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ABSTRACTS – INVITED LECTURES

Session 1: Genetic aspects of pituitary tumors

S1.1
Clinical and genetic aspects of familial isolated pituitary adenomas

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The most frequent conditions that are associated with inherited/familial pituitary adenomas are familial isolated pituitary adenoma (FIPA) and multiple endocrine neoplasia type 1 (MEN1), which together account for up to 5% of pituitary adenomas. FIPA occurs in kindreds with ≥2 related individuals affected with pituitary adenomas in the absence of MEN1 or other syndromic causes. To date, one important genetic cause of FIPA has been identified, namely, inactivating mutations or deletions in the aryl hydrocarbon receptor interacting protein (AIP) gene. FIPA is the most frequent clinical presentation of AIP mutations, but other significant at-risk populations, including young patients with pituitary adenomas and those with gigantism. This talk traces the current state of knowledge regarding the clinical features of FIPA and the particular genetic, pathological and clinical characteristics of pituitary adenomas due to AIP mutations.

S1.2
How *AIP* mutations cause aggressive pituitary adenomas

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Germline mutations of the tumor suppressor gene *aryl hydrocarbon receptor-interacting protein* (*AIP*) predispose to hereditary pituitary neoplasia. *AIP* mutations are mostly associated with somatotropinomas (~78%), although cases with prolactinomas, non-functioning (NFPA) adenomas and Cushing's syndrome have also been reported. The patients with *AIP* mutations are typically young (mean age at diagnosis ~25 years) and do not necessary have a strong family history of the disease. *AIP* associated pituitary tumors often have an aggressive disease profile, with large and invasive adenomas that poorly respond to available treatments, such as somatostatin analogues.

The exact molecular mechanisms by which inactivated *AIP* exerts its tumor promoting action in the pituitary have been unclear. The AIP protein has several well-characterized interaction partners, through which it has a potential to affect a large number of different pathways, such as the cyclic adenosine monophosphate (cAMP) and various nuclear receptor signaling pathways. As cAMP is a mitogenic factor selectively in somatotrophs, the role of AIP in cAMP signaling provides a conceivable mechanism of how inactivation of this tumor suppressor contributes to pituitary tumor development. To date, however, the exact role of AIP in cAMP signaling has been incompletely defined.

To better understand the role of *AIP* in pituitary pathogenesis, gene expression microarrays were performed to examine differences in expression profiles between *Aip* wildtype (WT) and knockout (KO) mouse embryonic fibroblast (MEF) cell lines. The pathway analyses underscored the potential involvement of *Aip* in the regulation of G-protein coupled receptor (GPCR) - cAMP signaling cascade, and in a variety of physiological events such as developmental processes, immune-inflammatory responses, and cellular proliferation and differentiation. cAMP immunoassay experiments revealed that that intracellular cAMP levels were two- to three-fold higher in *Aip* KO cells compared with the WT cells. Although we were not able to measure intracellular cAMP concentrations in *AIP*-mutated somatotropinomas due to the lack of fresh tumor material, we demonstrated that *Aip* silencing elevates cAMP levels also in a GH/PRL-secreting rat pituitary adenoma cell line GH3. In an attempt to clarify the role of the guanine nucleotide (GTP) binding protein alpha (Gα) subunits in the dysregulation of cAMP synthesis induced by *Aip* deficiency, we examined whether siRNA silencing of *Gα* - subfamily protein loci has an effect on cAMP levels in MEF cell lines. We observed that *Aip* deficiency leads to constitutive activation of cAMP synthesis through defective inhibitory $G\alpha_{i\text{-}2}$ and $G\alpha_{i\text{-}3}$ proteins that normally inhibit cAMP synthesis. In addition, $G\alpha_i$ immunostainings revealed that $G\alpha_{i\text{-}2}$ protein expressions were clearly reduced in human and mouse *AIP*-associated somatotropinomas, indicating defective $G\alpha_i$ signaling in these tumors. By contrast, all prolactinomas showed prominent $G\alpha_i$ protein levels, irrespective of *Aip* mutation status.

In conclusion, our study provides evidence that defective $G\alpha_i$ signaling resulting in constitutive activation of cAMP synthesis seems to be a major contributor to the development of *AIP*-associated somatotropinomas. Although further studies are necessary to clarify the exact molecular basis of the impaired $G\alpha_i$ pathway, the new insights into the signaling cascades altered by *AIP*-deficiency are important - especially considering that both somatostatin and dopamine signaling are mediated through $G\alpha_i$ protein coupling receptors.

S1.3
Micro-RNAs in pituitary adenoma pathology: culprits or bystanders?

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Several hypotheses have been postulated concerning development and progression of pituitary tumors. Molecular studies identified many mutations and/or abnormal mRNA/protein expression patterns and, recently, microRNA (miRNA) dysregulation has also been taken into consideration as a possible pathogenic mechanism. The miRNAs represent highly conserved, noncoding RNAs that regulate gene expression, causing a reduced mRNA transcription or translation. miRNA may importantly affect cell differentiation, apoptosis, and proliferation, being possibly involved in human tumor development, since they can act as both oncogenes and tumor suppressor genes Nearly 10 years ago, miR-15a and miR-16-1 have been demonstrated to be downregulated in pituitary adenomas, suggesting for the first time the role of miRNAs in pituitary tumorigenesis. Subsequently, different miRNA expression patterns have been identified as differentially expressed (down- or up-regulated) between pituitary adenomas and normal pituitary. Several studies also indicated that miRNAs signatures may represent useful diagnostic and prognostic biomarkers. Specific miRNA may also be future therapeutic targets, especially in the settings of pituitary adenomas sub-types, such as ACTH-secreting pituitary adenomas, that are difficult to treat and lack specific and effective therapeutic approaches. In these settings, the field of miRNAs research may offer new therapeutic targets, which investigation may unravel the mechanisms underlying pituitary adenoma development and progression. Therapeutic interventions aiming at regulating such newly identified pathways may help in achieving disease control.

S1.4
Pituitary gland in MEN1 syndrome: from histopathology to prognosis

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Familial cases of pituitary adenomas (PA) represent up to 5% of all PA, with 2.7% related to multiple endocrine neoplasia type 1 (MEN1) and nearly 2.5% related to the clinical entity familial isolated pituitary adenomas (FIPA). Mutations in the tumour suppressor gene *MEN1* predispose to MEN1, an autosomal dominant inherited disease, characterized by the presence of tumours in at least two endocrine tissues. These tumours include including PA that occur in ~40% of MEN1 cases (the prevalence of PA in MEN1 varies between 16-65%depending on the methodology), with an age of onset between 12 to 83 years (mean 38 years). Whereas hyperparathyroidism is frequently reported as the first manifestation of MEN1 syndrome, pituitary disease is the first lesion diagnosed in about 15% of *MEN1*-mutated patients. *MEN1* mutations do not play an important role in the pathogenesis of sporadic PA. Indeed, the systematic study of MEN1 gene mutations in large series of patients harbouring PA report frequencies of <5%. Furthermore, somatic mutations of MEN1 gene are found in only 3.5% of pituitary tumors. A Brazilian study in 2014 reveals a frequency of 7.7% of MEN1 in a group of 144 patients with PA and in a France–Belgium multicenter study, MEN1 mutations are identified in 3.4% of sporadic PA (macroadenoma) diagnosed before 30 years of age, without hypercalcemia. The frequency reaches 6.5% in the pediatric population (age ≤18 years old). In the previous France–Belgium multicentre studies, the mean age of patients with PA as the initial manifestation of MEN1 is significantly lower (33.9±14 years) than that of patients with other initial tumor manifestations (41.6±14 years). The delay between initial and subsequent MEN1 endocrine lesions is significantly longer when pituitary disease was the initial MEN1 manifestation (9.0±8.1 years) than when the initial manifestation was intestinal and pancreatic tumours (4.1±4.0 years) or hyperparathyroidism (5.2±5.1 years). The frequency of pituitary macroadenomas is significantly higher in MEN1 patients than in non-MEN1 subjects (85% vs. 42%) and clinical manifestations related to the size of PA are significantly more frequent in MEN1 patients than in non-MEN1 subjects (29% vs. 14%). Clinical control of pituitary hypersecretion is much less frequent in MEN1 patients than in non-MEN1 subjects (42% vs. 90%). A gender-related difference is also found. The prevalence and probability of developing pituitary tumours are significantly greater in females than in males. Although PA are larger and more often invasive in MEN1 patients than in sporadic, malignant tumours are not more frequent. Interestingly, three cases of pituitary carcinomas were reported in patients with MEN1: a female with sporadic MEN1 and gonadotroph pituitary carcinoma, a male with prolactin-producing carcinoma with familial MEN1 and a male with a thyrotroph carcinoma in sporadic MEN1. Usual guidelines for care of PA cannot be used for PA in MEN1 patients and follow-up must be very careful and prolonged for life.

Considering the histopathology of PA associated with MEN1 syndrome, prolactinomas are the most frequent (62%) followed by somatotropinomas (9%), clinically non-functioning (15%) and Cushing disease/corticotroph adenoma (4%). In 2008, Trouillas et al. study compared 77 surgically removed MEN1 PA (from 211 MEN1 patients of French GTE registry) with 2509 unselected non-MEN1 sporadic PA. The authors concluded that MEN1 tumours occurred in younger patients and were larger, more invasive and aggressive. A higher frequency of multiple adenomas (4% vs. 0.1%) and hyperplasia (4% vs. 0%) was found in the MEN1 patients versus sporadic. The frequency of functional PA was identical in the two groups (72% MEN1 vs. 64% non-MEN1), but the proportion of plurihormonal adenomas was significantly higher in the MEN1 group with very unusual associations: PRL and ACTH or PRL and FSH or plurihormonal GH adenomas. Although, division among the different histotypes of adenomas was not statistically different from the control population. Large PA were more frequent in the MEN1 group than in controls (excess of macroprolactinomas (84%) in MEN1 patients) but mitotic activity and the proliferation index (Ki67-MIB1>3%) were not significantly different. Histologic invasion was found in 31% of MEN1 PA and in 14% of controls (P<0.02).

In summary, PA associated with MEN1 syndrome are usually diagnosed at earlier ages than sporadic PA and occur more frequently in females. PA in MEN1 have a higher degree of aggression and invasiveness, a higher prevalence of macroadenomas and are more often resistant to medical therapy, with higher tumour recurrence rates. From a histological perspective, MEN1 PA are more frequently plurihormonal and mixed tumours. Furthermore, pituitary hyperplasia and multiple pituitary tumours are more frequently found in MEN1 than in sporadic tumours. Pituitary tumours associated with MEN1 syndrome may differ markedly from sporadic tumours, and these

differences are potentially helpful in the differential diagnosis between these entities.

S1.5
Clinical features and therapeutic outcomes in aggressive pituitary adenomas and carcinomas

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In absence of specific pathological markers aggressive pituitary adenoma and carcinomas were clinically defined. Indeed, only tumors associated with craniospinal or systemic metastases are considered as carcinomas, and multirecurrent pituitary tumor resistant to conventional treatment are classified as aggressive pituitary tumors.
Most pituitary carcinomas are secretory, secreting PRL (36% cases) or ACTH (30% cases) in the majority of cases. Non-functioning tumors are less frequent (23% cases). Unfortunately, pituitary carcinomas are generally associated with a poor prognosis with a median survival of 12 months.
Specific biomarkers that can distinguish between clinically aggressive and nonaggressive pituitary adenomas have not yet been identified, although a recent classification taking into account the association of tumor invasion and cell cycle markers might be of value. Indeed, a potential malignancy in pituitary tumors could reasonably be suspected based on the association of the following pathological signs: invasion, neoangiogenesis, vascular invasion, abnormal mitoses, very high index of Ki-67 >10% and p53 >5%.
In absence of consensual prognostic criteria the therapeutic strategies applied to such tumors are based on individual clinical follow-up without any preventive therapies. Temozolomide, an oral alkylating drug, demonstrated encouraging results in a limited number of cases of pituitary aggressive tumors or carcinoma. However, long term efficacy is unknown indicating that there is a need for new targeted therapies allowing a more personalized therapeutic strategy based on clinical and pathological characteristics.

