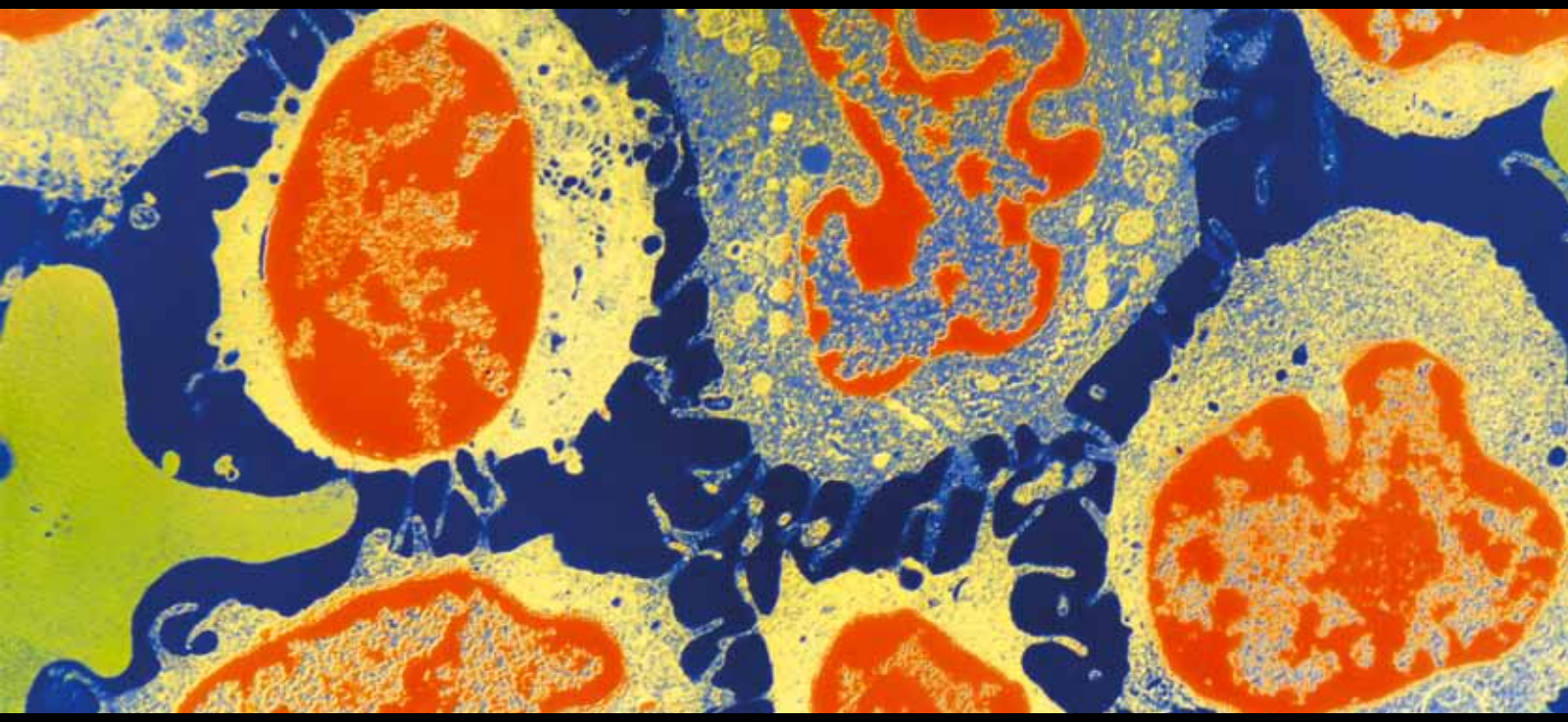
Journal of Oncology

New Perspectives on Diagnostics, Prognosis, and Therapy in Aggressive Endocrine Tumours

Guest Editors: Marialuisa Appetecchia, Bruce H. R. Wolffenbuttel, and Saadi Al Jadir



New Perspectives on Diagnostics, Prognosis, and Therapy in Aggressive Endocrine Tumours

Journal of Oncology

New Perspectives on Diagnostics, Prognosis, and Therapy in Aggressive Endocrine Tumours

Guest Editors: Marialuisa Appetecchia,
Bruce H. R. Wolffenbuttel, and Saadi Al Jadir

Editorial Board

Contents

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 820938, 2 pages
doi:10.1155/2012/820938

Editorial

New Perspectives on Diagnostics, Prognosis, and Therapy in Aggressive Endocrine Tumours

Marialuisa Appetecchia,[1] Bruce H. R. Wolffenbuttel,[2] and Saadi Al Jadir[3]

[1] *Endocrinology Unit, Regina Elena National Cancer Institute, 53 Elio Chianesi Street, 00144 Rome, Italy*
[2] *Department of Endocrinology, University Medical Center Groningen, Groningen, The Netherlands*
[3] *Endocrinology & Diabetes Unit, Department of Internal Medicine, Fujairah Hospital, Fujairah, UAE*

Correspondence should be addressed to Marialuisa Appetecchia, appetecchia@ifo.it

Received 6 November 2012; Accepted 6 November 2012

The research process is not entirely complete and sounds until the results are validated and transmitted to an appropriate target audience through modern publication.

Only through well-designed publication of results in established peer-reviewed journals or scientific congresses can new ideas and research findings be disseminated and effectively incorporated into clinical practice and eventually improvement in physician's performance and patient's health outcome.

We had ensured that all published reports of research have been reviewed by suitably qualified reviewers (e.g., including statistical review where appropriately needed).

Therefore, we are intended in our journal to achieve successful medical writing in order to serve as the optimal pathway to advance medical knowledge, promote critical thinking, incite scientific debate by involving a lot of expertise in our publications, and maintain the high degree of scientific and professional quality besides considering the continuing progress in different fields, modern medicine.

Endocrine tumors are a mixed group of diseases in which neoplastic cells are found in tissues of the endocrine system, which includes the thyroid, adrenal, pancreas, parathyroid, and pituitary glands.

Endocrine-related neoplasm has been enlarged to the domain of endocrine system to neural tissue as the embryonic development is closely interrelated; therefore, neuroendocrine tumors will be literally included in our researches, neuroendocrine tumors (Merkel cell, islet cell, MCT, pheochromocytoma, carcinoids etc.).

Most of these tumors appear as sporadic on one hand, and on other they might be clusters or familial part of genetic syndrome like MEN1, MEN2, or other more rare disorders.

These tumors ordinarily secrete hormones of the glands that have arisen from, and sometimes they are nonsecretors, majority are benign and others are malignant; some of these neoplasms tend to have indolent course, others are short and aggressive, as their clinical presentation and management vary accordingly. It is not surprising to find these tumors in unusual or ectopic sites that eventually rendered their topography and presentation difficult.

Last decades had shown a great development and tremendous successes in eruption of variety of biochemical; immunochemical; imaging techniques and most of these tumors showed their biological activity before their size could be detected by the conventional diagnostic testing. Currently, most of endocrine neoplasms can be successfully localized and subsequently have made their managements feasible.

In this special issue, we had tried to incorporate many aspects in studying these tumors including genetics behaviors, diagnosis, clinical course, new modalities in diagnosis, management, and surgical approaches as well as chemotherapy and specific palliative procedures in metastatic disease.

Clinical studies and review articles in this special issue have demonstrated the most recent updates in diagnosis and new treatment strategies, besides the enormous improvement in molecular biology of some relatively uncommon tumors that certainly will be of utmost importance to the practicing practitioners.

Thyroid cancer is the most common endocrine tumor. Besides standard treatment for differentiated thyroid cancer, the research for new therapies in advanced nonmedullary and medullary thyroid carcinomas, with the advent of tyrosine kinase inhibitors as well as antiangiogenic inhibitors, those patients could have an advantage with new target therapy.

In adrenocortical carcinoma radical surgery is considered the therapy of choice in the first stages of ACC. Mitotane, an adrenolytic drug with significant toxicity and unpredictable therapeutic response, is used in the treatment of ACC. Although, treatment for this aggressive cancer is still ineffective. Over the past years, the growing interest in ACC has contributed to the development of therapeutic strategies in order to contrast the neoplastic spread.

The efficacy of radiolabeled somatostatin analogues in patients with advanced neuroendocrine tumors had been illustrated in one article and clearly had exhibited that overall tumor response rate was appreciable, and PRRT is a promising perspective for patients with advanced NETs.

Cushing's syndrome whenever surgery is not curative, management of patients requires a major effort to control excess cortisol and associated symptoms. A multidisciplinary approach should be adopted. For aggressive ACTH dependent, several drugs are able to reduce cortisol levels. Their mechanism of action involves blocking adrenal steroidogenesis and novel chemotherapeutic agents (temozolomide and tyrosine kinase inhibitors) which have a significant activity against aggressive pituitary or ectopic tumors.

Recent genetic studies of malignant pheochromocytomas/paragangliomas have highlighted the main pathways involved in pathogenesis, thus suggesting the use of targeted therapy which, nevertheless, still has to be validated. Large collaborative studies on tissue specimens and clinical trials in large cohorts of patients are necessary to achieve better therapeutic tools and improve patient prognosis.

Review article about insulinoma had highlighted many aspects on this small neoplasm, including biological activity, new emerging imaging techniques in localizing the tumor especially in resectable ones, molecular pathogenesis of malignant disease, and treatment modalities for aggressive course, and put consequent hypoglycemia under control.

Merkel cell carcinoma is a rare and aggressive neuroendocrine tumor of the skin. Wide surgical excision must be associated with radiotherapy in early stages. In advanced disease, chemotherapy is the standard option despite the short duration of responses and poor quality of life.

Multiple endocrine neoplasias (MENs) are clinical inherited syndromes affecting different endocrine glands. Three different patterns of MEN syndromes can occur (MEN 1, MEN 2A, and MEN 2B). MEN 1 is characterized by the neoplastic transformation of the parathyroid glands, pancreatic islets, anterior pituitary, and gastrointestinal tract. Therapeutic approaches are different according to the different endocrinopathies. In MEN 2 syndromes, the medullary thyroid cancer is almost invariably present and can be associated with pheochromocytoma and/or multiple adenomatosis of parathyroid glands with hyperparathyroidism. Although surgery is the main option, nevertheless, 30% of MTC patients, especially in MENs 2B and 2A, are not cured by surgery. Recently, developed molecular therapeutics that target the RET pathway have shown very promising activity in clinical trials of patients with advanced MTC.

There is growing evidence of the role of IGF system dysregulation in endocrine neoplasms, and we will discuss the possible implications of these findings for tumor prevention and treatment, with a major focus on cancers from the thyroid, adrenal, and ovary, which are the most extensively studied. However, multiple molecular abnormalities of the IGF system frequently occur in endocrine neoplasms and may have a role in tumorigenesis as well as in tumor progression and resistance to therapies.

Marialuisa Appetecchia
Bruce H. R. Wolffenbuttel
Saadi Al Jadir

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 705036, 15 pages
doi:10.1155/2012/705036

Review Article

Genetic and Clinical Features of Multiple Endocrine Neoplasia Types 1 and 2

C. Romei, E. Pardi, F. Cetani, and R. Elisei

Department of Endocrinology and Metabolism, University of Pisa, 56124 Pisa, Italy

Correspondence should be addressed to R. Elisei, rossella.elisei@med.unipi.it

Received 30 July 2012; Accepted 16 September 2012

Academic Editor: Marialuisa Appetecchia

Multiple endocrine neoplasia (MEN) are clinical inherited syndromes affecting different endocrine glands. Three different patterns of MEN syndromes can occur (MEN 1, MEN 2A, and MEN 2B). MEN syndromes are very rare, affect all ages and both sexes are equally affected. MEN 1 is characterized by the neoplastic transformation of the parathyroid glands, pancreatic islets, anterior pituitary, and gastrointestinal tract. Heterozygous *MEN 1* germline mutations have been detected in about 70–80% of patients with MEN 1. The mutations are scattered throughout the entire genomic sequence of the gene. MEN 1 patients are characterized by variable clinical features, thus suggesting the lack of a genotype-phenotype correlation. Therapeutical approaches are different according to the different endocrinopathies. The prognosis is generally good if adequate treatment is provided. In MEN 2 syndromes, the medullary thyroid cancer (MTC) is almost invariably present and can be associated with pheochromocytoma (PHEO) and/or multiple adenomatosis of parathyroid glands with hyperparathyroidism (PHPT). The different combination of the endocrine neoplasia gives origin to 3 syndromes: MEN 2A, MEN 2B, and FMTC. The clinical course of MTC varies considerably in the three syndromes. It is very aggressive in MEN 2B, almost indolent in the majority of patients with FMTC and with variable degrees of aggressiveness in patients with MEN 2A. Activating germline point mutations of the *RET* protooncogene are present in 98% of MEN 2 families. A strong genotype-phenotype correlation has been observed and a specific *RET* mutation may be responsible for a more or less aggressive clinical course. The treatment of choice for primary MTC is total thyroidectomy with central neck lymph nodes dissection. Nevertheless, 30% of MTC patients, especially in MEN 2B and 2A, are not cured by surgery. Recently, developed molecular therapeutics that target the *RET* pathway have shown very promising activity in clinical trials of patients with advanced MTC. MEN 2 prognosis is strictly dependent on the MTC aggressiveness and thus on the success of the initial treatment.

1. Introduction

The term multiple endocrine neoplasia (MEN) defines clinical inherited syndromes affecting different endocrine glands, each with its own characteristic pattern [1, 2]. In some cases, the tumors are malignant, in others, benign. Benign or malignant tumors of nonendocrine tissues occur as components of some of these tumor syndromes.

Three different patterns of MEN syndromes can occur (MEN 1, MEN 2A, and MEN 2B) with some new variants such as MEN 4, which is considered a variant of MEN 1 and the familial medullary thyroid cancer (FMTC), which is considered a variant of MEN 2A [3, 4]. These syndromes are familial and caused by inherited genetic mutations, which have been discovered within the last 20 years [5].

2. Multiple Endocrine Neoplasia Type 1

2.1. Definition. Multiple endocrine neoplasia type 1 syndrome (MEN 1, OMIM no. 131100), also known as Wermer's syndrome because of the description in 1954 by Dr. Paul Wermer of a pluriglandular dysfunction transmitted as a dominant trait, is characterized by simultaneous neoplastic transformation of multiple endocrine tissues, typically the parathyroid glands, pancreatic islets, and anterior pituitary. The case of an acromegalic patient with three enlarged parathyroid glands and a pituitary adenoma was indeed firstly described in 1903 and, after small case reports, Underdahl, Woolner and Black in 1953 described a series of 8 patients with various combinations of pituitary, parathyroid, and pancreatic islet adenomas [6].

This disorder is strongly suspected either in patients with endocrinopathies of at least 2 of the 3 main affected glands (i.e., parathyroid, enteropancreatic, and pituitary tumors) or in patients with at least one endocrinopathy in one of these organs and a first-degree relative who is affected by one of these tumors (familial MEN 1). Patients with features of MEN 1 syndrome but without a family history of MEN 1 are affected by a sporadic form of MEN 1. MEN 1 syndrome presents a wide spectrum of more than 20 endocrine and nonendocrine associated manifestations other than the classic endocrinopathies, including adrenocortical, gastric, thymic or bronchial tumors, foregut carcinoids, visceral and cutaneous lipomas, meningiomas, facial angiofibromas, concurring to different phenotypic presentations (Figure 1) [7–9]. Thyroid tumors are also frequently associated, but this association should be considered likely casual for the high incidence of thyroid abnormalities in the general population. Various clinical cases report rare combinations of less common tumors of MEN 1 and these atypical cases are also known as MEN 1 "phenocopy variants."

The most frequent MEN 1-associated endocrinopathy, occurring in nearly 100% of patients by the age of 50 yrs, is primary hyperparathyroidism (PHPT), characterized by the synchronous or asynchronous development of multiglandular parathyroid hyperplasia with a benign course, while extremely rare is the occurrence of parathyroid carcinoma (PC), being only six cases of PC associated with MEN 1 in the literature [10]. Tumors of the parathyroid are often the first manifestation of MEN 1 in more than 85% of patients, with a typical age of onset of 20–25 yrs [11–13].

Gastroenteropancreatic endocrine (GEP) tumours, most arising in the pancreas as nonfunctioning neuroendocrine tumours or insulinomas, develop in up to 70–80% of MEN 1 patients, and gastrinoma represents, together with foregut carcinoids, the major cause of morbidity and mortality in MEN 1, because of its high rate of metastasis [17, 18] (Figure 1). The lesions range from microadenomas to macroadenomas, and to metastatic carcinomas. These tumors arise after the age of 40 yrs. Gastrinomas account for more than 50% of all GEP tumors and are typically small (<5 mm), multiple, mainly located in the duodenum and rarely in the pancreas. In the latter case it is difficult to distinguish these lesions from concomitant nonfunctioning pancreatic tumors (NFPTs).

The prevalence of pituitary tumors in MEN 1 ranges between 10% and 60%, being the prolactinoma the commonest MEN 1-related pituitary adenoma, although other pituitary tumors have been described so far (Figure 1). The majority of tumors are microadenomas (<10 mm). The mean age ± SD of onset has been reported to be 38 ± 15 yrs. Pituitary tumors are generally more invasive, symptomatic, with a higher prevalence of macroadenomas and a worse response to treatment than the sporadic counterparts [19, 20].

Foregut carcinoids, especially of the lung and thymus, are generally aggressive tumors and associated with a very high lethality. Adrenal tumors follow a benign course in most MEN 1 cases, and the majority are bilateral, hyperplastic, and nonfunctional [21]. Lipomas, both cutaneous and visceral, are present in about one-third of MEN 1 patients. Multiple facial angiofibromas occur in 40–80% of MEN 1 patients. Collagenomas are also common. These cutaneous lesions may be helpful for presymptomatic diagnosis of MEN 1 carriers.

2.2. Epidemiology. MEN 1 is rare, occurring in about one of 30,000 individuals, with an estimated prevalence of 2-3 per 100,000. The disorder affects all ages with a range of 5–81 yrs and both sexes equally [22]. A recent multicenter study analyzed 734 cases of MEN 1 and reported a different phenotype expression of the MEN 1 disease between males and females, in particular the prevalence of pancreatic tumors was higher in males than in females, while the opposite happened for the pituitary tumors. Thymic tumors were exclusively found in men. There was no significant gender difference in the prevalence and the probability of developing PHPT, adrenal and bronchial tumors in contrast to sporadic counterparts or in the proportion of positive genetic tests [23].

MEN 1-affected patients do not belong to particular geographical area, and there are no racial or ethnic preferences. No risk factors are known.

2.3. Pathogenesis. In 1988, linkage analysis studies in affected families placed the *MEN 1* gene within a 2 Mb interval in 11q13 and subsequently loss of heterozygosity [24] studies narrowed the location of the gene to a 600 kb interval [25]. The candidate gene, *MEN 1*, was finally identified by positional cloning in 1997 [26]. Combined LOH studies by microsatellite analysis in tumor tissues of MEN 1 patients and pedigree studies of large kindred supported a tumor suppressor function of the *MEN 1* gene suggesting the mechanism of biallelic inactivation firstly described by Knudson for the gene of retinoblastoma [27].

The *MEN 1* gene consists of 10 exons, the first of which is untranslated, spanning 7.2 kb of genomic sequence and encoding a protein, menin, of 610 amino acids, that does not present homologies to any other known proteins. *MEN 1* mRNA is expressed at a similar level in endocrine and nonendocrine organs, leaving unexplained the basis for endocrine predominance of neoplasia. Menin is a nuclear protein whose binding to the AP1 transcription factor JunD suggests a role in transcriptional regulation. The interaction with several partners and its participation in a variety of mechanisms, including regulation of cell proliferation and differentiation, apoptosis, endocrine/metabolic functions and the maintenance of genomic stability by DNA repair, have been so far reported [28]. The tumor suppressor nature of *MEN 1* gene is best achieved by menin-mediated inhibition of cell proliferation through multiple mechanisms such as (a) the interaction of menin with histone-modifying enzymes (MLL, EZH2, and HDACs) that affect gene transcription; (b) the interaction with various transcription factors, such as JunD, NF-κB, PPARγ, and VDR, to induce or suppress gene transcription; (c) the inhibition of cellular proliferation via TGF-β signaling and Wnt/β-catenin signaling pathways; (d) the repression of pro-proliferative factors (IGFBP-2, IGF2,s

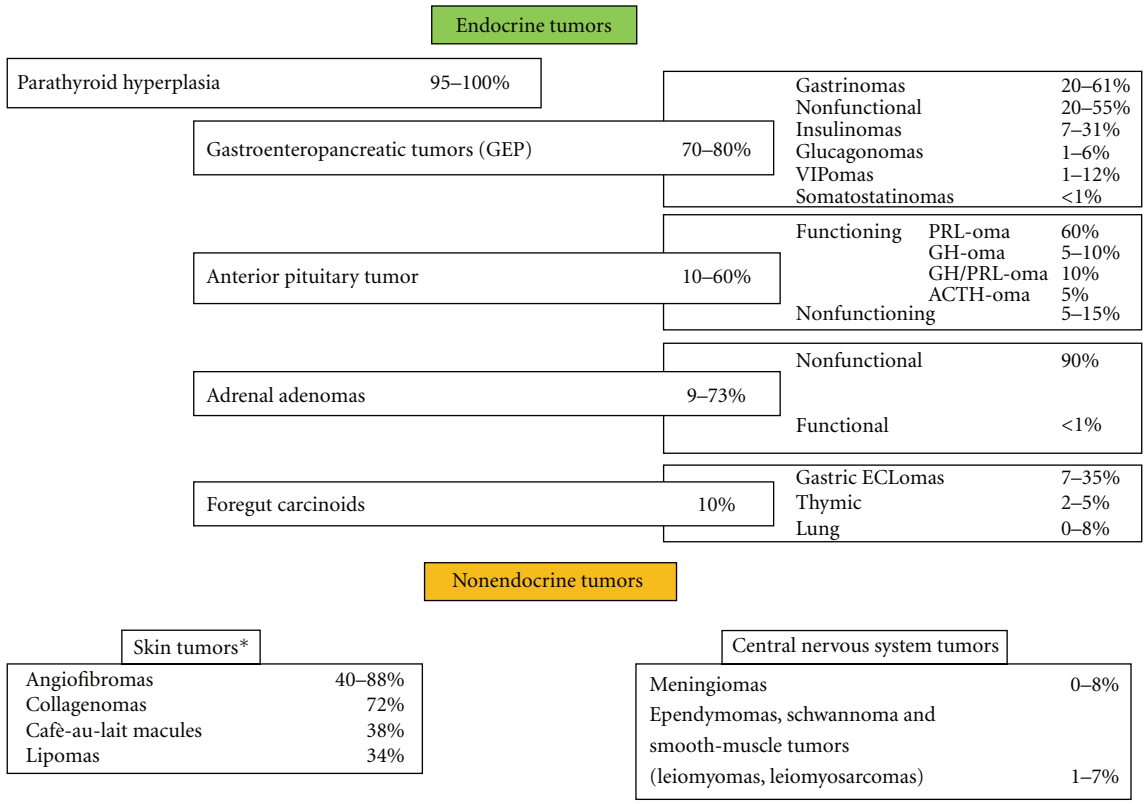


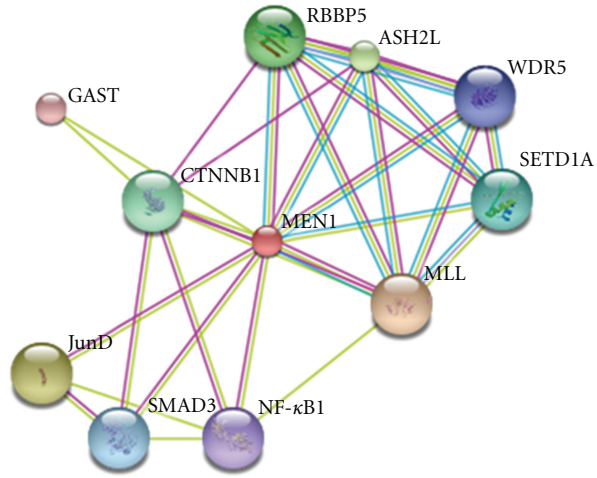
Figure 1: Prevalence of MEN 1 endocrine and nonendocrine manifestations. *Data obtained by a study on a series of 74 patients with MEN1 [8] and on a study determining the frequency of skin lesions in a series of 32 patients with MEN1 [9] Abbreviations: VIPoma-vasoactive intestinal peptide secreting tumor; PRLoma, prolactin secreting tumor; GHoma, growth hormone secreting tumor; ACTH, adrenocorticotropic hormone secreting tumor, ECLoma, enterochromaffin-like tumour.

and PTHrP) involved in endocrine tumors; (e) the direct effect on cell cycle progression (Figure 2) [29]. The recent described crystal structure of the human menin should help us to better explain the opposite effects of the protein in the transcription process [30].

Heterozygous *MEN 1* germline mutations have been detected in about 70–80% and 30% in patients with familial and sporadic MEN 1, respectively. The mutations are scattered throughout the entire genomic sequence of the gene, consistent with the lack of mutational hot spots. More than 1336 different germline and sporadic *MEN 1* gene mutations have been reported so far from the cloning of the gene [31]. More than 70% of *MEN 1* mutations lead to truncated form of the protein, confirming a loss-of-function mechanism.

To date, murine models of MEN 1 syndrome have been generated by disrupting different parts of the murine *MEN 1* gene localized on chromosome 19. The homozygous status shows a lethal phenotype at embryonic level, while the heterozygous mutant mice have a phenotype similar to the human MEN 1 disease, with a survival rate significantly lower than the wild-type mice, and with pancreatic islets lesions ranging from hyperplasia to insulin-producing islet cell tumors as the first manifestation [32]. Lesions of the parathyroid, pituitary, and adrenal glands occur later, and in addition to the typical MEN 1-associated endocrine tumors, these mice also develop tumors of the gonads and the thyroid. All the major tumors typically exhibit multistage tumor progression with metastatic potential [33, 34].

The variable clinical expression between MEN 1 patients and relatives of the same family sharing the same genetic defect suggests the lack of a genotype-phenotype correlation (Figure 3) [35]. The lack of a correlation between the genetic status and the phenotypic expression could be due to either additional genetic events or epigenetic factors. A variant of the classic MEN 1 syndrome, known as MEN 1-Burin or "prolactinoma variant" of MEN 1, has a characteristic phenotype, such as a unusual higher incidence of carcinoid and pituitary tumors, all prolactinomas, a very low incidence of pancreatic endocrine tumors, and a late onset PHPT compared with families with typical MEN 1. Initially four large MEN 1-Burin kindreds were identified in the Canadian Newfoundland area and share a common nonsense mutation in the *MEN 1* gene, suggesting the existence of a founder mutation [36]. Following the original report, similar families have also been described in Japan, Brazil, USA, and Mauritius carrying different nonsense or frameshift mutations suggesting that there is not a common *MEN 1* mutation in all MEN 1-Burin families [37, 38]. In addition, some kindreds may develop only PHPT, and this condition is referred to as familial isolated hyperparathyroidism (FIHP). Up to date mutations of the *MEN 1* gene, mostly missense, have been detected in 42 FIHP families [24, 39].



Abbreviations

MLL:	Myeloid/lymphoid or mixed-lineage leukemia
JunD:	JunD protooncogene (transcription factor)
ASH2L:	Histone methyltransferase complex subunit ASH2
RBBP5:	Retinoblastoma-binding protein 5
CTNNB1:	Catenin (cadherin-associated protein), beta 1
SETD1A:	SET domain containing 1A
SMAD3:	SMAD family member 3
WDR5:	WD repeat domain 5
NF-κB1:	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
GAST:	Gastrin.

Figure 2: Predicted functional partners of menin generated by the protein interaction database STRING v.9.0. Evidences for these associations derive from experiments (pink lines), homology data (violet lines), predictions by text data mining (green lines), and information obtained from databases (light blue lines). The loss of one or more of these interactions might contribute to the development of MEN 1 syndrome by different mechanisms.

Approximately 20–30% of MEN 1 patients do not have *MEN 1* mutations, suggesting that other tumor susceptibility genes may be involved in the pathogenesis of this syndrome. A germline nonsense mutation in the human *CDKN1B* gene, encoding p27 protein, a negative regulator of cell cycle progression [40], has indeed been identified in a MEN 1 proband with acromegaly and PHPT, and a first-degree relative carrier with renal angiomyolipoma. The search for *CDKN1B* mutations in MEN 1 kindred started after the identification of a germline mutation of the *CDKN1B* gene in a rat colony affected by a variant of both MEN 1 and MEN 2 human syndromes, named MEN X [41]. This strain of rats developed multiple endocrine tumors, involving anterior pituitary adenoma, adrenal pheochromocytoma, thyroid C-cell hyperplasia, parathyroid and pancreatic islet cells hyperplasia. So far, germline mutations in the coding as well as in 5′ untranslated region of *CDKN1B* gene have been detected in other six MEN 1 kindred negative to *MEN 1* gene mutation testing [42, 43]. The predicted role in tumor predisposition of the *CDKN1B* mutations has been addressed with analyses *in vitro* and studies of protein localization and expression. This syndrome has been designated as MEN 4 (OMIM no. 610755).

2.4. Diagnosis. A clinical diagnosis of MEN 1 is made in individuals who have developed two or more of the classic MEN 1-associated tumors and in patients who have one classic MEN 1-related tumor and a family history of MEN 1. The biochemical diagnosis of PHPT, prolactinoma, and secreting endocrine tumors of the GEP tract in known or suspected MEN 1 is the same as for sporadic tumors (Table 1). Presymptomatic MEN 1 is biochemically detectable virtually one-two decades prior to full-blown phenotype, when symptoms are often related with the hormone hypersecretion or mass effect due to the growth of the tumor. Imaging studies on PHPT do not influence the indications for surgery [44]. Magnetic resonance imaging (MRI) is the test of choice for pituitary tumors [45]. Computed tomography (CT) and MRI are sensitive to detecting pancreatic endocrine tumors, adrenal, thymic, and lung carcinoids. Esophagogastroduodenoscopy with biopsy is recommended in patient with hypergastrinemia to detect peptic ulcer disease and carcinoids. In asymptomatic patients with MEN 1 endoscopic ultrasound (EUS) study is the most sensitive procedure to detect small ($\leq$10 mm) pancreatic lesions [46]. For the identification of metastases of pancreatic tumors, the procedure of choice is the somatostatin receptor

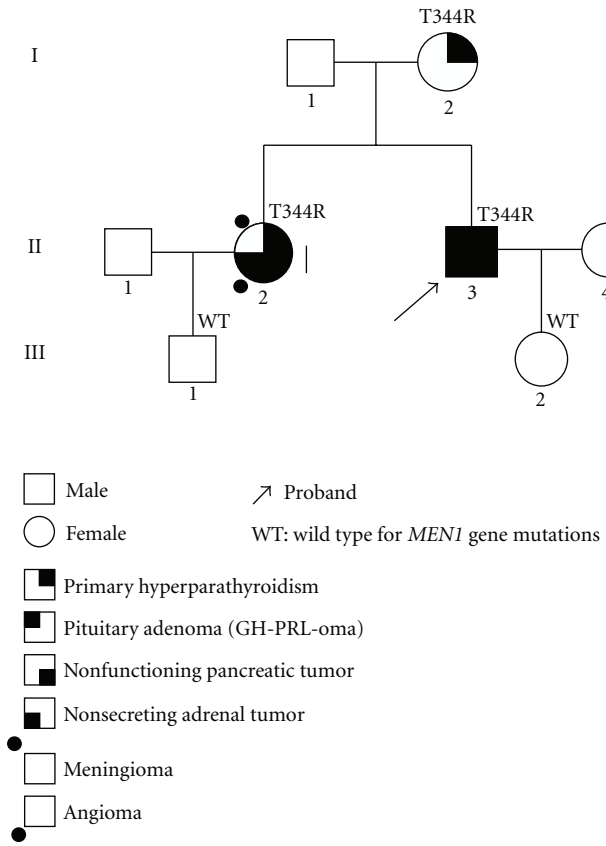


Figure 3: Pedigree of an Italian kindred positive for a germline MEN 1 missense mutation (T344R) in exon 10 (unpublished data). Generation numbers are represented by Roman numerals and individual numbers are in Arabic numerals. The proband, II-3, presents all the main MEN 1 endocrine manifestations; the sister, II-2, does not present pituitary lesions, but has nonendocrine lesions, such as a tumor of CNS and an angioma. Proband's mother, I-2, has only primary hyperparathyroidism, supporting the lack for a genotype-phenotype correlation.

scintigraphy. The imaging test schedule of MEN 1-affected patients is summarized in Table 1 [3, 47].

2.4.1. Genetic Testing. Mutation analysis of the *MEN 1* gene may be used to confirm the clinical diagnosis, provide a genetic diagnosis in difficult cases, and screen asymptomatic relatives. The genetic testing of asymptomatic family members should be offered in early childhood since the first MEN 1 manifestations may occur by the age of 5 yrs [48]. *MEN 1* germline mutation testing should be offered to relatives of MEN 1 patients before biochemical and imaging screening examinations in order to exclude MEN 1 tumors. *MEN 1* gene testing can be helpful when clinical diagnosis is inconclusive; however but a suspicion of MEN 1 exists. The genetic analysis of the entire coding region and splice sites fails to detect *MEN 1* mutation in about 30% of typical MEN 1 kindred. If *MEN 1* mutation is not detected, testing for large gene deletions, haplotype analysis of *MEN 1* locus, or analysis of other genes should be considered [47].

2.4.2. Screening Program of Tumor Expression in MEN 1 Gene Carriers. In contrast to the clinical importance of *RET* sequence testing in MEN 2, presymptomatic gene diagnosis has not been established to improve morbidity and mortality in MEN 1. Clinical practice guidelines on the management of *MEN 1* gene carriers who have not yet developed the disease have recently been published [47]. Briefly, annual biochemical screening should include the following measurements.

(i) PHPT: intact PTH and albumin-corrected total serum calcium or ionized serum calcium by age 8.

(ii) Pituitary tumors: serum prolactin and insulin growth factor 1 (IGF-1) by age 5.

(iii) Insulinoma: serum fasting glucose and insulin by age 5.

(iv) Gastrinoma: gastrin, gastric acid output, and secreting stimulated gastrin.

(v) Other GEP tumors: proinsulin, glucagon, and plasma chromogranin A before the age of 10 yrs. Biochemical tests for adrenal lesions are not recommended unless the presence of symptoms or signs of functioning tumors and/or the detection of tumors with a diameter >10 mm on imaging.

Diagnostic imaging procedures are recommended for the identification of pituitary tumors (MRI every 3 yrs), GEP

Table 1: Biochemical and imaging screening for tumors in MEN 1 patients.

Tumor	Annual biochemical tests	Imaging tests
Parathyroid	Calcium, PTH	—
GEP	Gastrin, glucagon, vasointestinal polypeptide, pancreatic polypeptide, chromogranin A, insulin, fasting glucose	MRI, CT, and endoscopic ultrasound (annually), gastroscopy with biopsy every 3 yr in patients with hypergastrinemia
Pituitary	PRL, IgF1	MRI (every 3–5 yrs)
Adrenal	—[1]	CT or MRI (every 3 yrs)
Thymus and lung	—	CT or MRI (every 1-2 yrs)

[1] Biochemical test should be performed in patients with tumors greater than 1 cm or with clinical features.

tumors (with the exception of gastrinoma and insulinoma) (MRI, CT, or EUS annually), adrenal lesions (MRI or CT annually), thymic and bronchial carcinoids (CT or MRI every 1-2 yrs).

2.5. Therapy

2.5.1. PHPT. The optimal surgical approach is controversial. Approaches include either subtotal parathyroidectomy (PTx) (removal of 7/8 of the parathyroid tissue) with cryopreservation of parathyroid tissue, or total PTx and autologous parathyroid tissue graft in the forearm [49, 50].

At initial surgery, transcervical near total thymectomy is also recommended [3] since it may cure thymic carcinoids or prevent their development; in addition, the thymus is a common site for parathyroid tumors in MEN 1 patients with recurrent PHPT. Minimally invasive PTx is usually not recommended for the typical multiglandular involvement. Involvement of a highly experienced surgeon is crucial to optimal outcome. There are reports showing that the recurrence rate of PHPT in MEN 1 for procedures less than subtotal PTx were 8%, 31%, and 63% at 1, 5, and 10 years, respectively [51]. However, when subtotal or total PTx was performed, the rate of recurrence was 5%, 20%, and 39% at 1, 5, and 10 years, respectively. Rapid intraoperative PTH (iPTH) measurement can be helpful to prevent a persistent PHPT after glands removal [52]. Total PTx guided by iPTH monitoring and followed by autograft to the forearm led to a 10% of recurrences in the autografted parathyroid after a mean time of years after surgery [53].

2.5.2. GEP Tumors

Gastrinoma. The therapy in MEN 1-associated gastrinoma aims for the treatment of acid hypersecretion and the resection of the tumor [54]. However, surgical versus nonsurgical management of gastrinoma in MEN 1 syndrome is still controversial since successful outcome of surgery is rare. When surgery is not possible, the medical treatment may include somatostatin analogs, interferon-alpha, and chemotherapy. Proton pump inhibitors or H2-receptor blockers are able to reduce gastric acid output in these patients.

Other GEP Tumors. The surgical approach for asymptomatic NFPT in MEN 1 is controversial. The choice between a preserving pancreatic-duodenectomy or a more aggressive approach depends on the estimated risk for the development of metastatic disease, the size of the lesions, and the functioning nature of the tumour [55]. Surgery is usually indicated for insulinoma. Somatostatin analogs, radionuclide therapy, biotherapy, and chemotherapy may be used in inoperable tumors [47]. In cases of inoperable or metastatic well-differentiated tumors, sunitinib or everolimus may be considered [56].

2.5.3. Pituitary Tumors. Treatment of pituitary tumors in MEN 1 is identical to that in sporadic tumors. Dopamine agonists, especially cabergoline, are the preferred treatment of PRL-secreting tumors. Transsphenoidal surgery is the treatment of choice in GH-secreting tumors with a success rate of 50–70%. Somatostatin analogs (octreotide and lanreotide) are considered the current medical treatment of choice of GH-secreting tumors and are able to normalize the serum levels of GH and IgF1 in $\geq$50% of patients. Dopamine agonists can be used in mixed GH-PRL secreting tumors and in cases of tumors resistant to somatostatin analogs. Surgery is the treatment of choice in ACTH-secreting pituitary tumors. Radiation therapy can be used in cases of persistent or recurrent disease.

2.5.4. Adrenal Tumors. Treatment of adrenal tumors in MEN 1 is similar to that for sporadic tumor. Surgery is the treatment of choice in functioning tumors and nonfunctioning tumors with significant growth over a 6-month interval, suspicious radiological features, and greater than 4 cmin size [57].

2.5.5. Thymic, Lung, and Gastric Neuroendocrine Tumors. The treatment of choice for thymic and lung carcinoids is surgery. When surgery is not possible, chemotherapy and radiotherapy should be considered.

The optimal therapy of gastric carcinoids is controversial. Endoscopic excision or partial/total gastrectomy is required for tumors >10 mm. Lesions <10 mm can be monitored by endoscopy [47].

