islets and its co- localization with insulin in the beta cell secretory granules. Diabetologia 1990; 33:250-252.

86. Terazono K, Yamamoto H, Takasawa S et al. A novel gene activated in regenerating islets. J Biol Chem 1988; 263:2111-2114.

87. Watanabe T, Yonekura H, Terazono K, Yamamoto H, Okamoto H. Complete nucleotide sequence of human reg gene and its expression in normal and tumoral tissues. The reg protein, pancreatic stone protein, and pancreatic thread protein are one and the same product of the gene. J Biol Chem 1990; 265:7432-7439.

88. Vinik AI, Pittenger GL, Pavlic-Renar I. Role of growth factors in pancreatic endocrine cells. Growth and differentiation. Endocrinol Metab Clin North Am 1993; 22(4):875-887.

89. Ahlman H, Wangberg B, Nilsson O. Growth regulation in carcinoid tumors. Endocrinol Metab Clin North Am 1993; 22(4):889-915.

90. Chaudhry A, Funa K, Oberg K. Expression of growth factor peptides and their receptors in neuroendocrine tumors of the digestive system. Acta Oncol 1993; 32(2):107-114.

91. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26(4):239-257.

92. Perry RR, Kang Y, Greaves B. Effects of tamoxifen on growth and apoptosis of estrogen-dependent and -independent human breast cancer cells. Ann Surg Oncol 1995; 2(3):238-245.

93. Rotello RJ, Lieberman RC, Purchio AF, Gerschenson LE. Coordinated regulation of apoptosis and cell proliferation by transforming growth factor beta 1 in cultured uterine epithelial cells. Proc Natl Acad Sci U S A 1991; 88(8):3412-3415.

94. Szende B, Zalatnai A, Schally AV. Programmed cell death (apoptosis) in pancreatic cancers of hamsters after treatment with analogs of both luteinizing hormone-releasing hormone and somatostatin. Proc Natl Acad Sci U S A 1989; 86(5):1643-1647.

95. Wang GG, Yao JC, Worah S et al. Comparison of genetic alterations in neuroendocrine tumors: frequent loss of chromosome 18 in ileal carcinoid tumors. Mod Pathol 2005; 18(8):1079-1087.

96. Kytola S, Hoog A, Nord B et al. Comparative genomic hybridization identifies loss of 18q22-qter as an early and specific event in tumorigenesis of midgut carcinoids. Am J Pathol 2001; 158(5):1803-1808.

97. Choi IS, Estecio MR, Nagano Y et al. Hypomethylation of LINE-1 and Alu in well-differentiated neuroendocrine tumors (pancreatic endocrine tumors and carcinoid tumors). Mod Pathol 2007; 20(7):802-810.

98. Larsson C, Skogseid B, Oberg K, Nakamura Y, Nordenskjold M. Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. Nature 1988; 332(6159):85-87.

99. Thakker RV, Bouloux P, Wooding C et al. Association of parathyroid tumors in multiple endocrine neoplasia type 1 with loss of alleles on chromosome 11. N Engl J Med 1989; 321(4):218-224.

100. Brandi ML, Aurbach GD, Fitzpatrick LA et al. Parathyroid mitogenic activity in plasma from patients with familial multiple endocrine neoplasia type 1. N Engl J Med 1986; 314(20):1287-1293.

101. Marx SJ, Vinik AI, Santen RJ, Floyd JC, Jr., Mills JL, Green J. Multiple endocrine neoplasia type I: assessment of laboratory tests to screen for the gene in a large kindred. Medicine (Baltimore) 1986; 65:226-241.

102. Owerbach D, Bell GI, Rutter WJ, Brown JA, Shows TB. The insulin gene is located on the short arm of chromosome 11 in humans. Diabetes 1981; 30(3):267-270.

103. Eisenbarth GS, Shimizu K, Bowring MA, Wells S. Expression of receptors for tetanus toxin and monoclonal antibody A2B5 by pancreatic islet cells. Proc Natl Acad Sci U S A 1982; 79:5066-5070.

104. McLeod M, Fukuuchi A, Warnock M, Tuteta A, Vinik A. Mechanisms of stimulatory and inhibitory effects of somatostatin on cell proliferation in rat insulinoma (RIN m5F). Cell Line . 1991.