S1.6
What we know and don't know about the pathophysiology of MEN 1 pituitary adenomas

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Tumors arising from the different cell types of the anterior pituitary are mostly benign adenomas that are either non-functioning or that continuously secrete the characteristic hormone of their cell-of-origin - prolactin (PRL), growth hormone (GH), adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH). Pituitary tumors, microadenomas or macroadenomas, can occur sporadically or as a part of at least four familial tumor syndromes: Carney complex, MEN1, MEN4 and FIPA.
The multiple endocrine neoplasia type 1 (MEN1) syndrome is caused by heterozygous germline inactivating mutations in the tumor suppressor gene *MEN1* that encodes menin, followed by somatic loss/inactivation of the remaining normal *MEN1* allele (LOH at 11q13) in specific tissues. MEN1 patients present with tumors in at least two of the three main endocrine organs associated with this syndrome (parathyroid, anterior pituitary and endocrine pancreas). Among MEN1 tumors 5% are non-functioning pituitary adenomas, but most MEN1 pituitary adenomas are hormonally active; the hormones secreted and the penetrance of such adenomas at age 40 are as follows: PRL (20%), GH+PRL (5%), GH (5%), and ACTH (2%). The prevalence of *MEN1* mutations is 70% in MEN1 families that show tumors in all 3 characteristic endocrine organs, and 60% in similar cases with all 3 tumors but no family history of MEN1. However, *MEN1* mutations are infrequently observed in MEN1-like familial cases with tumors only in the parathyroid (10%) or rarely observed in familial cases with only pituitary adenoma (0.1%). Unlike biallelic somatic inactivation of *MEN1* in 30-40% of sporadic parathyroid adenomas and pancreatic neuroendocrine tumors (PNETs), somatic *MEN1* mutations are observed only in 1-5% of sporadic pituitary adenomas. This raises the question whether pituitary adenomas have other mechanisms to silence *MEN1* such as from mutations in non-coding regions or functional inactivation of its encoded protein menin. Therefore, investigating the regulation of *MEN1* and menin, and the biological functions of menin in specific pituitary cells could provide insights into the pathophysiology of MEN1-associated and sporadic pituitary adenoma.
Mouse models have been informative in understanding MEN1 pituitary adenoma tumorigenesis and for exploring therapeutic interventions. Conventional homozygous knockout of *Men1* in the germline of mice causes early embryonic lethality (E10.5-E14.5), and heterozygous knockout causes endocrine tumors similar to the human MEN1 syndrome (parathyroid adenoma, insulinoma, and prolactinoma). Interestingly, tissue-specific knockout of *Men1* in pancreatic islet β-cells using Cre-recombinase driven by the rat insulin promoter (RIP-Cre) shows not only insulinoma in the pancreas but also prolactinoma in the pituitary. Note that Cre-recombinase expression from the RIP-Cre construct is also seen in pituitary cells. Nevertheless, these data highlight the importance of menin as a tumor suppressor in pituitary lactotrophs. The mouse models of MEN1 have been useful to study the effect of menin replacement and anti-angiogenesis inhibitors on pituitary adenomas. In other mouse models, a MEN1-like phenotype has been observed from targeted deletion of some cell cycle regulators that act downstream of menin (CDKN1B/p27 and CDKN2C/p18). Menin contains 610 amino acids, has a molecular mass of 67 kDa, is predominantly nuclear, and has been shown to play important roles in diverse biological pathways through interactions with various proteins such as those involved in transcriptional regulation. Whether mouse models could help to decipher which of these interactions are relevant to pituitary adenomas remains to be explored.
Menin's role in transcriptional regulation has been well studied from its interactions in the MLL1/MLL2 protein complexes that trimethylate histone-H3 at lysine-4 (H3K4me3), a mark for active gene transcription. H3K4me3-dependent menin targets in pituitary cells are not known. Using mouse embryonic stem cells from the conventional *Men1* knockout mouse model, and from other *in vivo* cell culture and *in vitro* studies we found that menin loss leads to loss of H3K4me3 at the *Dlk1-Meg3* locus and reduced expression of Meg3. MEG3 is a paternally imprinted long non-coding RNA that has been proposed to act as a tumor suppressor by activating the p53 and Rb pathways. Interestingly, sporadic pituitary adenomas show significantly reduced levels of MEG3 mRNA due to somatic epigenetic silencing and loss of gene expression from the *DLK1-MEG3* locus on chromosome 14q32. This raises the question whether reversal of epigenetic silencing mechanisms to activate MEG3 expression can be a potential therapeutic option for pituitary adenoma treatment. These data show how different paths lead to the same target genes for pituitary adenoma pathogenesis. However, which targets of MEG3 are affected in MEN1 pituitary adenomas and whether they are mutated, aberrantly expressed or inappropriately localized in

sporadic pituitary adenomas that lack *MEN1* mutations remains to be determined.
We propose that a combined analysis of the genes and pathways discovered from studies of pituitary adenomas that occur sporadically and those associated with tumor susceptibility syndromes such as MEN1 can be instrumental in understanding the pathophysiology of these tumors.

Session 2: MEN 1 and hyperparathyroid syndromes

S2.2
The calcium sensing receptor (CaSR) and disordered of Ca2+-sensing: from the gene to the patient

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Despite the near constancy of the level of extracellular Ca2+ (Ca2+o) in blood and related extracellular fluids, little attention was paid for many years to how Ca2+o was regulated in such a precise fashion, particularly the mechanism(s) that sensed Ca2+o. In contrast, there had been intense interest in the role of intracellular calcium (Ca2+i) as a ubiquitous second messenger regulating myriad processes in essentially all cells. Ca2+i was known to vary over a wide range during cellular activation thereby modulating cellular function via specific Ca2+o-binding "trigger" proteins, such as calmodulin. Since Ca2+o in blood varies by only a few percent under normal circumstances, it wasn't clear what type of mechanism could sense such small changes in Ca2+o. The parathyroid cell by virtue of the exquisite sensitivity of Ca2+o-regulated PTH release to its primary physiological ligand, the extracellular calcium ion, is the body's quintessential Ca2+o-sensing cell and became a useful model for studying this process further. Increases in Ca2+o triggered changes in intracellular second messengers in this cell type that are highly reminiscent of those evoked by various G protein-coupled receptors (GPCRs) in other cells, particularly activation of phospholipase C and pertussis toxin-sensitive inhibition of adenylate cyclase. The rapid, high Ca2+o-evoked, PLC-mediated increase in the cytosolic Ca2+ (Ca2+i) concentration provided a signature of the putative G protein-coupled Ca2+o-sensing receptor (CaSR) that was employed to isolate the CaSR by expression cloning in X. laevis oocytes. Sequencing of the gene documented that the CaSR belongs to a subfamily of the GPCRs, the family C (or family 3) receptors, which includes, for example, the CaSR, metabotropic glutamate receptors, GABAB receptors, and taste receptors. All are thought on the basis of molecular modeling or direct structural determination (of the mGluRs) to reside on the cell surface with a large extracellular domain (ECD) folded into a Venus flytrap (VFT)-like structure, a seven transmembrane motif and an intracellular C-terminal tail. The VFT of the family C receptors contains the main determinants of the binding of the respective receptors' physiological ligands. The marked positive cooperativity of the CaSR (Hill number of 3-4) relative to the other family C receptors begged the question of how many calcium-binding sites there are in the CaSR and how they interact to generate the CaSR's so-called positive homotropic cooperativity (the binding of a given ligand to its binding site increases the affinities of other binding sites on a protein for the same ligand). Studies using site-directed mutagenesis predicted the presence of a key binding site for Ca2+ (Site 1) in the crevice between the two lobes of the CaSR's VFT. Since the receptor functions as a dimer, interactions between these sites in the two monomers could elicit positive cooperativity, albeit with a Hill number of < 2, since only two sites are involved. Algorithms seeking Ca2+-binding sites in silico based on their possessing an appropriate geometry of oxygen-containing amino acids (e.g., aspartate, glutamate, serine, threonine, etc.) have identified as many as 4 additional predicted calcium-binding sites in the CaSR's ECD. When these sites are mutated, impairment of Ca2+o-sensing ensues, supporting their physiological relevance. Molecular Dynamic simulations have demonstrated "connectivity" between these sites, such that they move in the same or opposite directions during spontaneous movements of the protein (i.e., have correlated motions). Other regions of the molecule, in contrast, move randomly with respect to one another. A key role for molecular connectivity in engendering positive cooperativity is also supported by the capacity of L-phenylalanine, binding to its binding pocket adjacent to Ca2+-binding site 1, to enhance the positive cooperativity of the wild type CaSR for binding Ca2+ as well as to partially "rescue" the functions of mutant receptors harboring inactivating mutations in any one of these other Ca2+-binding sites. Ongoing studies are addressing the structural basis for how binding of calcium to its various biologically relevant Ca2+-binding sites produces conformational changes that activate the receptor.
An area of intense interest since the cloning of the CaSR has been the pathogenesis of so-called disorders of Ca2+o-sensing, in which alterations in the CaSR's structure and/of function enhance or diminish its responsiveness to the Ca2+o signal. Reduced responsiveness produces a rightward shift in the relationship between the level of Ca2+o and CaSR-mediated biological responses, while enhanced sensitivity left shifts this relationship. For instance, various types of inactivating mutations in the receptor or in its downstream G protein, G11, can decrease its responsiveness to Ca2+o [familial hypocalciuric hypercalcemia (FHH1 or 2, respectively) or neonatal severe hyperparathyroidism [NSHPT], which are usually caused by, respectively, heterozygous or homozygous inactivating mutations. The parathyroid hyperplasia in individuals with homozygous inactivating mutations of the CaSR has provided formal proof that the receptor tonically inhibits parathyroid cellular hyperplasia. Conversely, activating mutations in the CaSR or G11 enhance cellular responsiveness to Ca2+o and cause varying degrees of hypocalcemia in autosomal dominant hypocalcemia (ADH1 or 2), with or without the clinical and biochemical features of Bartter's syndrome (Bartter's type 5). Phenocopies of these genetic disorders arise from inactivating or activating antibodies to the CaSR that bind to the receptor's ECD and produce hyper- and hypocalcemia similar to the biochemical features caused by inactivating and activating mutations, respectively. Much more common are conditions that alter responsiveness of the CaSR-mediated, Ca2+o-sensing pathways without changing the receptor's primary structure. These include diverse forms of primary, secondary, and tertiary hyperparathyroidism. Much remains to be learned about how alterations in the receptor's level of expression and/or of other proteins participating in its signal transduction [i.e., G proteins (G11 and Gq), and caveolins, for instance] lead to defective Ca2+o-sensing, with attendant hypersecretion of PTH and/or excessive parathyroid cellular proliferation.
Even prior to the cloning of the CaSR, Nemeth and coworkers utilized bovine parathyroid cells as a bioassay to develop drugs potentiating or inhibiting the activation of the putative CaSR by Ca2+o, so-called calcimimetics and calcilytics, respectively. The third generation calcimimetic, cinacalcet, the only one currently FDA-approved CaSR-

active drug, has proven useful in various forms of hyperparathyroidism, particularly severe secondary hyperparathyroidism. As noted below, calcimimetics and calcilytics hold promise for the treatment of dysfunction of the CaSR in other tissues, including neoplastic conditions in which the CaSR participates in the malignant process.
Germane to this meeting, the CaSR has been shown, since it was initially isolated, to be expressed in a wide variety of tissues and to participate in numerous processes uninvolved in Ca2+o homeostasis. Accumulating evidence, for instance, supports roles for the CaSR in promoting or inhibiting the development and progression of cancer. In "unfavorable" neuroblastomas, silencing of the CaSR by genetic and epigenetic mechanisms and reactivating it induces ERK1/2-dependent apoptosis, i.e. promotes cancer. Conversely, in the parathyroid cell, as noted above, inactivation of the receptor in individuals with homozygous inactivating mutations or in mice with genetic deletion of the CaSR, can cause marked parathyroid hyperplasia owing to removal of the inhibitory effect of the CaSR on parathyroid cellular proliferation. Ongoing studies are addressing the possibility that appropriate use of CaSR-based therapeutics could have a useful role in the chemoprevention (i.e., in colon cancer) or treatment of various forms of cancer, although this field remains in its infancy.

S2.3
Genetics of parathyroid tumors

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Parathyroid tumours, resulting in primary hyperparathyroidism, may occur as part of a complex syndrome, e.g. as part of a multiple endocrine neoplasia (MEN) syndrome, or as an isolated endocrinopathy, which is referred to as familial isolated hyperparathyroidism (FIHP). Parathyroid tumours associated with complex syndromes, which may be inherited as autosomal dominant traits, comprise: MEN1, a disorder characterised by the combined occurrence of tumours of the parathyroids, pancreatic islets and anterior pituitary; MEN2 (also referred to as MEN2a) a disorder characterised by the combined occurrence of medullary thyroid carcinoma (MTC), phaeochromocytoma and parathyroid tumours; MEN3 (also referred to as MEN2b), a disorder characterised by the combined occurrence of MTC and phaeochromocytoma with rare occurrence of parathyroid tumours, but instead an association with a Marfanoid habitus, mucosal neuromas, medullated corneal fibres, and intestinal autonomic ganglion dysfunction leading to multiple diverticula and megacolon; MEN4, a disorder characterised by the combined occurrence of parathyroid and anterior pituitary tumours in association with tumours of the gonads, adrenals and kidneys; and the hyperparathyroidism-jaw-tumour (HPT-JT) syndrome, a disorder characterised by the combined occurrence of parathyroid tumours, which are often carcinomas, and ossifying jaw-fibromas in association with uterine tumours, renal tumours and rarely pancreatic adenocarcinomas, testicular mixed germ cell tumours and Hurthle cell thyroid adenomas. These syndromic disorders associated with parathyroid tumours are due to mutations as follows: MEN1 is caused by abnormalities of a tumour suppressor, menin, located on chromosome 11q13, which is involved in transcriptional regulation, genome stability, cell division and proliferation; MEN2 and MEN3 are due to mutations of RET, which encodes a tyrosine kinase receptor (TKR); MEN4 is due to mutations of CDNK1B which encodes the cyclin-dependent kinase inhibitor (CKI) p27kip1; and HPT-JT is due to mutations of parafibromin, also known as cell division cycle 73 (CDC73), which has a role in a key transcriptional regulatory complex that interacts with RNA polymerase II. Parathyroid tumours occurring as an isolated endocrinopathy, referred to as FIHP, may be due to heterozygous mutations of menin, parafibromin or the calcium-sensing receptor (CaSR), which is a G-protein coupled receptor (GPCR). Homozygous or compound heterozygous mutations of the CaSR, may also result in neonatal severe primary hyperparathyroidism. Moreover, it is important to note that ~10% of patients presenting, below the age of 45 years, with non-familial (sporadic) parathyroid tumours may also have a de novo germline mutation of menin, parafibromin or CaSR, and this has implications for their future management, in requiring screening for the occurrence of tumours associated with the specific syndrome, as well as for screening their children who may inherit the germline mutation. In summary, parathyroid tumours may occur as part of the hereditary syndromes of MEN1, MEN2, MEN3, MEN4 and HPT-JT, or as an isolated endocrinopathy due to mutations of menin, parafibromin or CaSR.