2.6. Prognosis. The prognosis is generally good if adequate treatment is provided for parathyroid, pancreatic, and pituitary tumors. Pancreatic endocrine tumours associated with MEN 1 are less malignant than sporadic tumors and

carry a better prognosis, with a median survival of 15 years compared to 5 years for patients with sporadic tumors. This may reflect more indolent disease or earlier diagnosis [58].

3. Multiple Endocrine Neoplasia Type 2

3.1. Definition. Multiple endocrine neoplasia type 2 syndrome (MEN 2) is characterized by the association of benign and malignant endocrine neoplasia with other nonendocrine diseases. In all syndromes, the medullary thyroid cancer (MTC), originating from C cells is present and can be associated with pheochromocytoma (PHEO) and/or multiple adenomatosis of parathyroid glands with hyperparathyroidism (PHPT). The different combination of the endocrine neoplasia with or without nonendocrine diseases gives origin to 3 different syndromes: MEN 2A, MEN 2B, and FMTC, this latter being considered as a variant of MEN 2A.

Although MEN 2 was firstly detected in the 19th century at the University Hospital of Freiburg, Germany [59], the association of an MTC and an PHEO in a single patient (Sipple's syndrome) was firstly described in 1961 [60, 61]. However, the entire entity of MEN 2A was recognized only in 1968 in a family with PHEO, MTC, PHPT, and Cushing's disease [62].

MEN 2A (OMIM 171400) syndrome is the most common form. Almost all affected patients develop MTC which is usually multifocal, bilateral and almost invariably associated with C-cells hyperplasia. Fifty percent of MEN 2A patients are at risk of developing PHEO which, although frequently asynchronous, is usually involving both adrenal glands. About 25% of MEN 2A patients can also develop PHPT [63]. MTC is generally the first manifestation of MEN2A and develops between the ages of 5 to 25 years [16]. PHEO usually presents after MTC or concomitantly; however, it has been reported as the first sign of the syndrome in 13–27% of MEN 2A cases [64, 65]. In some cases, Hirschsprung' s disease (HSCR) [66, 67], a congenital disease characterized by the aganglionosis of the gut and/or cutaneous lichen amyloidosis [68–70], a pruritic lichenoid skin lesion usually located in the interscapular region, is associated with MEN 2A (Table 2).

MEN 2B syndrome (OMIM 162300) is the least common but the most aggressive form of MEN 2 (5–10% of all cases) [71]. Patients rarely become adults since the metastatic lesions of MTC develop and progress very rapidly. In MEN 2B patients, MTC is associated with PHEO in 45–50% of cases, while an association with PHPT was never described. Typically, almost 100% of MEN 2B patients develop mucosal neuromas, bumpy lips, ganglioneuromatosis of the gastrointestinal tract, and a Marfanoid habitus [72] (Table 2).

Familial MTC (FMTC; OMIM 155240) is considered the mildest variant of MEN 2 since in patients with FMTC there is a strong predisposition to develop MTC but a very low incidence of the other clinical manifestations of MEN 2A [73]. It has been diagnosed more frequently in recent years (35–40% of all cases), and particularly after the introduction of the genetic test [74, 75]. The clinical diagnosis of FMTC can only be posed when four or more family members across at least 2 or more generations have isolated MTC [3, 4, 68]. In the absence of these criteria, to prove that a subject has an FMTC, it is necessary to demonstrate the presence of a germline *RET* mutation [3]. Whereas MEN 2A and 2B are clinically very well defined, the lack of specific clinical features and/or familial history makes the diagnosis of FMTC relatively difficult, thus generating an underestimation of FMTC prevalence within families, especially in series where no genetic test for *RET* mutation has been performed. From the discovery of the first kindred affected by MTC, it was clear that these syndromes are inherited with an autosomal-dominant mendelian mechanism. For this reason, 50% of first-degree relatives of the index case (i.e., parents, siblings, and children) may be affected.

3.2. Epidemiology. MEN 2 syndrome is a very rare disease. To have a better idea of the rarity of the disease, one can consider that MEN 2 syndrome represents 25% of all MTC cases and that MTC represents only 5–10% of all thyroid malignancies, which represent only 1% of all human malignancies. Thus, the overall prevalence of MEN 2 syndromes is very low, accounting for about 0.02-0.03% of all human tumors. The total prevalence of all MEN2 variants has been estimated approximately 1/30,000 individuals [4].

The relative prevalence of the 3 syndromes reported in the first International *RET* consortium in 1994 [68] (Figure 4(a)) was significantly different from that reported in more recent studies [76] (Figure 4(b)). In particular, this change has been observed after the introduction of the *RET* genetic screening which allowed to recognize several cases of hidden FMTC.

3.3. Clinical Manifestation. The clinical appearance of MTC in MEN 2 syndromes is that of a thyroid nodular disease, similar to that of the sporadic form with the exception that it is usually bilateral, multicentric, and associated with C cell hyperplasia, which is considered a preneoplastic lesion. The clinical course of MTC varies considerably in the three syndromes. It is very aggressive and almost invariably unfavourable in MEN 2B, with affected patients rarely surviving after the adolescence. It is almost indolent in the majority of patients with the FMTC and shows variable degrees of aggressiveness in patients with MEN 2A. It is the only malignant tumor and the most severe disease of the syndrome so that in the majority of cases the prognosis of the disease is mainly related with the prognosis of the MTC.

An age-related progression to MTC has been described with younger age of onset for MEN 2B (youngest reported 0.6 year), older age for FMTC (usually adult age > 20 years), and intermediate age (starting from 1.5 years, but childhood age is the most prevalent) [16] (Table 3).

Up to 70% of MTC patients have already cervical lymph node metastases at the diagnosis [77] and this is a unfavorable prognostic factor for the cure of the disease. About 30%, mainly belonging to MEN 2B and, to a lesser extent, to MEN 2A, have already distant metastasis at the time of diagnosis and this is an unfavorable prognostic factor

Table 2: Prevalence of tumoral diseases in MEN 2 syndromes.

Phenotype	MTC (%)	PHEO (%)	HPT (%)	Nonendocrinological associated pathologies (%)
MEN 2A	95	50	25	Cutaneous lichen amyloidosis (10%) Hirschsprung's disease (2%)
MEN 2B	95	50		Mucosal neuromas (100%) Marfanoid habitus (100%) Ganglioneuromatosis of the gastrointestinal tract (60%)
FMTC	100			

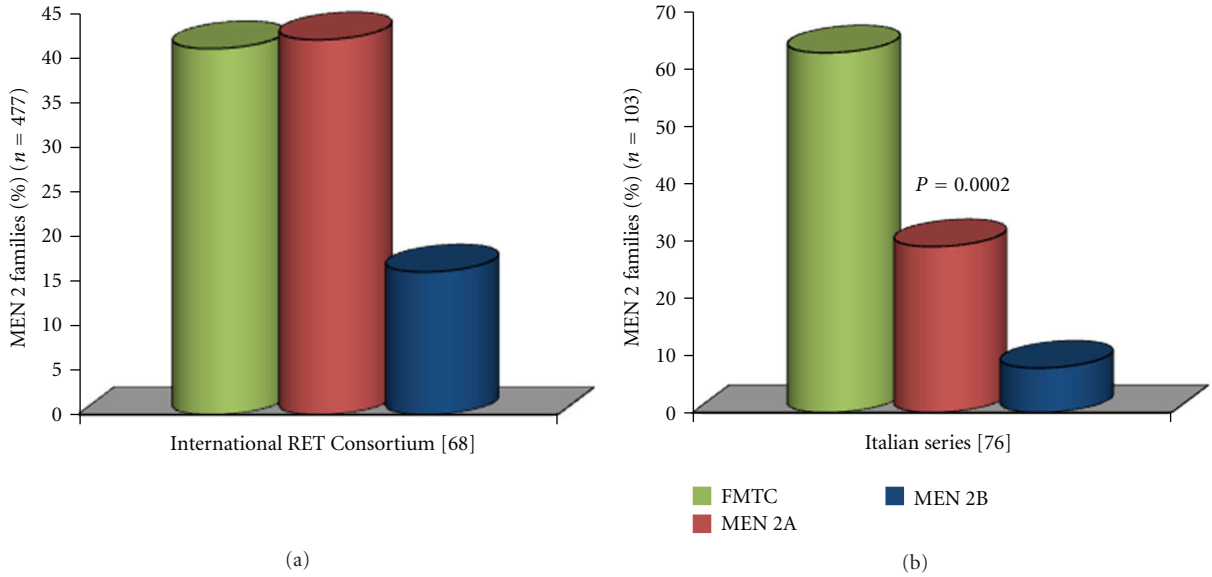


Figure 4: Prevalence rates of the three MEN 2 phenotypes in the International RET Consortium series (a) and in an Italian series (b). A higher prevalence of the FMTC phenotype was observed in the Italian series with respect to that reported by the International Consortium, which was based on cases collected up to 1994-1995.

for the survival although they have a median survival of 5–10 years.

MTC is usually the first neoplastic manifestation in most MEN2 kindred because of its earlier and overall higher penetrance. With few exceptions, PHEO and PHPT are usually discovered few years after the MTC diagnosis. Both PHEO and PHPT are benign diseases, but, when present, they can severely affect the patient with severe hypertension or unexpected hypertensive crisis and hypercalcemia, respectively.

Both CLA and mucosal and/or corneal nerves neurinomas associated with a Marfanoid habitus are strongly suggestive of MEN 2A or MEN 2B, respectively.

3.4. Pathogenesis. During the 80s, genetic linkage analysis localized the MEN 2 gene into the centromeric region of chromosome 10. In 1993, *RET* germline mutations were recognized as the causative molecular alterations in MEN 2 syndromes [78–80]. The *RET* protooncogene is a 21-exon gene and encodes for a tyrosine kinase transmembrane receptor located on chromosome 10q11.2. The receptor is composed of an extracellular domain (EC), with a distal cadherin-like region and a juxtamembrane cystein-rich region, a transmembrane domain (TM) and an intracellular domain with tyroisine-kinase activity (TK). In physiological conditions, the activation of the *ret* protein is secondary to its dimerization due to the interaction with one of its ligands. Four different ligands have so far been recognized: the glial cell-line derived neutrophilic factor (GDNF), neurturin (NTN), persepin (PNS) and artemin (ART). The interaction is mediated by a ligand-specific coreceptor (e.g., the GFRα-1 is the co-receptor for the GDNF). The dimerization of *ret* protein induces the autophosphorylation of the TK domain and the activation of downstream signaling pathways.

Activating germline point mutations of the *RET* proto-oncogene are causative events in MEN 2A, MEN 2B, and FMTC. *RET* mutations have been found to be widely distributed not only among the 5 cysteine codons 609, 611, 618, 620, and 634 but also in other noncysteine codons, such as codon 804 in exon 14, codon 883 in exon 15, and others. These widely spread non cysteine mutations are mainly associated with FMTC phenotype [74–76]. Virtually, all the mutations reported up to now

Table 3: Age of onset of endocrine tumors according to RET Mutation MEN 2 database. Data from ARUP Scientific Resource for Research and Education.

RET codon	MTC (years)	PHEO (years)	HPT (years)
533	21	34	
609	4	19 (C609S)	38
611	6	30	40
618	5	19	41
620	5	19	
630	1		32
634	0.8	5	10
768	9	59	
790	10	28	
791	15	38	
804	6	28	9
883	10		
891	9	46	17
918	0.17	12	

are present on public databases (http://www.hgmd.cf.ac.uk; http://www.arup.utah.edu/database/MEN2). Their prevalence, which is clearly different in different countries [14–16], is reported in Table 4.

After the introduction of genetic screening in the diagnostic procedures of patients affected with apparently sporadic MTC, new mutations were found, especially in noncysteine-rich regions [74, 81, 82], that were mainly associated with FMTC [76]. Sometimes these new mutations are very rare, present only in a few families and a few family members, raising doubts as to whether they represent the driving force of the tumoral disease or result from the genetic screening associated with MTC [83, 84].

Apart from genetic alterations, no risk factors have been associated with the development of MEN 2 syndrome.

3.5. Genotype-Phenotype Correlation. The MEN 2 syndromes are characterized by a strong genotype-phenotype correlation and a specific RET mutation may be responsible for a particular phenotype and a more or less aggressive clinical course. This close association was firstly identified in an early study of 477 families affected by MEN 2 [68] and confirmed by several other studies. This correlation can be summarized as follows.

(a) approximately 98% of families with MEN 2A have a germline RET mutation in exon 10 or 11 [4, 68, 74]. Mutations at codon 634 (exon 11) is the most frequently found in typical MEN 2A families (87%): in this case the 3 endocrinopathies (i.e., MTC, PHEO and PHPT) are usually present both in the same subject and in several family members; mutations of cysteine residues at codons 609, 611, 618, and 620 are usually present in the other MEN 2A cases in which the combination of the 3 endocrinopaties is less common [4, 85, 86];

(b) germline *RET* mutations are found in approximately 95% of families with FMTC [76, 85]. These mutations are mainly affecting the non cysteine codons located at exons 5, 8, 13, 14 and 15 with 20% to 30% of mutations located at one of the five cysteine residues (codons 609, 611, 618, 620, and 634). A different geographic distribution has been reported especially for cysteine and non cysteine mutations [15, 16, 76] (Table 4);

(c) about 95% of individuals with the MEN 2B phenotype have a single point mutation in the tyrosine kinase domain of the *RET* gene at codon 918 in exon 16, which substitutes a threonine for methionine (M918T) [68]. Another mutation at codon 883 in exon 15, A883F, has been identified in several affected individuals without a M918T mutation. Tandem *RET* mutations of codons 805, 806, and 904 in cis configuration with the V804M mutation have also been reported in individuals with MEN 2B [87, 88]. Taken together, *RET* mutations have been found in more than 98% of individuals with MEN 2B.

The genotype-phenotype correlation clearly indicates that not all mutations confer the same aggressiveness to MTC. A similar evidence is for the different levels of disease penetrance. The American Thyroid Association recently categorized the *RET* mutations into four levels of risk (Table 5); these levels are of great usefulness for the identification of the therapeutic and follow-up strategies [4].

3.6. Genetic Testing. All patients affected by MTC, both those with a familial history of MEN 2 and those with an apparently sporadic form, must undergo a germline *RET* protooncogene analysis. The major reason to test apparently sporadic MTC is the evidence that 5–10% of these cases are indeed "hereditary" cases since they harbor a germline *RET* mutation [89].

When a germline mutation is found, all first-degree relatives should be submitted to *RET* analysis to distinguish "gene carriers" from "nongene carriers." The *RET* gene carriers are at very high risk to develop MTC and they must be submitted to a diagnostic and therapeutic strategy which is very much conditioned by the ATA level of risk of the mutation. Recently, a greater importance has been recognized to serum calcitonin measurement for planning the timing of thyroidectomy which should be either prophylactic or very precocious when the tumor is still intrathyroid [90]. In nongene carriers the risk to develop MTC is similar to that of the general population and they should not be submitted to any further specific test.

The genetic screening activity should be accompanied by genetic counseling that should involve specific figures such as the geneticist, who will explain the particular type of transmissibility of the disease, the endocrinologist, who will explain the particular type of pathology and the risk of developing the different endocrine disorders, and possibly a psychologist to address issues arising from the knowledge of being a "gene carrier."

Table 4: Different prevalence of RET germline mutations in hereditary MTC in different European countries.

RET mutation	Italy ($n = 246$) [14]	Germany ($n = 141$) [15]	Euromen ($n = 145$) [16]
Cys634	86 (34.9%)	57 (40%)	98 (67.6%)
Val804	52 (21.1%)	9 (6.4%)	3 (2.1%)
Ser891	23 (9.3%)	3 (2.2%)	3 (2.1%)
Met918	20 (8.1%)	21 (15%)	4 (2.8%)
Cys618	15 (6.0%)	7 (5%)	10 (6.9%)
Glu768	9 (3.6%)	2 (1.4%)	1 (0.8%)
Cys620	9 (3.6%)	10 (7%)	10 (6.9%)
Leu790	8 (3.2%)	17 (12%)	7 (4.8%)
Cys609	6 (2.4%)	1 (0.7%)	1 (0.8%)
Cys630	4 (1.6%)	1 (0.7%)	1 (0.8%)
Cys611	1 (0.4%)	2 (1.4%)	4 (2.8%)
Tyr791	1 (0.4%)	10 (7%)	3 (2.1%)
Ala883	1 (0.4%)	0	0
Cys515	1 (0.4%)	0	0
Lys666	1 (0.4%)	0	0
Met848	1 (0.4%)	0	0
Ser904	1 (0.4%)	0	0
Thr338	1 (0.4%)	0	0
Asp631	0	1 (0.7%)	0
No mutations	6 (2.4%)	0	0

Table 5: Classification of RET mutations according to ATA risk level.

ATA risk level	RET codons
A (low)	768, 790, 791, 804, 649, 891
B (medium)	609, 611, 618, 620, 630, 631
C (high)	634
D (highest)	918, 883

As stated above, the identification of the type of mutation also gives information about the possible phenotype suggesting the diagnostic and therapeutic strategy to be followed. Although all cases of hereditary MTC should be evaluated for the possibility of developing PHEO or PHPT, some of them are more likely to manifest these diseases while others will never develop them or in a late stage of the disease.

3.7. Clinical Diagnosis. Clinical evaluation of MEN 2 patients consists in the measurement of basal and/or pentagastrin-(Pg-) stimulated serum calcitonin (CT), neck ultrasound, and fine needle aspiration of thyroid nodule if present. To rule out the presence of an PHEO, an abdominal ultrasound should be performed accompanied by the measurement of both plasmatic and urinary epinephrine and norepinephrine; whenever possible, the measurement of metanephrines is better recommended for their higher sensitivity. Serum PTH, calcium, and vitamin D measurement should be always performed for the diagnosis of PHPT. The physical examination of these patients is also important particularly in MEN 2B syndrome because the phenotype is quite typical being characterized by Marfanoid habitus, mucosal and/or corneal nerves neurinomas. The presence of an itchy/dark spot in the interscapular region should rise the question of a possible CLA that is highly suggestive of MEN 2A.

3.8. Conventional Therapy. The treatment of choice for primary MTC, both sporadic or hereditary, is total thyroidectomy with systematic dissection of all lymph nodes of the central compartment. Total thyroidectomy is necessary as MTC is multicentric in 65–90% of patients in MEN 2 and extensive central lymph node dissection has been reported to improve survival and recurrence rates compared to less aggressive procedures [91, 92]. Lymph node dissection of laterocervical compartments is not performed on principle but only when the neck ultrasound suggests the presence of metastatic nodes.

Endoscopic adrenal-sparing surgery has become the method of choice for the surgical therapy of PHEO [93]. In cases with an asynchronous development of PHEO, the adrenal gland without PHEO can be preserved, but the patient must be aware that the probability to repeat the surgical treatment in the near future is very high. The advantage of a monolateral adrenal surgery is the possibility to avoid substitutive therapy until the second surgery will be performed.

The parathyroid glands are frequently found to be enlarged at the time of the thyroidectomy for MTC and should, therefore, be carefully evaluated. The goal in MEN 2 patients with PHPT is to excise the enlarged glands and to leave at least one apparently normal parathyroid gland intact.

If all glands are enlarged, a subtotal parathyroidectomy or total parathyroidectomy with autotransplantation should be performed. In patients with persistent or recurrent PHPT, the long-term oral administration of calcimimetic drugs as cinacalcet to achieve long-term reductions in serum calcium and PTH concentration should be considered.

3.9. Prophylactic or Precocious Thyroidectomy in RET Gene Carrier. Prophylactic thyroidectomy is advised in gene carriers to guarantee a definitive cure in these subjects. Four different risk levels (from A, the lowest, to D the highest) for *RET* mutations have been suggested by the American Thyroid Association task force, which developed the most recent guidelines for the management of MTC patients [4]. According to these guidelines, these levels of risk, which are related to the clinical aggressiveness of the corresponding MTC, should be taken into consideration when planning surgical treatment. In particular patients with a level D, *RET* mutation (i.e., Met918Thr) should be treated as soon as possible in the first year of life; patients with level B and C mutations (located in exons 10, 11, 13, 14, and 15) should be operated on before 5 years of age; only for patients with a level A mutation (exon 8 and 5 mutations), total thyroidectomy can be delayed after five years of age or until the CT positivity.

Recently, some evidences in big series of *RET* gene carriers demonstrated that gene carriers with undetectable levels of basal CT have an almost null risk to have already developed the MTC [90, 94, 95]. Moreover, a serum Ct <30–40 pg/mL is always associated to an intrathyroidal micro-MTC without any evidence of lymph node metastases. Taking into account these observation, Elisei et al. [90] designed a study in which they operated on only *RET* gene carriers on the basis of basal and stimulated CT. According to their results, the time of surgical treatment could be personalized and safely planned when the stimulated serum CT becomes positive at the annual control, independently from the type of *RET* mutation and its associated level of risk. Of course, both cysteine *RET* mutations and older age are risk factors for having an earlier positive result for either basal or Pg-stimulated serum CT. For these reasons, the follow-up controls should be more or less frequent in cysteine or noncysteine *RET*-mutated gene carriers, respectively. This strategy obviously implies a high compliance of the *RET* gene carriers to the scheduled followup with the advantage that young children can be treated later, sometime even after the puberty, close to the adulthood.

3.10. Target Therapy for Persistent MTC. Thirty percent of MTC patients, especially in MEN 2B and 2A, are not cured by surgery. They remain affected and can develop, if not already present at the time of the diagnosis, distant metastasis in the lungs, liver, bone and, more rarely, brain. Several studies demonstrated that conventional therapies, such as chemotherapy and radiotherapy, did not determine any clinical benefit [96, 97]. Until few years ago, patients with advanced and progressive MTC were "orphan" of drugs. Recently, developed molecular therapeutics that target the *RET* pathway have shown very promising activity in clinical trials of patients with advanced MTC [98]. In the majority of cases, the drug is a multityrosine kinase inhibitor (TKI) with the ability to block not only *ret* butalsoone or more of the vascular endothelial growth factor receptors (VEGF-R) as well as C-MET and/or C-KIT or FLT3 and/or other kinases. Vandetanib has been recently approved both by FDA (Food and Drug Administration) and EMA (European Medical Agency) for the treatment of advanced and progressive MTC. Other TKIs, such as sorafenib, sunitinib, motesanib, lenvatinib, AND cabozantinib, are still under investigation either in official phase II/III clinical trials or in "off-label" studies [99]. Although very promising, further studies and longer followup are needed to better evaluate the clinical benefits in terms of progression-free survival and overall survival as compared to the discomfort determined by the side effects which is not negligible. Among several, the most severe and intolerable side effects are anorexia, weight loss, and fatigue, which are difficult to be controlled. Others, such as hypertension or skin lesions can be managed with standard care procedures. A list of drugs used in ongoing clinical trials is reported in Table 6.

Table 6: Drugs used in ongoing clinical trials for the treatment of advanced MTC and other thyroid tumors.

Drug	Molecular target
Axitinib	VEGFR, PDGFRβ, C. Kit
Gefitinib	EGFR
Imatinib	VEGFR, RET, BCR-ABL
Motesanib	VEGFR, RET, PDGFRβ, C. Kit
Sorafenib	VEGFR, RET, RET/PTC, BRAF, PDGFRβ, C. Kit
Sunitinib	VEGFR, RET, RET/PTC, PDGFRβ
Vandetanib	VEGFR, RET, RET/PTC, EGFR
XL184	VEGFR, RET, PDGFRβ

4. Conclusions

MEN syndromes are genetic disease transmitted with an autosomal dominant trait. Although rare, they caught the attention of both endocrinologists and geneticists and much information has been collected in the last decades. We know the genetic alterations of both MEN 1 and MEN 2, how they are transmitted, their prevalence, and the relationship between genotype and phenotype. Much is also known about clinical features and possible treatments. Despite all, information still remain to discover the genetic of MEN cases who are orphan of *MEN 1* or *RET* genes germline mutations.

References

[1] J. A. Carney, "Familial multiple endocrine neoplasia syndromes: components, classification, and nomenclature," *Journal of Internal Medicine*, vol. 243, no. 6, pp. 425–432, 1998.

[2] D. J. Marsh and O. Gimm, "Multiple endocrine neoplasia: types 1 and 2," *Advances in Oto-Rhino-Laryngology*, vol. 70, pp. 84–90, 2011.

[3] M. L. Brandi, R. F. Gagel, A. Angeli et al., "Consensus: guidelines for diagnosis and therapy of MEN type 1 and type 2," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 12, pp. 5658–5671, 2001.

[4] R. T. Kloos, C. Eng, D. B. Evans et al., "Medullary thyroid cancer: management guidelines of the American Thyroid Association," *Thyroid*, vol. 19, no. 6, pp. 565–612, 2009.

[5] N. Wohllk, H. Schweizer, Z. Erlic et al., "Multiple endocrine neoplasia type 2," *Best Practice and Research*, vol. 24, no. 3, pp. 371–387, 2010.

[6] J. A. Carney, "Familial multiple endocrine neoplasia: the first 100 years," *American Journal of Surgical Pathology*, vol. 29, no. 2, pp. 254–274, 2005.

[7] B. Asgharian, Y. J. Chen, N. J. Patronas et al., "Meningiomas may be a component tumor of multiple endocrine neoplasia type 1," *Clinical Cancer Research*, vol. 10, no. 3, pp. 869–880, 2004.

[8] B. Asgharian, M. L. Turner, F. Gibril, L. K. Entsuah, J. Serrano, and R. T. Jensen, "Cutaneous tumors in patients with multiple endocrine neoplasm type 1 (MEN1) and gastrinomas: prospective study of frequency and development of criteria with high sensitivity and specificity for MEN1," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 11, pp. 5328–5336, 2004.

[9] T. N. Darling, M. C. Skarulis, S. M. Steinberg, S. J. Marx, A. M. Spiegel, and M. Turner, "Multiple facial angiofibromas and collagenomas in patients with multiple endocrine neoplasia type 1," *Archives of Dermatology*, vol. 133, no. 7, pp. 853–857, 1997.

[10] C. del Pozo, L. García-Pascual, M. Balsells et al., "Parathyroid carcinoma in multiple endocrine neoplasia type 1. case report and review of the literature," *Hormones*, vol. 10, no. 4, pp. 326–331, 2011.

[11] F. Cetani, E. Pardi, S. Borsari, and C. Marcocci, "Molecular pathogenesis of primary hyperparathyroidism," *Journal of Endocrinological Investigation*, vol. 34, no. 7, supplement, pp. 35–39, 2011.

[12] C. R. C. Pieterman, L. T. Van Hulsteijn, M. Den Heijer et al., "Primary hyperparathyroidism in MEN1 patients: a cohort study with longterm follow-up on preferred surgical procedure and the relation with genotype," *Annals of Surgery*, vol. 255, no. 6, pp. 1171–1178, 2012.

[13] C. Eller-Vainicher, I. Chiodini, C. Battista et al., "Sporadic and MEN1-related primary hyperparathyroidism: differences in clinical expression and severity," *Journal of Bone and Mineral Research*, vol. 24, no. 8, pp. 1404–1410, 2009.

[14] C. Romei, S. Mariotti, L. Fugazzola et al., "Multiple endocrine neoplasia type 2 syndromes (MEN 2): results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes," *European Journal of Endocrinology*, vol. 163, no. 2, pp. 301–308, 2010.

[15] K. Frank-Raue, W. Höppner, A. Frilling et al., "Mutations of the ret protooncogene in German multiple endocrine neoplasia families: relation between genotype and phenotype," *Journal of Clinical Endocrinology and Metabolism*, vol. 81, no. 5, pp. 1780–1783, 1996.

[16] A. Machens, P. Niccoli-Sire, J. Hoegel et al., "Early malignant progression of hereditary medullary thyroid cancer," *New England Journal of Medicine*, vol. 349, no. 16, pp. 1517–1525, 2003.

[17] D. H. Schussheim, M. C. Skarulis, S. K. Agarwal et al., "Multiple endocrine neoplasia type 1: new clinical and basic findings," *Trends in Endocrinology and Metabolism*, vol. 12, no. 4, pp. 173–178, 2001.

[18] F. Tonelli, F. Giudici, G. Fratini, and M. L. Brandi, "Pancreatic endocrine tumors in multiple endocrine neoplasia type 1 syndrome: review of literature," *Endocrine Practice*, vol. 17, supplement 3, pp. 33–40, 2011.

[19] B. Delemer, "MEN1 and pituitary adenomas," *Annales d'Endocrinologie*, vol. 73, no. 2, pp. 59–61, 2012.

[20] L. V. Syro, B. W. Scheithauer, K. Kovacs et al., "Pituitary tumors in patients with MEN1 syndrome," *Clinics*, vol. 67, supplement, pp. 43–48, 2012.

[21] S. Schaefer, M. Shipotko, S. Meyer et al., "Natural course of small adrenal lesions in multiple endocrine neoplasia type 1: an endoscopic ultrasound imaging study," *European Journal of Endocrinology*, vol. 158, no. 5, pp. 699–704, 2008.

[22] R. V. Thakker, "Multiple endocrine neoplasia type 1 (MEN1)," *Best Practice & Research*, vol. 24, no. 3, pp. 355–370, 2010.

[23] P. Goudet, C. Bonithon-Kopp, A. Murat et al., "Gender-related differences in MEN1 lesion occurrence and diagnosis: a cohort study of 734 cases from the Groupe d'étude des Tumeurs Endocrines," *European Journal of Endocrinology*, vol. 165, no. 1, pp. 97–105, 2011.

[24] S. Miedlich, T. Lohmann, U. Schneyer, P. Lamesch, and R. Paschke, "Familial isolated primary hyperparathyroidism—a multiple endocrine neoplasia type 1 variant?" *European Journal of Endocrinology*, vol. 145, no. 2, pp. 155–160, 2001.

[25] C. Larsson, B. Skogseid, K. Oberg, Y. Nakamura, and M. Nordenskjold, "Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma," *Nature*, vol. 332, no. 6159, pp. 85–87, 1988.

[26] S. C. Chandrasekharappa, S. C. Guru, P. Manickam et al., "Positional cloning of the gene for multiple endocrine neoplasia-type 1," *Science*, vol. 276, no. 5311, pp. 404–406, 1997.

[27] A. G. Knudson, "Two genetic hits (more or less) to cancer," *Nature Reviews Cancer*, vol. 1, no. 2, pp. 157–162, 2001.

[28] K. Balogh, A. Patócs, L. Hunyady, and K. Rácz, "Menin dynamics and functional insight: take your partners," *Molecular and Cellular Endocrinology*, vol. 326, no. 1-2, pp. 80–84, 2010.

[29] T. Wu and X. Hua, "Menin represses tumorigenesis via repressing cell proliferation," *American Journal of Cancer Research*, vol. 1, no. 6, pp. 726–39, 2011.

[30] J. Huang, B. Gurung, B. Wan et al., "The same pocket in menin binds both MLL and JUND but has opposite effects on transcription," *Nature*, vol. 482, no. 7386, pp. 542–546, 2012.

[31] M. C. Lemos and R. V. Thakker, "Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene," *Human Mutation*, vol. 29, no. 1, pp. 22–32, 2008.

[32] J. S. Crabtree, P. C. Scacheri, J. M. Ward et al., "A mouse model of multiple endocrine neoplasia, type 1, develops multiple endocrine tumors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 3, pp. 1118–1123, 2001.

[33] P. Bertolino, W. M. Tong, D. Galendo, Z. Q. Wang, and C. X. Zhang, "Heterozygous Men1 mutant mice develop a range of endocrine tumors mimicking multiple endocrine neoplasia type 1," *Molecular Endocrinology*, vol. 17, no. 9, pp. 1880–1892, 2003.

[34] J. S. Crabtree, P. C. Scacheri, J. M. Ward et al., "Of mice and MEN1: insulinomas in a conditional mouse knockout," *Molecular and Cellular Biology*, vol. 23, no. 17, pp. 6075–6085, 2003.

[35] C. J. Lips, K. M. Dreijerink, and J. W. Höppener, "Variable clinical expression in patients with a germline MEN1 disease

gene mutation: clues to a genotype-phenotype correlation," *Clinics*, vol. 67, supplement, pp. 49–56, 2012.

[36] S.-E. Olufemi, J. S. Green, P. Manickam et al., "Common ancestral mutation in the MEN1 gene is likely responsible for the prolactinoma variant of MEN1 (MEN1(Burin)) in four kindreds from Newfoundland," *Human Mutation*, vol. 11, no. 4, pp. 264–269, 1998.

[37] C. Kong, S. Ellard, C. Johnston, and N. R. Farid, "Multiple endocrine neoplasia type 1Burin from Mauritius: a novel MEN 1 mutation," *Journal of Endocrinological Investigation*, vol. 24, no. 10, pp. 806–810, 2001.

[38] J. D. M. Lourenco, R. A. Toledo, I. I. Mackowiak et al., "Multiple endocrine neoplasia type 1 in Brazil: MEN1 founding mutation, clinical features, and bone mineral density profile," *European Journal of Endocrinology*, vol. 159, no. 3, pp. 259–274, 2008.

[39] F. Cetani, E. Pardi, A. Giovannetti et al., "Genetic analysis of the MEN1 gene and HPRT2 locus in two Italian kindreds with familial isolated hyperparathyroidism," *Clinical Endocrinology*, vol. 56, no. 4, pp. 457–464, 2002.

[40] N. S. Pellegata, L. Quintanilla-Martinez, H. Siggelkow et al., "Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 42, pp. 15558–15563, 2006.

[41] N. S. Pellegata, "MENX," *Annales d'Endocrinologie*, vol. 73, no. 2, pp. 65–70, 2012.

[42] S. Molatore and N. S. Pellegata, "The MENX syndrome and p27: relationships with multiple endocrine neoplasia," *Progress in Brain Research*, vol. 182, pp. 295–320, 2010.

[43] I. Marinoni and N. S. Pellegata, "p27kip1: a new multiple endocrine neoplasia gene?" *Neuroendocrinology*, vol. 93, no. 1, pp. 19–28, 2011.

[44] N. Nilubol, L. Weinstein, W. F. Simonds et al., "Preoperative localizing studies for initial parathyroidectomy in MEN1 syndrome: is there any benefit?" *World Journal of Surgery*, vol. 36, no. 6, pp. 1368–1374, 2012.

[45] B. Vergès, F. Boureille, P. Goudet et al., "Pituitary disease in MEN type 1 (MEN1): data from the France-Belgium MEN1 multicenter study," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 2, pp. 457–465, 2002.

[46] C. Barbe, A. Murat, B. Dupas et al., "Magnetic resonance imaging versus endoscopic ultrasonography for the detection of pancreatic tumours in multiple endocrine neoplasia type 1," *Digestive and Liver Disease*, vol. 44, no. 3, pp. 228–234, 2012.

[47] R. V. Thakker, P. J. Newey, G. V. Walls et al., "Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1)," *Journal of Clinical Endocrinology and Metabolism*, vol. 97, no. 9, pp. 2990–3011, 2012.

[48] C. A. Stratakis, D. H. Schussheim, S. M. Freedman et al., "Pituitary macroadenoma in a 5-year-old: an early expression of multiple endocrine neoplasia type 1," *Journal of Clinical Endocrinology and Metabolism*, vol. 85, no. 12, pp. 4776–4780, 2000.

[49] T. Carling and R. Udelsman, "Parathyroid surgery in familial hyperparathyroid disorders," *Journal of Internal Medicine*, vol. 257, no. 1, pp. 27–37, 2005.

[50] J. Waldmann, C. L. López, P. Langer, M. Rothmund, and D. K. Bartsch, "Surgery for multiple endocrine neoplasia type 1-associated primary hyperparathyroidism," *British Journal of Surgery*, vol. 97, no. 10, pp. 1528–1534, 2010.

[51] D. M. Elaraj, M. C. Skarulis, S. K. Libutti et al., "Results of initial operation for hyperparathyroidism in patients with multiple endocrine neoplasia type 1," *Surgery*, vol. 134, no. 6, pp. 858–865, 2003.

[52] F. Tonelli, S. Spini, M. Tommasi et al., "Intraoperative parathormone measurement in patients with multiple endocrine neoplasia type I syndrome and hyperparathyroidism," *World Journal of Surgery*, vol. 24, no. 5, pp. 556–563, 2000.

[53] F. Tonelli, T. Marcucci, G. Fratini, M. S. Tommasi, A. Falchetti, and M. L. Brandi, "Is total parathyroidectomy the treatment of choice for hyperparathyroidism in multiple endocrine neoplasia type 1?" *Annals of Surgery*, vol. 246, no. 6, pp. 1075–1082, 2007.

[54] P. V. Dickson, T. A. Rich, Y. Xing et al., "Achieving eugastrinemia in MEN1 patients: both duodenal inspection and formal lymph node dissection are important," *Surgery*, vol. 150, no. 6, pp. 1143–1152, 2011.

[55] F. Triponez, D. Dosseh, P. Goudet et al., "Epidemiology data on 108 MEN 1 patients from the GTE with isolated nonfunctioning tumors of the pancreas," *Annals of Surgery*, vol. 243, no. 2, pp. 265–272, 2006.

[56] K. Öberg, "Neuroendocrine tumors of the digestive tract: impact of new classifications and new agents on therapeutic approaches," *Current Opinion in Oncology*, vol. 24, no. 4, pp. 433–440, 2012.

[57] A. Germain, M. Klein, and L. Brunaud, "Surgical management of adrenal tumors," *Journal of Visceral Surgery*, vol. 148, no. 4, pp. e250–e261, 2011.

[58] P. Goudet, A. Murat, C. Binquet et al., "Risk factors and causes of death in men1 disease. a gte (groupe d'etude des tumeurs endocrines) cohort study among 758 patients," *World Journal of Surgery*, vol. 34, no. 2, pp. 249–255, 2010.

[59] H. P. H. Neumann, A. Vortmeyer, D. Schmidt et al., "Brief report: evidence of MEN-2 in the original description of classic pheochromocytoma," *New England Journal of Medicine*, vol. 357, no. 13, pp. 1311–1315, 2007.

[60] J. H. Sipple, "Multiple endocrine neoplasia type 2 syndromes: historical perspectives," *Henry Ford Hospital Medical Journal*, vol. 32, no. 4, pp. 219–222, 1984.

[61] H. R. Keiser, M. A. Beaven, J. Doppman, S. Wells, and L. M. Buja, "Sipple's syndrome: medullary thyroid carcinoma, pheochromocytoma, and parathyroid disease. Studies in a large family. NIH conference," *Annals of Internal Medicine*, vol. 78, no. 4, pp. 561–579, 1973.

[62] A. L. Steiner, A. D. Goodman, and S. R. Powers, "Study of a kindred with pheochromocytoma, medullary thyroid carcinoma, hyperparathyroidism and Cushing's disease: multiple endocrine neoplasia, type 2," *Medicine*, vol. 47, no. 5, pp. 371–409, 1968.

[63] J. R. Howe, J. A. Norton, S. A. Wells, C. Proye, G. B. Talpos, and S. E. Carty, "Prevalence of pheochromocytoma and hyperparathyroidism in multiple endocrine neoplasia type 2A: results of long-term follow-up," *Surgery*, vol. 114, no. 6, pp. 1070–1077, 1993.

[64] W. B. Inabnet, P. Caragliano, D. Pertsemlidis et al., "Pheochromocytoma: inherited associations, bilaterality, and cortex preservation," *Surgery*, vol. 128, no. 6, pp. 1007–1012, 2000.