105. Larsson C, Weber G, Teh BT, Lagercrantz J. Genetics of multiple endocrine neoplasia type 1. Ann N Y Acad Sci 1994; 733:453-463.

106. Mozell EJ, Cramer AJ, O'Dorisio TM, Woltering EA. Long-term efficacy of octreotide in the treatment of Zollinger-Ellison syndrome. Arch Surg 1992; 127(9):1019-1024.

107. Donis-Keller H, Dou S, Chi D et al. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 1993; 2(7):851-856.

108. Carlson KM, Dou S, Chi D et al. Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. Proc Natl Acad Sci U S A 1994; 91(4):1579-1583.

109. Hofstra RM, Landsvater RM, Ceccherini I et al. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 1994; 367(6461):375-376.

110. Perren A, Anlauf M, Komminoth P. Molecular profiles of gastroenteropancreatic endocrine tumors. Virchows Arch 2007; 451 Suppl 1:S39-S46.

111. Duerr EM, Mizukami Y, Ng A et al. Defining molecular classifications and targets in gastroenteropancreatic neuroendocrine tumors through DNA microarray analysis. Endocr Relat Cancer 2008; 15(1):243-256.

112. Zikusoka MN, Kidd M, Eick G, Latich I, Modlin IM. The molecular genetics of gastroenteropancreatic neuroendocrine tumors. Cancer 2005; 104(11):2292-2309.

113. Arnold R. Diagnosis and differential diagnosis of hypergastrinemia. Wien Klin Wochenschr 2007; 119(19-20):564-569.

114. Oberg K, Jelic S. Neuroendocrine gastroenteropancreatic tumors: ESMO clinical recommendation for diagnosis, treatment and follow-up. Ann Oncol 2009; 20 Suppl 4:150-153.

115. Oberg K. The genetics of neuroendocrine tumors. Semin Oncol 2013; 40(1):37-44.

116. Jiao Y, Shi C, Edil BH et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science 2011; 331(6021):1199-1203.

117. Elsasser SJ, Allis CD, Lewis PW. Cancer. New epigenetic drivers of cancers. Science 2011; 331(6021):1145-1146.

118. Berkovic MC, Jokic M, Marout J, Radosevic S, Zjacic-Rotkvic V, Kapitanovic S. IL-2 -330 T/G SNP and serum values-potential new tumor markers in neuroendocrine tumors of the gastrointestinal tract and pancreas (GEP-NETs). J Mol Med (Berl) 2010; 88(4):423-429.

119. Berkovic MC, Jokic M, Marout J, Radosevic S, Zjacic-Rotkvic V, Kapitanovic S. IL-6-174 C/G polymorphism in the gastroenteropancreatic neuroendocrine tumors (GEP-NETs). Exp Mol Pathol 2007; 83(3):474-479.

120. Chan AO, Kim SG, Bedeir A, Issa JP, Hamilton SR, Rashid A. CpG island methylation in carcinoid and pancreatic endocrine tumors. Oncogene 2003; 22(6):924-934.

121. Massironi S, Sciola V, Peracchi M, Ciafardini C, Spampatti MP, Conte D. Neuroendocrine tumors of the gastro-entero-pancreatic system. World J Gastroenterol 2008; 14(35):5377-5384.

122. Franko J, Feng W, Yip L, Genovese E, Moser AJ. Non-functional neuroendocrine carcinoma of the pancreas: incidence, tumor biology, and outcomes in 2,158 patients. J Gastrointest Surg 2010; 14(3):541-548.

123. Cigrovski BM, Catela IT, Marout J, Zjacic-Rotkvic V, Kapitanovic S. Interleukin 1beta gene single-nucleotide polymorphisms and susceptibility to pancreatic neuroendocrine tumors. DNA Cell Biol 2012; 31(4):531-536.

124. Bissette G, Manberg P, Nemeroff CB, Prange AJ, Jr. Neurotensin, a biologically active peptide. Life Sci 1978; 23(22):2173-2182.

125. Blackburn AM, Bryant MG, Adrian TE, Bloom SR. Pancreatic tumours produce neurotensin. J Clin Endocrinol Metab 1981; 52(4):820-822.