S2.4
Medical Management of primary hyperparathyroidism

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Primary hyperparathyroidism (PHPT) is the main endocrinopathy associated with MEN1 syndrome (MIM#131100), in more than 90% of individuals, with a penetrance reaching 100% within 50 years of age. PHPT in MEN1 is characterized by on age of onset is between 20 and 25 years that is three decades earlier than the one reported in the sporadic, non-syndromic, form of PHPT and it is generally supported by multiple parathyroid tumors with overproduction of parathyroid hormone (PTH) manifesting clinically with hypercalcaemia. Typically PHPT in MEN1 remains asymptomatic for a long time, or occurs with a reduction in bone mass at the age of 35 years. The classical signs and symptoms related to chronic hypercalcaemia are present in MEN1 patients with lethargy, trough, confusion, anorexia, nausea/vomiting, dieresis, dehydration, hypercalciuria, kidney stones, increased bone resorption and fracture risk, hypertension, and reduction in QT interval.
Neck surgery, the elective care of PHPT, although the optimum timing has not been defined. Conventional open bilateral exploration with subtotal parathyroidectomy (at least 3.5 glands) or total parathyroidectomy is recommended and, autotransplantation may be considered while minimally invasive parathyroidectomy is usually not recommended because multiple glands arc typically affected. One recommendation is that parathyroidectomy be reserved for symptomatic hypercalcemic patients and that asymptomatic hypercalcemic patients do not undergo parathyroid surgery but have regular assessment for symptom onset and complications, at which time subtotal parathyroidectomy with transcervical thymectomy, should be undertaken. It is important to emphasize that the literature has reported a relatively high incidence of hypoparathyroidism after neck surgery in MEN1 patients.
Conversely a recurrence of PHPT occurs in approximately 50% of cuparathyroid individuals with MEN1 syndrome 8-12 years after subtotal parathyroidectomy. For these reasons a pharmacological approach in early diagnosed MEN1 patients with PHPT would certainly the welcome.
To date there is little clinical experience on the use of Cinacalcet in hereditary PHPT in which a genetically determined susceptibility to the growth of abnormal parathyroid tissue is present at birth and information on the

effect of the drug on growth and function of parathyroid tissue in MEN1 is limited. The presentation will review what published up to now, with additional unpublished data.

S2.5
Management of primary hyperparathyroidism in pregnancy: why and when?

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There are profound changes in mineral metabolism during pregnancy. The requirement for calcium by the foetus is met by an increase in the active form of vitamin D, calcitriol, and an increase in calcium absorption. It is also met by an increase in bone turnover and bone loss, particularly from the axial skeleton. The serum calcium decreases because of a decrease in serum albumin and in the third trimester, the level of parathyroid hormone increases. These metabolic consequences of pregnancy can help mask the diagnosis of primary hyperparathyroidism. The total calcium needs to be adjusted for the albumin level or else the serum ionized calcium measured. The optimal localization is ultrasound (to avoid any irradiation) and this may allow minimally invasive surgery. It is usually recommended that surgery is performed in the second trimester. Many complications of primary hyperparathyroidism have been reported including an increased rate of spontaneous abortions and stillbirth. There is suppression of the foetal parathyroidism and this results in neonatal hypocalcacemia and tetany that might last a few months. It was thought that these complications were historical – in the past, primary hyperparathyroidism was much more severe than today. Nonetheless, recent case series have reported such pregnancy complications. There has been no clinical trial to evaluate the benefit of a surgical approach but this is still the usual recommendation. There is the possibility of the use of cinecalcet and there is a case report of its use, but there are calcium sensing receptors in both the placenta and foetus and so that may not be a safe approach.

S2.6
Hyperparathyroidism - Jaw Tumor Syndrome: current aspects in diagnosis and treatment

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Primary hyperparathyroidism (pHPT) is a common endocrine disorders, which arises sporadically in most cases and in a familial setting in a minority of patients (<10%). In familial variants, it is mostly associated with other endocrine neoplasms, including multiple endocrine neoplasia (MEN) type 1 or 2; less frequently it may be found without any association. Familial nonMEN pHPT includes several variants; among them, HPT-jaw tumor syndrome (HPT-JT) may occur. HPT-JT Syndrome (OMIM #145001) is a rare autosomal dominant syndrome with incomplete penetrance and variable expression. It is characterized by single/multiple parathyroid tumors occurring at an earlier age, a relatively high prevalence of carcinomas and atypical adenomas, ossifying fibromas of mandible and/or maxilla, and less frequently, a variety of renal lesions and uterine tumors. To date, less than 400 cases from less than 100 families have been reported. It was first described in 1990, but the genetic marker has been identified only in 2002; for these reasons, its real incidence is still unknown and might be possibly underestimated.
Pathogenesis. HPT-JT is linked to germ-line inactivating mutations in the tumor suppressor gene CDC 73 (former HRPT2), which encodes for Parafibromin, a ubiquitously expressed protein with antiproliferative properties. Several reports have demonstrated a loss of Parafibromin expression in HPT-JT related adenomas and in sporadic parathyroid carcinoma carrying somatic CDC73 mutations. No genotype-phenotype correlations for CDC73 mutations have been formally established to date. However, it has been suggested that missense mutations are more likely to be associated with the disease without typical associated features (familial isolated pHPT), whereas mutations causing gross Parafibromin disruption are more likely associated with the classical HPT-JT phenotype.
Clinical features. pHPT is the main finding of HPT-JT syndrome; it is found in almost 100% of mutation carriers. The prevalence increases with age; the onset is typically in early adulthood; the earliest reported age is 7 years and the earliest parathyroid carcinoma has been found at the age of 20. However, onset may be delayed until the sixth decade, and some older healthy mutation carriers have been reported. pHPT is usually mild, but, in case of parathyroid carcinoma, severe hypercalcemic crisis may occur. pHPT in HPT-JT is usually caused by a single benign parathyroid adenoma (differently from other variants of Familial disease), which is often cystic or has atypical histologic features. In approximately 10% of cases, a parathyroid carcinoma may be found. Multiglandular involvement occurs rarely at initial surgery; a second parathyroid tumor may occur metachronously years to decades after appearance of the first tumor. The frequent single-gland involvement supports the hypothesis that CDC73 is an oncosuppressor gene and that biallelic inactivation is required for tumor development. The rather low prevalence of multiglandular parathyroid involvement, either synchronous or metachronous, suggests that CDC73 germ-line mutations might achieve a predisposition to the neoplastic progression; a second hit or other genetic or epigenetic events should be necessary to the development of parathyroid tumors.
Instead of the denomination, jaw tumors are evident only in a minority of cases (10-20%), possibly leading to underdiagnosis of the disease. Ossifying fibromas of the mandible or maxilla may present as an enlarging visible or palpable mass, whereas others are detected only on jaw x-ray. Although benign, these tumors can disrupt normal dentition, and be of significant cosmetic concern. Tumors may occasionally be bilateral/multifocal and may recur and continue to enlarge if not treated. They usually appear to be radiographically radiolucent, and develop before the third decade of life. They must be differentiated by the brown tumors associated with severe pHPT, that resolve after curative parathyroidectomy.
Several non-endocrine tumors may be also present in HPT-JT patients. Uterine involvement is the second most common clinical feature (60% of cases). Some of these tumors (leyomiomas, endometrial hyperplasia, adenomyosis) are very common also in the general population; others (adenosarcomas, adenofibromas, multiple adenomyomatous polyps) are less frequent. Interestingly, most of these tumors appear to have a common embryological origin from the mesodermal Mullerian duct system. The absence of Parafibromin expression also in uterine polyps seems to support the pathogenetic role for CDC73 mutations in HPT-JT-related uterine involvement.
Renal involvement may be found in 15-20% of HPT-JT patients. Hamartomas, polycystic disease, Wilms tumors, and adenocarcinomas have been reported. Cysts have also been observed in association with rare solid tumors, which were histologically similar to mixed epithelial-stromal tumors or adult mesoblastic nephroma, or hamartomatous-type tumors.

Finally, pancreatic adenocarcinoma, testicular mixed germ cell tumors, thyroid and colon carcinomas have been reported but it is not clear that these tumors are present in a higher frequency in HPT-JT syndrome than in the general population.
Diagnosis. The diagnosis of HPT-JT must be confirmed by genetic testing. CDC73 germline analysis should be performed in cases of Familial pHPT with negative genetic testing for MEN1, in case of personal and/or familial history of HPT-JT syndrome (ossifying jaw fibroma or other associated conditions, such as Wilms tumor or other genitourinary disease); pHPT with cystic, atypical, and/or malignant parathyroid histology; in children diagnosed with ossifying fibroma(s) of the maxilla or mandible; in pHPT with absence of nuclear parafibromin staining in parathyroid tumor as demonstrated by immunohistochemistry; pHPT with young-onset (age <45 yrs) disease; multiglandular or recurrent pHPT.
Following initial diagnosis, it is necessary to establish the extent of the disease evaluating pHPT features (measurement of PTH, serum calcium concentration and daily calciuria, bone density and preoperative localizing imaging by neck ultrasonography and MIBI scintigraphy, CT and/or MR); jaw tumors (panoramic jaw x-ray); renal lesions (renal ultrasound examination or MR) and uterine tumors (pelvic ultrasound examination, CT or MR) for women starting at reproductive age.
Treatment. The optimal surgical approach to pHPT in HPT-JT syndrome has not yet been established and remains controversial. Extensive parathyroid surgery (bilateral neck exploration and total parathyroidectomy) has been proposed in the past, because of the risk of multiglandular involvement and malignancy, but, because of the recent finding of frequent single-gland involvement and lower risk of parathyroid carcinoma, selective parathyroidectomy has become more popular, especially when preoperative imaging techniques localize concordantly a single affected gland. Under these conditions, a focused and minimally invasive parathyroidectomy might be proposed in these cases in the same setting of sporadic HPT, with the potential advantage of causing lower risk of hypoparathyroidism and minimal tissue trauma, facilitating reoperations in case of recurrent hyperparathyroidism. In fact, the risk of recurrent and/or new disease exists; therefore, regular lifelong serum testing for biochemical evidence of hyperparathyroidism is recommended. In case of suspicion of parathyroid carcinoma (large and infiltrating parathyroid neoplasm, extremely elevated serum calcium and iPTH levels), an en bloc resection including the ipsilateral thyroid lobe should be performed, taking to prevent fracture of the tumor, which could seed the local area. Jaw tumors should be treated surgically by complete resection, which may not be possible in all cases. Patients should be followed closely because of the possibility of recurrence. No treatment guidelines for renal and uterine manifestations associated with HPT-JT syndrome exist; patients should be managed on a case-by-case basis.
Follow up. CDC73 healthy mutation carriers and affected patients must undergo appropriate surveillance. Measure serum concentrations of calcium, PTH, neck ultrasonography should be performed every 6 months beginning at around age five to ten years. Jaw tumors need Rx dental imaging at least every five years, starting at age of ten. Monitor for kidney lesions is performed by renal ultrasound examination and /or MRI at least every five years, starting at the age of diagnosis. Uterine involvement must be followed up by annual pelvic ultrasound examination and/or CT or MR in addition to regular gynecologic care, starting at reproductive age and for lifelong monitoring. Because of the risk of multiple tumors and various organs involvement, a systematic, multidisciplinary, and prolonged work-up is required.
Conclusion. HPT-JT is a rare disease related with inactivating mutations of CDC73. Penetrance of mutations is high, but disease expression may be incomplete. Loss of Parafibromin expression is a distinctive feature of the disease and may be used to select patients and relatives for further genetic analysis. The most common clinical presentation is pHPT that may occur with a frequent single-gland involvement and a relatively increased risk of parathyroid malignancy. Other nonendocrine tumors are also frequently diagnosed, such as uterine and renal neoplasms. Regardless of the denomination of the syndrome, jaw tumors may occur rarely. Selective parathyroidectomy may be an effective strategy, but a prolonged follow-up is required because of the risk of recurrences and malignancies. A systematic investigation is also required because of associated tumors.