[65] J. M. Rodriguez, M. Balsalobre, J. L. Ponce et al., "Pheochromocytoma in MEN 2A syndrome. Study of 54 patients," *World Journal of Surgery*, vol. 32, no. 11, pp. 2520–2526, 2008.

[66] M. Verdy, A. M. Weber, and C. C. Roy, "Hirschsprung's disease in a family with multiple endocrine neoplasia type 2," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 1, no. 4, pp. 603–607, 1982.

[67] M. B. Verdy, M. Cadotte, and W. Schurch, "A French Canadian family with multiple endocrine neoplasia type 2 syndromes,"

Henry Ford Hospital Medical Journal, vol. 32, no. 4, pp. 251–253, 1984.

[68] C. Eng, D. Clayton, I. Schuffenecker et al., "The relationship between specific ret proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2: international RET mutation consortium analysis," *Journal of the American Medical Association*, vol. 276, no. 19, pp. 1575–1579, 1996.

[69] D. T. Donovan, M. L. Levy, B. R. Alford et al., "Familial cutaneous lichen amyloidosis in association with multiple endocrine neoplasia type 2A: a new variant," *Henry Ford Hospital Medical Journal*, vol. 37, no. 3-4, pp. 147–150, 1989.

[70] R. F. Gagel, M. L. Levy, D. T. Donovan, B. R. Alford, T. Wheeler, and J. A. Tschen, "Multiple endocrine neoplasia type 2a associated with cutaneous lichen amyloidosis," *Annals of Internal Medicine*, vol. 111, no. 10, pp. 802–806, 1989.

[71] W. J. Cunliffe, P. Hudgson, J. J. Fulthorpe et al., "A calcitonin-secreting medullary thyroid carcinoma associated with mucosal neuromas, marfanoid features, myopathy and pigmentation," *The American Journal of Medicine*, vol. 48, no. 1, pp. 120–126, 1970.

[72] M. Brauckhoff, A. Machens, S. Hess et al., "Premonitory symptoms preceding metastatic medullary thyroid cancer in MEN 2B: an exploratory analysis," *Surgery*, vol. 144, no. 6, pp. 1044–1051, 2008.

[73] J. R. Farndon, G. S. Leight, and W. G. Dilley, "Familial medullary thyroid carcinoma without associated endocrinopathies: a distinct clinical entity," *British Journal of Surgery*, vol. 73, no. 4, pp. 278–281, 1986.

[74] R. Elisei, C. Romei, B. Cosci et al., "Brief report: RET genetic screening in patients with medullary thyroid cancer and their relatives: experience with 807 individuals at one center," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 12, pp. 4725–4729, 2007.

[75] P. Niccoli-Sire, A. Murat, V. Rohmer et al., "Familial medullary thyroid carcinoma with noncysteine RET mutations: phenotype-genotype relationship in a large series of patients," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 8, pp. 3746–3753, 2001.

[76] C. Romei, B. Cosci, G. Renzini et al., "RET genetic screening of sporadic medullary thyroid cancer (MTC) allows the preclinical diagnosis of unsuspected gene carriers and the identification of a relevant percentage of hidden familial MTC (FMTC)," *Clinical Endocrinology*, vol. 74, no. 2, pp. 241–247, 2011.

[77] M. S. Cohen and J. F. Moley, "Surgical treatment of medullary thyroid carcinoma," *Journal of Internal Medicine*, vol. 253, no. 6, pp. 616–626, 2003.

[78] H. Donis-Keller, S. Dou, D. Chi et al., "Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC," *Human Molecular Genetics*, vol. 2, no. 7, pp. 851–856, 1993.

[79] L. M. Mulligan, J. B. J. Kwok, C. S. Healey et al., "Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A," *Nature*, vol. 363, no. 6428, pp. 458–460, 1993.

[80] C. Eng, D. P. Smith, L. M. Mulligan et al., "Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours," *Human Molecular Genetics*, vol. 3, no. 2, pp. 237–241, 1994.

[81] M. F. Erdogan, A. Gürsoy, G. Özgen et al., "Ret proto-oncogene mutations in apparently sporadic Turkish medullary thyroid carcinoma patients: Turkmen study," *Journal of Endocrinological Investigation*, vol. 28, no. 9, pp. 806–809, 2005.

[82] C. Eng, L. M. Mulligan, D. P. Smith et al., "Low frequency of germline mutations in the RET protooncogene in patients with apparently sporadic medullary thyroid carcinoma," *Clinical Endocrinology*, vol. 43, no. 1, pp. 123–127, 1995.

[83] G. Orgiana, G. Pinna, A. Camedda et al., "A new germline RET mutation apparently devoid of transforming activity serendipitously discovered in a patient with atrophic autoimmune thyroiditis and primary ovarian failure," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 10, pp. 4810–4816, 2004.

[84] B. Cosci, A. Vivaldi, C. Romei et al., "In silico and in vitro analysis of rare germline allelic variants of RET oncogene associated with medullary thyroid cancer," *Endocrine-Related Cancer*, vol. 18, no. 5, pp. 603–612, 2011.

[85] J. R. Hansford and L. M. Mulligan, "Multiple endocrine neoplasia type 2 and RET: from neoplasia to neurogenesis," *Journal of Medical Genetics*, vol. 37, no. 11, pp. 817–827, 2000.

[86] K. M. Zbuk and C. Eng, "Cancer phenomics: RET and PTEN as illustrative models," *Nature Reviews Cancer*, vol. 7, no. 1, pp. 35–45, 2007.

[87] A. Miyauchi, H. Futami, N. Hai et al., "Two germline missense mutations at codons 804 and 806 of the RET proto-oncogene in the same allele in a patient with multiple endocrine neoplasia type 2B without codon 918 mutation," *Japanese Journal of Cancer Research*, vol. 90, no. 1, pp. 1–5, 1999.

[88] F. H. Menko, R. B. Van Der Luijt, I. A. J. De Valk et al., "Atypical MEN type 2B associated with two germline RET mutations on the same allele not involving codon 918," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 1, pp. 393–397, 2002.

[89] K. Frank-Raue, S. Rondot, and F. Raue, "Molecular genetics and phenomics of RET mutations: impact on prognosis of MTC," *Molecular and Cellular Endocrinology*, vol. 322, no. 1-2, pp. 2–7, 2010.

[90] R. Elisei, C. Romei, G. Renzini et al., "The timing of total thyroidectomy in RET gene mutation carriers could be personalized and safely planned on the basis of serum calcitonin: 18 Years experience at one single center," *Journal of Clinical Endocrinology and Metabolism*, vol. 97, no. 2, pp. 426–435, 2012.

[91] A. Machens, S. Hauptmann, and H. Dralle, "Increased risk of lymph node metastasis in multifocal hereditary and sporadic medullary thyroid cancer," *World Journal of Surgery*, vol. 31, no. 10, pp. 1960–1965, 2007.

[92] C. F. Russell, J. A. Van Heerden, and G. W. Sizemore, "The surgical management of medullary thyroid carcinoma," *Annals of Surgery*, vol. 197, no. 1, pp. 42–48, 1983.

[93] M. K. Walz and P. F. Alesina, "Single access retroperitoneoscopic adrenalectomy (SARA)-one step beyond in endocrine surgery," *Langenbeck's Archives of Surgery*, vol. 394, no. 3, pp. 447–450, 2009.

[94] G. S. K. Lau, B. H. H. Lang, C. Y. Lo et al., "Prophylactic thyroidectomy in ethnic Chinese patients with multiple endocrine neoplasia type 2A syndrome after the introduction of genetic testing," *Hong Kong Medical Journal*, vol. 15, no. 5, pp. 326–331, 2009.

[95] V. Rohmer, G. Vidal-Trecan, A. Bourdelot et al., "Prognostic factors of disease-free survival after thyroidectomy in 170 young patients with a RET germline mutation: a multicenter study of the Groupe Français d'Etude des Tumeurs Endocrines," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 3, pp. E509–E518, 2011.

[96] S. I. Sherman, "Advances in chemotherapy of differentiated epithelial and medullary thyroid cancers," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 5, pp. 1493–1499, 2009.

[97] S. I. Sherman, "Cytotoxic chemotherapy for differentiated thyroid carcinoma," *Clinical Oncology*, vol. 22, no. 6, pp. 464–468, 2010.

[98] S. A. Wells Jr., B. G. Robinson, R. F. Gagel et al., "Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial," *Journal of Clinical Oncology*, vol. 30, no. 2, pp. 134–141, 2012.

[99] M. L. Gild, M. Bullock, B. G. Robinson, and R. Clifton-Bligh, "Multikinase inhibitors: a new option for the treatment of thyroid cancer," *Nature Reviews Endocrinology*, vol. 7, no. 10, pp. 617–624, 2011.

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 391629, 10 pages
doi:10.1155/2012/391629

Review Article

New Treatment in Advanced Thyroid Cancer

Dario Giuffrida, Angela Prestifilippo, Alessia Scarfia, Daniela Martino, and Stefania Marchisotta

Department of Medical Oncology, Mediterranean Institut of Oncology, Via Penninazzo, 7, 95029 Viagrande, Italy

Correspondence should be addressed to Dario Giuffrida, dariogiuffrida@netscape.net

Received 25 June 2012; Revised 14 August 2012; Accepted 15 August 2012

Academic Editor: Marialuisa Appetecchia

Thyroid cancer is the most common endocrine tumor. Thyroidectomy, radioactive iodine, and TSH suppression represent the standard treatment for differentiated thyroid cancer. Since chemotherapy has been shown to be unsuccessful in case of advanced thyroid carcinomas, the research for new therapies is fundamental. In this paper, we reviewed the recent literature reports (pubmed, medline, EMBASE database, and abstracts published in meeting proceedings) on new treatments in advanced nonmedullary and medullary thyroid carcinomas. Studies of many tyrosine kinase inhibitors as well as antiangiogenic inhibitors suggest that patients with thyroid cancer could have an advantage with new target therapy. We summarized both the results obtained and the toxic effects associated with these treatments reported in clinical trials. Reported data in this paper are encouraging, but further trials are necessary to obtain a more effective result in thyroid carcinoma treatment.

1. Introduction

Thyroid cancer is rare, but is the most prevalent endocrine malignancy tumor. In 2002, in the USA 141,000 cases occurred and 35,300 deaths were estimated [1]. Among different parts of the world there is a 10-fold difference in incidence for women, but only a 3-fold difference for men [2].

The differences between the sexes declines after the middle age, but still three out of four cases arise in women. The most well-established cause of thyroid cancer is the exposure to ionizing radiations, particularly during childhood. Iodine deficiency influences thyroid function directly as well as indirectly, through a reduction of thyroid hormones levels and a consequent increase in TSH secretion. Chronic iodine deficiency is firmly established as a risk factor for goiter and follicular thyroid cancer, while some aetiological studies suggested that iodine supplementation programmes could increase the incidence of papillary thyroid cancer by inducing iodine excess. Supplementation effects are likely to be confused by diagnostic procedures improvement and therefore there may be not a biological background at the basis of this phenomenon [3]. Thyroid cancer is a heterogeneous disease that is classified into differentiated thyroid carcinoma (DTC), anaplastic thyroid carcinoma (ATC) and medullary thyroid carcinoma (MTC). DTC and ATC together are classified as nonmedullary thyroid cancer (NMTC). DTCs are the most common histotype (85%), and include papillary (70%) and follicular (10%–15%) as well as subtypes like Hurthle cell carcinomas. Although activating point mutations of the TSH receptor have been discovered in 60–70% of benign toxic adenomas, a pathogenetic role for these mutations in malignant transformation has been excluded or rarely reported [4]. In the last two decades, the molecular basis of thyroid cancer have been well characterized and the critical genetic pathways involved in the development of specific tumors histotype have been elucidated. Around 20–25% of thyroid medullary carcinomas can be attributed to genetic factors [5]. In particular, germ-line mutations in the RET gene are responsible for the hereditary tumour syndrome (i.e., multiple endocrine neoplasia type 2, MEN 2) which includes three subgroups, MEN 2A, MEN 2B, and

familial medullary thyroid carcinoma (FMTC), depending on the tissue involved. Follicular cell proliferation and function is physiologically regulated by thyroid-stimulating hormone (TSH). Most of the DTC are slowly progressive and frequently cured with adequate surgical management and radioactive iodine (131-I) ablation therapy (RAI), when identified at an early stage. Metastatic DTC that is untreatable by surgery or refractory to radioactive iodine therapy is associated with poor survival. MTC and, especially, ATC metastasize up to the 50% of diagnosticated cases, giving a worst prognosis. ATC is one of the most aggressive neoplasm in humans with a mortality rate over 90% and a mean survival of 6 months after diagnosis [6, 7]. Standard treatments in some cases of advanced differentiated thyroid cancer and medullary thyroid cancer (radiotherapy and/or chemotherapy) have been unsatisfactory and therefore new therapies are necessary. In the past decade, multiple clinical trials have been carried out thanks to an increased knowledge of the biological basis of thyroid cancer and to development of new treatments that target biological substrates. This paper will focus on current clinical trials and recent therapies on specific target involved in thyroid carcinogenesis.

2. Molecular Target Therapy in Advanced Thyroid Cancer

Recent advances in molecular biology resulted in significant improvement in our understanding of the pathogenesis of thyroid carcinoma

Gene rearrangements involving the RET and TRK proto-oncogenes have been demonstrated as causative events specific for a subset of the papillary histotype. Recently, another oncogene, BRAF, has been specifically associated with PTC with a frequency around 40%. Mutated forms of the H-ras, K-ras, and N-ras oncogenes are found in differentiated thyroid cancer, but the same mutation are also described in benign thyroid lesion.

RET-activating point mutations have been found exclusively in medullary thyroid carcinoma (MTC) and these mutations are observed in both sporadic MTC and FMTC.

All the identified mutation on RAS, RET, TRK, and BRAF genes involve MAP kinase activation. An abnormal activation of this pathway is one of the most studied mechanisms of thyroid tumorigenesis. In a lower percentage, other abnormalities have been reported to be involved in thyroid tumorigenesis such as DNA methylation [8] and gene deletions in chromosomes 11q13 and 3p [9].

RAS-activation induces cell division and inhibits cell differentiation. The expression of p21, the RAS-encoded protein, plays an important role in the intracellular signal transduction from the cell surface to the nucleus where it is able to activate genes expression that induces cell proliferation [10]. In thyroid neoplastic cell proliferation RAS role is still poorly known. It has been hypothesized that activated p21 could interact with some thyroid-specific transcription factors such as TTF1 or PAX-8 [11]. RAS activating point mutations have been found in 3 hot spots localized in the codons 12, 13, and 61. RAS oncogene point mutations account for nearly 40% of benign and malignant follicular thyroid tumours while they are rare in the papillary histotype [12, 13]. Interestingly, RAS mutations are more frequent in thyroid tumors of subjects living in countries where iodine intake is inadequate [14].

The RET proto-oncogene is located on chromosome 10q11-2. It encodes for a tyrosine kinase transmembrane receptor involved in the activation of the MAP kinase cascade. The proto-oncogene is normally expressed in a variety of neural cell lineages including thyroid C cells and adrenal medulla but it is not expressed, or it is expressed at very low levels, in normal thyroid follicular cells [15]. RET oncogene activation may be generated either by a fusion rearrangement of the tyrosine kinase domain of RET gene and the 5′ domain of other genes [16] or by activating point mutations [17]. RET/PTC rearrangements have been reported only in PTC [18] and in some cases of benign follicular adenomas [19]. Activating RET-point mutations have been exclusively found in MTC [17]. Several RET/PTC rearrangements have been described and all of them are characterized by the fusion of the RET tyrosine kinase domain with a housekeeping gene triggering the constitutive RET expression in the follicular cell [20–22]. RET/PTC rearrangements are related to ionizing radiation exposure which is a well-recognized risk factor for PTC. The evidence of an increasing incidence of RET/PTC rearrangements in childhood post-Chernobyl thyroid carcinomas [23] and the possibility of determining RET/PTC rearrangements *in vitro* in thyroid cells experimentally exposed to ionizing radiation [24] is a clear proof in favour of a causative connection between radiation exposure and these chromosomal alterations. Despite this evidence, RET/PTC rearrangements have also been reported in unirradiated thyroid lesions [25]. The prevalence of RET/PTC rearrangements in thyroid tumors of patients who had no history of neck irradiation ranges from 2.5 to 35% among different series [16, 23, 26–30]. The identification of RET/PTC rearrangements in microPTCs suggests that this is an early event in thyroid carcinogenesis [29]. On the other hand, RET/PTC positive tumors do not show a tendency of progression to poorly or undifferentiated tumor phenotype [31]. Germline RET point mutations in MTC are mainly localized in the tyrosine kinase domain and in the cysteine domain of the gene. Recently several other noncysteine mutations have been described, usually correlated with less aggressive phenotypes [32]. The point mutation determines a constitutive activation of the tyrosine kinase receptor and, as consequence, a continuous stimulus to cell proliferation. In thyroid tumors alteration of RET pathway have been found not only on mutation/overexpression of RET gene, but have been attributed to downstream protein.

Recently, an activating mutation of the B isoform of the Raf kinase gene, located on exon 15, which results in a valine to glutamic acid substitution at amino acid 600 ($BRAF^{V600E}$ mutation) has been found to be the most common mutation in PTC (Figure 1). [33] This mutation has a key role in leading to a constitutively activated state of the gene and thus tumorigenesis. Recently, $BRAF^{V600E}$ has emerged as a promising prognostic factor in the risk stratification of PTC and it has showed an association between *BRAF* mutation

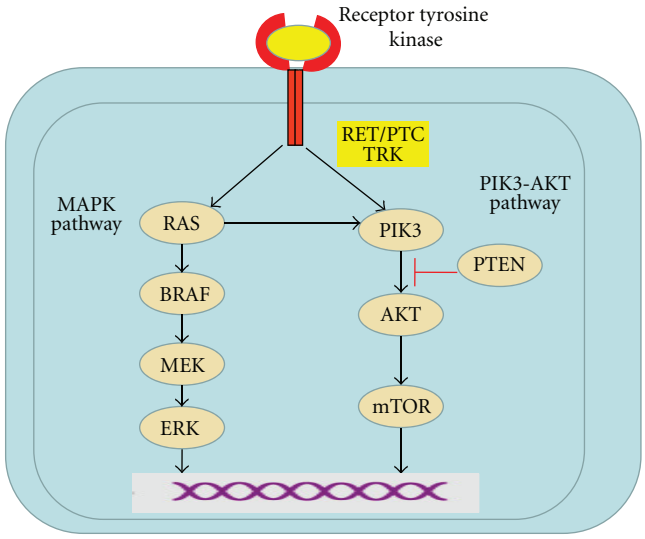


Figure 1: signaling pathways in thyroid cancer.

and high-risk clinical-pathological characteristics of PTCs [34].

3. Standard Treatment of Thyroid Cancer

Radioiodine (131-I) therapy has been used in the treatment of patients with well-differentiated tumors (papillary or follicular). Thyroid cancer tissue has a unique ability to uptake iodine from blood. Like iodine, radioiodine is uptaken and concentrated in thyroid follicular cells by specific membrane transporters. Compared with normal thyroid follicular cells, thyroid cancer cells have reduced expression of the transporter, which may account for the low 131-I uptake in thyroid cancer tissue.

131-I causes acute thyroid-cell death by emission of short path-length (1 to 2 mm) beta rays. 131-I uptake by thyroid tissue can be visualized by gamma radiation scanning. 131-I must be uptaken by thyroid tissue to be effective, resulting in an absence of response in patients whose thyroid cancers do not concentrate iodide, for example, patients with medullary cancer, lymphoma, or anaplastic cancer. Indications for 131-I administration after thyroidectomy in patients with differentiated thyroid cancer include ablation of residual normal thyroid tissue, adjuvant therapy of subclinical micrometastatic disease, and treatment of clinically apparent residual or metastatic thyroid cancer. The efficacy of radioiodine for both scanning and treatment depends upon patient preparation, tumor-specific characteristics, sites of disease, and dose [33, 35–37].

4. New Treatment Modalities in Thyroid Cancer

In a near future, Tyrosine Kinase Inhibitors (TKIs) may open a new era in the radioactive iodine refractory DTC and advanced MTC patients treatment. However, the published clinical trials are relatively limited compared to other malignancies and there is only one reported phase III trial in thyroid cancers and many others phase III are ongoing. The difficulty in enrollment of an adequate number of patients to these clinical trials may be a possible reason for this. It may be possible to overcome this difficulty by multi-institutional trials. On the other hand, there is no proof yet that TKIs improve overall survival. Moreover, having a relatively high number of significant undesirable effects, (see Table 1) patients must be selected carefully before starting the therapy. Randomised clinical trials for several agents are ongoing.

We examined the results and the adverse events for each TKIs used in thyroid-cancer-targeted therapy, reported in literature.

4.1. Sunitinib (SU1248). Sunitinib is a multitargeted tyrosine kinase inhibitor (TKI). Targets of the drug include vascular endothelial growth factor receptor (VEGFR) types 1 and 2, platelet-derived growth factor receptors, c-KIT, FLT3, and RET. The inhibitory effect of the drug on VEGF and RET makes it a rational candidate for the therapy of DTC and MTC. Somatic mutations of the proto-oncogene RET are critical in the development of MTC. In addition, elevated serum levels of vascular endothelial growth factor are also associated with poor prognosis in papillary carcinoma of the thyroid.

Sunitinib is currently approved for the therapy of renal cell carcinoma and gastrointestinal stromal tumor (GIST) on an intermittent treatment schedule. Actually the effect of sunitinib on DTC and MTC patients has been reported only on phase II trials, as phase III trials are absent.

Preliminary results from an open-label phase II trial in patients with progressive DTC or MTC reported partial response in 13% of 31 DTC patients, and disease stabilization in 68% of DTC and 83% of MTC patients [38]. Treatment consisted of 6-week cycles of sunitinib malate 50 mg everyday on a 4-week on/2-week off schedule. Primary endpoint was clinical response rate evaluated by RECIST and biochemical response rate.

The most common drug-related adverse events included fatigue (79%), diarrhea (56%), palmar-plantar erythrodysesthesia (53%), neutropenia (49%), and hypertension (42%). Grade 3-4 toxicity included neutropenia (26%), thrombocytopenia (16%), hypertension (16%), fatigue (14%), palmar-plantar erythrodysesthesia (14%), and gastrointestinal tract events (14%) [38]. Additionally, in an open-label phase II trial in patients with progressive DTC or MTC 18 patients were enrolled (3 MTC, 15 DTC) [39]. Treatment consisted of sunitinb 37.5 mg daily until tumor progression or prohibitive toxicity. The primary endpoint was response rate per RECIST criteria. Secondary endpoints included FDG-PET scan response rate (defined as 20% reduction from baseline SUV) after 7 days of treatment, toxicity, overall survival, duration of response, and time-to-tumor progression. Preliminary results showed that 44% of patients had FDG-PET response. All these patients had DTC. Grade 3 toxicities included neutropenia (28%), leukopenia (17%), anemia (6%), thrombocytopenia (6%), fatigue (11%), hand-foot syndrome (11%), pain (11%), gastrointestinal bleeding

Table 1: Most frequent (all grade) adverse events of tyrosine kinase inhibitors used in thyroid cancer.

Adverse event	Sunitinib [37, 39]	Sorafenib [41–45]	Vandetanib [47–49]	Motesanib [50]	Axitinib [53]	Pazopanib [55]	Lenvatinib [58]
Hypertension	22%	48%	33%	27%	28%	—	64%
Diarrhea	37%	77%	57%	41%	48%	73%	45%
Fatigue	45%	48%	43%	41%	50%	78%	55%
Weight loss	—	54%	30%	22%	25%	64%	43%
Nausea	—	22%	37%	26%	33%	73%	44%
Hand-foot skin reaction	35%	91%	—	—	15%	—	—
Rash	—	73%	46%	—	15%	75%	—

(11%), diarrhea (6%), mucositis (6%), and atrial fibrillation (6%) of the patients. There have been no grade 4 toxicities.

Recently, in a phase II study, sunitinib was administered at a dose of 37.5 mg/day in continuous schedule [40]. Thirty-five patients were evaluated with sunitinib; twenty-four patients underwent evaluation by FDG-PET both at baseline and after 7 days of sunitinib therapy.

Eight of 29 patients with DTC and 3 of 6 patients with MTC achieved a RECIST response (response rate, 28% and 50% for DTC and MTC, resp.). There were 1 complete response (3%) and 10 partial responses (28%). In addition, 16 patients (46%) had stable disease.

The median time to progression (TTP) was 12.8 months, and the decline in the uptake of fluorodeoxyglucose (FDG) at 7 days of treatment with sunitinib was superior in those patients who subsequently achieved positive radiological response (by RECIST criteria).

The most common toxicities seen included fatigue (11%), neutropenia (34%), hand/foot syndrome (17%), diarrhea (17%), and leukopenia (31%). One patient on anticoagulation died of gastrointestinal bleeding.

Tumors were highly metabolically active by FDG-PET, with median lesion SUV of 7.9, indicating an aggressive phenotype. In fact the presence of FDG-avid tumors is strongly predictive of a more aggressive course of the disease and associated with a 5-year OS of less than 50% [41].

Carr et al. [40] attempted to correlate the results of a FDG-PET scan one week after therapy initiation with a subsequent response to therapy, based on data showing that a decline in FDG uptake could be an early indicator of response in other diseases treated by sunitinib.

It was observed that there is a significant association between average SUV percent change and RECIST response. Patients with partial/complete response and stable disease had a significant decline in average SUVs compared with patients with progressive disease. This could provide a very useful method to predict treatment benefit, particularly when using an expensive therapy in a clinical situation where stable radiologic disease is of unclear significance. It is possible, and perhaps likely, that an FDG-PET done later than 1 week from treatment initiation would have been a better predictor of benefit and may merit further investigation.

Another open question is about type of schedule of Sunitinib. In fact, it was administered at a dose of 37.5 mg/day in a continuous schedule, while in renal cell carcinoma and gastrointestinal stromal tumor (GIST) Sunitinib is currently approved on an intermittent treatment schedule.

Therefore phase III clinical trials are necessary to define their accurate clinical benefit and the best schedule of treatment.

4.2. Sorafenib (Bay 43-9006). Sorafenib (BAY 43-9006) is an oral, small-molecule TKI targeting VEGF receptors 2 and 3, RET (including most mutant forms that have been examined), and BRAF. In preclinical studies, sorafenib prevented the growth of the TPC1- and TT-cell lines, which contain the RET/PTC1 and C634W RET mutations, respectively.

The effect of sorafenib on DTC and MTC patients has been reported on 4 nonrandomized phase II studies which used a dose of sorafenib 800 mg/day as a single agent in patients with DTC refractory to radioactive iodine. At the moment no phase III trials have been reported.

In the 30 patients treated by the group of Gupta-Abramson et al. a median PFS of 18.4 months was achieved: 7 (23%) patients achieving an objective radiological partial response and 16 patients (53%) achieving disease stabilization of more than 6 months [42].

In a more recent study, similar results were observed in 41 patients with PTC. In these patients, the objective radiological response rate was 15%, and disease stabilization was observed in 56% of patients [43]. The median PFS was 15 months.

In another study, a total of 34 patients with thyroid cancer were treated (15 MTC, 19 DTC) with an objective response rate of 25% in DTC and 18% in MTC at 12 months [44]. In a more recent study conducted in 32 DTC patients, the partial response rate achieved was 25%, and a

stabilization of disease was observed in 34% of patients at 26 weeks [45]. Most adverse effects occurring in these 4 studies were consistent with the already-known safety profile of the drug; the majority of toxicities found were grade I and II and easily manageable with a delay or dose reduction of sorafenib administration. Taken together, these results formed the scientific basis for the launch of a phase III registration termed DECISION (Study of Sorafenib in Metastatic or Locally Advanced, Refractory Patients with Thyroid Cancer RAI). The study compared the administration of sorafenib versus placebo in 380 patients with radioiodine-refractory DTC with PFS as the primary endpoint (NCT00984282). This study has just completed recruitment, and results are awaited with interest.

The anti-RET activity of sorafenib makes MTC a potential therapeutic target for this drug as well.

Preliminary results have been reported from open-label phase II study in patients with metastatic MTC [46]. Although partial response was observed in only 6% of patients with sporadic MTC, stable disease lasting more than 6 months was reported in 62%. A high frequency of side effects was noted, including flushing, diarrhea, weight loss, alopecia, hand-foot syndrome, and rash. Severe adverse events included a pulmonary embolus, hypokalemia, hypertension, hyponatremia, joint pain, and thrombocytopenia.

Anticipating synergy between sorafenib's ability to inhibit MAPK signaling and the RAS-blocking effects of the farnesyltransferase inhibitor tipifarnib, a phase I trial was performed of the combination of these drugs. The maximum tolerated doses of sorafenib and tipifarnib were 200 and 100 mg twice daily, respectively. In the 22 patients with DTC treated, median PFS was 20 months [47].

4.3. Vandetanib (ZD6474). Vandetanib is a small-molecule tyrosine kinase inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2), epidermal growth factor receptor (EGFR), and rearranged-during-transfection (RET-) dependent signaling.

MTC is a rare disease for which vandetanib was granted orphan-drug designation and for which there were previously no approved therapies. In the majority of cases of MTC, there is activation of the RET proto-oncogene, and both VEGFR and EGFR signaling pathways may also contribute to the pathogenesis.

On the basis of the preclinical demonstration that vandetanib inhibited most RET-point mutations, a multicenter, open-label phase II trial studied the efficacy of the drug in patients with metastatic familial forms of MTC. Thirty patients were enrolled, starting therapy with vandetanib, 300 mg daily. Confirmed partial response was reported in 21% of these patients, the median duration of response at data cutoff was 10.2 months. Calcitonin levels dropped by more than 50% in most patients (80%), but blocking RET may lead to a direct inhibition of calcitonin-gene expression, independent of tumor volume changes [48]. Adverse events were predominantly grade 1 or 2, and the most common events included diarrhea, fatigue, rash, and nausea. The most common grade 3 adverse events were QT prolongation and diarrhea, nausea, and hypertension. There were grade 4 adverse events of azotemia or muscle weakness, which were not considered by the investigator to be related to vandetanib. All of these events were managed with dose interruptions or reductions.

To assess the potential efficacy of a lower dose of vandetanib, Robinson and colleagues conducted a second single-arm phase II study in a similar population of patients with hereditary MTC to evaluate the activity of a 100 mg dose of vandetanib [49]. This study comprised 19 patients and demonstrated that the lower dose of vandetanib also has activity in this patient population. The objective tumor response rate was 16%, with a median duration of response of 6 months. The median PFS could not be determined because of an insufficient number of progression events. However, only 16% of the patients had a reduction in calcitonin levels of at least 50% from baseline.

Vandetanib 100 mg/d was well tolerated in the majority of patients in this study, most adverse events were of Common Terminology Criteria for Adverse Events (CTCAE) grade 1 or 2 and were manageable. Diarrhea, fatigue, and rash were the most common adverse events reported.

On the basis of the results of the phase II studies in hereditary MTC, Wells and colleagues initiated a randomized, placebo-controlled phase III study (ZETA) of vandetanib in patients with MTC. The ZETA study enrolled patients with both hereditary and sporadic MTC. A total of 331 patients were randomized to receive vandetanib 300 mg or placebo in a 2 : 1 ratio [50].

The ZETA study demonstrated a clinically significant benefit for vandetanib in prolonging PFS, with a statistically significant hazard ratio (HR) = 0.46 (95% confidence interval = 0.31–0.69; $P = 0.0001$). This HR represents a 54% reduction in the risk of progression for patients randomized to vandetanib. The median PFS for patients randomized to placebo was 19 months, whereas the median PFS for patients randomized to vandetanib was not reached but was estimated to be approximately 30 months. In addition to the benefits with respect to PFS, vandetanib also induced objective tumor responses in 45% of patients. Among the patients randomized to placebo, 13% (13 patients) had an objective tumor response according to the intention-treat analysis, but 12 of these 13 responses occurred only after the patients had switched over to open-label vandetanib. Significant decreases in calcitonin and CEA levels were seen in patients randomized to vandetanib, with 69% of patients on the vandetanib arm experiencing a calcitonin response (decline of at least 50% from baseline) and 52% having a CEA response, as compared to 3% and 2%, respectively, in those on placebo.

Almost all the patients randomized to vandetanib on the ZETA study experienced at least one adverse event, and 55% experienced an event of Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or higher. The most commonly reported side effects included rash (particularly photosensitivity), diarrhea, fatigue, and nausea, whereas the most severe toxicities included asymptomatic QT interval prolongation, rash, and diarrhea. The most

common side effect of vandetanib in the study was diarrhea, which could have been difficult to distinguish from disease-related diarrhea in some cases.

In conclusion, vandetanib has clinical antitumor activity in patients with advanced or metastatic hereditary MTC and in April 2011, the US Food and Drug Administration (FDA) approved it for the treatment of symptomatic or progressive medullary thyroid cancer (MTC) in patients with unresectable locally advanced or metastatic disease.

4.4. Motesanib (AMG 706). Motesanib is an oral inhibitor of multiple kinases,including VEGFR-1, 2, and 3 as well as the wild and mutant forms of the membrane receptor RET. In a phase I trial a 50% overall response rate was observed in patients with advanced thyroid carcinoma. Based on these results, a multicenter phase II trial was initiated, testing the efficacy of motesanib therapy in patients with progressive or symptomatic MTC. In this study the, median progression free survival was 40 weeks. Of 91 patients with progressive or symptomatic MTC who initiated therapy, only 2% had a confirmed partial response, but another 48% had stable disease for at least 24 weeks. The most common adverse events found at any grade were diarrhea (41%), hypertension (27%), fatigue (41%), and weight loss (22%) [51].

4.5. XL281. XL281 is a small molecule with potential antineoplastic activity specifically inhibits RAF kinases, located downstream from RAS in the RAS/RAF/MEK/ERK kinase signaling pathway, which may result in reduced proliferation of tumor cells. RAS mutations may result in constitutive activation of the RAS/RAF/MEK/ERK kinase signaling pathway, and have been found to occur frequently in human tumors. Preliminary data with the oral administration of this compound described prolonged a stable disease in 5 patients with PTC; of the 2 patients whose tumor were substained to contain BRAF mutations, both remained stable after more than 1 year of therapy [52].

4.6. Axitinib (AG013736). Axitinib (AG-013736) is an oral inhibitor that effectively blocks VEGF receptors at subnanomolar concentrations, but notably not the RET kinase.

One of five patients with thyroid carcinoma included in a phase I trial experienced tumor shrinkage, which however, was not qualified as a PR [53]. A phase II trial by Cohen et al. [54] studied the efficacy of axitinib in advanced or metastatic thyroid carcinoma of any histology ($n = 60$). A PR was seen in 30% of the patients. Stable disease lasting more than 16 weeks was reported in 38%. Objective responses were noted in all histological subtypes with a PR rate of 31% in patients with DTC and 18% in patients with MTC. Median PFS was 18.1 months. Common adverse events included diarrhea, hypertension, fatigue, decreased appetite, nausea, dysphonia, hand-foot syndrome, weight decreased, vomiting, and asthenia.

Exploratory analyses of soluble biomarkers showed increases in serum VEGF levels, a recognized phenomenon of effective angiogenesis inhibition. Given the absence of inhibitory activity against RET or other mutated kinases that are oncogenic in thyroid carcinoma, the efficacy of axitinib suggests that VEGFR-mediated angiogenesis is likely the primary mechanism by which the other anti-VEGFR inhibitory agents function. Currently ongoing is a multicenter, open-label phase II study to determine the efficacy of axitinib in patients with metastatic DTC refractory to doxorubicin, or if doxorubicin therapy is contraindicated.

4.7. XL184. XL184 is a small molecule designed to inhibit multiple tyrosine kinases receptors, specifically MET and VEGFR2. MET is a tyrosine kinase receptor that plays a key role in cellular proliferation, migration, and invasion as well as angiogenesis These biological processes contribute to the transformation, progression, survival, and metastasis of cancer cells. The MET pathway is frequently activated in tumors through MET amplification, mutation, and overexpression, as well as through overexpression of its ligand HGF. Expression of VEGF has been observed in a variety of cancers and has been associated with the stimulation and growth of new blood vessels to support the tumor. MET and VEGFR2 are important driving forces in angiogenesis, implicated in the ability of tumors to overcome hypoxia following angiogenesis inhibition. A phase I study was conducted in patients with metastatic solid malignant tumors including 37 MTC. The endpoint of the study included a dose escalation, the analysis of XL184 pharmacokinetics, safety, and RECIST response. Ten patients with MTC achieved partial response. Additionally 41% of MTC patients had stable disease for at least 6 months. Patients responsiveness was independent to the RET mutation status, an indication that the drug is active in patients without RET-activating mutations. A phase III trial, comparing XL184 with placebo, is ongoing [55].

4.8. Pazopanib (GW 786034). Pazopanib is a potent and selective multitargeted receptor tyrosine kinase inhibitor of VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-a/β, and c-Kit that blocks tumor growth and inhibits angiogenesis. It has been approved for renal cell carcinoma by the U.S. Food and Drug Administration. Pazopanib may also be active in ovarian cancer and soft tissue sarcoma. Pazopanib also appears effective in the treatment of non-small-cell lung carcinoma and thyroid cancer. In a phase II study, pazopanib administered at a dose of 800 mg/day induced a radiographic response rate of 49% in 37 patients with DTC who had disease progression over the previous 12 months. Progression-free survival was 11.8 months. The most frequent toxiticies found were fatigue (78%), skin rash (75%), diarrhea (73%), and nausea (73%) [56].

4.9. Lenvatinib (E7080). Lenvatinib is an oral tyrosine kinase inhibitor targeting VEGFR1-3, FGFR1-4, RET, KIT, and PDGFRβ [57, 58].

It is a synthetic, orally available inhibitor of vascular endothelial growth factor receptor 2 (VEGFR2, also known as KDR/FLK-1) tyrosine kinase with potential antineoplastic activity. Lenvatinib blocks VEGFR2-activation by VEGF, resulting in inhibition of the VEGF-receptor-signal-transduction pathway, decreased vascular endothelial cell

migration and proliferation, and vascular endothelial cell apoptosis; thus inhibits both VEGFR2 and VEGFR3 kinases.

In a phase II trial, 58 patients with refractory DTC were treated [59] with a starting dose of Lenvatinib 24 mg once daily in 28-day cycles untill disease progression. Primary end-point was response rate (RR) by RECIST.

Patients receiving prior VEGFR-directed treatment ($n = 17$) had an RR of 41%; while patients with not prior VEGFR-directed treatment ($n = 41$) had an RR of 54%. Median PFS was 12.6 months.

However, dose reduction was required in 35% of patients, and 23% of them discontinued treatment due to toxicity. The most frequent grade 3 or 4 toxicities that led to dose reductions were hypertension (10%), proteinuria (10%), decreased weight (7%), diarrhea (10%), and fatigue (7%).

This results formed the scientific basis for the launch of a phase III trial in which DTC refractory to radioactive iodine were randomized to receive lenvatinib or placebo.

Moreover, recently, therapeutic strategies have been investigated to study the ability of the proteasome inhibitor bortezomib to inhibit growth in ATC cell lines. Bortezomib was used as a single agent or in combination with TNF-related apoptosis-induced ligand to obtain the destruction of chemoresistant neoplastic thyrocytes and may represent a promising therapeutic agent in the treatment of ATC [60].