126. Sundler F, Alumets J, Hakanson R. Peptides in the gut with dual distribution in nerves and endocrine cells. In: Bloom SR, editor. In Gut Hormones. Churchill Livingston, 1978.

127. Wynick D, Williams SJ, Bloom SR. Symptomatic secondary hormone syndromes in patients with established malignant pancreatic endocrine tumors. N Engl J Med 1988; 319(10):605-607.

128. Lloyd RV. Practical markers used in the diagnosis of neuroendocrine tumors. Endocr Pathol 2003; 14(4):293-301.

129. Martinez A, Montuenga LM, Springall DR, Treston A, Cuttitta F, Polak JM. Immunocytochemical localization of peptidylglycine alpha-amidating monooxygenase enzymes (PAM) in human endocrine pancreas. J Histochem Cytochem 1993; 41(3):375-380.

130. Curry WJ, Barkatullah SC, Johansson AN et al. WE-14, a chromogranin a-derived neuropeptide. Ann N Y Acad Sci 2002; 971:311-316.

131. Steel JH, Bishop AE. Molecular approaches to neuroendocrine pathology. Cancer Metastasis Rev 1997; 16(1-2):179-205.

132. Scopsi L, Sampietro G, Boracchi P, Collini P. Argyrophilia and chromogranin A and B immunostaining in patients with sporadic medullary thyroid carcinoma. A critical appraisal of their prognostic utility. J Pathol 1998; 184(4):414-419.

133. DeLellis RA. Proliferation markers in neuroendocrine tumors: useful or useless? A critical reappraisal. Verh Dtsch Ges Pathol 1997; 81:53-61.

134. DeLellis RA. The neuroendocrine system and its tumors: an overview. Am J Clin Pathol 2001; 115 Suppl:S5-16.

135. Eriksson B, Oberg K, Stridsberg M. Tumor markers in neuroendocrine tumors. Digestion 2000; 62 Suppl 1:33-38.

136. Lamberts SW, Hofland LJ, Nobels FR. Neuroendocrine tumor markers. Front Neuroendocrinol 2001; 22(4):309-339.

137. Vinik A, Woltering E, O'Dorisio T, Go V, Mamikunian G. Neuroendocrine Tumors: A Comprehensive Guide to Diagnosis and Management. 5th ed. InterScience Institute, 2012.

138. Mirakhur B, Pavel ME, Pommier RF et al. Biochemical Response to Lanreotide in Neuroendocrine Tumors: Pooled Analysis of 2 Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 2017; Submitted.

139. Reubi JC, Heitz PU, Landolt AM. Visualization of somatostatin receptors and correlation with immunoreactive growth hormone and prolactin in human pituitary adenomas: evidence for different tumor subclasses. J Clin Endocrinol Metab 1987; 65(1):65-73.

140. Vinik A, Gomez-Panzani E, Wolin EM, Audry A, on behalf of ELECT investigators. ELECT: A phase 3 study of efficacy and safety of lanreotide Autoge/Depot (LAN) treatment for carcinoid syndrome in patients with neuroendocrine tumors (NETs). ASCO-GI Symposium January 16-18, 2014. 2014.

141. Raymond E, Dahan L, Raoul JL et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med 2011; 364(6):501-513.

142. Vinik AI, Raymond E. Pancreatic neuroendocrine tumors: approach to treatment with focus on sunitinib. Therap Adv Gastroenterol 2013; 6(5):396-411.

143. Strosberg J, El-Haddad G, Wolin E et al. Phase 3 Trial of 177Lu-Dotatate for Midgut Neuroendocrine Tumors. N Engl J Med 2017; 376(2):125-135.

144. Vinik E, Silva MP, Vinik AI. Measuring the relationship of quality of life and health status, including tumor burden, symptoms, and biochemical measures in patients with neuroendocrine tumors. Endocrinol Metab Clin North Am 2011; 40(1):97-109, viii.

145. Strosberg JR, Halfdanarson TR, Bellizzi AM et al. The North American Neuroendocrine Tumor Society Consensus Guidelines for Surveillance and Medical Management of Midgut Neuroendocrine Tumors. Pancreas 2017; 46(6):707-714.