L1: Historical Lecture

L1.2
Jakob Erdheim (1874-1937) - pioneer of endocrine pathology

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In 1900, when Jakob Erdheim completed his medical studies at the University of Vienna sub auspiciis imperatoris and started a career with the prolific pathologist Anton Weichselbaum (1845-1920), pathology had been enriched with microscopic and developmental anatomy, microbiology and physiology. Endocrinology was to emerge from research on nervous, osseous and glandular systems with emphasis on cranio-cervical centres. The endemic goiter not only provided a classical model for neuropsychiatric localization, but also fostered surgical technique and 1909 yielded a Nobel Prize for a surgeon, Billroth's student Theodor Kocher (1841-1917). Findings on pathophysiology and regular and radiographic anatomy of the hypophysis led to efficient transsphenoidal neurosurgical approaches. The academic environment encompassed the surgeons Hermann Schloffer (1868-1937), Oskar Hirsch (1877-1965) and Ottokar v. Chiari (1853-1918), the radiologist Arthur Schüller (1874-1957), and the endocrinologist Artur Biedl (1869-1933). Erdheim's graduation lecture addressed comparative anatomy of the brain. His first publication was on branchiogenic organs in humans (1901). With his painstaking description of secretory granules in the thyroid, parathyroid and pituitary glands, using novel dyes, he introduced ultrastructural anatomy into endocrine pathology, described MEN1 syndrome, and recognized the causation of acromegaly by eosinophilic pituitary adenoma (1903). His extensive description of his eponymous tumor (1904) contributed to the understanding of craniopharyngeoma. With his 1906 article Tetania parathyreopriva (1906) he participated in the difficult discussion about the parathyroid glands in bone pathology which later resulted in Felix Mandl's (1892-1957) first removal of a parathyroid adenoma 1925. 1908 Erdheim was promoted associate professor, and 1913 prosector at the St. Anna pediatric Hospital. During the First World War, Erdheim served in mobile antiinfectious hospitals in the Balkans and in Galicia, which 1923 prompted an invitation to Palestine as expert for treatment of malaria. From 1924 to his death from myocardial infarction, Jakob Erdheim headed the Department of Pathology in Vienna's first and most ambitious municipal hospital centre in Lainz, in exchange with the founder of this department, Weichselbaum's student Rudolf Maresch (1868-1936) who was appointed to the University Department of Pathology. One of Erdheim's collaborators in Lainz was Friedrich Feyrter (1895-1973) who later established the concept of the diffuse neuroendocrine

system. US academic guest William Chester (1903-1974) 1931 published a rare variant of histiocytosis, later eponymized by H. L. Jaffé as Erdheim-Chester-disease. Erdheim also made major contributions to vascular pathology, describing in depth idiopathic medionecrosis of the aorta (1929), completing O. Gsell's 1928 first description, and, as a leitmotif, to bone pathology. In Lainz, temporal bone pathologist Otto Mayer (1876-1936), with his callus theory (1931) perfected Adam Politzer's (1835-1920) magnum opus on otosclerosis. Lainz radiologist August Schönfeld (1874-1936), pioneer of radiation protection, like Erdheim had been trained under Weichselbaum. Anonymously but substantially, Jakob Erdheim contributed to Albert Müller-Deham's (1881-1971) autopsy based renewal of modern geriatrics (1937).
Around 1900, endocrinology emerged from multidisciplinary innovation, finally reconciling solidism with humorism. Jakob Erdheim in many ways contributed to this development.

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Session 3: MEN 1 and the pancreas

S3.1
Indication for surgery in MEN1 neuroendocrine pancreatic tumors: Biology

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Multiple endocrine neoplasia type 1 is characterized by tumor involvement of multiple endocrine organs including the parathyroids, pituitary, duodenum, stomach, lung and thymus. Besides this multifocality of the disease MEN1 associated pancreatic neuroendocrine tumors (pNET) are additionally characterized by multifocality in the pancreas. While macroscopically an average of 5 pNET [5] is described in patients undergoing operation, the number of microscopically detectable tumors is much higher [2]. The entire pancreas is involved by multiple microadenomas and monohormonal cell clusters, as well in islets, interstitially and in ducts [6]. These lesions have been known as dysplastic islets [4] but have been shown to be true neoplasms because of the very frequent loss of the second MEN1 allele by fluorescence in situ hybridization [6]. This multifocality explains for the high frequency of local recurrence [5]. Besides these neoplastic lesions hyperplastic changes of islets are aalso observed: these could represent pre-neoplastic changes or be an effect of MEN1 haplo-insuficciency without direct relation to the neoplasms [6]. A similar situation has been described in the duodenum with multiple primary gastrinomas (and fewer somatostatin producing tumors) as well as a hyperplasia of G-cells [1, 2].
These data suggest that most of MEN1 associated pNET remain stable in the state of microadenomas and only very few tumors get to the state of clinical detectability and possibly even less to the state of clinical relevance. It is unclear what mechanisms lead to progression in a subset of pNET, potentially this progression involves secondary mutations such as DAXX/ATRX mutations. In the setting of MEN1 disease, these mutations have been shown to occur exclusively in rare MEN1 associated pNET, and these tumors were larger than 3 cm [3].
In summary it seems that pNET in MEN1 patients develop from multiple clonally unrelated tumors. Only a subset of these tumors will develop the ability to grow to a detectable size and to become clinically relevant, however, we do not understand the underlying mechanism well enough to predict which tumors will undergo progression.

S3.2
Indication for surgery in MEN1 neuroendocrine pancreatic tumors: Size

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Introduction: The management of small non-functional neuro-endocrine tumors of the pancreas in MEN1 patients is still controversial, some authors recommending an aggressive resection as soon as tumors are evidenced, others recommending a conservative approach for NF-PET < 2 cm in the absence of other aggressive features.
Previous investigations from the GTE (Endocrine Tumor Study Group) demonstrated that that the risk to develop metastasis and/or death was very small in MEN1 patients with small NF-PET and that these patients had similar survival than MEN1 patients without NF-PET. However, the follow-up of patients with small NF-PET was only 3.3 years in the non-operated group of 50 patients. We present the long-term follow-up of these patients
Method: MEN1 patients with initially non-operated NF-PET < 2 cm, included in our previous investigations were prospectively followed.
Results: Fifty patients had NF-PET < 2 cm and were initially non-operated. At the end of our previous study (2003), 1 patient had died of thymic tumor and 3 were lost to follow-up leaving 46 patients for long-term follow-up. For these 46 patients, the total follow-up time (from the date of NT-PET diagnosis to end of this study) was 10.3 ± 4.3 years and the additional follow-up (from the end of our previous study to the end of this study) 7.5 ± 3.0 years. Only one patient was lost to follow-up (alive with 4 NF-PET, the biggest of 1.7 cm, after 2 years of additional follow-up), the 45 other patients could be followed. At the end of follow-up, one patient died of metastatic NF-PET at age 67 and one patient died of another cause at age 76. Thirty patients had stable disease after a total follow-up of 9.8 ± 3.8 years, 4 patients had increase in size of their biggest tumor but are still not operated, 1 developed a gastrinoma, one developed an insulinoma but were not operated and 7 were operated upon either because of secretion (1 insulinoma, 1 gastrinoma and 1 VIPoma) or because of increase in size of the tumor. All 7 operated patients are alive without persistent or recurrent disease.
Conclusions: In our study, only 16% of the patients needed surgery for the management of their NF-PET over a follow-up time of 10 years and only 1 patient died of NF-PET. In our opinion, an initial conservative management of MEN1 patients with small NF-PET is feasible and safe and therefore, size is an important indication for conservative versus surgical management of NF-PET in MEN1 patients.

S3.3
Indication for surgery in MEN1 neuroendocrine pancreatic tumors: Molecular genetic aspects

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Pancreatic tumors are responsible for the majority of MEN1-related deaths. Indications for surgery in non-metastatic patients aim either at controlling endocrine secretion or at preventing distant metastatic spread. Patients with insulinoma or rare tumors such as glucagonoma, vipoma or somatostatinoma are good candidates for surgery. Indications for surgery in cases of Zollinger-Ellison syndrome have remained a matter of debate. Indeed, the risk of metastatic spread does not depend on the size of the gastrinoma. In most cases, these tumors are small, multiple and located in the duodenum but difficult to localize. Various surgical techniques have been proposed but none have shown any proven advantage in terms of survival when compared with a conservative medical approach. There are still no determinant pejorative prognosis factors that could be used as an indication for surgery in patients at risk for metastases. Non-secreting pancreatic tumors are frequently associated with synchronous metastases when the size of the tumor reaches 2 centimeters. Nevertheless, metastases may also occur in smaller tumors. Consequently, there is no clear-cut attitude regarding indications for surgery when the tumor is small. The previous presentation demonstrated that a conservative approach is possible in cases of tumors smaller than 2 centimeters. Other parameters may also be taken into account such as rapidity of growth, histological pattern when available, the patient's health status or other associated indications for surgery. Recently published genetic data have shown that MEN1 mutations in the JunD interacting domain increases tumor aggressiveness and decreases survival in MEN1 patients. Another study demonstrated that belonging to the O blood group increases the risk of death from MEN1 pancreatic neuroendocrine tumor. Unfortunately, no multivariate analysis is available to assess the relative impact of these genetic factors among other already known prognosis factors. Therefore, they cannot be used as primary decision-making criteria for surgery. Nevertheless, using them when indications for surgery are highly debatable makes sense.

S3.4
Resection (Thompson procedure) or total pancreatectomy, asymptomatic tumors

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MEN-1 is an autosomal dominant inherited syndrome, with significant variability in its clinical expression. It is classically characterized by tumors of the parathyroid glands, pancreatic islets, and anterior pituitary gland; with detailed study it is now known that other tissues are often involved also, including thymus and bronchi (carcinoids), subcutaneous fat (lipomas), thyroid gland, adrenal glands and skin. The disease is characterized by near complete penetrance and variable expressivity.
Management of the pancreatic disease can risk both complications from treatment and progression of disease. While this syndrome can seem to provide a confusing series of possibilities and diseases, separating the patient management into the management of functional endocrine tumors and the management of the oncologic aspects of tumor growth can help to clarify the appropriate steps in patient care.

The Decision to intervene
The most controversial issue in MEN-1 management is probably the decision to intervene in the pancreatic disease. All macroscopic tumors of the MEN-1 pancreas are potentially malignant, and neither tumor size nor radiological findings or peptide production can safely be used as markers of malignancy. Pancreatic endocrine lesions develop during the third decade, and time may be an important risk factor for mutagenetic events essential for malignant transformation. If intervention can be performed safely, the prevention of death from metastatic islet cell tumor requires pancreatic surgery before frank clinical syndromes occur, in relatively young MEN-1 patients, based on active surveillance programs.

Surveillance
Surveillance for pancreatic endocrine tumors in patients with the MEN-1 mutation is a critical part of the management of adult patients. The options for surveillance include (1) hormone measurements; (2) cross-sectional imaging (3) somatostatin-receptor scintigraphy, and (4) endoscopic ultrasonography. Although pancreatic endocrine tumors can occur in people with an MEN-1 mutation during childhood, they are almost always insulinomas that declare themselves by symptoms. Surveillance for these tumors is not necessary until adulthood.

Operative management
The operative management must be individualized for each patient based upon his or her pattern of disease. The principles are complete tumor resection and preservation of pancreatic function, by preserving as much grossly normal pancreas as possible, while minimizing the morbidity of the procedure. In practice, this often results in the subtotal resection of the distal pancreas, and enucleation of tumors in the head of the pancreas and duodenum. This operation removes the gross disease in the distal pancreas, while preserving most of the pancreatic mass in the head, and avoiding the need for a pancreatic anastamosis. For patients with bulky disease in the head of the pancreas, however, which may not be amenable to enucleation, a better option may be pancreaticoduodenectomy, and enucleation of any tumors in the tail. For all patients with gastrinoma, the duodenum should be opened and submucosal tumors resected. Occasional patients may have disease that can be completely removed without formal pancreatic resection. Total pancreatectomy is rarely indicated, or necessary, to meet the goal of complete tumor resection.
Regardless of which strategy is initially employed, the fact that the pancreaticoduodenal disease of MEN-1 is multifocal and progressive has important implications for long-term outcomes. This issue is amplified by the strategy of early detection and early treatment which implies patients have their initial pancreatic operation earlier in life than if they were to be observed until the development of locally advanced disease. Should PETs recur in the pancreatic tail after a pancreaticoduodenectomy, reoperation would involve difficult exposure for enucleation or takedown of the pancreaticojejunostomy for completion pancreatectomy. Should PETs recur in the pancreatic head after the Thompson operation, then repeat enucleation or completion pancreatectomy and duodenectomy would be required without the need to construct a pancreaticojejunostomy. Neither scenario is simple and each requires a careful understanding of both the disease and the operative risks.

Conclusions

Pancreatic endocrine tumors in MEN-1 are the most likely cause of death in gene carriers. Strategies for identifying disease and managing it effectively include integrated screening programs using biochemical and image-based approaches. Operative resection should be considered for any patient with imageable tumor, with the main goals of the operation being the complete resection of grossly evident tumor, and the preservation of pancreatic function. Reoperations to manage the disease over the patient's lifetime must be tailored to both the patient and the disease.