5. Discussion

Standard treatment for differentiated thyroid cancer is based on total thyroidectomy, radioactive iodine, and TSH suppression. Despite the generally good prognosis of differentiated thyroid carcinoma, about 20% of patients will develop metastatic disease which fails to respond to radioactive iodine, exhibiting a more aggressive behavior.

Systemic chemotherapies for advanced or metastatic nonmedullary and medullary thyroid carcinomas have been of only limited effectiveness. For patient with differentiated or medullary carcinomas unresponsive to conventional treatments, novel therapies are needed to improve disease outcomes.

Aberrations in RET/PTC-RAS-RAF-MAPK pathway are present in a high percentage of thyroid cancer, as well as angiogenesis switch alterations and involvement of other receptor tyrosine kinases, such as VEGFR or c-Met. Because of the oncogenic roles of activated BRAF, RET, and RET/PTC kinases, the hypothesis that specific targeting of these kinases could block tumor growth was suggested. Targeted agents against the VEGF receptor and the MAP kinase pathway are amongst the most promising thus far (see Table 2) [61].

Although most small-molecule VEGF receptor antagonists also inhibit RET, the efficacy of axitinib and pazopanib to induce objective responses in the absence of any significant anti-RET activity suggests that RET may not be as important a target for therapy as VEGFR. Unfortunately, eventual progression despite antiangiogenic VEGFR blockade suggests emergence of alternate pathways to promote tumor growth and metastasis.

The aim of the introduction of these targeted therapies is to extend life duration while assuring a good quality of life. Toxicities of many of these new therapies, although less life-threatening than cytotoxic chemotherapies, are common and can be dose limiting, and clinicians should be familiar with recognizing and managing the side effects if they intend to use these agents.

While significant progress has been made in understanding some of the mechanisms underlying tumorigenesis and in translating that knowledge into various treatment modalities, numerous challenges remain in testing targeted therapies against refractory thyroid cancer.

Selecting a primary endpoint for phase II and III trials is difficult. Although the Response Evaluation Criteria in Solid Tumors (RECIST) is a methodology for standardizing the reporting of therapeutic response categories in cancer patients target therapies often produce a cytostatic, rather than cytotoxic response, in which case tumor shrinkage may not be seen, even in cases of highly effective therapy.

This has led many Phase II trials to revert to progression-free survival (PFS), rather than response rate (RR), as the primary imaging metric of efficacy. However, determining progression times and rates, rather than response rates, requires longer monitoring periods (especially in cases of effective therapies). Actually, no novel treatment has been demonstrated to advance the time of survival for patients with thyroid cancer.

Thus, objective responses using RECIST or PFS as an endpoint in phase II trials or overall survival as an endpoint in a phase III trial may not be optimal.

Likewise, many of the studies are measuring serum levels of thyroglobulin, calcitonin, or CEA to determine if these biomarkers may be used as an additional tool to evaluate response to therapy. As seen in the studies previously described, however, these markers are only partially usefull and may not be a reliable indicator of disease responsiveness. Further studies are needed, to understand the relationship between targeted molecular therapies and their direct effects on the synthesis or secretion of tumor-marker proteins.

Moreover another challenge is selecting appropriate patients for phase II and III clinical trials. An argument can be made to restrict eligibility of patients into clinical trials to those with PD in the 6 or 12 months prior to study entry so that attribution of SD as an objective response to targeted therapy may be interpretable. Furthermore, patients with an overall indolent cancer may be spared the toxicities of targeted therapies. A significant limitation of this approach, however, is that patients diagnosed at an advanced stage with severe or symptomatic tumor burden who desperately need therapy may not be eligible for the trials due to inability to prove PD at the study entry.

Additionally new studies should point out the possibility to use politherapy than monotherapy and cytotoxic chemotherapies in combination with target therapy to obtain more response that has not completely been reached in any of the actual trials.

However, the published clinical trials are relatively sparse compared to other malignancies and there is only one published phase III trial yet in thyroid cancers. A possible

Table 2: Summary of results of the most important clinical trials conducted in advanced thyroid carcinoma.

Drug	Target	Type of study (ref)	Histology	No. of patients	PR (%)	SD (%)
Sunitinib	VEGFR 1-2 PDGF, RET,	Phase II [37]	DTC	31	13%	68%
	c-KIT, FLT3	Phase II [39]	DTC (29), MTC (6)	35	31%	46%
Sorafenib	VEGFR 1-2 PDGF, RET RAF MAPK	Phase II [41]	DTC	30	23%	68%
		Phase II [42]	DTC	41	15%	56%
		Phase II [43]	MTC (15) /DTC (19)	34	15%	74%
		Phase II [44]	DTC	32	25%	34%
		Phase II [45]	MTC	15	6%	62%
Vandetanib	VEGFR 1-2 EGFR, RET	Phase II [47]	MTC	30	21%	53%
		Phase II [48]	MTC	19	16%	53%
		Phase III [49]	MTC	231	44%	20%
Motesanib	VEGFR 1-2-3 EGFR, RET	Phase II [50]	MTC	91	2%	48%
Axitinib	VEGF	Phase II [53]	MTC (11) /DTC (45) Other (4)	60	30%	38%
XL 184	VEGF, MET, RET, c-KIT , FLT3	Phase I [54]	MTC	37	29%	41%
Pazopanib	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-c-Kit	Phase II [55]	DTC	37	49%	
Lenvatinib	VEGFR1-3, FGFR1-4, RET, KIT PDGFRβ	Phase II [58]	DTC	58	50%	

reason is the difficulty in accrual of enough number of patients to these clinical trials.

It may be possible to overcome this difficulty by multi-institutional trials recruiting patients from several centers and working in multidisciplinary team (medical oncologist, endocrinologist, specialist in nuclear medicine, radiologist, surgeon, phatologist, molecular biologist, etc.) to enlarge the number of patients in clinical studies, to optimize the aim of protocols, to improve the characterization of tumor tissues, and to improve the tolerance of treatment.

References

[1] J. Ferlay, F. Bray, P. Pisani, and D. M. Parkin, "Cancer incidence, mortality and prevalence worldwide," GLOBOCAN 2002 IARC CancerBase No.5, version 2.0. IARC Press, Lyon, France, 2004.

[2] M. P. Curado, B. Edwards, H. R. Shin et al., *Cancer Incidence in Five Continents*, vol. 9, IARC Scientific Publications No. 160, Lyon, France, 2007.

[3] L. Dal Maso, C. Bosetti, C. La Vecchia, and S. Franceschi, "Risk factors for thyroid cancer: an epidemiological review focused on nutritional factors," *Cancer Causes and Control*, vol. 20, no. 1, pp. 75–86, 2009.

[4] F. Cetani, M. Tonacchera, A. Pinchera et al., "Genetic analysis of the TSH receptor gene in differentiated human thyroid carcinomas," *Journal of Endocrinological Investigation*, vol. 22, no. 4, pp. 273–278, 1999.

[5] J. Liska, V. Altanerova, S. Galbavy, S. Stvrtina, and J. Brtko, "Thyroid tumors: histological classification and genetic factors involved in the development of thyroid cancer," *Endocrine Regulations*, vol. 39, no. 3, pp. 73–83, 2005.

[6] D. Giuffrida and H. Gharib, "Current diagnosis and management of medullary thyroid carcinoma," *Annals of Oncology*, vol. 9, no. 7, pp. 695–701, 1998.

[7] D. Giuffrida and H. Gharib, "Anaplastic thyroid carcinoma: current diagnosis and treatment," *Annals of Oncology*, vol. 11, no. 9, pp. 1083–1089, 2000.

[8] K. Matsuo, S. H. Tang, K. Zeki et al., "Aberrant DNA methylation in human thyroid tumors," *The Journal of Clinical Endocrinology & Metabolism*, vol. 77, pp. 991–995, 1993.

[9] K. Matsuo, S. H. Tang, and J. A. Fagin, "Allelotype of human thyroid tumors: loss of chromosome 11q13 sequences in follicular neoplasms," *Molecular Endocrinology*, vol. 5, no. 12, pp. 1873–1879, 1991.

[10] K. Giehl, "Oncogenic Ras in tumour progression and metastasis," *Biological Chemistry*, vol. 386, no. 3, pp. 193–205, 2005.

[11] V. Gire and D. Wynford-Thomas, "RAS oncogene activation induces proliferation in normal human thyroid epithelial cells

without loss of differentiation," *Oncogene*, vol. 19, no. 6, pp. 737–744, 2000.

[12] N. R. Lemoine, E. S. Mayall, F. S. Wyllie et al., "High frequency of ras oncogene activation in all stages of human thyroid tumorigenesis," *Oncogene*, vol. 4, no. 2, pp. 159–164, 1989.

[13] H. G. Suarez, J. A. Du Villard, M. Severino et al., "Presence of mutations in all three ras genes in human thyroid tumors," *Oncogene*, vol. 5, no. 4, pp. 565–570, 1990.

[14] Y. F. Shi, M. J. Zou, H. Schmidt et al., "High rates of ras codon 61 mutation in thyroid tumors in an iodide-deficient area," *Cancer Research*, vol. 51, no. 10, pp. 2690–2693, 1991.

[15] M. Santoro, M. Grieco, R. M. Melillo, A. Fusco, and G. Vecchio, "Molecular defects in thyroid carcinomas: role of the RET oncogene in thyroid neoplastic transformation," *European Journal of Endocrinology*, vol. 133, no. 5, pp. 513–522, 1995.

[16] M. Grieco, M. Santoro, M. T. Berlingieri et al., "PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas," *Cell*, vol. 60, no. 4, pp. 557–563, 1990.

[17] M. D. Castellone and M. Santoro, "Dysregulated RET signaling in thyroid cancer," *Endocrinology and Metabolism Clinics of North America*, vol. 37, no. 2, pp. 363–374, 2008.

[18] A. Fusco, M. Grieco, and M. Santoro, "A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases," *Nature*, vol. 328, no. 6126, pp. 170–172, 1987.

[19] A. Wirtschafter, R. Schmidt, D. Rosen et al., "Expression of the RET/PTC fusion gene as a marker for papillary carcinoma in Hashimoto's thyroiditis," *Laryngoscope*, vol. 107, no. 1, pp. 95–100, 1997.

[20] M. Grieco, A. Cerrato, M. Santoro, A. Fusco, R. M. Melillo, and G. Vecchio, "Cloning and characterization of H4(D10S170), a gene involved in RET rearrangements in vivo," *Oncogene*, vol. 9, no. 9, pp. 2531–2535, 1994.

[21] M. A. Pierotti, M. Santoro, R. B. Jenkins et al., "Characterization of an inversion on the long arm of chromosome 10 juxtaposing D10S170 and RET and creating the oncogenic sequence RET/PTC," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 5, pp. 1616–1620, 1992.

[22] M. Santoro, N. A. Dathan, M. T. Berlingieri et al., "Molecular characterization of RET/PTC3; A novel rearranged version of the RETproto-oncogene in a human thyroid papillary carcinoma," *Oncogene*, vol. 9, no. 2, pp. 509–516, 1994.

[23] Y. E. Nikiforov, J. M. Rowland, K. E. Bove, H. Monforte-Munoz, and J. A. Fagin, "Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children," *Cancer Research*, vol. 57, no. 9, pp. 1690–1694, 1997.

[24] C. M. Caudill, Z. Zhu, R. Ciampi, J. R. Stringer, and Y. E. Nikiforov, "Dose-dependent generation of RET/PTC in human thyroid cells after in vitro exposure to γ-radiation: a model of carcinogenic chromosomal rearrangement induced by ionizing radiation," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 4, pp. 2364–2369, 2005.

[25] R. Elisei, C. Romei, T. Vorontsova et al., "RET/PTC rearrangements in thyroid nodules: studies in irradiated and not irradiated, malignant and benign thyroid lesions in children and adults," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 7, pp. 3211–3216, 2001.

[26] I. Bongarzone, M. A. Pierotti, N. Monzini et al., "High frequency of activation of tyrosine kinase oncogenes in human papillary thyroid carcinoma," *Oncogene*, vol. 4, no. 12, pp. 1457–1462, 1989.

[27] A. Bounacer, R. Wicker, B. Caillou et al., "High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumors from patients who had received external radiation," *Oncogene*, vol. 15, no. 11, pp. 1263–1273, 1997.

[28] M. Santoro, F. Carlomagno, I. D. Hay et al., "Ret oncogene activation in human thyroid neoplasms is restricted to the papillary cancer subtype," *Journal of Clinical Investigation*, vol. 89, no. 5, pp. 1517–1522, 1992.

[29] G. Viglietto, G. Chiappetta, F. J. Martinez-Tello et al., "RET/PTC oncogene activation is an early event in thyroid carcinogenesis," *Oncogene*, vol. 11, no. 6, pp. 1207–1210, 1995.

[30] M. . Zou, Y. Shi, N. R. Farid et al., "Low rate of ret proto-oncogene activation (PTC/retTPC) in papillary thyroid carcinomas from Saudi Arabia," *Cancer*, vol. 73, pp. 176–1780, 1994.

[31] G. Tallini, M. Santoro, M. Helie et al., "RET/PTC oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumor phenotypes," *Clinical Cancer Research*, vol. 4, no. 2, pp. 287–294, 1998.

[32] P. Niccoli-Sire, A. Murat, V. Rohmer et al., "Familial medullary thyroid carcinoma with noncysteine RET mutations: phenotype-genotype relationship in a large series of patients," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 8, pp. 3746–3753, 2001.

[33] R. De Maria, R. Elisei, D. Giuffrida, and A. Machens, Thyroid cancer guidelines, 2011, http://www.startoncology.net/site/.

[34] M. Xing, "BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications," *Endocrine Reviews*, vol. 28, no. 7, pp. 742–762, 2007.

[35] S. Tezelman and O. H. Clark, "Current management of thyroid cancer," *Advances in Surgery*, vol. 28, pp. 191–221, 1995.

[36] D. S. Cooper, G. M. Doherty, B. R. Haugen et al., "Management guidelines for patients with thyroid nodules and differentiated thyroid cancer," *Thyroid*, vol. 16, no. 2, pp. 109–142, 2006.

[37] C. Jimenez, M. I. Hu, and R. F. Gagel, "Management of medullary thyroid carcinoma," *Endocrinology and Metabolism Clinics of North America*, vol. 37, pp. 481–496, 2008.

[38] E. E. W. Cohen, B. M. Needles, K. J. Cullen et al., "Phase 2 study of sunitinib in refractory thyroid cancer," *Journal of Clinical Oncology*, vol. 26, abstract 6025, 2008.

[39] B. Goulart, L. Carr, R. G. Martins et al., "Phase II study of sunitinib in iodine refractory, well-differentiated thyroid cancer (WDTC) and metastatic medullary thyroid carcinoma (MTC)," *Journal of Clinical Oncology*, vol. 26, abstract 6062, 2008.

[40] L. L. Carr, D. A. Mankoff, B. H. Goulart et al., "Phase II study of daily sunitinib in FDG-PET-positive, iodine-refractory differentiated thyroid cancer and metastatic medullary carcinoma of the thyroid with functional imaging correlation," *Clinical Cancer Research*, vol. 16, no. 21, pp. 5260–5268, 2010.

[41] R. J. Robbins, Q. Wan, R. K. Grewal et al., "Real-time prognosis for metastatic thyroid carcinoma based on 2-[^{18}F]fluoro-2-deoxy-D-glucose-positron emission tomography scanning," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 2, pp. 498–505, 2006.

[42] V. Gupta-Abramson, A. B. Troxel, A. Nellore et al., "Phase II trial of sorafenib in advanced thyroid cancer," *Journal of Clinical Oncology*, vol. 26, no. 29, pp. 4714–4719, 2008.

[43] R. T. Kloos, M. D. Ringel, M. V. Knopp et al., "Phase II trial of sorafenib in metastatic thyroid cancer," *Journal of Clinical Oncology*, vol. 27, no. 10, pp. 1675–1684, 2009.

[44] M. Ahmed, Y. Barbachano, A. Riddell et al., "Analysis of the efficacy and toxicity of sorafenib in thyroid cancer: a phase II study in a UK based population," *European Journal of Endocrinology*, vol. 165, no. 2, pp. 315–322, 2011.

[45] H. Hoftijzer, K. A. Heemstra, H. Morreau et al., "Beneficial effects of sorafenib on tumor progression, but not on radioiodine uptake, in patients with differentiated thyroid carcinoma," *European Journal of Endocrinology*, vol. 161, no. 6, pp. 923–931, 2009.

[46] E. T. Lam, M. D. Ringel, R. T. Kloos et al., "Phase II clinical trial of sorafenib in metastatic medullary thyroid cancer," *Journal of Clinical Oncology*, vol. 28, no. 14, pp. 2323–2330, 2010.

[47] M. E. Cabanillas, R. Kurzrock, S. I. Sherman et al., "Phase I trial of combination sorafenib and tipifarnib: the experience in advanced differentiated thyroid cancer (DTC) and medullary thyroid cancer (MTC)," *Journal of Clinical Oncology*, vol. 28, supplement, abstract 5586, 2010.

[48] S. A. Wells Jr., J. E. Gosnell, R. F. Gagel et al., "Vandetanib for the treatment of patients with locally advanced or metastatic hereditary medullary thyroid cancer," *Journal of Clinical Oncology*, vol. 28, no. 5, pp. 767–772, 2010.

[49] B. G. Robinson, L. Paz-Ares, A. Krebs, J. Vasselli, and R. Haddad, "Vandetanib (100 mg) in patients with locally advanced or metastatic hereditary medullary thyroid cancer," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 6, pp. 2664–2671, 2010.

[50] S. A. Wells Jr., B. G. Robinson, R. F. Gagel et al., "Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial," *Journal of Clinical Oncology*, vol. 30, pp. 134–141, 2012.

[51] M. J. Schlumberger, R. Elisei, L. Bastholt et al., "Phase II study of safety and efficacy of motesanib in patients with progressive or symptomatic, advanced or metastatic medullary thyroid cancer," *Journal of Clinical Oncology*, vol. 27, no. 23, pp. 3794–3801, 2009.

[52] L. Santarpia, L. Ye, and R. F. Gagel, "Beyond RET: potential therapeutic approaches for advanced and metastatic medullary thyroid carcinoma," *Journal of Internal Medicine*, vol. 266, no. 1, pp. 99–113, 2009.

[53] H. S. Rugo, R. S. Herbst, G. Liu et al., "Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: pharmacokinetic and clinical results," *Journal of Clinical Oncology*, vol. 23, no. 24, pp. 5474–5483, 2005.

[54] E. E. W. Cohen, L. S. Rosen, E. E. Vokes et al., "Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study," *Journal of Clinical Oncology*, vol. 26, no. 29, pp. 4708–4713, 2008.

[55] R. Kurzrock, S. I. Sherman, D. W. Ball et al., "Activity of XL184 (cabozantinib), an oral tyrosine kinase inhibitor, in patients with medullary thyroid cancer," *Journal of Clinical Oncology*, vol. 29, no. 19, pp. 2660–2666, 2011.

[56] K. C. Bible, R. C. Smallridge, W. J. Maples et al., "Phase II trial of pazopanib in progressive, metastatic, iodine-insensitive differentiated thyroid cancers," *Proceedings of the American Society of Clinical Oncology*, vol. 27, abstract 3521, 2009.

[57] J. Matsui, Y. Funahashi, T. Uenaka, T. Watanabe, A. Tsuruoka, and M. Asada, "Multi-kinase inhibitor E7080 suppresses lymph node and lung metastases of human mammary breast tumor MDA-MB-231 via inhibition of vascular endothelial growth factor-receptor (VEGF-R) 2 and VEGF-R3 kinase," *Clinical Cancer Research*, vol. 14, no. 17, pp. 5459–5465, 2008.

[58] H. Glen, D. Boss, T. R. Evans et al., "A phase I dose finding study of E7080 in patients with advanced malignancies," *Journal of Clinical Oncology*, vol. 25, Supplement, ASCO Annual Meeting Proceedings, no. 18, abstract 14073, 2007.

[59] S. I. Sherman, B. Jarzab, M. E. Cabanillas et al., "Phase II trial of the multitargeted kinase inhibitor E7080 in advanced radioiodine (RAI)-refractory differentiate thyroid cancer (DTC)," *Journal of Clinical Oncology*, abstract 5503, 2011.

[60] C. Conticello, L. Adamo, R. Giuffrida et al., "Proteasome inhibitors synergize with tumor necrosis factor-related apoptosis-induced ligand to induce anaplastic thyroid carcinoma cell death," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 5, pp. 1938–1942, 2007.

[61] E. Grande, J. J. Diez, C. Zafon et al., "Thyroid cancer: molecular aspects and new therapeutic strategies," *Journal of Thyroid Research*, vol. 1, article 10, 2012.

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 234726, 11 pages
doi:10.1155/2012/234726

Review Article

Adrenocortical Carcinoma: Current Therapeutic State-of-the-Art

Amir H. Lebastchi, John W. Kunstman, and Tobias Carling

Department of Surgery, Yale Endocrine Neoplasia Laboratory, Yale School of Medicine, 333 Cedar Street, TMP202, Box 208062, New Haven, CT 06520, USA

Correspondence should be addressed to Tobias Carling, tobias.carling@yale.edu

Received 26 May 2012; Revised 13 August 2012; Accepted 27 August 2012

Academic Editor: Bruce H. R. Wolffenbuttel

Adrenocortical carcinoma (ACC) is a rare, aggressive malignancy that generally conveys a poor prognosis. Currently, surgical resection is considered the lone curative treatment modality. In addition, the low prevalence of ACC has limited effective clinical trial design to develop evidence-based approaches to ACC therapy. The proper role of radio- and chemotherapy treatment for ACC is still being defined. Similarly, the molecular pathogenesis of ACC remains to be fully characterized. Despite these challenges, progress has been made in several areas. After years of refinement, an internationally accepted staging system has been defined. International collaborations have facilitated increasingly robust clinical trials, especially regarding agent choice and patient selection for chemotherapeutics. Genetic array data and molecular profiling have identified new potential targets for rational drug design as well as potential tumor markers and predictors of therapeutic response. However, these advances have not yet been translated into a large outcomes benefit for ACC patients. In this paper, we summarize established therapy for ACC and highlight recent findings in the field that are impacting clinical practice.

1. Introduction

Adrenocortical carcinoma (ACC) is a rare, aggressive malignancy that features a correspondingly poor prognosis. The pathogenesis of ACC is poorly understood, especially at the molecular level, as the rarity of the disease makes comprehensive study difficult. As a result, therapeutic options for ACC are currently limited, with medical and radiation therapy remaining complementary to surgery, which is currently the lone curative modality for ACC. Additionally, apart from surgery, treatment for ACC has never been standardized due to the lack of large randomized trials. However, ACC therapy is now evolving. Novel research and the increasing quality of clinical trials may improve available treatment options and outcomes for ACC patients as novel chemotherapeutic agents are introduced and long-standing drug regimens are reassessed.

An overall incidence of 0.5–2 per 1 million cases of ACC have been reported annually worldwide [1, 2]. ACC shows a slight female gender preference and a bimodal age distribution with the first peak in children less than five years of age and the second peak in the fourth to fifth decade of life. Most cases of ACC are sporadic, although some familial cancer syndromes, such as Li-Fraumeni and Beckwith-Wiedemann syndromes, are associated with an increased incidence of ACC [3]. Approximately 60% of patients present with symptoms of excess hormone secretion, most commonly in the form of cortisol hypersecretion (most commonly, hypercortisolism: Cushing's syndrome), with or without virilization due to accompanying androgen excess. Progression is rapid, generally with less than 12 months elapsing from the first clinical changes to advanced Cushing's syndrome [4, 5]. Interestingly, hormonal secretion patterns can vary according to size, differentiation, and stage of the tumor. In cases without clinical hormone overactivity, the most common presentation is related to tumor growth and encroachment on the surrounding viscera, with symptoms such as abdominal discomfort, back pain, and nausea or vomiting. Despite this, overproduction of hormonal precursors is detectable in virtually all cases of ACC, due to defective steroidogenesis within the tumor.

Adrenocortical malignancy can be, regardless of biochemical activity, notoriously difficult to diagnose. In tumors confined to the adrenal gland, the diagnosis may be unclear even after pathological assessment following surgical resection; a widely validated scoring classification (Weiss criteria) is employed in such cases to improve accuracy of diagnosis [6]. On computed tomography (CT), ACC can demonstrate central tumor necrosis, calcifications, and also tends to be larger and more heterogeneous. Reliance on size alone can be misleading, as the widely utilized 4 cm cutoff has a sensitivity of only 81% for ACC. However, ACCs exhibit a significantly higher density on noncontrast CT than adenomas, with a specificity for differentiating adenoma from carcinoma of 100% and 96.9% using 10 and 20 Hounsfield unit cutoffs, respectively [7–9]. Steroid profiling, which is distinct from routine biochemical analysis for adrenal hormone production, is another promising method for differentiating adrenocortical adenomas (ACAs) from ACCs. By using gas chromatography/mass spectrometry to analyze the steroid profiles in 24-hour urine samples of patients with ACCs or ACAs versus control patients, Arlt et al. have identified several metabolites with diagnostic utility. In a retrospective study, their algorithm demonstrated a sensitivity and specificity of 88% for differentiating ACC and ACA when using the nine metabolites identified to have the most diagnostic significance, which exceeds the accuracy of CT alone [10].

Survival for patients with ACC is poor and related to stage at time of diagnosis, which is often advanced. Up to 70% of patients present with extra-adrenal disease [11]. Overall cancer-specific mortality (CSM) rates have been reported between 16% and 38% [1, 12, 13]. Five-year survival for patients with disease confined to the adrenal gland is size-dependent and varies from 61 to 82%. Those with distant metastases at diagnosis have a five-year survival of only 18% [14]. Improved radiographic imaging and surveillance of incidentally discovered adrenal masses have resulted in earlier detection and earlier staging at diagnosis.

2. Staging

The TNM staging system is considered the most important tool in prognostic stratification and therapy planning and stratifies cancer patients by survival based on clinical status [2, 12–14]. However, due to the low incidence of ACC and resultant inability to validate ACC staging and survival with any statistical reliability, no TNM classification was available for ACC until recently. Multiple different staging systems were used prior to that time, the most widespread being devised in 1978 by Sullivan, who modified the original McFarlane staging system [2, 15–17]. In 2004, the International Union Against Cancer (UICC) and the World Health Organization (WHO) proposed a new staging system based on Sullivan-McFarlane criteria (Table 1) [18].

To evaluate the UICC system, a large European study examined 416 patients and found a low correlation between five-year disease-specific survival and stage for patients with stage II and III disease. A new staging system was therefore proposed by the European Network for the Study of Adrenal Tumors (ENS@T) consisting of the following major changes.

Table 1: Comparison UICC and ENSAT staging systems for ACC.

Stage	UICC/WHO 2004	ENSAT 2008
I	T1, N0, M0	T1, N0, M0
II	T2, N0, M0	T2, N0, M0
III	T1-2, N1, M0	T1-2, N1, M0
	T3, N0, M0	T3-4, N0-1, M0
IV	T1–4, N0-1, M1	T1–4, N0-1, M1
	T3, N1, M0	
	T4, N0-1, M0	

T1: tumor ≤ 5 cm; T2: tumor > 5 cm; T3: tumor infiltration in surrounding tissue; T4: tumor infiltration in adjacent organs [ENSAT additionally the presence of a tumor thrombus in the Vena Cava or Vena Renalis]; N0: absence of positive lymph nodes; N1: presence of positive lymph nodes; M0: absence of distant metastases; M1: presence of distant metastasis.

(1) Existence of a thrombus in the inferior vena cava or adjacent renal veins upstages the tumor to T4, with a corresponding grading of stage III.

(2) Stage IV tumors are exclusively defined as those with distant metastases.

The ENS@T criteria have been validated in a large North American trial evaluating 573 patients, with a statistically significant difference in cancer-specific mortality now observed between stage II and III patients when the new criteria were applied. Furthermore, 3-year accuracy in predicting CSM rates improved for all patients regardless of stage from 79.5% to 83.0%, when the ENS@T rather than UICC criteria were employed.

3. Surgery

3.1. Established Therapy. In newly diagnosed cases of ACC, feasibility of surgical resection is the most important contributor to overall survival. While successful treatment of ACC requires a multidisciplinary approach, complete surgical resection is mandatory if possible for patients presenting with stage I to stage III disease. The goal of surgery is R0 resection of the tumor and any involved tissues or viscera in an en bloc fashion. Patients undergoing successful resection have a five-year survival of 40–50%, while median survival of unresectable patients is less than one year [17, 34, 35]. When stratified by stage at time of resection, 5-year disease-specific survival was found to be 82% for stage I disease, 58% for stage II, 55% for stage III, and 18% for stage IV [14].

There is consensus that resection should be performed by an experienced multidisciplinary team [36]. This is especially crucial in the management of patients with biochemically active tumors. Intraoperatively, maintaining tumor capsule integrity and preventing tumor spillage are key considerations [4]. Notably, presence of tumor thrombi and vascular invasion are not contraindications to resection. In cases with extensive vascular involvement, usage of cardiopulmonary

bypass can facilitate successful resection [37]. A transabdominal, open surgical approach allows maximal exposure. This facilitates en bloc excision of tumor and other involved organs, maintenance of the tumor capsule, and effective vascular control when necessary.

There is an ongoing debate on the role of lymphadenectomy. A retrospective analysis of the data from the German ACC registry by Reibetanz et al. indicated that locoregional lymph node dissection improved tumor staging and lead to a favorable oncologic outcome in patients with localized ACC [38]. For recurrent disease, reoperation with the goal of radical resection or tumor debulking is beneficial in those patients who are surgical candidates [35, 39]. During reoperation, complete resection is again crucial, resulting in a mean survival time of 74 months versus 16 months in those with incomplete resections [35]. In some patients that display unresectable disease at the time of diagnosis, debulking may be beneficial in tumors [36]. Debulking may provide relief from symptoms of hormonal excess and facilitate additional treatment options [20, 40]. Conversely, patients with widely metastatic disease or rapidly enlarging tumors at diagnosis are better managed with medical palliation only.

3.2. Emerging Trends. Since its introduction in 1992, laparoscopic adrenalectomy (LA) has become the treatment of choice for benign adrenal tumors due to improvements in postoperative analgesia use, cosmesis, and length of hospital stay [41, 42]. Resection of ACC via a laparoscopic approach, while technically feasible, remains highly controversial. Initial reports evaluating laparoscopic versus open adrenalectomy in ACC patients noted both higher rates of recurrence and shorter disease-free survival in those with laparoscopic resections [19, 33]. Notably, those undergoing laparoscopic resection demonstrated a substantially increased rate of local recurrence and peritoneal carcinomatosis compared to recurrences in those undergoing open resection (83% versus 43% in one study), suggesting loss of capsule integrity and port site seeding as potential causes of the poorer outcomes seen with laparoscopy [19]. Conversely, several more recent studies have found comparable outcomes between laparoscopic and open approaches in ACC resection (Tables 2 and 3). One study evaluating 152 patients undergoing either laparoscopic ($n = 35$) or open ($n = 117$) adrenalectomy for ACC found identical oncologic outcomes. However, it was limited to patients with tumor size ≤ 10 cm and 12 patients undergoing laparoscopic resection required conversion to an open approach [32]. An Italian study demonstrated similar findings in a cohort limited to patients with a stage I and II disease only [43]. In general, superior surgical outcomes following adrenalectomy are observed in centers with high-volume surgeons and considerable expertise; this is especially true in adrenalectomy for ACC [44, 45].

4. Radiotherapy

4.1. Established Therapy. Historically, radiation therapy (RT) has not been considered effective in treatment of primary ACC [46–49]. ACC is not an overly radiosensitive tumor, and the anatomical proximity to radiosensitive viscera such as the small bowel, kidney, and spinal cord has limited the clinical utility of radiotherapy. There is a well-defined role for RT in the treatment of metastatic ACC, especially in bony disease [50, 51]. Furthermore, radiotherapy can improve symptoms in patients with bulky abdominal tumors that are unresectable [52]. In a study of 91 patients in the German ACC registry, a response rate of 57% was noted in patients receiving palliative radiotherapy [53], and an investigation of the Dutch ACC registry showed that ACC can be radiosensitive and patients with advanced disease can benefit from it [54].

4.2. Emerging Techniques. The role of radiotherapy in an adjuvant therapy for ACC remains controversial. Improvements in technology and radiotherapy protocols (specifically, stereotactic body radiation therapy) have resulted in superior morbidity profiles compared to historical controls due to improved targeting and lower nontumor dosing. However, there is still no prospective evidence supporting radiotherapy in an adjuvant setting. The best current evidence advocates for the usage of radiotherapy for local control in unresectable disease and after resection in certain cases [55]. In general, adjuvant radiotherapy has been advocated as a means to reduce the high incidence of local recurrence observed in ACC. A recent North American study evaluating surgery alone versus surgery and radiotherapy found that those treated with surgery alone had an odds ratio for local recurrence of 4.7 versus those who received adjuvant radiation [56]. These data concur with a European study that also found increased local recurrence in the surgery alone group [57]. However, that study failed to demonstrate either a disease-free or overall survival benefit.

The lack of strong evidence supporting radiotherapy has resulted in a variety of treatment recommendations. The German ACC group currently recommends radiotherapy in the following cases [53]:

(1) all patients with incomplete (R1 or R2) or uncertain (Rx) resections,

(2) all patients with stage III disease regardless of resection adequacy,

(3) strong consideration in cases of >8 cm tumor size, Ki-67 index > 10%, and invasion of adjacent vasculature, even in cases of complete resection.

Prospective data are needed to fully define the role of radiotherapy in the adjuvant setting for ACC. Despite this, the available evidence supports treatment in patients with incomplete resection, stage III disease, or in the palliative setting.

5. Medical Therapy

Medical therapy for ACC takes two forms. Cytotoxic agents, of which mitotane (1,1-dichloro-2(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane) is the prototype, have been the mainstay of ACC chemotherapeutics for decades and have been studied in adjuvant, recurrent, and palliative settings.

Table 2: Retrospective series of open adrenalectomies.

Author	Total number of malignant cases	Mean tumor size (cm)	Duration of followup (months)	Recurrence rate	Comments
Gonzalez et al., 2005 [19]	133 ACC	NR	28	51%	Median survival duration: 34 months
Crucitti et al., 1996 [20]	91 ACC	NR	NR	15%	Median survival duration: 28 months
Terzolo et al. [21, 22]	55 ACC	10	67	90	Median survival duration: 52 months
	75 ACC	10	43	73	Median survival duration: 67 months
Icard et al., 2001 [17]	253 ACC	12	NR	NR	5 year survival: 38%
Kendrick et al., 2001 [23]	58 ACC	12.5	53	51	5 year survival: 37%

Table 3: Summary of series of laparoscopic adrenalectomies for adrenal malignancies.

Author	Total number of LA	Total number of malignant cases	Mean tumor size (cm)	Duration of followup (months)	Recurrence rate	Comments
Henry et al., 2002 [24]	233	6 ACC	7.4	47	17%	1 dead of disease
Porpiglia et al., 2004 [25]	205	6 ACC	6.9	30	0%	1 dead of cerebrovascular accident
Corcione et al., 2005 [26]	100	2 ACC	8.5	13.6	50%	Both patients alive, one has still disease
Gonzalez et al., 2005 [19]	*6*	*6 ACC*	*5.3*	*28*	*100%*	*2 are still alive with disease, remaining 4 died of disease*
Palazzo et al., 2006 [27]	391	3 MP	6.8	34	33%	1 dead of disease
Lombardi et al., 2006 [28]	79	4 ACC 3 MP	5.9	23	29%	4 alive disease free, 2 alive with disease and one dead of liver failure
Liao et al., 2006 [29]	210	4 ACC	6.2	39	25%	1 alive disease free, 1 alive with disease and 2 died of disease
Nocca et al., 2007 [30]	131	4 ACC	8.5	34	25%	3 alive disease free, 1 died of metastatic disease
Ramacciato et al., 2008 [31]	18	2 ACC	8.3	44	0%	Alive and disease free
Brix et al., 2010 [32]	35	35 ACC	6.2	39	77%	37% of patients died from ACC
Miller et al., 2010 [33]	17	17	7.0	36	20%	Not investigated, but authors concluded that the mean time to local recurrence was shorter in LA compared to the open group

ACC: adrenocortical cancer; MP: malignant pheochromocytoma; LA: laparoscopic adrenalectomy.

Disagreement persists over the most efficacious treatment regimen in each role. Biologic pharmaceuticals have recently been introduced into practice for ACC treatment based on rational selection of molecular targets. Numerous biologic agents are currently being evaluated in clinical trials.

6. Adjuvant Systemic Therapy

Mitotane is a derivate of the insecticide DDT and is directly toxic to the adrenocortical parenchyma. It has been the mainstay of systemic ACC treatment since the 1960s. Clinical response to mitotane is not universal, possibly due to the need for metabolic transformation of mitotane for therapeutic action [58]. The therapeutic index of mitotane is narrow; up to 80% of patients develop side effects, some of which can lead to cessation of therapy [59]. Nausea, emesis, and other gastrointestinal symptoms are most common, but neurologic toxicity can occur as well, especially at high dose ranges. A serum concentration of 14–20 mcg/mL is usually considered therapeutic [13, 60–62]. Despite this, the

optimum dose regimen is unknown. A therapeutic range can be achieved with a low-dose scheme designed to minimize toxicity [21]. However, most recurrences in the adjuvant setting occur less than six months postoperatively and the time to achieve effective serum levels is increased when utilizing a low-dose regimen [63, 64].

Currently, no prospective clinical trials exist evaluating adjuvant mitotane use. Several earlier observational studies did not report improved overall or disease-free survival with adjuvant mitotane use [65, 66], while more recent studies have noted a modest benefit [23, 67]. The best available evidence comes from a large European retrospective study evaluating 177 patients with ACC, with 47 patients receiving surgery and adjuvant mitotane and 130 receiving surgery alone. Recurrence-free survival was significantly improved in the treatment group. However, the surgery alone group had a higher incidence of advanced disease, which the authors controlled for by employing a multivariate statistical model which continued to demonstrate a benefit to mitotane use [22]. As such, the decision to administer adjuvant mitotane remains controversial. A panel of international experts in 2008 unanimously recommended adjuvant mitotane in patients with potential residual disease (R1 or Rx resection) or greater than 10% Ki67 positivity on pathologic examination [68]. Similarly, the same panel did not consider mitotane to be mandatory to patients with stage I or II disease who underwent histologically proven R0 resection with Ki67 indices less than 10%. The panel was undecided on whether to offer adjuvant therapy to stage III ACC patients following an R0 resection. Currently, a prospective randomized trial evaluating adjuvant mitotane use is recruiting patients in several European centers with the goal of improving future treatment algorithms.