S3.5
Surgical concepts of MEN1 gastrinoma

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The majority (~80%) of MEN1 gastrinomas occur in the duodenum and about 70-80% of MEN1 gastrinomas are malignant. The management of MEN1-gastrinoma is controversial and controlled randomized data are missing. Most groups recommend an operative approach only, if the tumor reaches 2-3 cm in size, since the risk for distant metastases then increases significantly. Conversely, we and others recommend surgery, if the biochemical diagnosis of ZES is unequivocal to decrease the probability of malignant spread provided a diffuse metastatic disease has been excluded by imaging.
Despite the timing of surgery, the extent of surgery is another controversial issue in MEN1-gastrinoma. This is especially true for duodenal gastrinomas, since it is considered an incurable disease. Most experts suggested that pancreatic gastrinomas should be either enucleated, if technically feasible, or removed by a formal pancreatic resection, both combined with a systematic lymphadenectomy. The most common operation for duodenal gastrinomas includes duodenotomy with excision of duodenal wall tumors, enucleation of any tumos of the pancreatic head, peripancreatic lymph-node dissection with (Thompson-procedure) or without distal pancreatectomy However, this procedure rarely provides cure with at most 30% of patients demonstrating a negative secretin test 5 years postoperatively. Therefore, we and other groups have chosen partial pancreaticoduodenectomy (PD) as the first line procedure for duodenal MEN1-ZES. There are several reasons for this strategy. First, since about 90% of MEN1-gastrinomas arise in the duodenum and proliferative gastrin cells in the normal duodenal mucosa are the precursors of these tumors, long-term cure is only possible, if the organ of origin is removed given the inherited predisposition. Second, PD allows radical treatment of the peripancreatic nodal lesions and of PNENs possibly prevalent in the head of the pancreas, thus clearing the whole gastrinoma triangle. Third, cephalic macroscopic PNENs can also be treated by PD by extending the procedure to include part of the pancreatic body. Fourth, a simple enucleation can be added to PD, if PNENs are found more distally in the pancreatic tail. Small cases series with 3 to 13 patients evaluating PD as initial procedure for MEN1-ZES showed a high rate of biochemical cure after median 3 years ranging from 77%-92%.
Imamura et al. recently described the pancreas preserving total duodenectomy (PPTD) as an alternative surgical procedure for duodenal MEN1-ZES. Five of 7 patients who underwent this procedure were biochemically cured after 2 to 6 years. The authors stated that PPTD is a less invasive procedure than PD with no complications in their series. However, they also noted, that dissection of regional lymph nodes may be incomplete by PPTD compared to PD. This might be the reason for the fact, that 2 of their 7 patients developed ZES recurrence due to liver or distant lymph node metastases within 3 years postoperatively. Based on these data the value of the PPTD for the treatment MEN1-ZES can yet not be determined.
Total pancreaticoduodenectomy is considered an overtreatment for MEN1-ZES by most experts, since the resulting briddle diabetes causes significant long-term morbidity. The procedure should be restricted to rare conditions such as duodenal ZES with diffuse large PNENs throughout the whole pancreas.

S3.6
Clinical features and surgical management of insulinomas

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Insulinoma is the most frequent functioning pancreatic endocrine tumor occurring in at least 15% of patients harbouring the MEN-1 mutation. Clinical features of MEN 1 insulinomas differ from sporadic counterpart in : 1) an earlier onset of symptoms; 2) the presence of primary hyperparathyroidism, anterior pituitary tumors or other functioning or non-functioning PNET; 3) possibility that hypoglicemia could be either controlled by the secretion of hyperglicemic hormones (i.e. increased GH by a pituitary adenoma) or worsened by hypoglicemic hormones (i.e. decreased ACTH secretion). Pathological characteristics of MEN 1 insulinomas are: 1) presence of multiple pancreatic micro- and macro-adenomas; 2) high probability of multiple pancreatic insulinomas (up to 30%); 3) a diameter inferior to 1 cm in at least 50% and inferior to 5 mm in at least 20% of the insulinomas; 4) a benign behaviour even if local invasion or metastasis originating from other PNETs can be present at surgery. The investigative procedures for localizing the MEN 1 insulinomas cannot be similar to those adopted for discovering the sporadic insulinoma. EUS is the most sensitive preoperative imaging technique either for pancreatic lesions as small as few mm in diameter, but cannot distinguish insulinoma from other PNETs. Identification of the site of the MEN 1 insulinomas could be possible by percutaneous portal venous sampling or by intra-arterial calcium stimulation test with hepatic sampling for the dosage of insulin and C-peptide, but the experience in MEN 1 patients are few and sometime can result in misleading informations. The EUS/FNA can be useful in localizing the insulinoma by immunohistochemestry of the biopsies. The histopathological characteristics of MEN 1 insulinomas make the treatment troublesome. For example it is still source of doubts if an insulinoma of only few mm in diameter is responsible of the preoperative, persistent or recurrent neuroglicopenic symptoms and how ascertain it. The potential multiplicity of the insulinoma in MEN 1 makes difficult the surgical procedure that could be to operate only the large tumor or to remove any detectable lesion. In contrast to sporadic insulinomas in whom simple pancreatic enucleation is usually curative, a conservative procedure in MEN 1 insulinomas failed most of the time. The main reason of the failure is the presence of residual insulinomas undistinguishable from other PNETS in preserved pancreas. The more adopted surgical treatment is the resection of pancreatic body and tail associated to the enucleation of macroadenoma eventually present in the pancreatic head. We personally choose to resect the most affected part of the pancreas through either duodenopancreatectomy or distal pancreatic resection on the basis of pre- and intra-operative assessment. Against enucleation it is also the possible risk of pancreatic duct injury when the lesions are deep within the pancreatic

head. However, enucleation can offer a valid alternative to resection, as a substantial part of the pancreas can be preserved decreasing the risk of post-operative diabetes. Today, intraoperative serum dosage of insulin or of insulin/glycaemia ratio can be useful to guide the extension of the pancreatic resection and to confirm the success of surgery. All these issues will be presented and discussed in this paper.

S3.7
Is laparoscopic surgery in patients with neuroendocrine pancreatic tumors associated with multiple endocrine neoplasia type 1 adequate?

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Surgery remains the only curative modality currently available for resectable NETs. The majority are located predominantly in the body and tail of the páncreas. This localización makes NETs suitable for the láparoscopic approach. Surgical management varíes with tumor type location and size. Small NETs may be treated with enucleation. Other functioning and non-functioning NETs associated to MEN1 should be managed with extended pancreatic resection including peripancreatic lymph node dissection. Some patients require repeated láparoscopic surgery. A variety of studies have confirmed that láparoscopy is associated with short hospital stay, minimal blood loss and few wound complications.

LS 1: Lunch Symposium: Medical treatment of advanced neuroendocrine pancreatic neoplasia

LS1.1
Molecular Subtypes of Pancreatic NETs and their Distinct Phenotypes

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Abstract not available.

LS1.2
Somatostatin analogs - what do we learn from the current clinical trials?

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Somatostatin (SST) analogues are widely being used for symptomatic control in patients with ectopic hormone/peptide production and have become a mainstay of treatment in patients with secretory NETs of various origins. A recent survey of clinical practice has shown a high rate of SST-use in patients with pNET irrespective of function, with 74% of all patients being given SST-analogues in the course of their disease. Interestingly, SST-analogues are being more likely used upfront (59% of patients at baseline), while treatment at progression was initiated in 47% of patients. As opposed to metastatic midgut NET, where the PROMID study has suggested a benefit in terms of time to progression (TTP) for therapy with long acting octrotide (OCT LAT) over placebo, this has not been substantiated by clinical data until recently in terms of antiproliferative efficacy. A retrospective analysis of 43 patients with metastatic pNET (19 functioning, 24 non-functioning) treated upfront with long acting octreotide (OCT LAR) published in 2013 has suggested OCT LAR as effective first line therapy in such patients. In this series, the median overall survival (OS) was 94 months (range; 14 - 216), with the median TTP being 13 months (range; 2 - 51). In keeping with prior data, a low rate of objective responses was reported with 3 patients showing partial remission according to RECIST criteria, while 25 patients had stable disease, resulting in a disease control rate of 65%. In this analysis, neither the length of disease duration before administration of OCT LAR nor remission status (SD, progressive disease or unknown) had an influence on the efficacy of treatment. In this analysis, patients with a Ki67 < 10% were shown to derive the maximum benefit from treatment when compared to patients with higher proliferative activity.
The recently completed CLARINET-trial (Controlled Study of Lanreotide Antiproliferative Response in Neuroendocrine Tumors) assessing the antiproliferative activity of lanreotide (LAN) autogel versus placebo in advanced non-functioning NETs is the largest randomized study conducted so far, and has included NETs of mid- and hindgut origin, but also pNET. In this study, patients were required to be treatment-naive, and all had to have a Ki-67 < 10% and a positive somatostatin-receptor assessment in vivo. Taken together, a highly significant prolongation of PFS was documented for the whole cohort of patients (18 months versus not reached in the LAN-chort), with an estimated rate of PFS at 2 years being 65% versus 33%. While the study was not powered for subgroup analyses, the large number of patients with pNET (n = 91) has prompted assessment of this cohort of patients. Interestingly, almost all patients had stable disease according to RECIST criteria at study entry, and 42 pNET patients were randomized to treatment with LAN autogel 120 mg every 4 weeks for a maximum of 2 years, while 49 patients were included on the placebo arm. In the LAN cohort, 18 events were seen in 42 patients as opposed to 31 in 49 placebo-patients, and the PFS was 12.1 months (CI:9.4 - 18.3) for the placebo arm, while it was not reached in the LAN-group. While the difference failed to reach statistical significance (in a trial that was not powered to detect such a difference), there was a clear trend with the hazard ratio being 0-58 (CI: 0.32 - 1.04) in favor or the therapy arm.
Taken together, these recent data demonstrate that SST-analogues are active in the treatment of patients with pNET and are able to slow tumor-growth with a favourable toxicity profile. As expected, however, the rate of objective remissions in terms of tumor regression were low, but a high rate of disease stabilisation could be achieved both in patients with progressive disease as well as stable disease at therapy onset. In view of this, the optimal timing of initiating therapy remains to be defined in further trials, though current data from the prospective trial suggest that early initiation of therapy might be beneficial. As no formal comparison with the targeted agents currently approved for therapy of progressive pNET has been performed, the optimal role as well as timing of SST analogues remain to be determined.

LS1.3
Sunitinib in the treatment of advanced pancreatic neuroendocrine tumors - current data

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Abstract not available.

Further information:

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N Engl J Med. 2011 Feb 10; 364(6):501-13. doi: 10.1056/NEJMoa1003825.

LS1.4
Everolimus in advanced pancreatic neuroendocrine tumors: the clinical experience

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The age-adjusted incidence of pancreatic neuroendocrine tumor (pNET) has risen from1 - 2 per million in the 1970s to 5.6 (95% CI, 5.1 - 6.2) per million in 2010.[1, 2] The prevalence of smaller pNETs in the general population, however, may be even higher. In an analysis of 11,472 autopsies performed at a Hong Kong hospital, pNETs were found in 0.1% of all cases.[3] This suggests majority of small asymptomatic pNETs carry a benign course and will remain clinically silent during the patient's lifetime. Advanced pNETs are incurable. While they follow a more indolent course, than pancreatic adenocarcinoma, most patients with metastatic disease will ultimately perish from the disease.
Recent advances have significantly improved our understanding of the molecular biology underlying pNETs. Genetic cancer syndromes associated with pNETs include multiple endocrine neoplasia type 1, tuberous sclerosis, neurofibromatosis, and von Hippel Lindau syndrome.[4] Tuberous sclerosis complex (TSC) is the endogenous inhibitor of mTOR. Loss of neurofibromatosis 1 gene also leads to constitutive mTOR activation. Exome-sequencing of sporadic pNETs identified three major groups of mutations involving MEN1, DAXX/ATRX, and the mTOR pathway.[5] Low protein expression of mTOR pathway components, TSC2 and PTEN, was associated with shortened PFS and OS among patients with pNETs.[6] Recent data also suggest that menin, the protein product of MEN1 gene, may also regulate the mTOR pathway through AKT.[7] These suggest that mTOR pathway dysregulation to be important in the development and malignant progression of pNETs.
Clinically, three human studies including 600 patients have evaluated the efficacy of oral mTOR inhibitor, everolimus, in pNET.[8-10] These studies have provided consistent data for the ability of everolimus to delay progression among patients with advanced pNET.

Proof-of-concept phase II study
The first of these studies is a single institution phase II study conducted at MD Anderson Cancer Center, patients with advanced pNETs with or without progression were treated with octreotide LAR 30 mg intramuscularly (IM) every 4 weeks along with everolimus at 5 or 10 mg orally (PO) daily (18). Among 30 evaluable patients with pNETs, a response rate of 27% (according to RECIST) and a median PFS of 11.6 months (95% CI, 7.1–16.1) were observed.[8] From the larger sample of 60 patients, which also included an additional 30 patients with carcinoid tumors (i.e., non-pNETs), a higher response rate was observed among patients treated with everolimus 10 mg daily (30%) compared with everolimus 5 mg daily (13%).
Evaluation from paired biopsy specimens showed that everolimus slowed tumor proliferation as demonstrated by reduced Ki-67 labeling.[8] In this study we also demonstrated that treatment with everolimus was associated with a decrease in p-S6 S240/244 (P = 0.003 compared to pre-treatment baseline), p-S6 235/236 (P = 0.02), and an increase in p-Akt. However, patients with RECIST response after everolimus were more likely to have an increase in p-Akt T308 from baseline than patients who did not achieve a radiologic response (P = 0.01).[11] Thus, we demonstrated that increase in p-Akt, which is an expected consequence of successful mTOR inhibition and p-S6 downregulation, does not preclude clinical benefit.

RADIANT-1: Global phase II studies in pNET progressive after cytotoxic chemotherapy
RADIANT-1 was an open-label phase II study that enrolled 160 patients from 11 countries. One hundred and fifteen patients who were not being treated with octreotide at study entry were assigned to stratum 1 (everolimus 10 mg PO daily), and 45 patients who were on octreotide LAR were assigned to stratum 2 (everolimus 10 mg PO daily and octreotide LAR IM every 28 days at prestudy dose [≤30 mg]) (19). Response rate was 9.6% (95% CI, 4.9% – 16.5%) for patients receiving everolimus and 4.4% (95% CI, 0.5% - 15.1%) among patients receiving everolimus plus octreotide LAR.[9] Median PFS by central radiology review was 9.7 months (95% CI, 8.3–13.3) for those receiving everolimus and 16.7 months (95% CI, 11.1–NA) for those receiving the combination of everolimus and octreotide LAR.
High baseline chromogranin A and neuron-specific enolase were prognostic of poor outcome.[12] Early biomarker response defined as a 30% decrease at week 4 from baseline was associated with improved PFS.