7. Systemic Therapy in Locally Advanced and Metastasized Disease

Standard of treatment in patients with unresectable or metastasized ACC previously consisted of mitotane alone or in combination with other cytotoxic drugs [36, 69]. Generally, prognosis is poor in this patient population, although reports of long-term survival exist [35, 70, 71]; it is unclear whether this is due to favorable tumor biology or therapeutic intervention. Studies investigating mitotane alone in these patients have demonstrated a response rate of 19–33%, but with minimal survival benefit in responders (9 months in the largest trial) [60, 62, 72]. Mitotane in combination with standard cytotoxic agents has been investigated, with the two most popular regimens of mitotane-streptozocin (M-Sz) and mitotane etoposide/doxorubicin/cisplatin (M-EDP) having previously been investigated in phase II trials. In separate studies, an objective responsive rate of 36% was observed in 22 patients with advanced ACC receiving M-Sz [73] and in 53% of 28 patients receiving M-EDP [74]. Recently, the FIRM-ACT (First International Randomized Trial in Locally Advanced and Metastatic ACC Treatment) trial was released, which is a landmark randomized controlled trial comparing M-Sz and M-EDP in 304 patients with advanced ACC [75]. The trial found M-EDP statistically superior to M-Sz in terms of objective tumor response (23.2 versus 9.2%, resp.), progression-free survival (5.0 versus 2.1 months), and proportion of patients without progression at one year (23.2% versus 9.2%). Tumor burden was monitored by serial CT scans every eight weeks. Overall survival at the time of the study's conclusion favored M-EDP (14.8 months versus 12.0 months) without reaching statistical significance. The study also included an elegantly designed nested trial evaluating each regimen as a second-line regimen, whereas patients with treatment failure in their original group were given the alternative regimen (but still underwent primary endpoint analysis in an intent-to-treat manner). As second-line therapies, the efficacy of both regimens was similar to their efficacy as first-line therapy, with M-EDP showing superior antitumor efficacy and progression-free survival. Notably, the authors found no statistically significant difference in quality of life or rate of adverse events between the two treatment groups. As such, selection of systemic treatment for recurrent or metastatic ACC should favor M-EDP as the first-line therapy of choice in the future.

8. Targeted Therapy

Ongoing research into the oncogenesis of ACC has increased knowledge of the molecular mechanisms of ACC tumor growth and also identified potential targets for drug development. Gene transcriptome analysis has elucidated significant differences between the gene expression profile of ACC and benign adrenocortical adenomas with a large number of genes demonstrating differential or tumor-specific expression [76–80]. Szabó et al. analyzed microarray data from several studies and found a significant upregulation of genes involved in the cell cycle, growth factors and receptors and, simultaneously, a downregulation of genes that play a role in steroidogenesis, metabolism, and cell transport in ACC compared to benign disease [81]. Alterations in expression of the insulin-growth factor genes (IGF-1 and -2) are one of the most common mutations in ACC and one of the earliest recognized [82, 83]. Furthermore, additional growth factors have been implicated in ACC such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), and vascular endothelial growth factor (VEGF). Many of the receptors for these ligands belong to the tyrosine kinases receptor superfamily, inhibitors of which are already in clinical use for other malignancies [84]. In general, initial results utilizing tyrosine kinase inhibitors and other targeted therapeutics for ACC have been unable to improve upon the current standard of care as yet [4, 85–88]. The following section summarizes the current experience with selected agents that are in clinical investigation or have been promising in preclinical studies. Active clinical trials utilizing targeted therapy for ACC are listed in Table 4.

8.1. IGF Antagonists. IGF-1 and -2 are implicated in ACC development through both the phosphoinositide-3-kinase (PI3 K)-Akt and the Ras-Raf-MAP kinase pathways. Genome-wide gene expression studies have identified that

Table 4: Ongoing Clinical Trials that test the Target Therapies.

Study	Target	ID	Purpose	Status
Mitotane with or without *IMC-A12* in treating patients with recurrent, metastatic, or primary adrenocortical cancer that cannot be removed by surgery	*IGF1R*	NCT00778817	This randomized phase II trial compares the combination of mitotane and IMC-A12 with mitotane alone in the treatment of recurrent, metastatic, or primary adrenocortical cancer that cannot be removed by surgery	Recruiting
A study of *OSI-906* in patients with locally advanced or metastatic adrenocortical carcinoma (GALACCTIC)	*IGF1R*	NCT00924989	A multicenter, randomized, double-blind, placebo-controlled, phase III study of single-agent OSI-906 in patients with locally advanced/metastatic adrenocortical carcinoma who received at least 1 but no more than 2 prior drug regimens	Ongoing not recruiting
Phase II trial of *ZD1839* (*Iressa*) in patients with nonresectable adrenocortical carcinoma	*VEGFR*	NCT00215202	This phase II trial investigates the effect of Iressa in patients with nonresectable adrenocortical cancer who have previously been treated with one other form of systemic therapy (either Mitotane or chemotherapy).	Completed
Phase II Study of *Axitinib* (AG-013736) With Evaluation of the VEGF-Pathway in Metastatic, Recurrent or Primary Unresectable Adrenocortical Cancer	*Multikinase* (i) *VEGFR* (ii) *PDGFR* (iii) *KIT*	NCT01255137	To evaluate the effectiveness of axitinib in individuals who have adrenocortical cancer that is inoperable and has not responded to standard treatments	Recruiting
Sunitinib in Refractory Adrenocortical Carcinoma (SIRAC)	*Multikinase* (i) *VEGFR* (ii) *PDGFR* (iii) *KIT*	NCT00453895	The primary objective of this trial is to estimate the response (defined as progression-free survival of $\geq$12 weeks) rate associated with Sunitinib treatment in patients advanced ACC progressing after cytotoxic chemotherapy	Unknown
Sorafenib Plus Paclitaxel in adreno-cortical-cancer patients (PAXO)	*Multikinase* (i) *RAF* (ii) *VEGFR* (iii) *PDGFR* (iv) *KIT*	NCT00786110	The aim of this phase II trial is to evaluate the clinical benefit and toxicity of the combination of Sorafenib plus metronomic chemotherapy in patients with locally advanced or metastatic ACC who progressed after first or second line chemotherapy.	Unknown
Clinical trial of *Dovitinib* in first-line metastatic or locally advanced non-resectable adrenocortical carcinom	*FGFRs*	NCT01514526	Non-randomized, phase II clinical trial, that investigates the use of Dovitinib in adult patients with metastatic or locally advanced non-resectable adrenocortical carcinoma, confirmed histologically	Recruiting
Cixutumumab in treating patients with relapsed or refractory solid tumors	*IGF1R*	NCT00831844	Phase II trial that studies the side effects and how well cixutumumab works in treating patients with relapsed or refractory solid tumors, including ACC	Recruiting

overexpression of IGF-2, the ligand to the IGF-1 receptor which activates both of the above pathways, is a very consistent molecular event in the development of ACC [76, 77, 82, 89, 90]. Haluska et al. investigated an anti-IGF-1R antibody (figitumumab) in a phase I study and found it to be well tolerated and achieved stability of disease in 8 out of 14 patients (57%) [91]. The GALACCTIC trial is a phase 3 randomized, double-blinded trial investigating OSI-906, a combined inhibitor of IGF-1R and insulin receptor(IR) in patients with locally advanced or metastatic ACC who failed at least one prior drug regimen. OSI-906 has shown an antineoplastic effect in vitro [92]. The study has reached its accrual goals and is currently ongoing.

8.2. mTOR Antagonists. The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that is involved in cell growth and proliferation that is activated in part by IGF-1R signaling via the aforementioned PI3 K-Akt pathway. The prototype mTOR inhibitor, sirolimus, has long been utilized as an antirejection drug following solid organ transplant, and mTOR inhibitors have recently been investigated in clinical trials for treating malignancies, such as renal cell carcinoma [93]. Given its association with the IGF-1R pathway known to be dysregulated in ACC, inhibition of mTOR seems an appropriate target for investigation in ACC treatment. Doghman et al. demonstrated that inhibition of mTOR signaling reduced adrenocortical tumor growth

in vitro and in an in vivo mouse model in 2010 [94]. Temsirolimus, a second-generation mTOR inhibitor in combination with cixutumumab, an anti-IGF-1R antibody, has been welltolerated in phase I trials in patients with advanced tumors, including ACC [95]. Additional clinical trials are ongoing (see Table 4).

8.3. Future Targets. The canonical Wnt/β-catenin signaling pathway, involved in both human development and homeostasis, is dysregulated in a large number of human disease processes [96]. Microarray analysis has demonstrated upregulation of this pathway in ACC, and *CTNNB1*, the gene encoding β-catenin, is frequently mutated in adrenocortical neoplasia [97, 98]. Furthermore, *CTNNB1,* the gene encoding β-catenin, has been found to be frequently mutated in both ACC and adrenocortical adenomas [99]. This common mutation in both benign and malignant adrenocortical neoplasia may indicate an early step in a common pathway of tumorigenesis. Berthon et al. demonstrated a clear link between constitutive β-catenin activation and adrenal cortex dysplasia in a transgenic murine model, resulting in malignant changes such as neovascularization and local tumor invasion [100]. However, drugs effectively targeting the Wnt pathway have been slow to develop and further research identifying specific genetic targets is needed.

Steroidogenic factor (SF)-1 is an orphan nuclear receptor that has a key role in normal endocrine and gonadal development, as well as regulating steroid production in the adrenal cortex via interactions with several cytochrome P450 steroid hydroxylases in the adrenal cortex [101]. Overexpression of SF-1 has been demonstrated in ACC, especially in pediatric cases [102, 103]. Interestingly, SF-1 has been demonstrated to have a dose-dependent effect on the induction of adrenocortical cell proliferation through changes in apoptosis and cell cycle control [104]. In a mouse model, SF-1 induced adrenal hyperplasia and tumor growth. Antagonists of SF-1 inverse agonists have been identified and demonstrated inhibition of adrenocortical cell proliferation and steroidogenesis [105].

8.4. Individualized Treatment. Giordano et al. first reported transcriptional profiling of ACC in 2003. Genetic profiling of individual tumors has many potential benefits, but especially relevant to treatment of ACC is the opportunity to predict response rates to certain pharmaceutical agents and determination of prognosis. Aside from improving patient care, a secondary benefit of such information would be segregation of patients into clinical trial subgroups with others that have their specific form of disease [97]. Several molecular markers of drug sensitivity in ACC have been identified and are under investigation, as standard ACC chemotherapy is associated with significant toxicity and variable response rates characteristics [106]. For instance, ERCC1, a DNA repair protein, has previously been shown to predict resistance to platinum-based chemotherapy regimens. Less than 50% of ACCs treated with a platinum-based regimen shows a clinical response, and ACC patients demonstrating a high rate of ERCC1 expression following platinum-based chemotherapy have a significantly shortened median survival time (8 versus 24 months in low-ERCC1 patients) [107]. Additionally, a phase II trial is underway for ACC utilizing a regimen including XR9576, an inhibitor of MDR1, expression of which has been implicated in other multidrug-resistant tumors [108, 109]. Ideally, as the cost of genetic sequencing continues to fall and knowledge of the implications of specific genetic changes on treatment response and outcome improves, treatment regimens can be optimized on an individualized basis.

9. Conclusion

ACC remains a rare malignancy that has seen little improvement in overall mortality over the past two decades. Until recently, standard of care was based only on individual opinion and occasionally expert consensus. Over the past decade, international collaboration has begun to improve the management of these patients and the molecular understanding of the disease. The development of standardized staging criteria and the gradual accrual of clinical trials in treating ACC, especially with the release of the first randomized phase III trial in ACC, exemplifies the ongoing progress in clinical care of these patients. Similarly, as the genetic understanding of adrenal tumorigenesis continues to progress, more targets for future drug development are identified and evaluated. As these discoveries are translated into clinical practice, ACC therapy will move beyond historical modalities to the benefit of patients everywhere.

References

[1] B. L. Wajchenberg, M. A. Albergaria Pereira, B. B. Medonca et al., "Adrenocortical carcinoma: clinical and laboratory observations," *Cancer*, vol. 88, no. 4, pp. 711–736, 2000.

[2] M. Fassnacht, C. Wittekind, and B. Allolio, "Current TNM classification systems for adrenocortical carcinoma," *Pathologe*, vol. 31, no. 5, pp. 374–378, 2010.

[3] P. S. H. Soon, K. L. McDonald, B. G. Robinson, and S. B. Sidhu, "Molecular markers and the pathogenesis of adrenocortical cancer," *Oncologist*, vol. 13, no. 5, pp. 548–561, 2008.

[4] M. Fassnacht and B. Allolio, "Clinical management of adrenocortical carcinoma," *Best Practice and Research*, vol. 23, no. 2, pp. 273–289, 2009.

[5] L. Zini, F. Porpiglia, and M. Fassnacht, "Contemporary management of adrenocortical carcinoma," *European Urology*, vol. 60, no. 5, pp. 1055–1065, 2011.

[6] L. M. Weiss, L. J. Medeiros, and A. L. Vickery, "Pathologic features of prognostic significance in adrenocortical carcinoma," *American Journal of Surgical Pathology*, vol. 13, no. 3, pp. 202–206, 1989.

[7] G. W. L. Boland, M. A. Blake, P. F. Hahn, and W. W. Mayo-Smith, "Incidental adrenal lesions: principles, techniques, and algorithms for imaging characterization," *Radiology*, vol. 249, no. 3, pp. 756–775, 2008.

[8] W. F. Young Jr., "Conventional imaging in adrenocortical carcinoma: update and perspectives," *Hormones and Cancer*, vol. 2, no. 6, pp. 341–347, 2011.

[9] A. H. Hamrahian, A. G. Ioachimescu, E. M. Remer et al., "Clinical utility of noncontrast computed tomography attenuation value (hounsfield units) to differentiate

adrenal adenomas/hyperplasias from nonadenomas: cleveland clinic experience," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 2, pp. 871–877, 2005.

[10] W. Arlt, M. Biehl, A. E. Taylor et al., "Urine steroid metabolomics as a biomarker tool for detecting malignancy in adrenal tumors," *The Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 12, pp. 3775–3784, 2011.

[11] A. T. Phan, "Adrenal cortical carcinoma-review of current knowledge and treatment practices," *Hematology/Oncology Clinics of North America*, vol. 21, no. 3, pp. 489–507, 2007.

[12] G. Lughezzani, M. Sun, P. Perrotte et al., "The European Network for the Study of Adrenal Tumors staging system is prognostically superior to the international union against cancer-staging system: a North American validation," *European Journal of Cancer*, vol. 46, no. 4, pp. 713–719, 2010.

[13] B. Allolio and M. Fassnacht, "Clinical review: adrenocortical carcinoma: clinical update," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 6, pp. 2027–2037, 2006.

[14] M. Fassnacht, S. Johanssen, M. Quinkler et al., "Limited prognostic value of the 2004 International Union Against Cancer staging classification for adrenocortical carcinoma: proposal for a Revised TNM Classification," *Cancer*, vol. 115, no. 2, pp. 243–250, 2009.

[15] E. Kebebew, E. Reiff, Q. Y. Duh, O. H. Clark, and A. McMillan, "Extent of disease at presentation and outcome for adrenocortical carcinoma: Have we made progress?" *World Journal of Surgery*, vol. 30, no. 5, pp. 872–878, 2006.

[16] M. Sullivan, M. Boileau, and C. V. Hodges, "Adrenal cortical carcinoma," *Journal of Urology*, vol. 120, no. 6, pp. 660–665, 1978.

[17] P. Icard, P. Goudet, C. Charpenay et al., "Adrenocortical carcinomas: surgical trends and results of a 253-patient series from the French Association of Endocrine Surgeons Study Group," *World Journal of Surgery*, vol. 25, no. 7, pp. 891–897, 2001.

[18] R. A. DeLellis, *Pathology and Genetics of Tumours of Endocrine Organs. World Health Organization Classification of Tumours*, IARC Press, Lyon, France, 2004.

[19] R. J. Gonzalez, S. Shapiro, N. Sarlis et al., "Laparoscopic resection of adrenal cortical carcinoma: a cautionary note," *Surgery*, vol. 138, no. 6, pp. 1078–1085, 2005.

[20] F. Crucitti, R. Bellantone, A. Ferrante et al., "The italian registry for adrenal cortical carcinoma: analysis of a multiinstitutional series of 129 patients," *Surgery*, vol. 119, no. 2, pp. 161–170, 1996.

[21] M. Terzolo, A. Pia, A. Berruti et al., "Low-dose monitored mitotane treatment achieves the therapeutic range with manageable side effects in patients with adrenocortical cancer," *Journal of Clinical Endocrinology and Metabolism*, vol. 85, no. 6, pp. 2234–2238, 2000.

[22] M. Terzolo, A. Angeli, M. Fassnacht et al., "Adjuvant mitotane treatment for adrenocortical carcinoma," *The New England Journal of Medicine*, vol. 356, no. 23, pp. 2372–2380, 2007.

[23] M. L. Kendrick, R. Lloyd, L. Erickson et al., "Adrenocortical carcinoma: surgical progress or status quo?" *Archives of Surgery*, vol. 136, no. 5, pp. 543–549, 2001.

[24] J. F. Henry, F. Sebag, M. Iacobone, and E. Mirallie, "Results of laparoscopic adrenalectomy for large and potentially malignant tumors," *World Journal of Surgery*, vol. 26, no. 8, pp. 1043–1047, 2002.

[25] F. Porpiglia, C. Fiori, R. Tarabuzzi et al., "Is laparoscopic adrenalectomy feasible for adrenocortical carcinoma or metastasis?" *BJU International*, vol. 94, no. 7, pp. 1026–1029, 2004.

[26] F. Corcione, L. Miranda, E. Marzano et al., "Laparoscopic adrenalectomy for malignant neoplasm: our experience in 15 cases," *Surgical Endoscopy and Other Interventional Techniques*, vol. 19, no. 6, pp. 841–844, 2005.

[27] F. F. Palazzo, F. Sebag, M. Sierra, G. Ippolito, P. Souteyrand, and J. F. Henry, "Long-term outcome following laparoscopic adrenalectomy for large solid adrenal cortex tumors," *World Journal of Surgery*, vol. 30, no. 5, pp. 893–898, 2006.

[28] C. P. Lombardi, M. Raffaelli, C. De Crea, and R. Bellantone, "Role of laparoscopy in the management of adrenal malignancies," *Journal of Surgical Oncology*, vol. 94, no. 2, pp. 128–131, 2006.

[29] C. H. Liao, S. C. Chueh, M. K. Lai, P. J. Hsiao, and J. Chen, "Laparoscopic adrenalectomy for potentially malignant adrenal tumors greater than 5 centimeters," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 8, pp. 3080–3083, 2006.

[30] D. Nocca, R. Aggarwal, A. Mathieu et al., "Laparoscopic surgery and corticoadrenalomas," *Surgical Endoscopy and Other Interventional Techniques*, vol. 21, no. 8, pp. 1373–1376, 2007.

[31] G. Ramacciato, P. Mercantini, M. L. Torre et al., "Is laparoscopic adrenalectomy safe and effective for adrenal masses larger than 7 cm?" *Surgical Endoscopy and Other Interventional Techniques*, vol. 22, no. 2, pp. 516–521, 2008.

[32] D. Brix, B. Allolio, W. Fenske et al., "Laparoscopic versus open adrenalectomy for adrenocortical carcinoma: surgical and oncologic outcome in 152 patients," *European Urology*, vol. 58, no. 6, p. e53, 2010.

[33] B. S. Miller, J. B. Ammori, P. G. Gauger, J. T. Broome, G. D. Hammer, and G. M. Doherty, "Laparoscopic resection is inappropriate in patients with known or suspected adrenocortical carcinoma," *World Journal of Surgery*, vol. 34, no. 6, pp. 1380–1385, 2010.

[34] J. E. Lee, D. H. Berger, A. K. El-Naggar et al., "Surgical management, DNA content, and patient survival in adrenal cortical carcinoma," *Surgery*, vol. 118, no. 6, pp. 1090–1098, 1995.

[35] R. D. Schulick and M. F. Brennan, "Long-term survival after complete resection and repeat resection in patients with adrenocortical carcinoma," *Annals of Surgical Oncology*, vol. 6, no. 8, pp. 719–726, 1999.

[36] D. E. Schteingart, G. M. Doherty, P. G. Gauger et al., "Management of patients with adrenal cancer: recommendations of an international consensus conference," *Endocrine-Related Cancer*, vol. 12, no. 3, pp. 667–680, 2005.

[37] L. Chiche, B. Dousset, E. Kieffer, and Y. Chapuis, "Adrenocortical carcinoma extending into the inferior vena cava: presentation of a 15-patient series and review of the literature," *Surgery*, vol. 139, no. 1, pp. 15–27, 2006.

[38] J. Reibetanz, C. Jurowich, I. Erdogan et al., "Impact of lymphadenectomy on the oncologic outcome of patients with adrenocortical carcinoma," *Annals of Surgery*, vol. 255, no. 2, pp. 363–369, 2012.

[39] R. Bellantone, A. Ferrante, M. Boscherini et al., "Role of reoperation in recurrence of adrenal cortical carcinoma: results from 188 cases collected in the Italian National Registry for Adrenal Cortical Carcinoma," *Surgery*, vol. 122, no. 6, pp. 1212–1218, 1997.

[40] P. Icard, Y. Chapuis, B. Andreassian, A. Bernard, and C. Proye, "Adrenocortical carcinoma in surgically treated patients: a retrospective study on 156 cases by the French Association of Endocrine Surgery," *Surgery*, vol. 112, no. 6, pp. 972–980, 1992.

[41] C. D. Smith, C. J. Weber, and J. R. Amerson, "Laparoscopic adrenalectomy: new gold standard," *World Journal of Surgery*, vol. 23, no. 4, pp. 389–396, 1999.

[42] I. S. Gill, "The case for laparoscopic adrenalectomy," *Journal of Urology*, vol. 166, no. 2, pp. 429–436, 2001.

[43] F. Porpiglia, C. Fiori, F. Daffara et al., "Retrospective evaluation of the outcome of open versus laparoscopic adrenalectomy for stage I and II adrenocortical cancer," *European Urology*, vol. 57, no. 5, pp. 873–878, 2010.

[44] C. P. Lombardi, M. Raffaelli, M. Boniardi et al., "Adrenocortical carcinoma: effect of hospital volume on patient outcome," *Langenbeck's Archives of Surgery*, vol. 397, no. 2, pp. 201–207, 2012.

[45] H. S. Park, S. A. Roman, and J. A. Sosa, "Outcomes from 3144 adrenalectomies in the United States: which matters more, surgeon volume or specialty?" *Archives of Surgery*, vol. 144, no. 11, pp. 1060–1067, 2009.

[46] R. D. Schulick and M. F. Brennan, "Adrenocortical carcinoma," *World Journal of Urology*, vol. 17, no. 1, pp. 26–34, 1999.

[47] J. P. Luton, S. Cerdas, L. Billaud et al., "Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy," *The New England Journal of Medicine*, vol. 322, no. 17, pp. 1195–1201, 1990.

[48] A. M. Hutter Jr. and D. E. Kayhoe, "Adrenal cortical carcinoma. Clinical features of 138 patients," *The American Journal of Medicine*, vol. 41, no. 4, pp. 572–580, 1966.

[49] M. B. Lipsett, R. Hertz, and G. T. Ross, "Clinical and pathophysiologic aspects of adrenocortical carcinoma," *The American Journal of Medicine*, vol. 35, no. 3, pp. 374–383, 1963.

[50] K. Cohn, L. Gottesman, and M. Brennan, "Adrenocortical carcinoma," *Surgery*, vol. 100, no. 6, pp. 1170–1177, 1986.

[51] B. Percarpio and A. H. Knowlton, "Radiation therapy of adrenal corticol carcinoma," *Acta Radiologica*, vol. 15, no. 4, pp. 288–292, 1976.

[52] S. E. Rodgers et al., "Adrenocortical carcinoma," *Surgical Oncology Clinics of North America*, vol. 15, no. 3, pp. 535–553, 2006.

[53] B. Polat, M. Fassnacht, L. Pfreundner et al., "Radiotherapy in adrenocortical carcinoma," *Cancer*, vol. 115, no. 13, pp. 2816–2823, 2009.

[54] I. G. C. Hermsen, Y. E. Groenen, M. W. Dercksen, J. Theuws, and H. R. Haak, "Response to radiation therapy in adrenocortical carcinoma," *Journal of Endocrinological Investigation*, vol. 33, no. 10, pp. 712–714, 2010.

[55] S. A. Milgrom and K. A. Goodman, "The role of radiation therapy in the management of adrenal carcinoma and adrenal metastases," *Journal of Surgical Oncology*, vol. 106, no. 5, pp. 647–650, 2012.

[56] A. Sabolch, M. Feng, K. Griffith, G. Hammer, G. Doherty, and E. Ben-Josef, "Adjuvant and definitive radiotherapy for adrenocortical carcinoma," *International Journal of Radiation Oncology Biology Physics*, vol. 80, no. 5, pp. 1477–1484, 2011.

[57] M. Fassnacht, S. Hahner, B. Polat et al., "Efficacy of adjuvant radiotherapy of the tumor bed on local recurrence of adrenocortical carcinoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 11, pp. 4501–4504, 2006.

[58] D. E. Schteingart, "Adjuvant mitotane therapy of adrenal cancer—use and controversy," *The New England Journal of Medicine*, vol. 356, no. 23, pp. 2415–2418, 2007.

[59] J. Lafemina and M. F. Brennan, "Adrenocortical carcinoma: past, present, and future," *Journal of Surgical Oncology*, vol. 106, no. 5, pp. 586–594, 2012.

[60] H. R. Haak, J. Hermans, C. J. H. Van de Velde et al., "Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients," *British Journal of Cancer*, vol. 69, no. 5, pp. 947–951, 1994.

[61] H. van Slooten, A. J. Moolenaar, A. P. Van Seters, and D. Smeenk, "The treatment of adenocortical carcinoma with o,p′-DDD: prognostic simplications of serum level monitoring," *European Journal of Cancer and Clinical Oncology*, vol. 20, no. 1, pp. 47–53, 1984.

[62] E. Baudin, G. Pellegriti, M. Bonnay et al., "Impact of monitoring plasma 1, 1-dichlorodiphenildichloroethane (o, p′DDD) levels on the treatment of patients with adrenocortical carcinoma," *Cancer*, vol. 92, no. 6, pp. 1385–1392, 2001.

[63] A. Faggiano, S. Leboulleux, J. Young, M. Schlumberger, and E. Baudin, "Rapidly progressing high o,p'DDD doses shorten the time required to reach the therapeutic threshold with an acceptable tolerance: preliminary results," *Clinical Endocrinology*, vol. 64, no. 1, pp. 110–113, 2006.

[64] F. Daffara, S. de Francia, G. Reimondo et al., "Prospective evaluation of mitotane toxicity in adrenocortical cancer patients treated adjuvantly," *Endocrine-Related Cancer*, vol. 15, no. 4, pp. 1043–1053, 2008.

[65] R. Vassilopoulou-Sellin, V. F. Guinee, M. J. Klein et al., "Impact of adjuvant mitotane on the clinical course of patients with adrenocortical cancer," *Cancer*, vol. 71, no. 10, pp. 3119–3123, 1993.

[66] B. Bodie, A. C. Novick, J. E. Pontes et al., "The Cleveland Clinic experience with adrenal cortical carcinoma," *Journal of Urology*, vol. 141, no. 2, pp. 257–260, 1989.

[67] A. A. Kasperlik-Zaluska, B. M. Migdalska, S. Zgliczyński, and A. M. Makowska, "Adrenocortical carcinoma. A clinical study and treatment results of 52 patients," *Cancer*, vol. 75, no. 10, pp. 2587–2591, 1995.

[68] A. Berruti, M. Fassnacht, E. Baudin et al., "Adjuvant therapy in patients with adrenocortical carcinoma: a position of an international panel," *Journal of Clinical Oncology*, vol. 28, no. 23, pp. e401–e402, 2010.

[69] S. Hahner and M. Fassnacht, "Mitotane for adrenocortical carcinoma treatment," *Current Opinion in Investigational Drugs*, vol. 6, no. 4, pp. 386–394, 2005.

[70] B. Wängberg, A. Khorram-Manesh, S. Jansson et al., "The long-term survival in adrenocortical carcinoma with active surgical management and use of monitored mitotane," *Endocrine-Related Cancer*, vol. 17, no. 1, pp. 265–272, 2010.

[71] I. Ilias, M. Alevizaki, G. Philippou, E. Anastasiou, and A. Souvatzoglou, "Sustained remission of metastatic adrenal carcinoma during long-term administration of low-dose mitotane," *Journal of Endocrinological Investigation*, vol. 24, no. 7, pp. 532–535, 2001.

[72] R. J. Gonzalez, E. P. Tamm, C. Ng et al., "Response to mitotane predicts outcome in patients with recurrent adrenal cortical carcinoma," *Surgery*, vol. 142, no. 6, pp. 867–875, 2007.

[73] T. S. Khan, H. Imam, C. Juhlin et al., "Streptozocin and o,p′DDD in the treatment of adrenocortical cancer patients: long-term survival in its adjuvant use," *Annals of Oncology*, vol. 11, no. 10, pp. 1281–1287, 2000.

[74] A. Berruti, M. Terzolo, P. Sperone et al., "Etoposide, doxorubicin and cisplatin plus mitotane in the treatment of advanced adrenocortical carcinoma: a large prospective phase II trial," *Endocrine-Related Cancer*, vol. 12, no. 3, pp. 657–666, 2005.

[75] M. Fassnacht, M. Terzolo, B. Allolio et al., "Combination chemotherapy in advanced adrenocortical carcinoma," *The*

New England Journal of Medicine, vol. 366, no. 23, pp. 2189–2197, 2012.

[76] T. J. Giordano, D. G. Thomas, R. Kuick et al., "Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis," *American Journal of Pathology*, vol. 162, no. 2, pp. 521–531, 2003.

[77] F. de Fraipont, M. El Atifi, N. Cherradi et al., "Gene expression profiling of human adrenocortical tumors using complementary deoxyribonucleic acid microarrays identifies several candidate genes as markers of malignancy," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 3, pp. 1819–1829, 2005.

[78] P. S. H. Soon, A. J. Gill, D. E. Benn et al., "Microarray gene expression and immunohistochemistry analyses of adrenocortical tumors identify IGF2 and Ki-67 as useful in differentiating carcinomas from adenomas," *Endocrine-Related Cancer*, vol. 16, no. 2, pp. 573–583, 2009.

[79] D. Velázquez-Fernández, C. Laurell, J. Geli et al., "Expression profiling of adrenocortical neoplasms suggests a molecular signature of malignancy," *Surgery*, vol. 138, no. 6, pp. 1087–1094, 2005.

[80] E. P. Slater, S. M. Diehl, P. Langer et al., "Analysis by cDNA microarrays of gene expression patterns of human adrenocortical tumors," *European Journal of Endocrinology*, vol. 154, no. 4, pp. 587–598, 2006.

[81] P. M. Szabó, V. Tamási, V. Molnár et al., "Meta-analysis of adrenocortical tumour genomics data: novel pathogenic pathways revealed," *Oncogene*, vol. 29, no. 21, pp. 3163–3172, 2010.

[82] C. Gicquel, X. Bertagna, V. Gaston et al., "Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors," *Cancer Research*, vol. 61, no. 18, pp. 6762–6767, 2001.

[83] C. Gicquel, M. L. Raffin-Sanson, V. Gaston et al., "Structural and functional abnormalities at 11p15 are associated with the malignant phenotype in sporadic adrenocortical tumors: study on a series of 82 tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 8, pp. 2559–2565, 1997.

[84] A. Arora and E. M. Scholar, "Role of tyrosine kinase inhibitors in cancer therapy," *Journal of Pharmacology and Experimental Therapeutics*, vol. 315, no. 3, pp. 971–979, 2005.

[85] M. Quinkler, S. Hahner, S. Wortmann et al., "Treatment of advanced adrenocortical carcinoma with erlotinib plus gemcitabine," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 6, pp. 2057–2062, 2008.

[86] S. Wortmann, M. Quinkler, C. Ritter et al., "Bevacizumab plus capecitabine as a salvage therapy in advanced adrenocortical carcinoma," *European Journal of Endocrinology*, vol. 162, no. 2, pp. 349–356, 2010.

[87] M. Fassnacht, M. C. Kreissl, D. Weismann, and B. Allolio, "New targets and therapeutic approaches for endocrine malignancies," *Pharmacology and Therapeutics*, vol. 123, no. 1, pp. 117–141, 2009.

[88] A. Berruti, A. Ferrero, P. Sperone et al., "Emerging drugs for adrenocortical carcinoma," *Expert Opinion on Emerging Drugs*, vol. 13, no. 3, pp. 497–509, 2008.

[89] C. Laurell, D. Velázquez-Fernández, K. Lindsten et al., "Transcriptional profiling enables molecular classification of adrenocortical tumours," *European Journal of Endocrinology*, vol. 161, no. 1, pp. 141–152, 2009.

[90] G. G. Fernandez-Ranvier, J. Weng, R. F. Yeh et al., "Identification of biomarkers of adrenocortical carcinoma using genomewide gene expression profiling," *Archives of Surgery*, vol. 143, no. 9, pp. 841–846, 2008.

[91] P. Haluska, F. Worden, D. Olmos et al., "Safety, tolerability, and pharmacokinetics of the anti-IGF-1R monoclonal antibody figitumumab in patients with refractory adrenocortical carcinoma," *Cancer Chemotherapy and Pharmacology*, vol. 65, no. 4, pp. 765–773, 2010.

[92] M. J. Mulvihill, A. Cooke, M. Rosenfeld-Franklin et al., "Discovery of OSI-906: a selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor," *Future Medicinal Chemistry*, vol. 1, no. 6, pp. 1153–1171, 2009.

[93] S. Faivre, G. Kroemer, and E. Raymond, "Current development of mTOR inhibitors as anticancer agents," *Nature Reviews Drug Discovery*, vol. 5, no. 8, pp. 671–688, 2006.

[94] M. Doghman, A. El Wakil, B. Cardinaud et al., "Regulation of insulin-like growth factor-mammalian target of rapamycin signaling by MicroRNA in childhood adrenocortical tumors," *Cancer Research*, vol. 70, no. 11, pp. 4666–4675, 2010.

[95] A. Naing, R. Kurzrock, A. Burger et al., "Phase I trial of cixutumumab combined with temsirolimus in patients with advanced cancer," *Clinical Cancer Research*, vol. 17, no. 18, pp. 6052–6060, 2011.

[96] B. T. MacDonald, K. Tamai, and X. He, "Wnt/β-catenin signaling: components, mechanisms, and diseases," *Developmental Cell*, vol. 17, no. 1, pp. 9–26, 2009.

[97] G. Assie, T. J. Giordano, and J. Bertherat, "Gene expression profiling in adrenocortical neoplasia," *Molecular and Cellular Endocrinology*, vol. 351, no. 1, pp. 111–117, 2012.

[98] B. Ragazzon, G. Assié, and J. Bertherat, "Transcriptome analysis of adrenocortical cancers: from molecular classification to the identification of new treatments," *Endocrine-Related Cancer*, vol. 18, no. 2, pp. R15–R27, 2011.

[99] F. Tissier, C. Cavard, L. Groussin et al., "Mutations of β-catenin in adrenocortical tumors: activation of the Wnt signaling pathway is a frequent event in both benign and malignant adrenocortical tumors," *Cancer Research*, vol. 65, no. 17, pp. 7622–7627, 2005.

[100] A. Berthon, I. Sahut-Barnola, S. Lambert-Langlais et al., "Constitutive β-catenin activation induces adrenal hyperplasia and promotes adrenal cancer development," *Human Molecular Genetics*, vol. 19, no. 8, pp. 1561–1576, 2010.

[101] B. P. Schimmer and P. C. White, "Minireview: steroidogenic factor 1: its roles in differentiation, development, and disease," *Molecular Endocrinology*, vol. 24, no. 7, pp. 1322–1337, 2010.

[102] B. C. Figueiredo, L. R. Cavalli, M. A. Pianovski et al., "Amplification of the steroidogenic factor 1 gene in childhood adrenocortical tumors," *The Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 2, pp. 615–619, 2005.

[103] M. A. D. Pianovski, L. R. Cavalli, B. C. Figueiredo et al., "SF-1 overexpression in childhood adrenocortical tumours," *European Journal of Cancer*, vol. 42, no. 8, pp. 1040–1043, 2006.

[104] M. Doghman, T. Karpova, G. A. Rodrigues et al., "Increased steroidogenic factor-1 dosage triggers adrenocortical cell proliferation and cancer," *Molecular Endocrinology*, vol. 21, no. 12, pp. 2968–2987, 2007.

[105] M. Doghman, J. Cazareth, D. Douguet, F. Madoux, P. Hodder, and E. Lalli, "Inhibition of adrenocortical carcinoma cell proliferation by steroidogenic factor-1 inverse agonists," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 6, pp. 2178–2183, 2009.

[106] M. J. Demeure, K. J. Bussey, and L. S. Kirschner, "Targeted therapies for adrenocortical carcinoma: IGF and beyond," *Horm Cancer*, vol. 2, no. 6, pp. 385–392, 2011.

[107] C. L. Ronchi, S. Sbiera, L. Kraus et al., "Expression of excision repair cross complementing group 1 and prognosis in adrenocortical carcinoma patients treated with platinum-based chemotherapy," *Endocrine-Related Cancer*, vol. 16, no. 3, pp. 907–918, 2009.

[108] P. Mistry, A. J. Stewart, W. Dangerfield et al., "In vitro and in vivo reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576," *Cancer Research*, vol. 61, no. 2, pp. 749–758, 2001.

[109] J. Walker, C. Martin, and R. Callaghan, "Inhibition of P-glycoprotein function by XR9576 in a solid tumour model can restore anticancer drug efficacy," *European Journal of Cancer*, vol. 40, no. 4, pp. 594–605, 2004.

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 749030, 9 pages
doi:10.1155/2012/749030

Clinical Study

Merkel Cell Carcinoma: A Retrospective Study on 48 Cases and Review of Literature

Fernando Cirillo,[1] Marco Vismarra,[1] Ines Cafaro,[2] and Mario Martinotti[1]

[1] *Department of General Surgery, General Surgery Unit, Rare Hormonal Tumors Group, Surgery of Rare Hormonal Tumors, Azienda Ospedaliera Istituti Ospitalieri, Viale Concordia 1, 26100 Cremona, Italy*
[2] *Radiotherapy Unit and Nuclear Medicine, Azienda Ospedaliera Istituti Ospitalieri, Viale Concordia, 1, 26100 Cremona, Italy*

Correspondence should be addressed to Fernando Cirillo, f.cirillo@neuroendocrini.it

Received 19 March 2012; Revised 28 June 2012; Accepted 13 August 2012

Academic Editor: Marialuisa Appetecchia

Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine tumor of the skin. Fourty-eight patients with MCC were observed at the Rare Hormonal Tumors Group of Cremona Hospital, 15 of these with unknown primary site. Due to rarity of Merkel cell carcinoma, clinical experience is generally limited. Data from our series confirm the current recommendations. Wide surgical excision must be associated with radiotherapy also in early stages in order to avoid local relapse and the rapid progression of disease. In advanced stages chemotherapy is the standard despite the short duration of responses and poor quality of life. The data of our series, characterized by a high demand for second opinion, offer some insight about the real rarity of the tumor, the difficulty of managing of disease in our country secondary to a wrong cultural approach to the problem, the indiscriminate use of molecules unnecessary and often expensive, the lack of protocols, and the presence of guidelines often ignored. This results in very poor survival associated with a very low quality of life, requiring to find the right direction towards a correct management of disease.