RADIANT-3: Pivotal placebo-controlled phase III study
RADIANT-3 enrolled 410 patients from 18 countries. Patients with advanced pNET and progressive disease were randomly assign to treatment with everolimus or matching placebo.[10] The use of somatostatin analogues was allowed in both arms as supportive care. The study demonstrated clinically and statistically significant benefit in PFS for patients receiving everolimus. Everolimus prolonged median PFS from 4.6 months (95% CI, 3.1 – 5.4) to 11 months (95% CI, 8.4 – 13.9) leading to a 65% risk reduction for progression compared to placebo (HR=0.35; 95% CI, 0.27 – 0.45; p <0.0001). Treatment also reduced the level of tumor-secreted hormones.

Real word management of pNET patients with everolimus
Over the past five years, three landmark phase III studies have brought new treatment options for patients with pNET. FDA approved agents include streptozocin, everolimus, and sunitinib. Recently, the somatostatin analogue, lanreotide, has also demonstrated significant anti-proliferative activity in a phase III study.[13] Surgical resection, regional therapy, and therapies not yet approved including peptide receptor radiotherapy, selective internal radiotherapy, and temozolomide offer additional options. There are no data yet to guide the optimal sequencing of therapy.
The choice of therapy at each decision point should take into account the aggressiveness of the tumor, the burden of disease, and any symptoms due to tumor burden or hormonal secretion. For example, for a patient with low volume, stable, and asymptomatic disease, observation or somatostatin analogues may be appropriate. Cytotoxic

chemotherapy may offer relief to a patient with bulky, progressive, and symptomatic disease. Everolimus or sunitinib can be suitable options for most patients in between the two extremes. The choice between everolimus and sunitinib can be considered based on the strength of published evidence, secretory status, and the matching of patient co-morbidities to the adverse event profile of the drug. For example, everolimus has been more extensively studied among treatment naïve patients and patients who have failed prior chemotherapy. It may also be preferred among patients with secretory (functional) tumors.
The most common, and often troublesome, adverse event experienced by patients receiving everolimus is apthous ulceration or stomatitis. In a recent meta-analysis of seven phase III studies, stomatitis within 8 weeks of starting therapy was associated with greater PFS benefit from everolimus therapy.[14] Similarly, in a separate meta-analysis of everolimus trials in oncology, higher blood trough level was also associated with lower risk of disease progression.[15] Together with phase II data showing better outcome for patients on 10 mg of everolimus instead of 5 mg, these studies demonstrate dose dependent activity of everolimus in pNET. Appropriate management and supportive measures for adverse events such as aphthous ulceration including the use of topical steroids and analgesics along with dose reduction where appropriate will help to ensure best outcome.
Additional analyses including germline genetic polymorphism and tumor exome sequencing of patients treated with pNET are underway to identify biomarkers of benefit. A placebo-controlled phase III study in the broader group non-functional NETs (RADIANT-4) has completed accrual and will determine the role of everolimus in non-functional NET originating from thorax and gastrointestinal tract.

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L2: Jakob Erdheim Honorary Lecture

L2.2
Monoclonality, polyclonality, and coupling in MEN1 and other endocrine tumors

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Background
Two of Jacob Erdheim's major research contributions were the recognition that parathyroidectomy caused thinning of bone (1), and that the parathyroids were enlarged in osteomalacia (2). These were several of the earliest insights that the parathyroids were involved in calcium/bone metabolism. Later studies progressed to primary hyperparathyroidism, secondary hyperparathyroidism, clonality, and other tumorigenic processes.
Secondary hormonal tumors are considered polyclonal (2). The originating stimulus acts from outside the cell and acts upon all the hormonal cells of the tissue. For example, in the parathyroids the extracellular stimulus to secondary proliferation may be hypocalcemia, low calcitriol, antibody to the extracellular calcium-sensing receptor, lithium, or radiation.
Primary hormone-secreting tumors, whether beginning in the germline or in somatic tissue, are now believed most to reflect an intracellular initiation in one or few tumor precursor cells and development after the initiating monoclonal or oligoclonal mutation (2). The initiating mutation confers growth advantage as, for example, within *MEN1, RET, VHL* or an SDH gene. Following initiation, the development involves years of silent growth advantage in the abnormal tissue. Eventually hormone excess becomes a major expression. Such a tumor, if benign, can be treated by excision, often leaving a remnant of normally-functioning hormonal tissue.
Primary hormonal tumor without increased proliferation (i.e. without monoclonality) is examined here but is rarely considered. One reason is that there are not good methods to establish polyclonality of tumor initiation for a primary process. Contamination by normal (polyclonal) tissue is difficult to exclude from specimens. Such a tumor is diffuse, polyclonal, and uncoupled. Uncoupling is defined here as an excess of hormone secretion without parallel increase of proliferation in the hormonal tissue.

Methods
I examine three selected syndromes and determine shared features that reflect their likely polyclonal basis. The syndromes are:
-familial hypocalciuric hypercalcemia (FHH).
-congenital diazoxide resistant hyperinsulinemic hypoglycemia (CDRI) and
- primary hyperaldosteronism type III with *G151EKCNJ5* mutation (HA-IIIb).
The central data are traditional indices of clinical expression and more recent indices of genetics, including mutation and molecular function by pathways.

Findings and conclusions
Important features by syndrome
FHH accounts for about 2% of all primary hyperparathyroidism (4). The hypercalcemia begins near birth, is usually asymptomatic, and is consistent with a long lifespan of continuous hypercalcemia. Two series showed that the parathyroid glands' average size is normal (5, 6). Subtotal parathyroidectomy is followed by persistence of hypercalcemia within 7 days (7). The cause is usually a mutation in the *CASR , GA11,* or *AP2S1* gene (8, 9, 10).
CDRI is the most frequent cause of neonatal hypoglycemia. Subtotal removal of the pancreas is followed by persistence or by insulin deficiency. Islet and particularly beta cell histology and size are mainly normal, but there is a variable increase in size of beta cell nuclei (11). The main cause is mutation in a gene for either of two subunits of the islet potassium channel (*ABCC8* encodes SUR1; or *KCNJ11* encodes Kir6.2) (12). Variants with later onset age, diazoxide responsiveness, and less severe, nonsyndromal hypoglycemia have been attributed to mutation the gene for any of 7 enzymes involved in glucose metabolism in the islet.
HA-IIIb shows early onset hyperaldosteronism with good response to spironolactone (13). Only 9 cases in 3 families have been identified so far. Normal sized adrenal cortex has been deduced from bilateral adrenalectomy in one case and from CAT scans in 6 cases. The cause is the same K+ channel mutation in each family,*KCNJ5 G151E. KCNJ5 G151R*, a different mutation of the same codon, is associated with increased proliferation in adrenal cortex, a

later onset, and milder hyperaldosteronism. A sporadic variant has been reported in two infants with
severe neurologic deficits; both had germline mutation of *CACNA1D*, the major plasma membrane calcium channel of the adrenal glomerulosa (14).
Shared features differ from features of a monoclonal tumor and characterize these three syndromes (with comment beneath each feature):
(1) Onset of hormone excess as early as near birth
The mutated tissue is sufficient in development and in mass to cause hormonal excess at or near birth (i.e. without a long interval of tissue growth).
(2) Resistance to remission from subtotal excision
Even a small remnant can sustain hormone excess. This points to secretory dysfunction.
(3) Hormonal tissue shows diffusely normal size and histology
This points to secretory dysfunction since a proliferative dysfunction would be simple to recognized by histology. The selective excess of secretion is uncoupled from any accompanying excess of proliferation.
(4) Hormonal tissue is neither nodular, adenomatous, nor malignant
The principal defect does not cause proliferative disturbances.
(5) Initiation from mutation in germline
With mutation in all hormonal cells of the tissue, over-expression may occur at early age.
(6) Same mutation does not progress to monoclonal tumor in germline or somatic tissue
This mutation does not have potential to cause additional mutations of a benign or malignant type and not in germline or somatic tissue
(7) The mutant gene is in the pathways from sensing of metabolite (calcium, glucose, potassium)
to secretion of the hormone regulating that metabolite
Pathway location is appropriate for secretory more than proliferative over-function (Table) (15)

TABLE. Likely functions of proteins, whose germline mutation is expressed as uncoupled secretory excess in a hormonal tissue.

Syndrome	Metabolite in Serum	Hormone	Mutated gene by likely function of its protein		
			Sensor of Serum Metabolite	Transducer of Serum Metabolite	Secretor of Hormone
Familial hypocalciuric hypercalcemia	Calcium	PTH	*CASR*	*GA11 AP2S1*	
Congenital diazoxide resistant hyperinsulinemic hypoglycemia	Glucose	Insulin			*ABCC8 KCNJ11*
Congenita hyperaldosteronism type IIIb (with *KCNJ5 G151E*)	Potassium	Aldosterone	*KCNJ5 G151E*		*CACNA1D*

These seven specific features establish a paradigm of hormone excess uncoupled from proliferation. Stated differently, the hormonal tissue has a dysfunction that is mainly in its hypersecretion, without a similar excess of proliferation. The evidences for polyclonality of the uncoupled secretory tissue are clinical, indirect and multiple. They include the mutation being in
the germline, the histologically diffuse involvement in the secretory tissue, and the lack of trend to progress to monoclonal lesions. Polyclonality alone does not cause hypersecretion. Even completely normal tissue is also polyclonal. The uncoupled hypersecretion must be an expression of the germline mutation. And other mutations such as in *R-TSH* in neonatal thyrotoxicosis might cause a polyconal excess with a more balanced increase in proliferation.
The uncoupled paradigm, centered in hormone secretory tissue, is unusual and has important differences from the standard monoclonal paradigm, at the clinical and molecular level (above). Furthermore, other genes and other syndromes with this previously unrecognized paradigm are likely to be identified.

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Session 5: MEN 2 and rare RET mutations / Medullary thyroid cancer

S5.1
Medullary thyroid cancer - The clinical case discussion - Clinical data

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Abstract not available.

S5.2
Medullary thyroid cancer - Cytological, biochemical and molecular findings

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The *RET* genetic screening is the most specific and sensitive test to identify subjects at risk to develop a hereditary medullary thyroid cancer (MTC). The *RET* gene exons 5, 8, 10, 11, 13, 14, 15 and 16 should be analyzed in all first degree family members of an index case using PCR and direct sequencing. When a germline mutation of *RET* gene is found the subject (i.e. gene carrier, GC) should undergo a series of clinical and biochemical evaluation to verify if the MTC is already present or not yet[1]. Many different *RET* gene mutations have been described so far and the majority of them have been demonstrated to be causative of MTC. Some new and rare *RET* germline mutations have been also found in the

constitutive DNA of some MTC patient, mainly apparently sporadic cases. Not all of these mutations are able to induce in vitro cell tumoral transformation and appear devoid of any tumorigenic activity. These *RET* variants of unknown significance (VUS) should be considered as non causative of hereditary MTC and no further analysis should be performed in these subjects: still remains the question of their biological significance[2].
The most frequent clinical manifestation of a hereditary medullary thyroid cancer (MTC) is a thyroid nodule. In this case a fine needle aspiration (FNA) of the nodule should be performed for both the cytological diagnosis, that could be improved by calcitonin immunocitochemistry, and the calcitonin (Ct) measurement in the wash out of the needle used for the FNA[3]. However, in many GC, when the genetic screening is early performed, it is likely that the thyropid nodule(s) is not yet present. In these cases, the measurement of serum Ct becomes a fundamental diagnostic procedure. If the serum Ct is in the normal range and up to 40 pg/ml (or < institutional cut-off, i.e. 20 pg/ml in the German series and 60 pg/ml in the Italian series) a microscopic MTC and/or C-cell hyperplasia (ICC) will be present at the histology[4-6]. No cases with central neck node metastases have been found in GC with these low serum level of Ct. These cases can be submitted to total thyroidectomy but they can avoid the central neck dissection that is mandatory when basal Ct is > than the institutional cut off. Of course the type of *RET* mutation is very important to plan the follow up of GC since they have different transforming activities with some of them (i.e. 918 and 634) being highly and rapidly transforming and others (i.e. 768, 791 and others) being very low and slowly transforming thus inducing the tumoral appearance in the adult life.