1. Introduction

Merkel cell carcinoma (MCC) is a rare and aggressive tumor of the skin described the first time by Toker, in 1972 [1] as a trabecular carcinoma of the skin, suggesting the origin from the cells of the sweat glands, with a later ultrastructural demonstration of neurosecretory granules that places the neoplasm from the Merkel cells [2]. At the beginning of the 1980s, other authors contributed to the discussion on the suitability of the term suggested by Toker, introducing a great variety of alternative names ("primitive small cell carcinoma of the skin," "malignant Merkel cell carcinoma," "Murky cell carcinoma," "Merkeloma") [3–8]. Finally, histochemical and histogenetic studies coined the term "neuroendocrine carcinoma of the skin" for this neoplasm placing it, therefore, in the large family of APUDomas [9–16], even if the true origin of the tumor from Merkel cell has not been proved definitively. It was also postulated the derivation of Merkel cell from the neural crest, the separation from the cells of Schwann, and the following migration from mesenchyma to epidermis as prolongation of the sensitive nerves of the derma [14]. MCC is currently considered deriving from an epithelial totipotent cell able to differentiate it both in neuroendocrine way, and as cheratinocita [15]. The presence of transitional cells similar both to the cheratinocytis and Merkel cells gives support to this theory.

The definite function of Merkel cell is not clear yet. The nearby contact with sensitive fibers would make to suppose a role of Merkel cell in the process of transition for some nervous stimulus; an influence is also possible on the secretion of neuropeptides as paracrine regulators on the near structures of the epidermis and adnexa [15]. Our observation of a consistent number of cases of MCC has induced us to a review of the literature in order to optimize the diagnostic and therapeutic approach to this tumor, and to emphasize management problems secondary to a cultural limitations that considers in our country MCC like a cancer of little

interest with a negative influence in terms of cost and survival.

2. Materials and Methods

At the Rare Hormonal Tumors Group, Department of General Surgery of Cremona Hospital, in the last 21 years we have observed 48 patients suffering from MCC in different stages of disease: stage I 28.2%, stage II 8.6%, stage III 26%, and stage IV 37% of cases. Most of the observed patients came from other institutions as a request for second opinion (at least 2/3), and for this reason the analysis of data cannot be homogeneous (Table 1). In order to stage the disease we have preferred to use the previous staging system from American Joint Committee on Cancer (AJCC) 2005 because more simple to use, and because the greatest part of the oldest literature refers to this.

There were 26 male (54.1%) and 22 female (45.8%) patients with slight male predominance. In our series MCC affects most frequently elderly patients with a mean age of 70.1 for male, and 71.2 for female (male plus female equal to 70.6, range from 52 to 95 years). MCC has been observed as a nodule of the skin in most of the cases. The extremities (40% of cases) were the most common site of incidence: follow unknown primary site (31%), head and neck (19%), buttock (8%), and trunk (1%). Half of cases had lesions with diameter <2 cm, and the other diameter equal to 2 cm or more, with a mean of 2.42 cm, and range from 0.5 to 8 cm. About proliferation cell index (ki67, MIB1 clone), we have separated the series in three different groups (ki67 10–30%, ki67 30–50%, and ki67 > 50%) in which percentage were 13.6% for the group 1, 22.7% for group 2, and 63.6% for group 3 (range from 22% to 90%). The group with the most elevated cell proliferation is prevailing, and this confirms that MCC is a particularly aggressive tumor. In our series we have observed 3 patients with associated rheumatoid arthritis, 3 with hepatitis virus C related, 1 transplanted, 1 with Kaposi sarcoma, and 4 patients with a personal history of tumor (1 non-Hodgkin lymphoma and 3 carcinomas). In 1 case we have observed MCC associated with squamous-cell carcinoma growing together [17]. About unknown primary site, we observed 15 patients (31%): 6 with lesion situated in the groin (40%), 5 in the buttock (33%), and in 1 case lesions were situated in axilla, thigh, vestibule of nose, and parotid gland, respectively.

Role of surgery was confirmed as fundamental for treatment of MCC, above all in early stage. In our series all the patients received surgical approach always: as radical and curative method in early stage, or as debulking for local relapse in advanced stages. Due to the different origin of patients, surgery was associated with other therapies: radiotherapy (12.5%), chemotherapy (10.4%), somatostatin analogues (8.3%), or more treatments together, as radiotherapy plus somatostatin analogues (8.3%), chemotherapy plus somatostatin analogues (6.2%), and radiotherapy plus chemotherapy plus somatostatin analogues (6.2%), radiotherapy plus chemotherapy (2%). In only 4% of cases other treatments were considered, as receptor radionuclide therapy, α interferon (IFN), and imiquimod.

3. Results and Discussion

The true incidence of MCC is unknown [18–20]. This tumor most frequently affects elderly patients over the age of sixty (range 7–95) [21], in 78.6% of cases [22], with a preference for women (M : F = 1 : 3) [22, 23]; MCC is most common in Caucasian populations, but occasionally is also present among blacks and Polynesians [15]. The most common site of the tumor is the skin of the head and neck (50%); in 40% of cases extremities are affected, and in 10% trunk and mucosa. Cases have also been reported of multiple sites of the disease [15, 23].

The markers normally expressed by this tumour are neuron-specific enolase (NSE) [24], chromogranins [25], and synaptophysin [26]. Vimentin and desmin are usually negative [27, 28]. Cytoplasmatic granules can be rich of vasoactive intestinal polypeptide (VIP), and of met-encephalin.

The neoplasm is typically presented as an isolated, raised or flat lesion, red-purplish in colour, with a shiny surface occasionally associated with nearby telangiectasias. The epidermis may be intact or ulcerated. The tumor can occasionally be pediculate [12, 29]. The size of the neoplasm can vary greatly, up to 15 cm in diameter, with an average of 3 cm at presentation [23].

In early stage MCC doesn't present specific characters, so that the differential diagnosis can result difficult: in fact MCC can be confused with the baso or spinocellular carcinoma, the pyogenic granuloma, the cheratoacantoma, the melanoma, the cutaneous linfoma, cutaneous metastasis from anaplastic carcinoma, carcinoid tumors, retinoblastoma, sarcoma of Ewing, and neuroblastoma [15]. A high incidence of the tumor (over 600 cases) was reported in transplanted patients with a mean of 53 years (range 33–78). MCC was observed after 5–286 months from transplant (average 91.5) with characteristics of greater aggressiveness probably secondary to the immunosuppression of the patient [30–32]. The immunosuppressive situation could be the cause of metastatic MCC also in an HIV patient [33]. In our series 3 cases reported of rheumatoid arthritis associated with MCC could be secondary to immunosuppression. Since rheumatoid arthritis is considered an autoimmune disease, it is possible a predisposition to MCC among elderly patients with immune defenses reduced because of the prolonged use of steroid molecules [34].

The staging of MCC considers a whole-body CT spiral scan because of the frequent high-proliferation index and poor differentiation of the tumor, with the aim of identifying metastatic involvement of soft tissues, sometimes associated with lytic bone lesions [35]. Positron Emission Tomography (^{18}F-FDG-PET-CT) is an highly useful whole-body-staging method compared to conventional imaging methods, also when used as a single procedure [36, 37]. OctreoScan, using a labelled analogue of somatostatin (^{111}In-Pentetreotide), is still considered an highly sensitive method also when compared with other conventional imaging techniques [38]. Laboratory diagnosis considers the plasmatic dosage of chromogranin A and NSE, more specific in posttreatment followup rather than during the stage of the tumor.

Table 1: Merkel cell carcinoma series (1990–2012).

Sex	Age	Site	Type	Size (cm)	Stage	Ki67% (MIB1)	ChrA staining	NSE staining	ChrA (ng/mL)	NSE (ng/mL)	Therapy	Survival (months)	Other
F	83	EXTR	NOD/ULC	3	III	—	NEG	POS	—	—	SURG + SMS	36	RA
M	52	EXTR	NOD	—	—	—	—	—	—	—	SURG + RT	2	
F	76	BUTTOCK	NOD	3	II	—	NEG	NEG	—	—	SURG	—	
F	75	EXTR	NOD	1	I	30	POS	POS	—	—	SURG + RT	29	RA
M	70	NS	—	3	III	>50	NEG	POS	—	—	SURG + SMS	8	HCV+
F	81	NS	—	1.5	III	—	—	—	—	—	SURG + RT + SMS	26	
F	83	HEAD	NOD	1.2	I	—	NEG	POS	177	5.4	SURG + RT	24	
M	55	BUTTOCK	NOD	3.5	IV	—	—	—	52.2	39.8	SURG + CHT	25	
M	80	TRUNK	NOD	2.2	II	>70	POS	POS	43.2	5.5	SURG + SMS	24	
F	74	HEAD	NOD/CYS	—	I	—	—	—	—	6.3	SURG + RT + SMS + α IFN	15	
M	70	EXTR	NOD	0.8	I	—	—	—	—	—	SURG	—	HCV+
F	72	HEAD	NOD	1	I	80	—	—	—	—	SURG	—	Ca breast, Ca lung
F	63	NS	—	5	III	—	—	POS	51	31.3	SURG	25	HCV+ Ca uterus
M	74	EXTR	NOD	1.5	I	—	POS	—	136	5.4	SURG	3	
M	70	NS	—	—	IV	—	—	—	—	—	SURG + CHT	12	
M	85	EXTR	NOD	8	III	25	POS	—	870	43	SURG + SMS	37	Kaposi sarcoma
F	61	EXTR	NOD	4.3	II	80	POS	POS	—	—	SURG	—	
M	76	HEAD	NOD	1.5	III	—	POS	POS	185	8.3	SURG	—	Ca rectus
F	84	EXTR	NOD	2.0	IV	—	POS	POS	—	—	SURG + CHT	48	
F	79	EXTR	NOD	1.5	I	—	—	—	—	—	SURG + RT	—	
M	76	EXTR	NOD	—	IV	—	—	—	—	—	SURG + CHT	12	
F	72	NS	—	4.5	IV	80	POS	—	70	81.2	SURG + CHT + SMS	58	
M	55	EXTR	NOD	1	III	—	NEG	—	—	—	SURG	—	
F	80	EXTR	NOD/ULC	1.7	I	80	POS	POS	—	—	SURG	—	
M	67	HEAD	NOD	0.5	I	—	—	—	—	—	SURG	—	
M	70	NS	—	—	IV	70	—	—	46	13	SURG + CHT	16	
M	70	EXTR	NOD	2	IV	40	—	—	99.3	17.8	SURG + RT + CHT + SMS	27	
F	61	NS	—	6	IV	50	—	—	50	21.3	SURG + CHT	—	
M	63	BUTTOCK	NOD	5	IV	50	POS	—	—	—	SURG	15	LNH
M	95	HEAD	NOD	1.2	I	70	POS	—	—	—	SURG	—	
M	80	HEAD	NOD	0.5	IV	—	—	—	46	8.9	SURG + RT + CHT + SMS + RMT	17	
M	74	NS	—	—	IV	—	—	NEG	—	—	SURG + RT + CHT + SMS	23	
F	69	EXTR	NOD	—	IV	—	—	—	—	—	SURG	—	
M	64	NS	—	3	II	60	—	POS	156	8.0	SURG	—	
F	60	HEAD	NOD	0.7	I	90	POS	POS	—	—	SURG	—	
M	89	BUTTOCK	NOD	—	IV	80	POS	—	760	86.2	SURG + RT + CHT	13	

Table 1: Continued.

Sex	Age	Site	Type	Size (cm)	Stage	Ki67% (MIB1)	ChrA staining	NSE staining	ChrA (ng/mL)	NSE (ng/mL)	Therapy	Survival (months)	Other
F	65	EXTR	NOD	—	—	—	—	—	—	—	SURG	—	
M	59	EXTR	NOD	1	III	—	POS	POS	—	—	SURG	6	
M	64	HEAD	NOD	1.1	III	60	POS	—	—	—	SURG	—	RA
F	59	EXTR	NOD	0.6	I	22	—	—	—	—	SURG	17	
M	75	NS	—	4	IV	—	—	—	—	—	SURG	6	HCV+
F	59	NS	—	1	I	40	POS	—	—	—	SURG	14	
F	78	EXTR	NOD	2.5	III	—	—	—	—	—	SURG	—	
F	60	NS	—	—	IV	—	—	—	—	—	SURG	—	Paraneoplastic polineuritis
M	69	EXTR	NOD	2	III	35	POS	POS	116	10	SURG + RT	5	Transpl
F	74	NS	—	6	IV	—	POS	—	700	102	SURG + CHT+ SMS	52	
M	58	NS	—	2.5	III	80	POS	POS	46.5	5.3	SURG + RT	—	
M	63	NS	—	1.2	IV	80	POS	—	1500	17.20	SURG + CHT + SMS	22	

AR: rheumatoid arthritis, ChrA: chromogranin A, 19–98 ng/mL, CHT: chemotherapy, EXTR: extremities, F: female, α IFN: alpha interferon, M: male, NS: no skin (unknown primary site), NSE: neuron-specific enolase, <12 ng/mL, RM: receptor radionuclide therapy, RT: radiotherapy, SMS: somatostatin analogues, SURG: Surgery, TRANS: transplanted.

Table 2: Merkel cell carcinoma staging system, 2005 [40].

Stage	TNM	OS 2 y	OS 5 y
Stage I	Primary < 2 cm (T1)	67%	81%
Stage II	Primary 2 cm or more (T2)	59%	67%
Stage III	Nodal disease (N1)	49%	52%
Stage IV	Systemic metastases (M1)	23%	11%

Patients affected by MCC can be classified using the last classification AJCC 2010, more online with other skin malignancies, although more complicated to use [39]. Because of this, the literature often refers to the previous staging system from AJCC 2005 [40], more simple to use, but making comparison is difficult with newer studies that consider the last classification. For this reason, we preferred to refer to the classification AJCC 2005 in order to give more homogeneity to our older cases staged by this classification (Table 2).

3.1. Surgery. In stage I and II, surgical is the treatment of choice represented by the excision of the primitive lesion [15, 41–44]. In order to avoid local recurrence, an adequate resection margin of at least 2 cm is required [45, 46]. A more wide excision provides a significant reduction in local recurrence rate by increasing the margin from 1 to 3 cm [15, 47, 48]. In our series of 8 cases from other institutions, a wide excision was not considered after histological examination causing a local relapse to distance. The necessity of elective lymph nodal treatment is controversial. Tumor size > 1 cm was found to be a poor prognostic factor [49], and 2 cm can be a significant cut off for poor prognosis [34, 40]. For these reasons, and also in relationship with our experience, we suggest that Sentinel Lymph Node Biopsy (SLNB) should always be considered [50]. SLNB detects MCC spread in one-third of patients understaged, and those who did not receive treatment that involved nodes [51]; this method identifies occult nodal metastases in 29% of patients with localized MCC [52]. About this method, in our series we have observed a higher sensibility using ^{18}F-scan rather than ^{99}Tc-scan. Finally, in absence of SLNB, adjuvant radiotherapy to the primary and nodal region should be delivered.

3.2. Radiotherapy. The greatest part of authors are in favor to consider adjuvant postoperative radiotherapy routinely. This choice is associated with a reduced risk of local recurrence [53, 54]. Radiant treatment (40–60 Gy) should follow surgical excision [55] in order to prevent the progression of disease in stage I and II with development of lymph nodal metastases in 40–73% of the cases and local relapse in 23–60% of the cases [56], with a disease free survival only up to 8 months [45]. In these cases surgical debulking can be associated with more sustained radiant regimes with survival in approximately 60% of cases [15], and a disease free survival from 3 to 30 months (average 8 months) [57]. The largest series from SEER data shows median survival for adjuvant radiotherapy up to 63 months compared with median survival without radiant therapy up to 45 months. Radiotherapy is associated with an increased survival particularly for primary lesions greater than 2 cm [58]. In another series from Canada and Australia, combined surgery and radiotherapy improves both loco-regional control and disease-free survival [49]. On the contrary, adjuvant chemotherapy does not reduce the rate of local relaps nor improve survival [59]. We have observed 10 cases from other institutions with local relapse due to the absence of prior radiant therapy that were in need of surgical debulking.

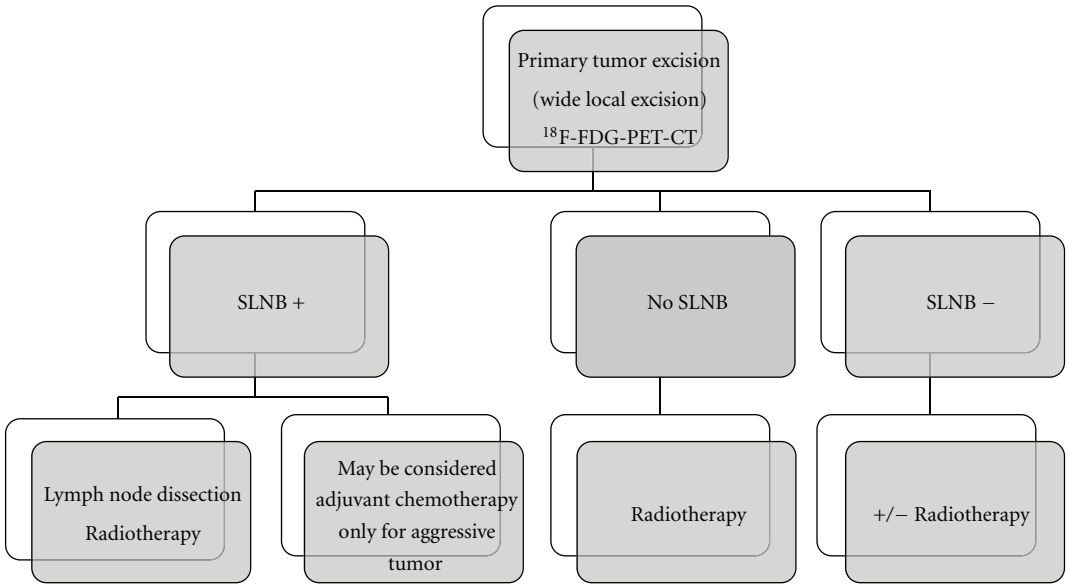


Figure 1: Algorithm for staging and treatment MCC.

3.3. Radiotherapy Alone. Radiotherapy as primary treatment is essential in cases of locally advanced tumors or invasion of critical structures with difficult resectability. It was reported, a study on 3 cases with complete response after primary radiotherapy, and the absence of local relapses for up to 3 years [60]. In a retrospective study there were no statistical differences and disease-free survival between two different groups (radiotherapy alone versus conventional therapy) [61]. In a series of 50 patients, lymph node radiation alone in metastatic nodes has resulted in a great percentage of local control compared with lymphadenectomy alone on both microscopic and palpable nodes, and no differences for overall survival [62].

3.4. Chemotherapy. Advanced disease is characteristic of stage IV. Chemotherapy treatment considers a wide range of molecules used both in monotherapy and in combination, as etoposide, carbo/cisplatin, doxorubicin, dacarbazine, vincristine, cyclophosphamide, and methotrexate. Chemotherapy shows a surprising objective response at beginning of treatment (61%) with a progressive drop during a second (45%), and a third line of therapy (20%) [63] with a very short duration, from 3.5 to 12 months [64, 65]. In the TROG study, synchronous carboplatin/etoposide plus radiation have been achieved high levels of locoregional control and survival [66], in contrast with a retrospective study from the same group [67].

3.5. Other Methods. Local infiltration of α-2b IFN [68], tumor necrosis factor (TNF) [69], hyperthermia in association with low doses of radiotherapy [70], or radiotherapy with TNF-α, IFN γ, and melphalan [71], have showed occasional remissions with relatively long, but anecdotal, disease-free survival. Among the immunomodulatory molecules, imiquimod combined with radiotherapy has suggested the possible use effective with a complete response up to 7 months in a case reported of MCC of the head [72]. About somatostatin analogues treatment, there is a few number of reports in literature. In one case of metastatic MCC from our series, the treatment with octreotide showed an immediate objective response with a moderate dose (1 mg/day subcutis), in absence of significant side effects and survival over 10 months from the start of therapy [73]; moreover, in 2 cases observed, OctreoScan was been able to determine a partial regression of local relapse, even before starting treatment with somatostatin analogues. In another case reported of local advanced and recurrent MCC of the head, treatment with lanreotide at the dose of 15 mg intramuscular every two weeks showed a favorable course after 17 months from the start of therapy [74]. In other case of metastatic MCC reported treated with octreotide has been observed a favorable course up to 3 years with a good quality of life [75]. Somatostatin analogues can play a role in the therapy of metastatic MCC, in alternative to chemotherapy, limited to selected cases with mild aggressive disease, and with significant density *in vivo* for somatostatin receptors. In our series, somatostatin analogues represent a wide slice in the treatment of MCC (29% of cases) in different modalities of association. Receptor radionuclide therapy is reported only in one case after relapse from MCC in a elderly patient, with a good response [76]. In our series we have treated only one elderly patient suffering from MCC with ^{177}Lu-DOTATATE (1.5 GBq), already submitted to other therapies, and probably in a too advanced stage to consent a response.

4. Conclusions

MCC is a highly aggressive cancer of the skin with 30% of mortality. The incidence in USA has increased threefold and became the second common cause of nonmelanoma skin cancer death [77]. The most common features were used to create a simple acronym: AEIOU (asymptomatic/lack of

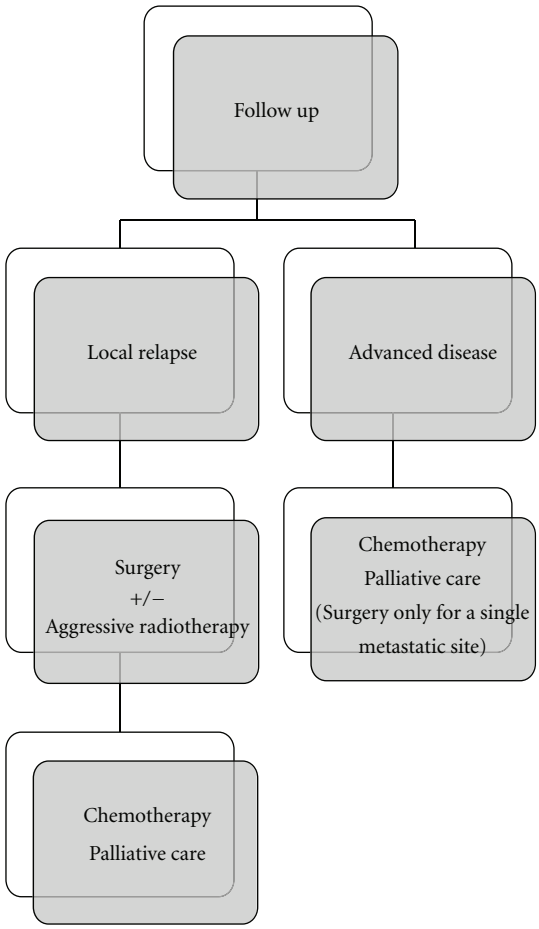


Figure 2: Algorithm for advanced or locally relapsed MCC.

tenderness, expanding rapidly <3 months, immunosuppression, older than 50, and location on an ultraviolet-exposed site). These criteria can allow as a clue in the diagnosis of MCC, with three or more criteria in 89% of cases in a series of 195 patients [78]. In relation to the current knowledge, the correct management for MCC is the aggressive and radical excision of the lesion in its early stage in order to reduce the rate of relapse, and to improve survival. Surgery is the mainstay of treatment for MCC when feasible. Outcome depends mainly on the early and wide excision [79], and on sequential radiotherapy, in order to avoid local relapse and/or progressive disease, as also confirmed from our personal observations. In this way, the role of SLNB is in our opinion fundamental also in stage I, given that size of lesion may not match the malignancy of the tumor. About chemotherapy, its role should be revisited with newer molecules including targeted agents. In this way, coexpression of KIT in a high percentage of MCC suggests an important role in Merkel cell transformation [80], so that the potential use of KIT kinase inhibitor-based therapies, as imatinib, should be also considered in metastatic MCC [81, 82].

The finding that polyomavirus (MCPyV) is frequently present in MCC (69–85% of cases) has been confirmed by several independent groups [83]. The integration of this virus before the tumor development supports a role for polyomavirus in tumorigenesis process [84]. In this way, prophylaxis with vaccination against Merkel cell polyomavirus should be possible in high-risk patients, in the future.

In our opinion, our series highlights a number of interesting aspects. The first concerns the number of patients observed. The great number of patients in our case series can suggest the consideration that the MCC, although considered a low-tumor incidence, it is not so quite rare. The second aspect concerns the cultural approach to the problem. Looking at the cases with advanced disease from other institutions, it is evident that the large number of patients to whom it was not proposed or wide surgical excision, or radiotherapy, or both: thus clearly demonstrates the lack of expertise in the management of MCC, and because of the high aggressiveness of MCC, it is subsequently assumed the highest rate of local relapses or metastatic disease. The third aspect relates to the timing in the management of MCC. We have observed several cases where the choice to remove the primary lesion was made after so many months from the onset of disease, and several cases with long latency between histological diagnosis and subsequent treatment decisions. In one case there was not even the histological examination of the primary lesion and in another even that of relapse. These observations are once again due to the lack of experience for MCC, but also towards a management too superficial in regard to a tumor too underestimated. The fourth point concerns the treatment of metastatic disease. Chemotherapy should be considered at present the standard treatment in advanced disease: but in our series we can observe the frequent use of different molecules (particularly somatostatin analogues) for patients from other institutions, which cannot be considered appropriate to control metastatic disease and even related symptoms. The fifth point relates to the lack of diagnostic and therapeutic protocols, a problem affecting almost the entire management of rare tumors. This question also involves the management of MCC and is highlighted by the large number of second opinion requests. The lack of protocols is partly covered by some guidelines (in Italy by the guidelines from ROL, Rete Oncologica Lombarda) in many cases not known and in many other cases disregarded. The sixth and final point concerns the last classification of MCC, which in our opinion is too complex with the result of a difficult staging, and the consequence of a therapeutic approach to disease not always easy, another reason that makes us still choose the previous staging system from AJCC 2005.

All these reasons lead clearly to the impossibility having concrete data of survival. In our series the survival rate was calculated considering the distance in time between first diagnosis and our last control of patient. Since the majority of patients we have considered as second opinion in different stages of disease, and the greatest part of these in advanced disease (stage IV) or in presence of local relapse, it is not possible to report the correct data of survival. Furthermore, in a significant part of cases from other institutions we were not able to get further information about the progress of disease. About MCC from unknown primary site (31% in

our series), survival appears very low (average 24 months) but conditioned by a very significant late diagnosis up to 18 months, and few treatment options [85]. Finally, we believe that the comprehensive evaluation of the patient integrated with imaging and laboratory parameters can allow to find the right direction for a balanced choice of therapy and not always immediately easy. It will nevertheless require a cultural change in the approach of MCC as in case of other rare tumors (Figures 1 and 2) [50, 86].

References

[1] C. Toker, "Trabecular carcinoma of the skin," *Archives of Dermatology*, vol. 105, no. 1, pp. 107–110, 1972.

[2] C. K. Tang and C. Toker, "Trabecular carcinoma of the skin: an ultrastructural study," *Cancer*, vol. 42, no. 5, pp. 2311–2321, 1978.

[3] C. Toker, "Trabecular carcinoma of the skin: a question of title," *American Journal of Dermatopathology*, vol. 4, no. 6, pp. 497–500, 1982.

[4] J. B. Stern, "'Murky cell' carcinoma (formerly trabecular carcinoma). A reply in which yet another name is revealed at the very end," *American Journal of Dermatopathology*, vol. 4, no. 6, pp. 517–519, 1982.

[5] A. M. Rywlin, "Malignant Merkel-cell tumor is a more accurate description than trabecular carcinoma," *American Journal of Dermatopathology*, vol. 4, no. 6, pp. 513–515, 1982.

[6] G. S. Sidhu, "What's in a name? Should it be Merkel cell neoplasm or trabecular carcinoma?" *American Journal of Dermatopathology*, vol. 4, no. 6, pp. 509–511, 1982.

[7] C. Urmacher, "Trabecular carcinoma is an accurate designation," *American Journal of Dermatopathology*, vol. 4, no. 6, p. 507, 1982.

[8] J. Rosai, "On the nature and nomenclature of a primary small carcinoma of the skin exhibiting endocrine (?Merkel cell) differentiation," *American Journal of Dermatopathology*, vol. 4, no. 6, pp. 501–505, 1982.

[9] R. K. Sibley, J. Rosai, and E. Foucar, "Neuroendocrine (Merkel cell) carcinoma of the skin. A histologic and ultrastructural study of two cases," *American Journal of Surgical Pathology*, vol. 4, no. 3, pp. 211–221, 1980.

[10] E. Silva and B. Mackay, "Neuroendocrine (Merkel cell) carcinomas of the skin: an ultrastructural study of nine cases," *Ultrastructural Pathology*, vol. 2, no. 1, pp. 1–9, 1981.

[11] C. De Wolf-Peeters, K. Marien, J. Mebis, and V. Desmet, "A cutaneous APUDoma or Merkel cell tumor? A morphologically recognizable tumor with a biological and histological malignant aspect in contrast with its clinical behavior," *Cancer*, vol. 46, no. 8, pp. 1810–1816, 1980.

[12] B. Frigerio, C. Capella, and V. Eusebi, "Merkel cell carcinoma of the skin: the structure and origin of normal Merkel cells," *Histopathology*, vol. 7, no. 2, pp. 229–249, 1983.

[13] A. G. E. Pearse, "The neuroendocrine (APUD) cells of the skin," *American Journal of Dermatopathology*, vol. 2, no. 2, pp. 121–123, 1980.

[14] K. Hashimoto, "The ultrastructure of the skin of human embryos. X. Merkel tactile cells in the finger and nail," *Journal of Anatomy*, vol. 111, no. 1, pp. 99–120, 1972.

[15] D. Ratner, B. R. Nelson, M. D. Brown, and T. M. Johnson, "Merkel cell carcinoma," *Journal of the American Academy of Dermatology*, vol. 29, no. 2, pp. 143–160, 1993.

[16] K. B. English, "Morphogenesis of haarscheiben in rats," *Journal of Investigative Dermatology*, vol. 69, no. 1, pp. 58–67, 1977.

[17] F. Cirillo, "Neuroendocrine tumors and their association with rare tumors: observation of 4 cases," *European Review for Medical and Pharmacological Sciences*, vol. 14, no. 7, pp. 577–588, 2010.

[18] A. Di Cataldo, G. Li Destri, R. A. Ciraldo RA et al., "Il carcinoma neuroendocrino a cellule di Merkel della cute," *Chirurgia*, vol. 5, pp. 527–530, 1982.

[19] T. Kivela and A. Tarkkanen, "The Merkel cell and associated neoplasms in the eyelids and periocular region," *Survey of Ophthalmology*, vol. 35, no. 3, pp. 171–187, 1990.

[20] L. L. Anderson, T. J. Phipps, and M. L. McCollough, "Neuroendocrine carcinoma of the skin (Merkel cell carcinoma) in a black," *Journal of Dermatologic Surgery and Oncology*, vol. 18, no. 5, pp. 375–380, 1992.

[21] T. Y. Chuang, W. P. D. Su, and S. A. Muller, "Incidence of cutaneous T cell lymphoma and other rare skin cancers in a defined population," *Journal of the American Academy of Dermatology*, vol. 23, no. 2 I, pp. 254–256, 1990.

[22] S. Zanardi, L. Canobbio, R. Torrisi, D. Cannata, and F. Boccardo, "Le neoplasie neuroendocrine. Aspetti nosografici, anatomopatologici e clinici (1)," *Argomenti Di Oncologia*, vol. 15, pp. 23–36, 1994.

[23] U. Meyer-Pannwitt, K. Kummerfeldt, P. Boubaris, and J. Caselitz, "Merkel cell carcinoma," *Langenbecks Archiv fur Chirurgie*, vol. 382, no. 6, pp. 349–358, 1997.

[24] J. Gu, J. M. Polak, and S. Van Noorden, "Immunostaining of neuron-specific enolase as a diagnostic tool for Merkel cell tumors," *Cancer*, vol. 52, no. 6, pp. 1039–1043, 1983.

[25] B. S. Wilson and R. V. Lloyd, "Detection of chromogranin in neuroendocrine cells with a monoclonal antibody," *American Journal of Pathology*, vol. 115, no. 3, pp. 458–468, 1984.

[26] J. P. Ortonne, J. P. Petchot-Bacque, P. Verrando, A. Pisani, G. Pautrat, and F. Bernerd, "Normal Merkel cells express a synaptophysin-like immunoreactivity," *Dermatologica*, vol. 177, no. 1, pp. 1–10, 1988.

[27] V. E. Gould, R. Moll, and I. Moll, "Neuroendocrine (Merkel) cells of the skin: hyperplasias, dysplasias, and neoplasms," *Laboratory Investigation*, vol. 52, no. 4, pp. 334–353, 1985.

[28] W. Hartschuh, E. Weihe, N. Yanaihara, and M. Reinecke, "Immunohistochemical localization of vasoactive intestinal polypeptide (VIP) in Merkel cells of various mammals: evidence for a neuromodulator function of the Merkel cell," *Journal of Investigative Dermatology*, vol. 81, no. 4, pp. 361–364, 1983.

[29] T. C. Chao, J. M. Park, H. Rhee, and J. A. Greager, "Merkel cell tumor of the back detected during pregnancy," *Plastic and Reconstructive Surgery*, vol. 86, no. 2, pp. 347–351, 1990.

[30] M. J. Veness, "Aggressive skin cancers in a cardiac transplant recipient," *Australasian Radiology*, vol. 41, no. 4, pp. 363–366, 1997.

[31] R. H. Williams, M. B. Morgan, I. M. Mathieson, and H. Rabb, "Merkel cell carcinoma in a renal transplant patient: increased incidence?" *Transplantation*, vol. 65, no. 10, pp. 1396–1397, 1998.

[32] I. Penn and M. R. First, "Merkel's cell carcinoma in organ recipients: report of 41 cases," *Transplantation*, vol. 68, no. 11, pp. 1717–1721, 1999.

[33] P. Samarendra, L. Berkowitz, S. Kumari, and R. Alexis, "Primary nodal neuroendocrine (Merkel cell) tumor in a oatient with HIV infection," *Southern Medical Journal*, vol. 93, no. 9, pp. 920–922, 2000.

[34] F. Cirillo, M. Buononato, G. Lima, I. Cafaro, and P. Alquati, "Clinical experience on eight cases of Merkel cell carcinoma," *Tumori*, vol. 89, no. 2, pp. 146–151, 2003.

[35] F. Eftekhari, S. Wallace, E. G. Silva, and R. Lenzi, "Merkel cell carcinoma of the skin: imaging and clinical features in 93 cases," *British Journal of Radiology*, vol. 69, no. 819, pp. 226–233, 1996.

[36] G. Maury, D. Dereure, A. Du-Thanh, D. Mariano-Goulart, and B. Guillot, "Interest of (18)F-FDG PET-CT scanning for staging and management of merkel cell carcinoma: a retrospective study of 15 patients," *Journal of the European Academy of Dermatology and Venereology*, vol. 25, no. 12, pp. 1420–1427, 2011.

[37] P. Peloschek, C. Novotny, C. Mueller-Mang et al., "Diagnostic imaging in Merkel cell carcinoma: lessons to learn from 16 cases with correlation of sonography, CT, MRI and PET," *European Journal of Radiology*, vol. 73, no. 2, pp. 317–323, 2010.

[38] S. W. J. Lamberts, E. P. Krenning, and J. C. Reubi, "The role of somatostatin and its analogs in the diagnosis and treatment of tumors," *Endocrine Reviews*, vol. 12, no. 4, pp. 450–482, 1991.

[39] American Joint Committee on Cancer Staging Handbook, *Merkel Cell Carcinoma*, Springer, New York, NY, USA, 2010.

[40] P. J. Allen, W. B. Bowne, D. P. Jaques, M. F. Brennan, K. Busam, and D. G. Coit, "Merkel cell carcinoma: prognosis and treatment of patients from a single institution," *Journal of Clinical Oncology*, vol. 23, no. 10, pp. 2300–2309, 2005.

[41] J. H. Raaf, C. Urmacher, and W. K. Knapper, "Trabecular (Merkel cell) carcinoma of the skin. Treatment of primary, recurrent, and metastatic disease," *Cancer*, vol. 57, no. 1, pp. 178–182, 1986.

[42] J. Tennvall, A. Biorklund, L. Johansson, and M. Akerman, "Merkell cell carcinoma: management of primary, recurrent and metastatic disease. A clinicopathological study of 17 patients," *European Journal of Surgical Oncology*, vol. 15, no. 1, pp. 1–9, 1989.

[43] S. Pilotti, F. Rilke, C. Bartoli, and A. Grisotti, "Clinicopathologic correlations of cutaneous neuroendocrine Merkel cell carcinoma," *Journal of Clinical Oncology*, vol. 6, no. 12, pp. 1863–1873, 1988.

[44] E. G. Silva, B. Mackay, H. Goepfert, M. A. Burgess, and R. S. Fields, "Endocrine carcinoma of the skin (Merkel cell carcinoma)," *Pathology Annual*, vol. 19, pp. 1–30, 1984.

[45] M. J. Ott, K. K. Tanabe, M. A. Gadd et al., "Multimodality management of Merkel cell carcinoma," *Archives of Surgery*, vol. 134, no. 4, pp. 388–393, 1999.

[46] H. Goepfert, D. Remmler, E. Silva, and B. Wheeler, "Merkel cell carcinoma (endocrine carcinoma of the skin) of the head and neck," *Archives of Otolaryngology*, vol. 110, no. 11, pp. 707–712, 1984.

[47] A. Yiengpruksawan, D. G. Coit, H. T. Thaler, C. Urmacher, and W. K. Knapper, "Merkel cell carcinoma: prognosis and management," *Archives of Surgery*, vol. 126, no. 12, pp. 1514–1519, 1991.

[48] C. L. Hitchcock, K. I. Bland, R. G. Laney, D. Franzini, B. Harris, and E. M. Copeland III, "Neuroendocrine (Merkel cell) carcinoma of the skin. Its natural history, diagnosis, and treatment," *Annals of Surgery*, vol. 207, no. 2, pp. 201–207, 1988.

[49] J. R. Clark, M. J. Veness, R. Gilbert, C. J. O'Brien, and P. J. Gullane, "Merkel cell carcinoma of the head and neck: is adjuvant radiotherapy necessary?" *Head and Neck*, vol. 29, no. 3, pp. 249–257, 2007.

[50] A. Assouline, P. Tai, K. Joseph, J. L. Dong Lian, C. Krzisch, and E. Yu, "Merkel cell carcinoma of skin—current controversies and recommendations," *Rare Tumors*, vol. 3, article e23, pp. 71–75, 2011.

[51] S. G. Gupta, L. C. Wang, P. F. Peñas, M. Gellenthin, S. J. Lee, and P. Nghiem, "Sentinel lymph node biopsy for evaluation and treatment of patients with Merkel cell carcinoma: the Dana-Farber experience and meta-analysis of the literature," *Archives of Dermatology*, vol. 142, no. 6, pp. 685–690, 2006.

[52] R. C. Fields, K. J. Busam, J. F. Chou et al., "Recurrence and survival in patients undergoing sentinel lymph node biopsy for merkel cell carcinoma: analysis of 153 patients from a single institution," *Annals of Surgical Oncology*, vol. 18, no. 9, pp. 2529–2537, 2011.

[53] W. H. Morrison, L. J. Peters, E. G. Silva, C. D. Wendt, K. Kian Ang, and H. Goepfert, "The essential role of radiation therapy in securing locoregional control of Merkel cell carcinoma," *International Journal of Radiation Oncology Biology Physics*, vol. 19, no. 3, pp. 583–591, 1990.