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S5.3
Medullary thyroid cancer - Functional Imaging

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Functional imaging with different tracers is not recommended for routine initial screening of medullary thyroid carcinoma (MTC) metastases in patients with a fine needle aspiration biopsy and/or calcitonin levels suggestive for MTC, but it may have a role in revealing MTC recurrences (both in sporadic and inherited forms of MTC) which are often difficult to detect using conventional imaging methods.
In particolar several PET tracers evaluating different metabolic pathways have been used for detecting MTC recurrence: the most used are fluorine-18 fluorodeoxyglucose (FDG) for glucose metabolism, fluorine-18 dihydroxyphenylalanine (FDOPA) for bioamines metabolism and somatostatin analogues labelled with Gallium-68 (Ga-DOTA-peptides) for somatostatin receptor expression.
FDG is a glucose analogue and the most used PET tracer in oncology because tumours usually have increased glucose metabolism. A recent meta-analysis demonstrated that the detection rate of FDG-PET or PET/CT in suspected recurrent MTC on a per patient-based analysis was 59%. Even if this detection rate is non-optimal, FDG-PET/CT could modify the patient management in a certain number of recurrent MTC because this method is often performed after negative conventional imaging studies. The detection rate of MTC lesions using FDG-PET/CT increases in patients with higher calcitonin and carcinoembryonic antigen (CEA) values and shorter calcitonin and CEA doubling times, suggesting that this imaging method could be very helpful in patients with more aggressive disease.
DOPA is an amino acid that is converted to dopamine by aromatic amino acid decarboxylase (AADC). FDOPA is taken up through ubiquitous transmembrane amino acid transporter systems that are significantly upregulated in neuroendocrine tumours, including MTC. Furthermore increased activity of metabolic pathways involving the enzyme AADC is a specific property of neuroendocrine tumours. A recent meta-analysis demonstrated that the detection rate of FDOPA-PET or PET/CT on a per patient and a per lesion-based analysis was 66% and 71%, respectively. These detection rates significantly increase in patients with higher calcitonin levels and shorter calcitonin doubling times.
Neuroendocrine tumours usually overexpress somatostatin receptors on their cell surface and this represents the rationale for using somatostatin analogues for diagnosis and therapy of these tumours. The experience with Ga-DOTA-peptides in MTC is quite limited. A recent study comparing FDOPA, FDG and Ga-DOTA peptides PET/CT in recurrent MTC showed a significantly lower sensitivity of Ga-DOTA-peptides PET/CT (33%) compared with FDOPA-PET/CT (72%). Other studies reported a complementary role of Ga-DOTA-peptides PET/CT compared with FDG-PET/CT in recurrent MTC. However, Ga-DOTA-peptides PET/CT could be a useful method in selecting patients suitable for consideration of targeted therapy using radiolabelled somatostatin analogues to treat metastatic lesions showing a high expression of somatostatin receptors.
In conclusion, to date, FDOPA seems to be the most useful PET tracer in detecting recurrent MTC based on rising levels of tumour markers. FDG-PET/CT and bone scintigraphy could be performed if FDOPA is not available. Anyway, the different PET tracers reflect different metabolic pathways and seem to show a complementary role in detecting recurrent sporadic or inherited MTC.

S5.4
Medullary thyroid cancer - Surgical perspectives

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Abstract not available.

S5.5
Medullary thyroid cancer - Medical perspectives

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See abstract S7.2 – page 17.

S5.6
New developments in the guidelines for management of MEN 2 and medullary thyroid carcinoma

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The American Thyroid Association (ATA) decided to update the first guidelines for the diagnosis and management of patients with MTC [1]. The current abstact focusses on some changes updated in the first revision of the original guidelines [2].

Two MEN2 syndromes (R, recommendation 2) There should be two MEN2 syndromes: MEN2A and MEN2B. Within MEN2A there should be four variants: Classical MEN2A (represented by the uniform presence of MTC and the less frequent occurrence of PHEO, or HPTH, or both), MEN2A with cutanous lichen amyloidosis, MEN2A with Hirschsprung's Disease, and FMTC (families or individuals with only MTC).

Three risk groups of RET mutations (R1). The current ATA risk categories should be changed. The current risk levels A and B should be combined into a moderate (MOD) risk category. The current risk level C should be changed to a high (H) risk category, and the current risk level D (with the exception of the A883F mutation and double mutations involving codon 804) should be changed to the highest (HST) risk category

Children in the ATA-HST category should have a thyroidectomy in the first year of life, perhaps even in the first months of life (R35). Children in the ATA-H category should have US of the neck and measurement of Ctn prior to thyroidectomy. Thyroidectomy should be performed typically at age 5 years, or earlier based on the presence of elevated serum Ctn levels (R36). Children in the ATA-MOD category should have US of the neck and measurement of Ctn prior to thyroidectomy. Timing of thyroidectomy should most often be based on serum Ctn levels, however, 6 month or annual evaluations may extend to several years or decades (R37).

Calcitonin (Ctn) becomes more important. The new Ctn assays are more robust and precise in defining the upper limit of reference range. Over the past decade commercial assays for measuring Ctn have progressed to the newest immunochemiluminometric assays (ICMAs) that are highly sensitive and specific for monomeric Ctn. Current reference ranges for serum Ctn vary with gender, being higher in men than women. Using these systems the 95th percentile for serum Ctn levels to be about 5 to 6.5 pg/mL and about 9 to 10 pg/mL in women and men, respectively. With ICMAs healthy individuals usually have serum Ctn levels less than 10 pg/mL. Some clinical investigators feel that the sensitivity of the ICMA assay is such that provocative testing is no longer necessary, others consider it useful in varying clinical situations.

- Accumulating evidence shows that Ctn levels can safely be used in determinig timing of thyroidectomy in RET carriers at least in the moderate RET mutation risk group. In ATA-H thyroidectomy should be performed typically at age 5 years, or earlier based on the presence of elevated serum Ctn levels. In ATA-Mod category timing of thyroidectomy should most often be based on serum Ctn levels (R36, 37).
- Ctn levels can be used to determine extent of surgery. A central neck dissection should be performed in patients with a serum Ctn level above 40 pg/mL or with imaging evidence of metastatic disease (R36). When preoperative imaging is positive in the ipsilateral lateral neck compartment but negative in the contralateral neck compartment, contralateral neck dissection

should be considered if the basal calcitonin level is greater than 200 pg/mL (R 27).

- Ctn influences surgical aims during follow-up. Postoperatively, the TNM classification and other data, such as the number of lymph node metastases, and the postoperative serum Ctn level are useful to predict outcome and to plan the long-term follow-up of patients (R 46). Patients who have had an inadequate total thyroidectomy and lymphadenectomy should be considered for reoperation to include a compartment oriented lymph node dissection if the preoperative basal serum CTN level is less than 1,000 pg/mL and 5 or fewer metastatic lymph nodes were removed at the initial surgery (R32). If Ctn levels are > 1000 pg/ml and more than 5 LN are positive surgical aim shifts to local control.
- Ctn influences imaging. If Ctn levels are < 150 pg/ml imaging often is negative and therefore: Patients with elevated postoperative serum Ctn levels less than 150 pg/mL should have a physical examination and US of the neck. If these studies are negative the patients should be followed with physical examination and US every 6 months (R48).
- Ctn/CEA doubling times are prognostic parameters and add in decision-making concerning targeted therapy. Patients with elevated postoperative serum Ctn levels should have Ctn and CEA serum levels measured a least every six months to determine the doubling times of the markers (R50). Tumor burden can be estimated from imaging studies and measurement of tumor markers. The growth rate of MTC can be derived from sequential imaging studies using response evaluation criteria in solid tumors (RECIST), which documents specific incremental increases or decreases in tumor size over time. The MTC growth rate can also be determined by measuring serum levels of Ctn and CEA over multiple time points to determine the rate at which the marker levels double. In patients with significant tumor burden and symptomatic or progressive disease according to RECIST tyrosine kinase inhibitors appear to be the most effective treatment modalities (R66).

Pheochromocytoma (PHEO) and Hyperparathyroidism (HPT) Screening for PHEO should begin by age 11 years for children in the ATA-H and ATA-HST categories and by age 16 years in children in the ATA-MOD category. Screening consists of measuring plasma free metanephrines and normetanephrines, or 24-hour urine metanephrines and normetanephrines. Adrenal imaging with CTS or MRI is indicated in patients with positive biochemical results (R38). For practical reasons, patients in the ATA-H and ATA-MOD categories should be screened for HPT at the time of screening for PHEO (R43).

Controversies The panel realizes that expert opinions are mixed regarding the routine use of determining serum Ctn levels in patients with multi-nodular goiters, and therefore recommends that physicians caring for patients with MTC decide whether the technique should be used in their clinic (R20). Controversies exist concerning the use of Ctn stimulation testing in determining the time of thyroidectomy in children who have inherited a mutated *RET* allele, in the evaluation of patients for persistent or recurrent MTC following thyroidectomy, and for detecting MTC in patients with nodular goiters.

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Session 6: New experimental and clinical developments

S6.1

Nuclear RET Increases Survival by Suppression of ATF4-mediated Apoptosis

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The RET proto-oncogene, a tyrosine kinase, is known for its essential role in cell survival. Germ- line missense mutations were found to cause multiple endocrine neoplasia type 2. However, the mechanism of RET functions in control of gene expression and its oncogenic activity remains elusive. Here, we report that activated RET represses the ATF4-mediated apoptotic gene transcription. Knockdown of RET in MTC-derived TT cells induces expression of the ATF4 target proapoptotic genes including *NOXA* and *PUMA,* and increases sensitivity to cisplatin-induced apoptosis. We show that RET physically interacts with and phosphorylates ATF4 at threonine residues, and negatively regulates its transcriptional activity through ubiquitination and degradation. Targeted mutations that block threonine phosphorylation induce the ATF4 stability and activity, resulting in stimulation of the *NOXA* and *PUMA* genes expression. We demonstrate that a dual-drug regimen using a tyrosine kinase inhibitor and an endoplasmic reticulum-associated protein degradation inhibitor that induces ATF4 expression can synergistically induce apoptotic cell death. Finally, low ATF4 expression in medullary thyroid cancer correlates with poor overall patient survival. These data support the development of novel therapies for the treatment of malignancies with RET abnormalities.

S6.2
Molecular profiling of intestinal NET: future application

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Abstract not available.

S6.3
Epigenetic regulation of Hedgehog signaling in MEN1 tumor syndrome

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Multiple endocrine neoplasia type 1 (MEN1), an inherited tumor syndrome, has susceptibility to pancreatic islet tumors. This syndrome results from mutations in the MEN1 gene, which encodes menin protein. While menin interacts with mixed lineage leukemia (MLL) protein, a histone H3 lysine 4 (H3K4) methyltransferase, the precise basis for how menin suppresses gene expression and proliferation of pancreatic beta cells remains poorly understood. We show that menin ablation enhances Hedgehog signaling, a pro-proliferative and oncogenic pathway, in murine pancreatic islets. Menin directly interacts with protein arginine methyltransferase 5 (PRMT5), a negative regulator of gene transcription. Menin recruits PRMT5 to the promoter of the Gas1 gene, a crucial factor for binding of Sonic Hedgehog (Shh) ligand to its receptor PTCH1 and subsequent activation of the Hedgehog signaling pathway, increases repressive histone arginine symmetric dimethylation (H4R3m2s), and suppresses Gas1 expression. Notably, MEN1 disease-related menin mutants have reduced binding to PRMT5, and fail to impart the repressive H4R3m2s mark at the Gas1 promoter, resulting in its elevated expression. Pharmacologic inhibition of Hedgehog signaling significantly reduces proliferation of insulinoma cells, and expression of Hedgehog signaling targets including Ptch1, in MEN1 tumors of mice. These findings unravel a novel link between menin and Hedgehog signaling whereby menin/PRMT5 epigenetically suppresses Hedgehog signaling. Moreover, menin also directly suppresses the activity of Gli1. Collectively, these results also suggest that the dysregulated Hedgehog pathway in MEN1 syndrome serves as a target for treating MEN1 tumors.

Session 7: Treatment of advanced endocrine tumors

S7.1
Treatment landscape of NET

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Abstract not available.

S7.2
Vandetanib - Current perspectives

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Vandetanib is a small molecule multi-kinase inhibitor that inhibits phosphorylation of RET, VEGF2 and EGF receptors. Encouraging results from a phase II studies of hereditary medullary thyroid carcinoma (MTC) led to a phase III study of hereditary and sporadic MTC that demonstrated an objective response rate of 46% vs. 13% in the placebo group and prolongation of progression-free survival from 19.3 to a predicted median of 30.5 months. At the time the study was reported, the survival data were immature and confounded by the cross-over design of the study. There has been no update of the survival data at this writing. As a requirement for approval the manufacturer was required to perform a comparison of vandetanib 300 mg/day with 150 mg/day to determine whether there is comparable efficacy and lower toxicity at the 150 mg/day dose. Although the study was recently completed, analysis of this study has not been completed.
A number of questions remain regarding the optimal use of this effective therapeutic agent. The speaker will attempt, using a case-based approach, to address several of these questions including duration of effect, use of this agent in patients with paratracheal metastasis and long-term tolerability

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S7.3
Cabozantinib - Current perspectives

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Patients with advanced metastatic medullary thyroid cancer (MTC) had no therapeutic options until a couple of years ago when vandetanib was approved by both FDA and EMA,[1,2]. However other tyrosine kinase inhibitors (TKI) have been tested in the last years and cabozantinib (XL184) is one of the most promising. It has been recently approved by FDA and EMA but it is still not worldwide available. Cabozantinib inhibits mainly three tyrosine kinase receptors: MET (hepatocyte growth factor [HGF] receptor), VEGFR2 (vascular endothelial growth factor receptor 2) and RET (REarranged during Transfection). These 3 receptors play important roles in the transformation of C cells and in the progression of MTC. Preclinical and clinical studies have demonstrated that Cabozantinib is able to inhibit tumor angiogenesis, invasiveness, metastatic diffusion and also the progression of bone lesions. The EXAM study (a phase 3 multicentric, randomized, double blind study) showed that the progression free survival time was significantly longer in patients treated with cabozantinib than with placebo (11.2vs 4.0 months, $p< 0.0001$)[2] . The analysis of prespecified subgroups according to the *RET* mutation status or the previous TKI administration showed a significant advantage in patients treatment with cabozantinib with respect to those treated with placebo in each group. Moreover, patients treated with cabozantinib showed a 28% of objective response with respect to 0% in the placebo group
Several side effects (SE) have been developed by patients treated with cabozantinib. The most frequent SE are fatigue, diarrhea, decreased appetite and nausea, weight loss, and palmar-plantar erythrodysaesthesia. However, the majority of them can be reduced or controlled by introducing specific drugs (i.e loperamide for diarrrea) or by reducing the daily dose of the drug[4].
According to these results cabozantinb represents a valid new and alternative therapeutic option for patients with advanced metastatic and progressive MTC.