[54] H. Medina-Franco, M. M. Urist, J. Fiveash, M. J. Heslin, K. I. Bland, and S. W. Beenken, "Multimodality treatment of merkel cell carcinoma: case series and literature review of 1024 cases," *Annals of Surgical Oncology*, vol. 8, no. 3, pp. 204–208, 2001.

[55] M. E. Marks, R. Y. Kim, and M. M. Salter, "Radiotherapy as an adjuvant in management of Merkel cell carcinoma," *Cancer*, vol. 65, no. 1, pp. 60–64, 1990.

[56] P. Savage, D. Constenla, C. Fisher, J. M. Thomas, and M. E. Gore, "The natural history and management of merkel cell carcinoma of the skin: a review of 22 patients treated at the Royal Marsden Hospital," *Clinical Oncology*, vol. 9, no. 3, pp. 164–167, 1997.

[57] R. M. Nathu, W. M. Mendenhall, and J. T. Parsons, "Merkel cell carcinoma of the skin," *Radiation Oncology Investigations*, vol. 6, pp. 233–239, 1998.

[58] P. Mojica, D. Smith, and J. D. I. Ellenhorn, "Adjuvant radiation therapy is associated with improved survival in merkel cell carcinoma of the skin," *Journal of Clinical Oncology*, vol. 25, no. 9, pp. 1043–1047, 2007.

[59] E. R. Kokoska, M. S. Kokoska, B. T. Collins, D. R. Stapleton, and T. P. Wade, "Early aggressive treatment for merkel cell carcinoma improves outcome," *American Journal of Surgery*, vol. 174, no. 6, pp. 688–693, 1997.

[60] M. A. Ashby, D. H. Jones, A. D. Tasker, and A. J. Blackshaw, "Primary cutaneous neuroendocrine (Merkel cell or trabecular carcinoma) tumour of the skin: a radioresponsive tumour," *Clinical Radiology*, vol. 40, no. 1, pp. 85–87, 1989.

[61] E. Pape, N. Rezvoy, N. Penel et al., "Radiotherapy alone for Merkel cell carcinoma: a comparative and retrospective study of 25 patients," *Journal of the American Academy of Dermatology*, vol. 65, no. 5, pp. 983–990, 2011.

[62] L. C. Fang, B. Lemos, J. Douglas, J. Iyer, and P. Nghiem, "Radiation monotherapy as regional treatment for lymph node-positive merkel cell carcinoma," *Cancer*, vol. 116, no. 7, pp. 1783–1790, 2010.

[63] E. Voog, P. Biron, J. P. Martin, and J. Y. Blay, "Chemotherapy for patients with locally advanced or metastatic Merkel cell carcinoma," *Cancer*, vol. 85, pp. 2589–2595, 1999.

[64] D. Sharma, G. Flora, and S. M. Grunberg, "Chemotherapy of metastatic Merkel cell carcinoma: case report and review of the literature," *American Journal of Clinical Oncology*, vol. 14, no. 2, pp. 166–169, 1991.

[65] J. Redmond III, J. Perry, P. Sowray, S. J. Vukelja, and N. Dawson, "Chemotherapy of disseminated merkel-cell carcinoma," *American Journal of Clinical Oncology*, vol. 14, no. 4, pp. 305–307, 1991.

[66] M. Poulsen, D. Rischin, E. Walpole et al., "High-risk Merkel cell carcinoma of the skin treated with synchronous carboplatin/etoposide and radiation: a Trans-Tasman Radiation Oncology Group Study-TROG 96:07," *Journal of Clinical Oncology*, vol. 21, no. 23, pp. 4371–4376, 2003.

[67] M. G. Poulsen, D. Rischin, I. Porter et al., "Does chemotherapy improve survival in high-risk stage I and II Merkel cell carcinoma of the skin?" *International Journal of Radiation Oncology Biology Physics*, vol. 64, no. 1, pp. 114–119, 2006.

[68] J. M. Durand, C. Weiller, M. A. Richard, I. Portal, and M. Mongin, "Treatment of Merkel cell tumor with interferon-alpha-2b," *British Journal of Dermatology*, vol. 124, no. 5, p. 509, 1991.

[69] Y. Hata, K. Matsuka, O. Ito et al., "Two cases of Merkel cell carcinoma cured by intratumor injection of natural human tumor necrosis factor," *Plastic and Reconstructive Surgery*, vol. 99, no. 2, pp. 547–553, 1997.

[70] S. J. Knox and D. S. Kapp, "Hyperthermia and radiation therapy in the treatment of recurrent Merkel cell tumors," *Cancer*, vol. 62, no. 8, pp. 1479–1486, 1988.

[71] A. F. T. Olieman, D. Liénard, A. M. M. Eggermont et al., "Hyperthermic isolated limb perfusion with tumor necrosis factor α, interferon gamma, and melphalan for locally advanced nonmelanoma skin tumors of the extremities: a multicenter study," *Archives of Surgery*, vol. 134, no. 3, pp. 303–307, 1999.

[72] M. Balducci, B. De Bari, S. Manfrida, G. R. D'Agostino, and V. Valentini, "Treatment of Merkel cell carcinoma with radiotherapy and imiquimod (Aldara): a case report," *Tumori*, vol. 96, no. 3, pp. 508–511, 2010.

[73] F. Cirillo, L. Filippini, G. F. Lima, G. Caresana, and P. Alquati, "Tumore a cellule di Merkel: segnalazione di un caso e trattamento con octreotide," *Minerva Chirurgica*, vol. 52, no. 11, pp. 1359–1365, 1997.

[74] M. Fakiha, P. Letertre, J. P. Vuillez, and J. Lebeau, "Remission of Merkel cell tumor after somatostatin analog treatment," *Journal of Cancer Research and Therapeutics*, vol. 6, no. 3, pp. 382–384, 2010.

[75] M. T. Grosse Perdekamp and M. H. Zarrabi, "Treatment of metastatic Merkel cell tumor with Octreotide: report of two cases and review of the literature," in *ASCO Annual Meeting*, vol. 20, abstract 235, 2001.

[76] G. Meier, C. Waldherr, R. Herrmann, H. Maecke, J. Mueller-Brand, and M. Pless, "Successful targeted radiotherapy with 90Y-DOTATOC in a patient with Merkel cell carcinoma: a case report," *Oncology*, vol. 66, no. 2, pp. 160–163, 2004.

[77] N. C. Hodgson, "Merkel cell carcinoma: changing incidence trends," *Journal of Surgical Oncology*, vol. 89, no. 1, pp. 1–4, 2005.

[78] M. Heath, N. Jaimes, B. Lemos et al., "Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features," *Journal of the American Academy of Dermatology*, vol. 58, no. 3, pp. 375–381, 2008.

[79] J. R. Howle, T. M. Hughes, V. Gebski, and M. J. Veness, "Merkel cell carcinoma: an Australian perspective and the importance of addressing the regional lymph nodes in clinically node-negative patients," *Journal of the American Academy of Dermatology*, vol. 67, no. 1, pp. 33–40, 2012.

[80] K. Krasagakis, S. Krüger-Krasagakis, J. Eberle, A. Tsatsakis, A. D. Tosca, and E. N. Stathopoulos, "Co-expression of KIT receptor and its ligand stem cell factor in merkel cell carcinoma," *Dermatology*, vol. 218, no. 1, pp. 37–43, 2008.

[81] L. Kondapalli, K. Soltani, and M. E. Lacouture, "The promise of molecular targeted therapies: protein kinase inhibitors in the treatment of cutaneous malignancies," *Journal of the American Academy of Dermatology*, vol. 53, no. 2, pp. 291–302, 2005.

[82] K. Krasagakis, I. Fragiadaki, M. Metaxari et al., "KIT receptor activation by autocrine and paracrine stem cell factor stimulates growth of merkel cell carcinoma in vitro," *Journal of Cellular Physiology*, vol. 226, no. 4, pp. 1099–1109, 2011.

[83] R. Houben, D. Schrama, and J. C. Becker, "Molecular pathogenesis of Merkel cell carcinoma," *Experimental Dermatology*, vol. 18, no. 3, pp. 193–198, 2009.

[84] V. Foulongne, O. Dereure, N. Kluger, J. P. Molès, B. Guillot, and M. Segondy, "Merkel cell polyomavirus DNA detection in lesional and nonlesional skin from patients with Merkel cell carcinoma or other skin diseases," *British Journal of Dermatology*, vol. 162, no. 1, pp. 59–63, 2010.

[85] I. Cozzolino, R. Zeppa, and P. Zeppa, "Lymph nodal Merkel cell carcinoma: primary tumor or metastasis from unknown primary site?" *Journal of Cutaneous Pathology*, vol. 38, no. 10, pp. 836–837, 2011.

[86] J. H. Ruan and M. Reeves, "A Merkel cell carcinoma treatment algorithm," *Archives of Surgery*, vol. 144, no. 6, pp. 582–585, 2009.

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 408131, 13 pages
doi:10.1155/2012/408131

Review Article

Current and Emerging Therapeutic Options in Adrenocortical Cancer Treatment

Antonio Stigliano,[1,2] Lidia Cerquetti,[1,2] Camilla Sampaoli,[1,2] Barbara Bucci,[1,2] and Vincenzo Toscano[1]

[1] *Endocrinology, Department of Clinical and Molecular Medicine, Sant'Andrea Hospital, Faculty of Medicine and Psychology, "Sapienza" University of Rome, Via di Grottarossa, 1035, 00189 Rome, Italy*
[2] *Research Center, San Pietro Hospital Fatebenefratelli, Via Cassia 600, 00189/Rome, Italy*

Correspondence should be addressed to Antonio Stigliano, antonio.stigliano@uniroma1.it

Received 23 April 2012; Revised 26 June 2012; Accepted 27 June 2012

Academic Editor: Marialuisa Appetecchia

Adrenocortical carcinoma (ACC) is a very rare endocrine tumour, with variable prognosis, depending on tumour stage and time of diagnosis. The overall survival is five years from detection. Radical surgery is considered the therapy of choice in the first stages of ACC. However postoperative disease-free survival at 5 years is only around 30% and recurrence rates are frequent. *o,p'*DDD (*ortho-*, *para'*-, dichloro-, diphenyl-, dichloroethane, or mitotane), an adrenolytic drug with significant toxicity and unpredictable therapeutic response, is used in the treatment of ACC. Unfortunately, treatment for this aggressive cancer is still ineffective. Over the past years, the growing interest in ACC has contributed to the development of therapeutic strategies in order to contrast the neoplastic spread. In this paper we discuss the most promising therapies which can be used in this endocrine neoplasia.

1. Introduction

Adrenocortical carcinoma (ACC) is a rare malignant disease with poor prognosis and an estimated incidence between 1 and 2 per million population annually [1–4]. The age distribution is reported as bimodal with a first peak in childhood and a second higher peak in the fourth and fifth decade [3, 4]. Genetic studies performed on ACC were focused on molecular alterations either at the germline level in rare familial diseases or at somatic level in sporadic tumors. These advances underline the importance of genetic alterations in ACC development and indicate various chromosomal regions (2, 11p15, 11q, 17p13) and genes (IGF-II, p53, β-catenin, etc.), potentially involved in ACC [5–11]. In particular, the monoclonality analysis indicates that tumor progression is the final result of an intrinsic genetic mutation, whereas polyclonality suggests that tumor cells are affected by local or systemic stimuli. Analysis of the pattern of X-chromosome inactivation in heterozygous female tissue shows that ACC consists of monoclonal populations of cells [12]. Molecular alterations lead to inactivation of the tumor suppressor genes and sequential activation of the oncogenes. The insulin growth factor II (IGF-II) system, located at 11p15, is heavily involved in ACC ethiopathogenesis [5]. Loss of heterozygosity (LOH) at chromosome region 11p15, associated with a higher risk of tumor recurrence, is more frequent in ACC than in adrenal adenomas [6]. The development of ACC may be due to an activation of the Wnt signaling pathway caused by germline mutations of the Adenomatous Polyposis Coli (APC) gene [7, 8]. Germline mutations in TP53 are identified in 70% of families with the Li-Fraumeni syndrome. This syndrome shows dominant inheritance and confers susceptibility to ACC and other tumor development [9, 10]. LOH at 17p13 of p53 has been consistently demonstrated in ACC but not in adrenocortical adenomas [6, 11]. The diagnosis of malignancy of adrenocortical tumors relies not only on careful clinical and biological investigations, but above all on improved of radiological imaging such as computerized tomography (CT) or magnetic resonance imaging (MRI) and more recently the use of ^{18}F-FDG PET to distinguish between benign and malignant lesions [13, 14]. Patients could present

signs or symptoms of steroid hormone excess or signs due to the presence of abdominal mass. Hormonal investigations demonstrate cortisol oversecretion in most ACCs. Some of these have a cosecretion of glucocorticoid and androgens. Androgen secreting ACCs in women induce hirsutism and virilization. On the contrary, estrogen-secreting adrenal tumors in males lead to gynecomastia and testicular atrophy [15]. High level of DHEA-S is another marker suggesting ACC, whereas decreased serum DHEA-S concentrations are suggestive of a benign adenoma [15]. ACC producing aldosterone is very rare and present with hypertension and pronounced hypokalemia [16]. Hormonally inactive ACCs usually present with gastrointestinal symptoms or back pain caused by a mass effect of the large tumor.

ACCs show variable prognosis, depending on tumour stage and time of diagnosis with a median overall survival longer than 5 years for patients with stage I and stage II, whereas in stage III and IV, it decreased [17, 18]. Frequency of metastasis associated with ACC varies depending on the study, ranging from 30% to 85% of patients with distant metastasis at the time of presentation [19].

The classification of ACC by the *International Union against Cancer* (UICC) and the *World Health Organization* (WHO) in 2004 is based on the *tumor, lymph node and metastasis* (TNM) criteria as described by Macfarlane [20] and later modified by Sullivan et al. [21]. Thereafter in 2008, the European Network for the Study of Adrenal Tumors (ENS@T) proposed a revision of this staging, in which stage III is defined by tumour infiltration in surrounding tissue or tumour thrombus in vena cava/renal vein or positive lymph nodes, and stage IV is defined only by the presence of distant metastases (Table 1) [22]. The ENSAT-staging system showed higher accuracy in predicting cancer-specific mortality risk than the 2004 UICC-staging system in the ACC prognosis (83% versus 79.5%). This is currently the best criteria staging of ACC [17, 23].

Unfortunately, ACC prognosis is very poor. It depends largely on the stage of tumor: the rate of survival at 5 years is estimated at 60% for patients with stage I cancer, 58% for stage II tumors, 24% for stage III tumors, and 0% for disease stage IV [24].

Patients with stage I and II of disease are amenable to potentially curative surgery. They had a prolonged survival compared to patients with stage III and IV [24]. Macfarlane [20] reported a median survival of 2.9 months for untreated carcinomas, whereas between 16% and 38% of those treated have a median survival at 5 years, depending on the series studied [25]. Patients with stage I and II of disease have a similar prognosis, which is much better than that seen in stage III and IV [22]. Median overall survival rate is 38% and 50%, respectively, for patients undergoing surgery [23]. The median survival in metastatic disease, however, is almost always less than 12 months from time of diagnosis [17]. Factors indicative of good prognosis are early diagnosis of disease (stages I and II) and complete tumor resection (R0).

However, available clinical series shows that diagnosis usually occurs in advanced stages of disease [25–27]. The complete surgical resection of the tumor, the diameter of the lesion, the secretory activity of the tumor, and some molecular markers are factors that can influence the prognosis. Significant reduction in survival is associated with ACC greater than 12 cm in diameter, even if completely resected [28, 29]. Cancer-producing cortisol excess or a mixture of cortisol and androgens, is reported to be worse compared to ACC secreting androgens alone or hormonal precursors [24, 30, 31]. Overexpression of IGF-II and topoisomerase 2A (TOPO 2A) [32] and loss of heterozygosity (LOH) on chromosome 11p15 and 17p13 loci [6] were identified as factors could potentially be used to predict a grade of malignancy. However, the prognostic value of most of these markers has not yet been established by prospective studies [29].

Table 1: Staging systems of adrenal cortical carcinoma (ACC) according to the criteria of the Union Internationale Contre Cancer (UICC) 2004 and the European Network for the Study of Adrenal Tumors (ENSAT) 2008.

Stage	UICC/WHO 2004	ENSAT 2008
I	T1, N0, M0	T1, N0, M0
II	T2, N0, M0	T2, N0, M0
III	T1-2, N1, M0 T3, N0, M0	T1-2, N1, M0 T3-4, N0-1, M0
IV	T1–4, N0-1, M1 T3, N1, M0 T4, N0-1, M0	T1–4, N0-1, M1

T1: tumor ≤5 cm; T2: tumor >5 cm; T3: tumor infiltration into surrounding tissue; T4: tumor invasion into adjacent organs or venous tumor thrombosis; N0: no positive lymph nodes; N1: positive lymph node(s); M0: no distant metastasis; M1: distant metastasis.

Therefore, the clinical picture and the prognosis of patients affected by ACC appear to be rather disappointing. Following recent data acquisition, it is now well-established opinion that ACC requires a multidisciplinary management. The crucial first therapeutic step is radical surgery, also in the incidence of isolated metastatic disease [2, 28, 33]. However, the most widely used medical therapy for patients unsuitable for surgery is treatment with mitotane, an insecticide derivative *o,p'*-DDD (*ortho, para'*, dichloro-, diphenyl-, dichloroethane) either alone or in combination with chemotherapeutic agents [24, 29, 30, 34–40]. Unfortunately, given the high toxic effects resulting from mitotane therapy, the response rates are rather low in ACC [25, 36, 41]. Several cytotoxic pharmacological agents, such as cisplatin, etoposide, doxorubicin/adriamycin, vincristine, 5-fluorouracil, and streptozotocin, have been used individually or in a combination regimen in the treatment of patients with late-stage ACCs [36, 41–43]. To date, the studies that have shown the highest rates of therapeutic response were the so-called "Italian" protocol, consisting of etoposide, doxorubicin, and cisplatin, with concomitant mitotane administration (EDP/M) [29]. A second active regimen is the combination of streptozotocin and mitotane [37]. These two therapeutic regimens were tested in the first randomized controlled trial Phase III started in 2004 for ACC (FIRM-ACT: First International Randomized Trial in Locally Advanced and

Metastatic Adrenocortical Carcinoma Treatment). The trial was started in 2004 in order to establish the gold standard in the treatment of locally advanced adrenal cortical carcinoma not amenable to surgical resection (resp., stages III and IV) and in patients with a poor life expectancy. The growing interest in this neoplasia has encouraged molecular biology and pharmacology studies. In recent years research efforts have focused on the identification of molecular biomarkers of this neoplasia. The purpose of these studies is to improve the diagnostic and therapeutic options in ACC. The aim of this paper is to describe the current treatment options in patients with ACC and establish innovative strategies for this cancer.

2. Surgery

Complete surgical removal of ACC represents the current treatment of choice for this tumor. The likelihood of achieving a healing is by radical surgery, especially in the lower stages of cancer (I and II). A disease-free resection margin (R0) is also an important predictor of long-term survival. However, locoregional recurrence or the appearance of distant metastases during the subsequent followup is common (85%) even after complete resection of the tumor [17, 22, 24, 33, 35, 44–46]. The probability of failure increases in advanced stage of disease. This happens when the lesion is greater than 12 centimeters in maximum diameter, with a high mitotic rate and intralesional hemorrhage [19, 47]. Therefore surgery is demanding and must be performed by a highly experienced surgical team using a laparotomic approach. The utmost care should be taken in order to reach a negative resection margin (R0) and to avoid tumor spillage in the abdominal cavity during its removal since this is unfavorable prognostic factor. The role of laparoscopy in the removal of an ACC has not yet been defined. Its influence on the prognosis of the disease is still unspecified, and there are no randomized trials which have compared the efficacy of laparotomic adrenalectomy versus laparoscopic adrenalectomy [29]. Recently, the role of laparoscopy in the surgical treatment of the ACC has been much debated. Some studies have shown that there was no difference in the oncologic outcome between the laparoscopic and the laparotomic approach in patients affected by ACC [48, 49]. Retrospective data from the German ACC Registry shows that the removal of regional lymph nodes significantly reduced tumor recurrence and disease-related death in patients with localized ACC [50].

Currently the laparotomic approach is considered more reliable in the instance of preoperative diagnosis of ACC. Due to the frequent invasion or the close adhesion of the ACC to adjacent organs, surgery often requires excision en bloc of the ipsilateral kidney, spleen, and a partial pancreatectomy in the case for left adrenal cancer and a partial hepatectomy for right adrenal cancer. Furthermore, removal of the abdominal lymph nodes is performed to remove ACC [29].

Metastatic disease debulking surgery, which removes as much of the tumor as possible, helps to reduce the blockage caused by a mass, usually large as well as the hormonal excess produced by the tumor. However, there is no significant effect of this therapy, since the median survival of patients with incomplete resection of the primary tumor or inoperable metastatic disease not removable by surgery appears to be less than 12 months [24, 33]. Surgical resection of recurrent disease is accepted as a treatment option of choice, as it is associated in a different series with an increase in median overall survival. However, a complete cure was rarely achieved. The surgical approach includes the removal of locoregional tumor recurrence and excision of isolated metastatic foci in the liver and lung [28].

3. Mitotane Treatment

Mitotane (1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-ethane or *o*',*p*'-DDD), is an isomer of the insecticide DDT. The first description about its use was made in 1948 when it was shown to produce adrenal atrophy in dogs [51]. This drug acts by inhibiting 11β-hydroxylation and P450 side chain cleavage in the mitochondria of steroidogenic cells, therefore blocking cortisol synthesis, decreasing both plasma and urine steroid levels [52]. Since then, mitotane has been used in the treatment either in the first or in advanced stages of ACC [34–40]. Mitotane exerts a specific cytotoxic effect on adrenal cortex cells, resulting in focal degeneration of the fasciculata and reticularis areas while the effects on glomerular are relatively scarce [53]. The effect of this adrenolytic drug is due to its metabolic activation via a hydroxylation reaction. Dehydrochlorination occurs with the production of an acylchloride. This reactive compound may bind covalently to intracellular macromolecules, and in particular to mitochondrial proteins, exerting their biological activity, or become the main inactive metabolite of mitotane (*o,p*'-DDA). Drug activation occurs primarily within the mitochondria in the adrenal cortex cells, while a small portion is subjected to the action of hepatic microsomal enzymes. Metabolic activation of mitotane in the liver induces the formation of two metabolites 1,1-(*o,p*'-dichlorodiphenyl)-2,2-dichloroethane (*o,p*'-DDA) and 1,1-(*o,p*'-dichlorodiphenyl) acetic acid (*o,p*'-DDE), deriving from β- or α-hydroxylation of *o,p*'-DDD respectively [54]. It has been demonstrated that *o,p*'-DDA represents the active metabolite of mitotane because the β-hydroxylation of *o,p*'-DDD results in an adrenolytic effect while the α-hydroxylation of *o,p*'-DDD results in a deactivation. On this basis it was hypothesized that the measurement of o,p'-DDA, in patients affected by ACC, can predict the response to mitotane [55]. The effect of increased hepatic metabolic activity is reflected in the reduction of the main urinary catabolite of cortisol and the increasing production of water-soluble polar metabolites of cortisol [39]. The effect of mitotane on the pharmacokinetics of other drugs is not fully understood. Recently, van Erp et al. have observed that mitotane was capable of inducing the activity of hepatic CYP3A4 potentially interfering with the therapeutic efficacy of other molecules including antineoplastic drugs [56], since many drugs are metabolized by CYP3A4. This aspect will

Table 2: Treatment protocols employed in the FIRM-ACT study.

Berruti and *coll.* protocol (EDP/M)	Every 28 days		
	(i) Day 1	40 mg/m^2	Doxorubicin
	(ii) Day 2	100 mg/m^2	Etoposide
	(iii) Day 3, 4	100 mg/m^2	Etoposide + 40 mg/m^2 cisplatin
	(iv) Daily		Mitotane with a blood level 14–20 mg/L
Khan and *e coll.* protocol (Sz/M)	Every 21 days		
	(i) Day 1–5	1 g	Streptozotocin
	(ii) Subsequently	2 g	Streptozotocin
	(iii) Daily		Mitotane with a blood level 14–20 mg/L

need to be considered in the treatment of patients with ACC and in the future design of clinical trials involving the use of chemotherapy in combination with mitotane.

Furthermore, mitotane inhibits the production of testicular androgens, acting as an antagonist on the progesterone and androgen receptors and as an agonist of the estrogen receptor [39]. Beside its adrenolytic effects, mitotane inhibits MDR-1/P-glycoprotein, a multidrug resistance protein, thus enhancing the effect of different chemotherapy drugs [30, 57, 58]. The drug is administered orally at a dose generally greater than 4 g/day, taking the therapeutic window between 14 and 20 μg/dL into account [35, 59, 60]. The plasmatic half-life is approximately 2-3 hours, gradually increasing from the beginning of therapy in relation to drug accumulation in adipose tissue [61]. The daily amount of drug absorbed is excreted in the urine as inactive metabolites, mainly in the form of *o,p*'-DDA, a lower percentage, however, is excreted in the feces. As mitotane accumulates in adipose tissue, the plasma elimination half-life is extremely long (18–159 days) [62].

In patients treated with mitotane, it is essential to establish replacement therapy with glucocorticoids (preferably hydrocortisone 50 mg/day) for the effect on the suppression of cortisol synthesis and the increase of its peripheral catabolism [39]. The major limitation in the use of this drug is linked to the appearance of significant side effects affecting the gastrointestinal tract with the onset of anorexia, nausea, vomiting, and diarrhea and CNS with lethargy, drowsiness, depression, vertigo and ataxia [17, 63]. In light of these findings, ACC-affected patients taking mitotane must be periodically subjected to the plasmatic assay of drug concentration. In addition, it is appropriate to have a close followup with clinical and diagnostic monitoring of liver function.

Therefore mitotane is a therapeutic indication in the treatment of inoperable ACC and in preparing for adrenalectomy. However, the high rate of recurrence of ACC justifies its use in adjuvant therapy following surgical resection [40, 64–66], in accordance with the concept of adjuvant therapy, providing the drug administration immediately after surgery [67]. The different considerations on the effectiveness of adjuvant therapy can be justified by the hypotheses that patients affected by ACC vary in their ability to metabolic drug transformation [37]. Currently, to evaluate the effectiveness of adjuvant therapy with mitotane, a randomized prospective study, called ADIUVO (http://www.adiuvo-trial.org/), is ongoing. This controlled trial provides the enrollment of patients randomized to mitotane treatment after ACC complete resection or followup without drug treatment.

4. Chemotherapy

Chemotherapy drugs alone or in combination with mitotane are employed due to the high aggressiveness and poor prognosis of ACC, especially when not amenable to surgical resection or when present in metastatic stage. This combination exploits the ability of mitotane to overcome the drug-resistance induced by P-glycoprotein, which is widely expressed in ACC. The chemotherapeutic agents used individually or in a combination regimen in the treatment of patients with ACC in advanced stages include cisplatin, etoposide, doxorubicin/adriamycin, vincristine, 5-fluorouracil, and streptozotocin [29, 30, 36–39]. Although the results are variable, there is some evidence that cisplatin, alone or in combination with etoposide, exerts a favorable therapeutic effect against ACC at an advanced stage. Bukowski et al. [36] evaluated the effectiveness of the combination of cisplatin with mitotane, achieving a complete response to therapy of 30%. Bonacci et al. [42], using a regimen that included the combination of cisplatin, etoposide, and mitotane, achieved an overall response of 33% while Burgess et al. [43], using a combination of cisplatin and etoposide without mitotane, obtained a response rate of 46%. Although Williamson et al. [41] administered the same protocol (cisplatin plus etoposide) without mitotane to patients with unresectable or metastatic ACC, they achieved a complete response of less than 11%.

To date, the studies that have shown the highest rates of therapeutic response were those performed by Khan et al. [37], and Berruti et al. [30] administered the combination of streptozotocin and mitotane and the combination of etoposide, doxorubicin and cisplatin (EDP) in repeated cycles, in association with mitotane, respectively (Table 2). In Berruti's study, which was based on a large prospective multicenter phase II trial, the EDP combination with mitotane was administered to 72 patients affected by ACC not amenable to surgery: 5 patients achieved a complete response to therapy and 30 a partial response, giving an

overall response rate of 48.6%. Khan's study, instead, evaluated the effectiveness of the combination of streptozotocin and mitotane in 22 patients with ACC, giving an overall response of 36.4% (1 patient with complete response and 7 with partial response). In light of these results, the International Consensus Conference on Adrenal Cancer of Ann Arbor recommended the use of these protocols as first-line regimens against metastatic ACC in 2003 [29]. The First International Randomized Trial in Locally Advanced and Metastatic Adrenocortical Carcinoma Treatment (FIRM-ACT) (http://www.firm-act.org/) was initiated in April of 2004, in order to establish the gold standard in therapy in advanced ACC not amenable to radical surgical resection (resp., stages III and IV), in patients with a life expectancy greater than 3 months. The FIRM-ACT was therefore initiated in order to make a comparison with Berruti et al. and Khan et al., chemotherapeutic protocols. The study provided the randomized administration of a combination EDP-mitotane every 4 weeks and streptozotocin-mitotane every 3 weeks in patients with advanced ACC. Patients with disease progression were treated with the alternative combination therapy. The primary objective of the trial was to compare the survival rates. Secondary objectives consisted of evaluating the quality of life, time to disease progression, response rate to therapy, duration of response, and disease-free survival in the two-regimen protocols. The FIRM-ACT study is now completed and the results recently published have shown that patients treated with EDP-mitotane combination had a significantly higher response rate than those treated with streptozotocin-mitotane. Unfortunately, no differences were observed in overall survival confirming the poor prognosis in patients affected by advanced ACC. No differences were found in the quality of life and adverse events in patients receiving the two therapeutic regimens [68].

5. Radiotherapy

The efficacy of radiotherapy in ACC has been largely debated. This neoplasm has indeed considered radio resistant for a long time, and many authors have noticed poor results in patients subjected to radiotherapy after surgical removal of the adrenal mass [67, 69]. In other studies, however, a response rate of approximately 42% of cases has been described [17]; moreover, it has also been demonstrated that radiotherapy reduced the risk of local failure by 4.7 times in a clinical study involving 58 patients [70].

Radiotherapy has also been used as palliative care treatment in ACC cases associated with bone metastasis [4, 29, 71]. Despite a certain difficulty in monitoring some parameters, it seems that ionizing radiation treatment was able to reduce metastatic size and symptoms in 57% of cases [27].

Radiation treatment as an adjuvant therapeutic option has been described to significantly reduce recurrence rates, thus suggesting a significant therapeutic potential. In particular, Fassnacht et al. reported that the probability of recurrence risk reduction was significantly higher in a group of patients treated with 45–55 Gy for five weeks after surgery, than in patients who did not undergo radiotherapy (79% versus 12%) [72].

Existing data regarding radiotherapy efficacy in ACC indicate that this treatment should be taken into consideration only after having carefully evaluated the clinical picture of every patient [27, 29]. In particular, radiotherapy is recommended when microscopic tumor residues are detectable after surgery (R1), whereas those patients who exhibit macroscopically visible residual tumours (R2) are advised to undergo a second operation. Radiotherapy is suitable also in cases where residual tumour dimensions are not known (RX) and when recurrence risk is high. Finally, patients with advanced disease and those with a stage III tumour with local lymph node invasion and no distant metastasis may benefit from adjuvant radiotherapy [27, 73].

Currently, no general guidelines exist for radiotherapy use in patients who have undergone complete tumour removal (R0), although this treatment is not usually not recommended when tumour dimensions are $\leq$8 cm. Instead, it can be considered for tumours with greater dimensions, blood vessels invasion (V1), and a Ki-67 index $\geq$ 20%, which are associated with a high recurrence risk [27, 65, 74].

Based on clinical observations, treatment planning should be individualized on the basis of patient characteristics however radiotherapy as adjuvant therapy should start as soon as possible, within 3 months from surgery [27, 65]. The optimum radiation protocol has not yet been defined in fact the highest total dose reported was 60 Gy, administered in daily fractions of 1.5–1.8 Gy over 5–7 weeks [75] however many studies recommended lower doses, ranging from 20 Gy to 55 Gy [27]. In general, radiotherapy treatment on tumors should be carried out for 5 to 6 weeks at doses of 1.8–2.0 Gy *per* fraction, with total amounts ranging from 40 Gy to 50/60 Gy [27, 75].

Combined treatment based on the association between radiotherapy and cytotoxic drugs, such as mitotane, is currently under investigation. Some *in vitro* studies, in fact, reported an inhibitory effect of mitotane *plus* ionizing radiations on ACC cell lines [76, 77]. A recent study by Salboch et al. [70] evaluated the effect of mitotane administration in ACC patients and found no differences in response rates in the surgery group or surgery and radiotherapy group after mitotane treatment (25% versus 20%, resp.) while other authors argue that radiotherapy efficacy might be ameliorated by concomitant administration of mitotane or other chemotherapeutic agents [78, 79]. According to some authors, mitotane treatment in association with radiotherapy is recommended for patients who underwent R1 and RX resection. However, mitotane doses should be <3 g/d, in order to prevent sever hepatic toxicity; moreover, levels of GOT, GPT, and bilirubin should be monitored every 2/3 weeks [74].

6. Targeted Therapies

Recent advances in the understanding of genetic alterations involved in ACC onset and progression led to the identification of several potential molecular targets for selective

therapy of ACC. Until recently, the main genetic modifications discovered in ACC cases involved oncosuppressor genes, such as *TP53*, *CDKN1C*, *MEN1,* and *CDKN2A*, and oncogenes such as *IGF2*, *RAS* and *CTNNB1*. Currently many other genes are under investigation in order to understand their usefulness for the development of new therapeutic strategies [67, 69, 80, 81].

Insulin-Like Growth Factor-2 Pathway. Overexpression of insulin-like growth factor-2 (IGF-2) represents the most important molecular event identified in ACCs, which occurs in more than 90% of cases [6, 82]. IGF-2 hypersecretion translates in an unchecked cell proliferation, due to the activation of PI3K/Akt/mTOR pathway through IGF-1R [83, 84]. Preclinical studies on cell and xenograft models demonstrated that NVP-AEW541, a small molecule inhibitor, and IMC-A12, a fully human monoclonal antibody, both targeting IGF-1R, were able to inhibit IGF-2 downstream pathway and to reduce cell proliferation. Moreover, the association of these molecules with mitotane strongly inhibited tumor growth in a synergistic way [71, 83].

Recently, two phase I studies demonstrated the efficacy of figitumumab and OSI-906 in inducing a partial tumor response in 57% and 33% of patients, respectively [71, 85, 86]. Figitumumab is an anti-IGF-1R monoclonal antibody, and OSI-906 is a small molecule tyrosine kinase inhibitor directed against IGF-1R. Moreover, an international phase III trial is currently in progress, in order to evaluate the feasibility and efficacy of OSI-906 for treatment of patients with ACC should end in 2013 [87].

Recent studies demonstrated the association between *IGF2* overexpression, mTOR hyper-activation and reduced expression of miR-99a and miR-100, whose function thus appeared to be the inhibition of these factors [88]. The role of mTOR in malignant tumors onset has been established by several studies, thus highlighting its important as a potential therapeutic target for ACC [71, 89]. The most important inhibitors of mTOR are rapamycin (sirolimus) and its derivatives everolimus (RAD001) and temsirolimus (CCI-779) [89]. A recent study demonstrated that pharmacologic inhibition of mTOR signaling by everolimus greatly reduced adrenocortical tumor cell growth both *in vitro* and *in vivo*, also confirming the importance of microRNA regulation of IGF-2/mTOR signalling cascade [88]. Moreover, a phase I clinical trial evaluating the effect of temsirolimus in combination with the anti-IGF-1R recombinant monoclonal antibody cixutumumab in advanced malignancies demonstrated a tumor reduction in 4 of 10 patients with ACC [71, 90].

Angiogenesis. Considering the great importance of angiogenesis and neovascularization for tumor proliferation and migration, new therapeutic strategies have been conceived. These approaches either prevent new blood vessel formation or disrupt existing tumor vasculature. In ACC, vascular endothelial growth factor (VEGF) is over-expressed and its levels appear to decrease after tumor removal, thus confirming its role in ACC growth and its importance as an efficient therapeutic target [57, 69, 71].

Despite the expanding interest in this field, clinical trials employing antiangiogenic drugs are quite inefficient; moreover, the results obtained to date are discouraging. A recent study involving 10 patients with advanced ACC who were treated with the monoclonal VEGF antibody bevacizumab (Avastin) in combination with the oral prodrug capecitabine as salvage therapy reported no objective response or stable disease. Moreover, this therapeutic regimen caused severe side effects which required treatment suspension in two cases [91]. Instead, a single case report described instead a partial response in a 40-year-old patient with advanced chemoresistant ACC taking 200 mg/d thalidomide [92].

More encouraging results have been obtained in clinical studies employing small-molecule tyrosine kinase inhibitors targeting VEGFR, such as sorafenib and sunitinib [71]. Sunitinib has been found to induce a strong adrenal toxicity in animal models, and a partial response to this treatment has been reported in a single patient with metastatic ACC, after failure of mitotane-based chemotherapy creating the necessary conditions for the beginning of a phase II trial with sunitinib as monotherapy for refractory ACCs [71, 93, 94]. The first demonstration of sorafenib efficacy in ACC treatment derives from a phase I trial employing sorafenib plus the farnesyltransferase inhibitor tipifarnib. This trial reported stable disease in two patients with advanced ACC [95]. Moreover, a single-case report described a sustained regression of metastatic lesions associated with a stage IV ACC after sorafenib administration [96]. Recently, a phase II study investigating the effects of sorafenib in combination with metronomic paclitaxel was conducted, in order to evaluate both the efficacy of sorafenib treatment and the potential of metronomic therapy in inhibiting tumor growth. Despite *in vitro* data suggesting that sorafenib was able to reduce viability of H295R cells, treatment of patients with advanced ACC was ineffective; moreover, paclitaxel administration did not increase the effect of sorafenib *in vitro* [71, 93, 97].

The antiangiogenic effect of rapamycin has been demonstrated *in vitro*, although the clinical data currently available is insufficient [57, 71]. Nevertheless, in a recent study, Gangadhar et al. observed a partial response in a patient with ACC treated with sirolimus and sunitinib in combination [98].

Finally the significance of heparanase-1 in ACC angiogenesis has recently been highlighted, suggesting that this protein could represent selective target treatment of ACC [99].

Tyrosine Kinase Inhibitors (TKIs). The identification of molecular targets for ACC is often achieved through microarray and transcriptome analyses, which allows the identification of some signalling pathways that are disrupted in this neoplasia [57, 84, 100]. Many of these pathways are hyperactivated by the overexpression of growth factors, such as IGF-II, EGF, and FGF, which is a frequent event

occurring in ACC. Thus novel therapeutic strategies are thus based on the inhibition of protein kinases involved in signal transduction, especially receptor tyrosine kinases. In addition to IGF-1R and VEGFR inhibitors, mentioned above, there has been a substantial investment in the development of inhibitors of other tyrosine kinase receptors, such as EGFR and PDGF (platelet-derived growth factor) receptor [57, 71].