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S7.4
Current aspects of a personalized treatment approach in advanced adrenocortical cancer

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Adrenocortical cancer (ACC) is a rare tumor with an overall poor but heterogeneous outcome. Genomics studies revealed subtype of adrenocortical tumors with different pattern of molecular alterations associated with different outcome. Recently, exome sequencing and SNP array analysis revealed recurrent alterations in known drivers (*CTNNB1*, *TP53*, *CDKN2A*, *RB1*, *MEN1*) and genes not previously reported in ACC (*ZNRF3*, *DAXX*, *TERT* and *MED12)*. *ZNRF3* is the gene the most frequently altered (1/5). The integration of various genomic analyses (mRNA and miRNA expression profiles; chromosomal and methylation alterations) led to the identification of two distinct molecular ACC subgroups with opposite outcome. One group of poor outcome ACC is characterized by numerous mutations and DNA methylation alterations, whereas the other ACC group with good prognosis display a specific deregulation of two miRNA clusters. Thus, aggressive and indolent ACC correspond to two distinct molecular entities, driven by different oncogenic alterations. This lead to a new vision of adrenocortical tumors classification based on molecular analysis. Following these genomics studies efforts are made to develop new molecular tools for adrenocortical tumors diagnosis and prognostication. How this would translate with the results of conventional pathology and clinical status in helpful hints for patient management will be discussed.

Session 9: Hereditary pheochromocytoma and paraganglioma

S9.1
Carney-Stratakis dyad and Carney Triad: an update

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Carney's triad (CTr) is a non-hereditary condition, primarily seen in young women, characterized by gastrointestinal stromal tumors (GIST), extra-adrenal paragangliomas and pulmonary chondromas. Most CTr patients present with GIST and pulmonary chondroma (incomplete CTr). A marked decrease in the enzymatic activity of succinate dehydrogenase (SDH) has been detected in these tumors, but the presence or absence of SDH mutations in CTr is controversial. Recently, we reported (in collaboration with Haller et al.) SDHC-specific methylation in CTr (Endocr Related Cancer 2014). A related disease, known as Carney-Stratakis Syndrome (CSS), has no female predisposition and presents with extra-adrenal paragangliomas and GIST tumors, without pulmonary chondromas. In distinction to CTr, CSS is hereditary due to germline SDH mutations in the enzyme's subunits (SDHA, SDHB, SDHC and SDHD) with corresponding decreased SDH activity in associated tumors. We have investigated 64 patients with CTr for germline mutations and/or deletions in the SDH subunit genes; six patients were found to have germline mutations in SDHA, SDHB or SDHC. All six patients had multifocal gastric GIST and pulmonary chondromas at diagnosis; two subsequently developed paragangliomas, confirmed by biopsy. One of the patients with CTr and a *SDHB* mutation had a nephew with the same mutation and a neuroblastoma, confirming a previously reported association of neuroblastoma with GIST(JMG 2009; 46:215, and AJMG 2010; 152A: 1531). Thus, a few CTr patients maybe more correctly classified as having CSS; the two disorders may be allelic. SDHC-specific
methylation is essential for CTr tumor development, but the responsible gene defect in CTr remains unknown.

S9.2
The role of imaging in screening for pheochromocytoma and paraganglioma

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The genomic landscape of pheochromocytoma and paraganglioma (Pheo/PGL) is being progressively defined and has potential implications for optimal imaging strategies for screening patients at potential risk of these tumours. Important considerations include the likelihood of disease developing (related to disease penetrance), the median age of onset, whether lesions are more prevalent in adrenal or extra-adrenal sites, the functional status of resulting tumours and type of catecholamine secreted as well as the likelihood of malignancy. It is now recognized that up to a third of all Pheo/PGL arise from germ-line mutations. Broadly, Pheo/PGL has been divided into pseudo-hypoxia and receptor tyrosine kinase (RTK) subgroups based on hierarchical gene clustering. However, even within these clusters there is wide variation in the clinical behaviour and presentation of disease. The pseudo-hypoxia cluster is represented by mutations in *VHL*, *SDHx*(A-D), *EGLN1* (PHD2) or *EPAS1* (HIF2a). The RTK cluster includes mutations in *RET*, *NF1*, *TMEM127* or *MAX*.

Although anatomical imaging, including CT, MRI and ultrasound, has been, and probably remains, the most widely advocated approach for detection of Pheo/PGL in patients at high risk of these conditions through the documented presence of heritable mutations or a relevant family history, there is increasing recognition of the opportunity to better stage and characterize disease using molecular imaging techniques. The use of radioactive tracers that target specific aspects of the biology of these tumours can have either purely diagnostic or combined diagnostic and therapeutic ("theranostic") roles. The mainstay of functional imaging and the only tracer widely used for both the diagnosis and therapy of Pheo/PGL is meta-iodo-benzylguanidine (MIBG), which is taken up by the epinephrine transporter and stored in neurosecretory granules. For diagnostic purposes it is usually labelled with I-123 or I-131 whereas higher administered activities of I-131 MIBG are typically used for therapy. However, it is also possible to leverage catecholamine transporters for PET/CT imaging, which typically has superior sensitivity to conventional gamma camera imaging for detection of small lesions due to higher spatial and contrast resolution. This is particularly relevant for the detection of metastatic lesions or in patients with elevated catecholamine levels but negative structural imaging. Agents include I-124 MIBG, C-11 hydroxyepedrine(HED), and F-18 flurodopamine (FDA). Like I-123 MIBG, these agents have relatively high specificity for Pheo/PGL but can have suboptimal sensitivity, especially for PGL of the head and neck region, which less often produce catecholamines being often derived from the parasympathetic nervous system. Precursor amino-acids involved in catecholamine synthesis, such F -18 -L-fluoro-dihydroxyphenylalanine (FDOPA), have also been evaluated. These agents are, however, not widely available.

Despite its widespread use, there are a number of drugs that impair catecholamine transport and further reduce the sensitivity and limit clinical utility of MIBG. Therefore, other aspects of tumour biology have been leveraged for imaging. Although non-specific, increased glycolytic metabolism has been shown to be a feature of many Pheo/PGL, especially those arising within the pseudo-hypoxia cluster. With the wide clinical availability of FDG PET/CT, this has become a practical alternative to I-123 MIBG SPECT/CT for detection and staging of these tumours. Recognition of the frequent expression of somatostatin receptors (SSTR) on Pheo/PGL has also led to the use of radiolabelled somatostatin analogues (SSA) for both diagnosis and therapy. In-111 pentetreotide (Octreoscan) is widely available for SSTR imaging but various SSAs have been labelled for PET imaging and also with therapeutic radionuclides, representing an alternative theranostic paradigm to that offered by I-123/I-131 MIBG.

It is obviously not practical to use all of these agents for screening for Pheo/PGL and to a large extent the choice of radiotracer will be determined by local availability and regulatory approvals. Accordingly, there are no generally agreed algorithms that inform the use of these agents. Nevertheless, it is possible to establish rational guidelines regarding for whom, when and with which agent screening should be considered. The genomic characteristics of Pheo/PGL syndromes provide the basis for clinical decisions in this regard. The European Association of Nuclear Medicine has developed such guidelines in 2010 and other groups have made similar suggestions. Fundamental principles underpinning these recommendations are the general availability of tracers, their diagnostic performance across the spectrum of benign and malignant, functional and non-functional tumours but increasingly also the understanding of the natural history of disease related to specific genomic background of the patient and the imaging phenotypes of the associated Pheo/PGL. At our facility, FDG PET/CT is generally the first screening test and is combined with either I-123 MIBG SPECT/CT or Ga-68 DOTA-octreotate (GaTate) PET/CT and sometimes both if metastatic disease is suspected. These agents provide an indication of potential suitability for I-131 MIBG or SSTR-targeted peptide receptor radionuclide therapy. Although this imaging paradigm is costly, there is increasing recognition of the heterogeneity that can exist in molecular imaging phenotype. More accurate characterisation of therapeutic targets allows much more rational selection and planning of treatments that are, themselves, more costly and that have associated morbidity.

Specific features of syndromic Pheo/PGL that inform the timing and type of imaging will be discussed with a focus on VHL, MEN2 (RET), NF1 and SDHx.

S9.3
Clinical experiences from the French pheochromocytoma and paraganglioma Network (PGL.EVA study)

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Following the identification of the first mutations in the SDHx genes at the beginning of the twenty first century, an active clinical research dedicated to improve knowledge about SDHx-related paragangliomas was led in France. To assess the main genotype-phenotype correlations in SDHx mutation carriers, the paraganglioma network (PGL.NET), granted by the GIS-Institut des Maladies Rares, was founded in 2002. Then, in order to perform the recommendations for the first clinical screening after the identification of a germline SDHx mutation, 238 subjects (124 SDHB, 96 SDHD and 18 SDHC mutation carriers) were prospectively enrolled in the French PGL.EVA multicentric study, granted by the French national cancer institute, and benefited from the same screening protocol in 22 different clinical centers. The combination of a head and neck gadolinium-enhanced magnetic resonance angiography, thoracic-abdominal-pelvic contrast-enhanced computed tomography, and somatostatin receptor scintigraphy detected 202 tumours in 96 subjects comprising 185 paragangliomas (PGL) (151 in the head and neck, 18 in the thorax, 16 in the abdominal or pelvic area) and 17 pheochromocytomas (PCC) with a diagnostic sensitivity superior to 90%. One or several lesions were diagnosed in 66.4% of the patients who were previously diagnosed for a PCC/PGL (index cases) and in 16.8% of the asymptomatic mutation carriers (relatives). More recently (2013), the French national registry for SDHx hereditary paraganglioma (PGL.R) was created. This registry will include each index patients diagnosed in France and will permit to determine the natural history of

the disease and to specify the recommendations for the long-term follow-up. New data, based on 3 years follow-up, of the patients included in the PGL.EVA cohort, concerning the penetrance of the disease, the biological phenotype and the imaging screening will be presented during the 14th International Workshop on Multiple Endocrine Neoplasia and other rare endocrine tumors, held in Vienna.

include proper characterization of MPP patients at large cancer referral centers with multidisciplinary teams; improved strategies to stratify patients prognostically and implementation of trials within national and international networks. Currently, several phase II trials are analyzing the anti-tumor role of angiogenic agents . Progress in the molecular characterization and staging of MPP constitutes the basis for significant treatment breakthroughs.

S9.4
A new paraganglioma-polycythemia syndrome: Clinical presentations, genetics, and pathogenesis

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Hypoxia-inducible factors (HIFs) are transcription factors controlling energy, erythropoiesis, and development. Paragangliomas/pheochromocytomas are catecholamine-producing tumors. The occurrence of two or more distinct types of tumors, one of them paraganglioma, is unusual in a patient, except in hereditary cancer syndromes. Polycythemia, a disease state in which the proportion of blood volume occupied by red cells and the red-cell mass increases, can be primary or secondary (mediated by circulating erythropoietin) often related to abnormalities in hypoxia-sensing pathways. Six unrelated patients were investigated with thorough clinical evaluation. Germline/tumor tissue DNAs were analyzed for hypoxia-inducible factor 2A (*HIF2A*) mutations. The patients were found to have polycythemia, multiple paragangliomas, and duodenal somatostatinomas by imaging or biochemistry with somatic gain-of-function *HIF2A* mutations. The *HIF2A* mutations of these patients were clustered adjacent to an oxygen-sensing proline residue, affecting HIF-2α interaction with the prolyl hydroxylase domain-2 containing protein (PHD2), decreasing the hydroxylation of HIF-2α and reducing its affinity for von Hippel-Lindau (VHL) protein and its degradation. An increase in the half-life of HIF-2α was associated with an up-regulation of the hypoxia-related genes *EPO*, *VEGFA*, *GLUT1*, and *END1* in tumors.
The present findings indicate the existence of a new syndrome with multiple paragangliomas and somatostatinomas associated with polycythemia.

S9.5
Malignant hereditary pheochromocytoma and paraganglioma: focus on therapeutics

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Mestastatic pheochromocytoma and paraganglioma (MPP) present clinicians with three major challenges: scarcity, complexity of characterization, and heterogeneous behavior and prognosis. As with the treatment of all neuroendocrine tumors, the control of hormonal symptoms and tumor growth are the main therapeutic objectives in MPP patients. A significant number of MPP patients still die from uncontrolled hormone secretion. In addition, the management of MPP remains palliative. Screening and control of loco regional hazards, including those related to bone metastases, constitute the main antitumor challenge of slowly progressive MPP. In patients with rapidly progressive MPP, dacarbazine-based chemotherapy and MIBG are the most frequently used options. Steps forward

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