In vitro studies demonstrated that suramin, an anti-parasitic drug known to inhibit the binding of growth factors (e.g., EGF, PDGF, TGF-β, FGF-β) to their receptors, was able to antagonize the ability of these factors to stimulate tumor cells' proliferation and reduced cortisol secretion [101]. Further studies, however, showed that the effect of suramin was partial and that the side effects were extremely severe. Therefore this drug is not currently used for ACC treatment [102].

Recently, a clinical study conducted on ten patients with advanced ACC treated with erlotinib, an EGFR inhibitor, in combination with gemcitabine reported very limited to no efficacy of this therapeutic regimen [103]. Similarly, Samnotra et al. observed a 0% response rate in a cohort of 19 patients with pathologically confirmed unresectable ACC treated with gefitinib (Iressa) as a second-line monotherapy [104]. The reasons of this therapeutic failure might be that EGFR is over-expressed in approximately 76% of ACC; however no mutations were found in the *EGFR* gene. In addition, EGFR expression does not represent a useful prognostic factor, questioning its real therapeutic value [57, 93, 105]. Similar observations were made regarding PDGFR, whose expression does not seem to be altered in ACC cases [57]. Consistently with that, a phase II study involving 4 patients with advanced ACC treated with oral imatinib mesylate, a PDGFR inhibitor, reported disease progression in three cases. In one case the side effects were so severe that treatment was suspended [106].

MDR/P-Glycoprotein. It has long been known that ACC is a chemoresistant tumor, and this fact seems to be related to the overexpression of the multidrug resistance protein MDR-1 (P-glycoprotein, Pgp), which is an ATP-dependent drug efflux pump [57, 58, 93]. Moreover, some MDR-1-independent mechanisms seem to be involved in ACC drug resistance, and excision repair cross-complementing group 1 (ERCC1) is also shown to play a role in the resistance to platinum-based treatment [57, 107].

So far, several compounds which can interfere with MDR-1 function have been so far identified. The MDR-1 inhibitor verapamil has been shown to improve chemosensitivity in leukemic and ovarian cancer, although its effectiveness in ACC has not been proven yet [70, 108]. Second- and- third generation MDR-1 modulators, including D-verapamil, valspodar (PSC833), an analogue of cyclosporine D, and tariquidar (XR9576), a P-glycoprotein drug efflux pump inhibitor, have been developed in order to strengthen the cotreatment with cytotoxic agents. However, the effect of these compounds is rather unsatisfying [57, 71]. Moreover, results from a preclinical study employing primary human ACC cells treated with doxorubicin and vincristine in association with Pgp antagonists verapamil, cyclosporine A, and its analogue SDZ PSC833 indicated that the resistance to chemotherapy in ACC is mediated by mechanisms other than Pgp [109].

Despite the results of clinical trials obtained on a small numbers of patients with metastatic disease seem discordant from the effects obtained *in vitro*, further studies are necessary to prove the efficacy of inhibitors of Pgp.

The chemosensitizing effect of mitotane has also been investigated. *In vitro* studies demonstrated that clinically achievable concentrations of *o,p*'-DDD could increase drug accumulation, due to the inhibition of Pgp-mediated drug efflux [58]. However, clinical trials employing doxorubicin, vincristine, and etoposide in combination with mitotane failed to demonstrate the effectiveness of this treatment [110, 111].

PPAR-γ Antagonists. The nuclear receptor PPAR-γ is highly expressed in the normal and neoplastic adrenal cortex and is implicated in the regulation of the IGF-2/IGF-1R signalling pathway, by inhibiting Akt activation [112, 113]. The use of PPAR-γ antagonists is complicated by the observation that thiazolidinediones (TZDs) can induce severe adverse effects, particularly on the cardiovascular system [113]; nevertheless these compounds were shown to be able to inhibit cell proliferation in ACC cell lines and xenograft models [112–116].

The molecular mechanism underlying the anti-proliferative and prodifferentiating effects of rosiglitazone, a member of the TZD class, in ACC has not been completely elucidated. However it has been demonstrated that both PPAR-γ-dependent and -independent pathways were activated by this drug, leading to growth arrest, cell death and decreased *VEGF* expression, which could be involved in the reduction of tumor infiltration and neovascularization [112, 114, 116].

Wnt/β-catenin pathway. The involvement of Wnt/β-catenin pathway in adrenocortical tumorigenesis is supported by the observation that in many adrenocortical tumors, both benign and malign, an accumulation of β-catenin protein was noticed [8, 69, 117]. Constitutive activation of this protein seems to be a main event leading to adrenocortical carcinogenesis; moreover nuclear localization of β-catenin represents a predictive factor for a worse prognosis in ACC cases [8, 117, 118].

Preclinical *in vitro* studies evaluated the effect of PKF115-584, a small molecule inhibitor of the T-cell factor (Tcf)/β-catenin complex, on β-catenin-dependent transcription and proliferation in H295R ACC cells, harbouring mutations in *CTNNB1* gene. Treatment with PKF115-584 inhibited cell proliferation and induced apoptosis in a dose-dependent way in H295R cells, but not in HeLa cells, thus indicating that targeting the Wnt/β-catenin pathway might be useful in the treatment of adrenocortical tumors [119]. CWP232291 is a compound which is able to promote β-catenin degradation. It also exhibited potent growth inhibitory activity

in several multiple myeloma cell lines and a phase I clinical study of CWP232291 in patients with relapsed or refractory acute myeloid leukemia is currently ongoing (http://www.clinicaltrials.gov/, trial ID NCT01398462) [120]. However, no clinical data is available at present concerning ACC; moreover, despite the great potential of Wnt/β-catenin inhibition for ACC treatment, the involvement of these factors in many fundamental cellular processes should always be considered during treatment planning, in order to prevent severe toxicity and adverse effects [120, 121].

Steroidogenic Factor-1. Steroidogenic factor-1 (SF-1) is a nuclear receptor involved in adrenal and gonadal development, steroidogenesis, and reproductive axis regulation. *SF-1* gene is frequently amplified and over-expressed in pediatric ACCs, whereas in adult carcinomas chromosomal abnormalities in chromosome 9 have been noticed. Moreover, patients showing higher levels of SF-1 expression seem to have a worse prognosis compared with those who express lower levels of this factor [122, 123]. It has previously been found that increased SF-1 dosage stimulates proliferation, decreases apoptosis of human adrenocortical cells, and induces ACTs in transgenic mice [124]. *In vitro* studies performed on H295R cells demonstrated that SF-1 gene silencing strongly affected TGF-β and Wnt/β-catenin signalling, suggesting the existence of a crosstalk between these pathways. Moreover, SF-1 knockdown induced a significant reduction of proliferation rate in treated cells compared to control cells and a reduction of cells in the S-phase [124, 125].

Recently, synthetic SF-1 inverse agonists have been identified and tested in ACC cell lines. Two members of the alkyloxyphenol class, AC-45594 and OOP, were shown to inhibit proliferation of H295R and SW-13 cells through a mechanism which did not seem to be selective for SF-1. On the contrary, members of the IsoQ class, SID7969543 (IsoQ A), and the number 31 and 32 compounds, selectively inhibited cell proliferation in conditions of increased SF-1 expression, strongly suggesting that the IsoQ drugs selectively target the activity of SF-1-related genes, thus representing a potential new class of compounds to be used in ACC treatment [126].

Gene Therapy and Immunotherapy. The use of gene therapy is an evolving approach for cancer treatment; nevertheless, poor results have so far been obtained in ACC. The aim of gene therapy would be to re-activate oncosuppressor genes and/or to inhibit oncogenes, whose expression is deregulated during tumor progression. The systemic administration of antisense oligonucleotides would represent an interesting approach for ACC therapy; moreover, an *in vitro* study demonstrated that a suicide vector, in which the herpes simplex virus thymidine kinase (HSV-TK) gene was driven by the CYP11B1 promoter with a P450scc enhancer element, was able to increase chemosensitivity in Y1 mouse adrenocortical cancer cells [57, 127].

Immunotherapy is a therapeutic approach based on the stimulation of the immune response against cancer cells. Recently, immunotherapy using dendritic cells has been proven to be safe and effective in inducing antitumor immune responses leading to tumor regression [128]. In a study conducted on two patients with metastasized hypersecretory ACC, the vaccination with autologous dendritic cells was able to induce antigen-specific Th1 immunity. However, no clinical advantage was observed [93, 129]. The main limitation of this therapy is the difficulty in identifying specific tumoral antigens; however some interesting targets for a specific immunotherapeutic approach in ACC might be represented by steroidogenic factor 1, surviving and steroidogenic acute regulatory protein (StAR) [94, 130].

Estrogen Pathways. Estrogens are produced by the enzyme aromatase using androgens as substrate, and Barzon et al. have shown that ACC are characterized by aromatase overexpression [131]. Then, it is possible that in ACC patients despite normal circulating estrogen levels a higher local estrogen production can occur. The classical mechanisms of estrogen action are mainly mediated by two members of the nuclear receptor superfamily, the estrogen receptor (ER) α and β [132]. In ACCs also has been demonstrated a differential expression of ERs [131, 133]. Another study demonstrated that hydroxytamoxifen controls proliferation of H295R cells by decreasing ERα and upregulating ERβ expression levels causing an increase in the expression of the pro-apoptotic factor FasL [134]. Very recent studies using H295R cells, demonstrated a central role for ERα in both E2- and IGF-II-dependent cell proliferation, suggesting that targeting this receptor could be effective in controlling ACC growth. The authors have demonstrated that IGFII is capable of activating ERα through phosphorylation in an estrogen-independent manner, suggesting that in ACC ERα can be activated also by IGF-II. In addition, exploiting the ability of H295R cells to generate xenografts in athymic nude mice, this preliminary data demonstrated an hypothetical role of the selective estrogen receptor modulator (SERM) tamoxifen to control ACC growth *in vivo* [135].

7. Summary and Conclusion

Despite advances in the biology of ACC, prognosis remains very poor. Although exciting results have been obtained in lab regarding new therapies, their application in clinical practice has been somewhat disappointing. The organization in the international study groups is certainly an important goal for research in the context of a rare malignancy. So far this has led to the conclusion of the FIRM-ACT and ADIUVO equipment. An early diagnosis is fundamental for the prognosis and for an improvement of the survival rate of patients affected by ACC. Recent contributions by transcriptomics and proteomics have identified specific biomarkers able to discriminate between more aggressive and less aggressive forms. However, the aggressiveness of this tumor still requires efforts in the identification of prognostic and therapeutics markers to use not only in clinical settings but also for the design of new specific drugs. Considering the biological heterogeneity of this malignancy, new therapeutic

strategies, such as a target therapy, could be the future in ACC treatment.

References

[1] National Cancer Institute, "Third national cancer survey: incidental data," DHEW publication no. (NIH) 75–787. NCI Monogr 41, 1975.

[2] A. P. B. Dackiw, J. E. Lee, R. F. Gagel, and D. B. Evans, "Adrenal cortical carcinoma," *World Journal of Surgery*, vol. 25, no. 7, pp. 914–926, 2001.

[3] M. Bielinska, H. Parviainen, S. Kiiveri, M. Heikinheimo, and D. B. Wilson, "Review paper: origin and molecular pathology of adrenocortical neoplasms," *Veterinary Pathology*, vol. 46, no. 2, pp. 194–210, 2009.

[4] A. Patalano, V. Brancato, and F. Mantero, "Adrenocortical cancer treatment," *Hormone Research*, vol. 71, no. 1, pp. 99–104, 2009.

[5] C. Gicquel, X. Bertagna, and Y. Le Bouc, "Recent advances in the pathogenesis of adrenocortical tumours," *European Journal of Endocrinology*, vol. 133, no. 2, pp. 133–144, 1995.

[6] C. Gicquel, X. Bertagna, V. Gaston et al., "Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors," *Cancer Research*, vol. 61, no. 18, pp. 6762–6767, 2001.

[7] H. Bläker, C. Sutter, M. Kadmon et al., "Analysis of somatic APC mutations in rare extracolonic tumors of patients with familial adenomatous polyposis coli," *Genes Chromosomes and Cancer*, vol. 41, no. 2, pp. 93–98, 2004.

[8] F. Tissier, C. Cavard, L. Groussin et al., "Mutations of β-catenin in adrenocortical tumors: activation of the Wnt signaling pathway is a frequent event in both benign and malignant adrenocortical tumors," *Cancer Research*, vol. 65, no. 17, pp. 7622–7627, 2005.

[9] C. Caron de Fromentel and T. Soussi, "TP53 tumor suppressor gene: a model for investigating human mutagenesis," *Genes Chromosomes and Cancer*, vol. 4, no. 1, pp. 1–15, 1992.

[10] M. Hisada, J. E. Garber, C. Y. Fung, J. F. Fraumeni, and F. P. Li, "Multiple primary cancers in families with Li-Fraumeni syndrome," *Journal of the National Cancer Institute*, vol. 90, no. 8, pp. 606–611, 1998.

[11] T. Yano, M. Linehan, P. Anglard et al., "Genetic changes in human adrenocortical carcinomas," *Journal of the National Cancer Institute*, vol. 81, no. 7, pp. 518–523, 1989.

[12] F. Beuschlein, M. Reincke, M. Karl et al., "Clonal composition of human adrenocortical neoplasms," *Cancer Research*, vol. 54, no. 18, pp. 4927–4932, 1994.

[13] M. A. Blake, J. M. A. Slattery, M. K. Kalra et al., "Adrenal lesions: characterization with fused PET/CT image in patients with proved or suspected malignancy—initial experience," *Radiology*, vol. 238, no. 3, pp. 970–977, 2006.

[14] I. Ilias, A. Sahdev, R. H. Reznek, A. B. Grossman, and K. Pacak, "The optimal imaging of adrenal tumours: a comparison of different methods," *Endocrine-Related Cancer*, vol. 14, no. 3, pp. 587–599, 2007.

[15] M. Fassnacht, W. Kenn, and B. Allolio, "Adrenal tumors: how to establish malignancy?" *Journal of Endocrinological Investigation*, vol. 27, no. 4, pp. 387–399, 2004.

[16] T. M. Seccia, A. Fassina, G. G. Nussdorfer, A. C. Pessina, and G. P. Rossi, "Aldosterone-producing adrenocortical carcinoma: an unusual cause of Conn's syndrome with an ominous clinical course," *Endocrine-Related Cancer*, vol. 12, no. 1, pp. 149–159, 2005.

[17] M. Fassnacht and B. Allolio, "Clinical management of adrenocortical carcinoma," *Best Practice and Research*, vol. 23, no. 2, pp. 273–289, 2009.

[18] G. Assié, G. Antoni, F. Tissier et al., "Prognostic parameters of metastatic adrenocortical carcinoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 1, pp. 148–154, 2007.

[19] A. Stojadinovic, R. A. Ghossein, A. Hoos et al., "Adrenocortical carcinoma: clinical, morphologic, and molecular characterization," *Journal of Clinical Oncology*, vol. 20, no. 4, pp. 941–950, 2002.

[20] D. A. Macfarlane, "Cancer of the adrenal cortex. The natural history, prognosis and treatment in a study of fifty-five cases," *Annals of the Royal College of Surgeons of England*, vol. 23, no. 3, pp. 155–186, 1958.

[21] M. Sullivan, M. Boileau, and C. V. Hodges, "Adrenal cortical carcinoma," *Journal of Urology*, vol. 120, no. 6, pp. 660–665, 1978.

[22] M. Fassnacht, S. Johanssen, M. Quinkler et al., "Limited prognostic value of the 2004 international union against cancer staging classification for adrenocortical carcinoma," *Cancer*, vol. 115, no. 2, pp. 243–250, 2009.

[23] G. Lughezzani, M. Sun, P. Perrotte et al., "The European Network for the Study of Adrenal Tumors staging system is prognostically superior to the international union against cancer-staging system: a North American validation," *European Journal of Cancer*, vol. 46, no. 4, pp. 713–719, 2010.

[24] P. Icard, P. Goudet, C. Charpenay et al., "Adrenocortical carcinomas: surgical trends and results of a 253-patient series from the French Association of Endocrine Surgeons Study Group," *World Journal of Surgery*, vol. 25, no. 7, pp. 891–897, 2001.

[25] M. D. Wooten and D. K. King, "Adrenal cortical carcinoma. Epidemiology and treatment with mitotane and a review of the literature," *Cancer*, vol. 72, no. 11, pp. 3145–3155, 1993.

[26] G. Abiven, J. Coste, L. Groussin et al., "Clinical and biological features in the prognosis of adrenocortical cancer: poor outcome of cortisol-secreting tumors in a series of 202 consecutive patients," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 7, pp. 2650–2655, 2006.

[27] B. Polat, M. Fassnacht, L. Pfreundner et al., "Radiotherapy in adrenocortical carcinoma," *Cancer*, vol. 115, no. 13, pp. 2816–2823, 2009.

[28] B. Allolio, S. Hahner, D. Weismann, and M. Fassnacht, "Management of adrenocortical carcinoma," *Clinical Endocrinology*, vol. 60, no. 3, pp. 273–287, 2004.

[29] D. E. Schteingart, G. M. Doherty, P. G. Gauger et al., "Management of patients with adrenal cancer: recommendations of an international consensus conference," *Endocrine-Related Cancer*, vol. 12, no. 3, pp. 667–680, 2005.

[30] A. Berruti, M. Terzolo, P. Sperone et al., "Etoposide, doxorubicin and cisplatin plus mitotane in the treatment of advanced adrenocortical carcinoma: a large prospective phase II trial," *Endocrine-Related Cancer*, vol. 12, no. 3, pp. 657–666, 2005.

[31] G. Abiven, J. Coste, L. Groussin et al., "Clinical and biological features in the prognosis of adrenocortical cancer: poor outcome of cortisol-secreting tumors in a series of 202 consecutive patients," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 7, pp. 2650–2655, 2006.

[32] T. J. Giordano, D. G. Thomas, R. Kuick et al., "Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis," *American Journal of Pathology*, vol. 162, no. 2, pp. 521–531, 2003.

[33] F. Crucitti, R. Bellantone, A. Ferrante et al., "The italian registry for adrenal cortical carcinoma: analysis of a multi-institutional series of 129 patients," *Surgery*, vol. 119, no. 2, pp. 161–170, 1996.

[34] D. Bergenstal, M. Lipsett, R. Moy, and R. Hertz, "Regression of adrenal cancer and suppression of adrenal function in men by o, p′-DDD," *Transactions of American Physicians*, vol. 72, p. 341, 1959.

[35] J. P. Luton, S. Cerdas, L. Billaud et al., "Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy," *The New England Journal of Medicine*, vol. 322, no. 17, pp. 1195–1201, 1990.

[36] R. M. Bukowski, M. Wolfe, H. S. Levine et al., "Phase II trial of mitotane and cisplatin in patients with adrenal carcinoma: a Southwest Oncology Group study," *Journal of Clinical Oncology*, vol. 11, no. 1, pp. 161–165, 1993.

[37] T. S. Khan, H. Imam, C. Juhlin et al., "Streptozocin and o,p′DDD in the treatment of adrenocortical cancer patients: long-term survival in its adjuvant use," *Annals of Oncology*, vol. 11, no. 10, pp. 1281–1287, 2000.

[38] D. E. Schteingart, "Conventional and novel strategies in the treatment of adrenocortical cancer," *Brazilian Journal of Medical and Biological Research*, vol. 33, no. 10, pp. 1197–1200, 2000.

[39] S. Hahner and M. Fassnacht, "Mitotane for adrenocortical carcinoma treatment," *Current Opinion in Investigational Drugs*, vol. 6, no. 4, pp. 386–394, 2005.

[40] M. Terzolo, A. Angeli, M. Fassnacht et al., "Adjuvant mitotane treatment for adrenocortical carcinoma," *The New England Journal of Medicine*, vol. 356, no. 23, pp. 2372–2380, 2007.

[41] S. K. Williamson, D. Lew, G. J. Miller et al., "Phase II evaluation of cisplatin and etoposide followed by mitotane at disease progression in patients with locally advanced or metastatic adrenocortical carcinoma: a Southwest Oncology Group Study," *Cancer*, vol. 88, no. 5, pp. 1159–1165, 2000.

[42] R. Bonacci, A. Gigliotti, E. Baudin et al., "Cytotoxic therapy with etoposide and cisplatin in advanced adrenocortical carcinoma," *British Journal of Cancer*, vol. 78, no. 4, pp. 546–549, 1998.

[43] M. A. Burgess, S. S. Legha, and R. V. Sellin, "Chemotherapy with cisplatinum and etoposide (UP16) for patients with advanced adrenal cortical carcinoma (ACC)," *Proceedings of the Annals of Society of Clinical Oncology*, vol. 12, p. 188, 1993.

[44] R. F. Pommier and M. F. Brennan, "An eleven-year experience with adrenocortical carcinoma," *Surgery*, vol. 112, no. 6, pp. 963–971, 1992.

[45] S. P. Hedican and F. F. Marshall, "Adrenocortical carcinoma with intracaval extension," *Journal of Urology*, vol. 158, no. 6, pp. 2056–2061, 1997.

[46] R. D. Schulick and M. F. Brennan, "Long-term survival after complete resection and repeat resection in patients with adrenocortical carcinoma," *Annals of Surgical Oncology*, vol. 6, no. 8, pp. 719–726, 1999.

[47] L. E. Harrison, P. B. Gaudin, and M. F. Brennan, "Pathologic features of prognostic significance for adrenocortical carcinoma after curative resection," *Archives of Surgery*, vol. 134, no. 2, pp. 181–185, 1999.

[48] S. Gaujoux, S. Bonnet, and B. Dousset, "Laparoscopic versus open adrenalectomy for adrenocortical carcinoma: surgical and oncologic outcome in 152 patients," *European Urology*, vol. 58, no. 4, pp. 609–615, 2010.

[49] F. Porpiglia, C. Fiori, F. Daffara et al., "Retrospective evaluation of the outcome of open versus laparoscopic adrenalectomy for stage I and II adrenocortical cancer," *European Urology*, vol. 57, no. 5, pp. 873–878, 2010.

[50] J. Reibetanz, C. Jurowich, I. Erdogan et al., "Impact of lymphadenectomy on the oncologic outcome of patients with adrenocortical carcinoma," *Annals of Surgery*, vol. 25, no. 2, pp. 363–369, 2012.

[51] A. A. Nelson and G. Woodard, "Adrenal cortical atrophy and liver damage produced in dogs by feeding," *Federation Proceedings*, vol. 7, no. 1, part 1, p. 277, 1948.

[52] Y. Touitou, A. Bogdan, and J. P. Luton, "Changes in corticosteroid synthesis of the human adrenal cortex in vitro, induced by treatment with o,p′-DDD for Cushing's syndrome: evidence for the sites of action of the drug," *Journal of Steroid Biochemistry*, vol. 9, no. 12, pp. 1217–1224, 1978.

[53] D. M. Bergenstal, R. Hertz, M. B. Lipsett, and R. H. Moy, "Chemotherapy of adrenocortical cancer with o, p′-DDD," *Annals of Internal Medicine*, vol. 53, no. 4, pp. 672–682, 1960.

[54] H. van Slooten, A. J. Moolenaar, A. P. van Seters, and D. Smeenk, "The treatment of adenocortical carcinoma with o,p'-DDD: prognostic simplications of serum level monitoring," *European Journal of Cancer and Clinical Oncology*, vol. 20, no. 1, pp. 47–53, 1984.

[55] I. G. Hermsen, M. Fassnacht, M. Terzolo et al., "Plasma concentrations of o,p′DDD, o,p′DDA, and o,p′DDE as predictors of tumor response to mitotane in adrenocortical carcinoma: results of a retrospective ENS@T multicenter study," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 6, pp. 1844–1851, 2011.

[56] N. P. van Erp, H. J. Guchelaar, B. A. Ploeger, J. A. Romijn, J. Den Hartigh, and H. Gelderblom, "Mitotane has a strong and a durable inducing effect on CYP3A4 activity," *European Journal of Endocrinology*, vol. 164, no. 4, pp. 621–626, 2011.

[57] L. S. Kirschner, "Emerging treatment strategies for adrenocortical carcinoma: a new hope," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 1, pp. 14–21, 2006.

[58] S. E. Bates, C. Y. Shieh, L. A. Mickley et al., "Mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (mdr-1/P-glycoprotein) which is also expressed by adrenocortical carcinomas," *Journal of Clinical Endocrinology and Metabolism*, vol. 73, no. 1, pp. 18–29, 1991.

[59] H. R. Haak, J. Hermans, C. J. H. Van de Velde et al., "Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients," *British Journal of Cancer*, vol. 69, no. 5, pp. 947–951, 1994.

[60] J. Bertherat, J. Coste, and X. Bertagna, "Adjuvant mitotane in adrenocortical carcinoma," *The New England Journal of Medicine*, vol. 357, no. 12, pp. 1256–1257, 2007.

[61] H. van Slooten, A. P. van Seters, D. Smeenk, and A. J. Moolenaar, "O,p′-DDD (mitotane) levels in plasma and tissues during chemotherapy and at autopsy," *Cancer Chemotherapy and Pharmacology*, vol. 9, no. 2, pp. 85–88, 1982.

[62] E. Baudin, G. Pellegriti, M. Bonnay et al., "Impact of monitoring plasma 1,1 dichlorodiphenildichloroethane (o,p′DDD) levels on the treatment of patients with adrenocortical carcinoma," *Cancer*, vol. 92, no. 6, pp. 1385–1392, 2001.

[63] A. T. Phan, "Adrenal cortical carcinoma-review of current knowledge and treatment practices," *Hematology/Oncology Clinics of North America*, vol. 21, no. 3, pp. 489–507, 2007.

[64] M. Wandoloski, K. J. Bussey, and M. J. Demeure, "Adrenocortical Cancer," *Surgical Clinics of North America*, vol. 89, no. 5, pp. 1255–1267, 2009.

[65] A. Lacroix, "Approach to the patient with adrenocortical carcinoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 11, pp. 4812–4822, 2010.

[66] A. A. Kasperlik-Zaluska, B. M. Migdalska, S. Zgliczynski, and A. M. Makowska, "Adrenocortical carcinoma. A clinical study and treatment results of 52 patients," *Cancer*, vol. 75, no. 10, pp. 2587–2591, 1995.

[67] B. L. Wajchenberg, M. A. Albergaria Pereira, B. B. Medonca et al., "Adrenocortical carcinoma: clinical and laboratory observations," *Cancer*, vol. 88, no. 4, pp. 711–736, 2000.

[68] M. Fassnacht, M. Terzolo, B. Allolio et al., "Combination chemotherapy in advanced adrenocortical carcinoma," *The New England Journal of Medicine*, vol. 366, no. 23, pp. 2189–2197, 2012.

[69] R. Libè, A. Fratticci, and J. Bertherat, "Adrenocortical cancer: pathophysiology and clinical management," *Endocrine-Related Cancer*, vol. 14, no. 1, pp. 13–28, 2007.

[70] A. Sabolch, M. Feng, K. Griffith, G. Hammer, G. Doherty, and E. Ben-Josef, "Adjuvant and definitive radiotherapy for adrenocortical carcinoma," *International Journal of Radiation Oncology Biology Physics*, vol. 80, no. 5, pp. 1477–1484, 2011.

[71] L. J. Tacon, R. S. Prichard, P. S. H. Soon, B. G. Robinson, R. J. Clifton-Bligh, and S. B. Sidhu, "Current and emerging therapies for advanced adrenocortical carcinoma," *Oncologist*, vol. 16, no. 1, pp. 36–48, 2011.

[72] M. Fassnacht, S. Hahner, B. Polat et al., "Efficacy of adjuvant radiotherapy of the tumor bed on local recurrence of adrenocortical carcinoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 11, pp. 4501–4504, 2006.

[73] L. Zini, F. Porpiglia, and M. Fassnacht, "Contemporary management of adrenocortical carcinoma," *European Urology*, vol. 60, no. 5, pp. 1055–1065, 2011.

[74] M. Fassnacht and B. Allolio, "What is the best approach to an apparently nonmetastatic adrenocortical carcinoma?" *Clinical Endocrinology*, vol. 73, no. 5, pp. 561–565, 2010.

[75] A. M. Markoe, W. Serber, B. Micaily, and L. W. Brady, "Radiation therapy for adjunctive treatment of adrenal cortical carcinoma," *American Journal of Clinical Oncology*, vol. 14, no. 2, pp. 170–174, 1991.

[76] L. Cerquetti, B. Bucci, R. Marchese et al., "Mitotane increases the radiotherapy inhibitory effect and induces G_2-arrest in combined treatment on both H295R and SW13 adrenocortical cell lines," *Endocrine-Related Cancer*, vol. 15, no. 2, pp. 623–634, 2008.

[77] L. Cerquetti, C. Sampaoli, D. Amendola et al., "Mitotane sensitizes adrenocortical cancer cells to ionizing radiations by involvement of the cyclin B1/CDK complex in G_2 arrest and mismatch repair enzymes modulation," *International Journal of Oncology*, vol. 37, no. 2, pp. 493–501, 2010.

[78] I. G. C. Hermsen, Y. E. Groenen, M. W. Dercksen, J. Theuws, and H. R. Haak, "Response to radiation therapy in adrenocortical carcinoma," *Journal of Endocrinological Investigation*, vol. 33, no. 10, pp. 712–714, 2010.

[79] D. F. Maluf, B. H. de Oliveira, and E. Lalli, "Therapy of adrenocortical cancer: present and future," *American Journal of Cancer Research*, vol. 1, no. 2, pp. 222–232, 2011.

[80] A. C. Latronico, E. M. Pinto, S. Domenice et al., "An inherited mutation outside the highly conserved DNA-binding domain of the p53 tumor suppressor protein in children and adults with sporadic adrenocortical tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 10, pp. 4970–4973, 2001.

[81] F. M. Barlaskar and G. D. Hammer, "The molecular genetics of adrenocortical carcinoma," *Reviews in Endocrine and Metabolic Disorders*, vol. 8, no. 4, pp. 343–348, 2007.

[82] A. de Reyniès, G. Assié, D. S. Rickman et al., "Gene expression profiling reveals a new classification of adrenocortical tumors and identifies molecular predictors of malignancy and survival," *Journal of Clinical Oncology*, vol. 27, no. 7, pp. 1108–1115, 2009.

[83] F. M. Barlaskar, A. C. Spalding, J. H. Heaton et al., "Preclinical targeting of the type I insulin-like growth factor receptor in adrenocortical carcinoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 1, pp. 204–212, 2009.

[84] B. Ragazzon, G. Assié, and J. Bertherat, "Transcriptome analysis of adrenocortical cancers: from molecular classification to the identification of new treatments," *Endocrine-Related Cancer*, vol. 18, no. 2, pp. R15–R27, 2011.

[85] C. P. Carden, S. Frentzas, M. Langham et al., "Preliminary activity in adrenocortical tumor (ACC) in phase I dose escalation study of intermittent oral dosing of OSI-906, a small-molecule insulin-like growth factor-1 receptor (IGF-1R) tyrosine kinase inhibitor in patients with advanced solid tumors," *Journal of Clinical Oncology*, vol. 27, no. 15, p. 3544, 2009.

[86] P. Haluska, F. Worden, D. Olmos et al., "Safety, tolerability, and pharmacokinetics of the anti-IGF-1R monoclonal antibody figitumumab in patients with refractory adrenocortical carcinoma," *Cancer Chemotherapy and Pharmacology*, vol. 65, no. 4, pp. 765–773, 2010.

[87] T. J. Giordano, "Adrenocortical tumors an integrated clinical, pathologic, and molecular approach at the university of Michigan," *Archives of Pathology and Laboratory Medicine*, vol. 134, no. 10, pp. 1440–1443, 2010.

[88] M. Doghman, A. El Wakil, B. Cardinaud et al., "Regulation of IGF—mTOR signalling by miRNA in childhood adrenocortical tumors," *Cancer Research*, vol. 70, no. 11, pp. 4666–4675, 2010.

[89] M. C. De Martino, P. M. van Koetsveld, R. Pivonello, and L. J. Hofland, "Role of the mTOR pathway in normal and tumoral adrenal cells," *Neuroendocrinology*, vol. 92, no. 1, pp. 28–34, 2010.

[90] A. Naing, R. Kurzrock, A. Burger et al., "Phase I trial of cixutumumab combined with temsirolimus in patients with advanced cancer," *Clinical Cancer Research*, vol. 17, no. 18, pp. 6052–6060, 2011.

[91] S. Wortmann, M. Quinkler, C. Ritter et al., "Bevacizumab plus capecitabine as a salvage therapy in advanced adrenocortical carcinoma," *European Journal of Endocrinology*, vol. 162, no. 2, pp. 349–356, 2010.

[92] R. Chacón, G. Tossen, F. S. Loria, and M. Chacón, "CASE 2. Response in a patient with metastatic adrenal cortical carcinoma with thalidomide," *Journal of Clinical Oncology*, vol. 23, no. 7, pp. 1579–1580, 2005.

[93] M. Fassnacht, M. C. Kreissl, D. Weismann, and B. Allolio, "New targets and therapeutic approaches for endocrine malignancies," *Pharmacology and Therapeutics*, vol. 123, no. 1, pp. 117–141, 2009.

[94] J. O. Lee, K. W. Lee, C. J. Kim et al., "Metastatic adrenocortical carcinoma treated with sunitinib: a case report," *Japanese Journal of Clinical Oncology*, vol. 39, no. 3, pp. 183–185, 2009.

[95] D. S. Hong, S. M. Sebti, R. A. Newman et al., "Phase I trial of a combination of the multikinase inhibitor sorafenib and the farnesyltransferase inhibitor tipifarnib in advanced

malignancies," *Clinical Cancer Research*, vol. 15, no. 22, pp. 7061–7068, 2009.

[96] C. Butler, W. M. Butler, and A. A. Rizvi, "Sustained remission with the kinase inhibitor sorafenib in stage IV metastatic adrenocortical carcinoma," *Endocrine Practice*, vol. 16, no. 3, pp. 441–445, 2010.

[97] A. Berruti, P. Sperone, A. Ferrero et al., "Phase II study of weekly paclitaxel and sorafenib as second/third-line therapy in patients with adrenocortical carcinoma," *European Journal of Endocrinology*, vol. 166, no. 3, pp. 451–458, 2012.

[98] T. C. Gangadhar, E. E. W. Cohen, K. Wu et al., "Two drug interaction studies of sirolimus in combination with sorafenib or sunitinib in patients with advanced malignancies," *Clinical Cancer Research*, vol. 17, no. 7, pp. 1956–1963, 2011.

[99] Y. Z. Xu, Y. Zhu, Z. J. Shen et al., "Significance of heparanase-1 and vascular endothelial growth factor in adrenocortical carcinoma angiogenesis: potential for therapy," *Endocrine*, vol. 40, no. 3, pp. 445–451, 2011.

[100] G. Assié, M. Guillaud-Bataille, B. Ragazzon, X. Bertagna, J. Bertherat, and E. Clauser, "The pathophysiology, diagnosis and prognosis of adrenocortical tumors revisited by transcriptome analyses," *Trends in Endocrinology and Metabolism*, vol. 21, no. 5, pp. 325–334, 2010.

[101] K. Dorfinger, H. Vierhapper, A. Wilfing et al., "The effects of suramin on human adrenocortical cells in vitro: suramin inhibits cortisol secretion and adrenocortical cell growth," *Metabolism*, vol. 40, no. 10, pp. 1020–1024, 1991.

[102] W. Arlt, M. Reincke, L. Siekmann, W. Winkelmann, and B. Allolio, "Suramin in adrenocortical cancer: limited efficacy and serious toxicity," *Clinical Endocrinology*, vol. 41, no. 3, pp. 299–307, 1994.

[103] M. Quinkler, S. Hahner, S. Wortmann et al., "Treatment of advanced adrenocortical carcinoma with erlotinib plus gemcitabine," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 6, pp. 2057–2062, 2008.

[104] V. Samnotra, R. Vassilopoulou-Sellin, A. T. Fojo et al., "A phase II trial of gefitinib monotherapy in patients with unresectable adrenocortical carcinoma (ACC)," *Journal of Clinical Oncology*, vol. 27, no. 18, p. 15527, 2007.

[105] P. Adam, S. Hahner, M. Hartmann et al., "Epidermal growth factor receptor in adrenocortical tumors: analysis of gene sequence, protein expression and correlation with clinical outcome," *Modern Pathology*, vol. 23, no. 12, pp. 1596–1604, 2010.

[106] D. J. Gross, G. Munter, M. Bitan et al., "The role of imatinib mesylate (Glivec) for treatment of patients with malignant endocrine tumors positive for c-kit or PDGF-R," *Endocrine-Related Cancer*, vol. 13, no. 2, pp. 535–540, 2006.

[107] C. L. Ronchi, S. Sbiera, L. Kraus et al., "Expression of excision repair cross complementing group 1 and prognosis in adrenocortical carcinoma patients treated with platinum-based chemotherapy," *Endocrine-Related Cancer*, vol. 16, no. 3, pp. 907–918, 2009.

[108] K. Takara, T. Sakaeda, and K. Okumura, "An update on overcoming MDR1-mediated multidrug resistance in cancer chemotherapy," *Current Pharmaceutical Design*, vol. 12, no. 3, pp. 273–286, 2006.

[109] H. Fridborg, R. Larsson, C. Juhlin et al., "P-glycoprotein expression and activity of resistance modifying agents in primary cultures of human renal and adrenocortical carcinoma cells," *Anticancer Research*, vol. 14, no. 3 A, pp. 1009–1016, 1994.

[110] N. Feller, K. Hoekman, C. M. Kuiper et al., "A patient with adrenocortical carcinoma: characterization of its biological activity and drug resistance profile," *Clinical Cancer Research*, vol. 3, no. 3, pp. 389–394, 1997.

[111] J. Abraham, S. Bakke, A. Rutt et al., "A phase II trial of combination chemotherapy and surgical resection for the treatment of metastatic adrenocortical carcinoma: continuous infusion doxorubicin, vincristine, and etoposide with daily mitotane as a P-glycoprotein antagonist," *Cancer*, vol. 94, no. 9, pp. 2333–2343, 2002.

[112] M. J. Betz, I. Shapiro, M. Fassnacht, S. Hahner, M. Reincke, and F. Beuschlein, "Peroxisome proliferator-activated receptor-γ agonists suppress adrenocortical tumor cell proliferation and induce differentiation," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 7, pp. 3886–3896, 2005.

[113] P. Ferruzzi, E. Ceni, M. Tarocchi et al., "Thiazolidinediones inhibit growth and invasiveness of the human adrenocortical cancer cell line H295R," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 3, pp. 1332–1339, 2005.

[114] G. Cantini, A. Lombardi, E. Piscitelli et al., "Rosiglitazone inhibits adrenocortical cancer cell proliferation by interfering with the IGF-IR intracellular signaling," *PPAR Research*, vol. 2008, Article ID 904041, 11 pages, 2008.

[115] M. Luconi, M. Mangoni, S. Gelmini et al., "Rosiglitazone impairs proliferation of human adrenocortical cancer: preclinical study in a xenograft mouse model," *Endocrine-Related Cancer*, vol. 17, no. 1, pp. 169–177, 2010.

[116] L. Cerquetti, C. Sampaoli, D. Amendola et al., "Rosiglitazone induces autophagy in H295R and cell cycle deregulation in SW13 adrenocortical cancer cells," *Experimental Cell Research*, vol. 317, no. 10, pp. 1397–1410, 2011.

[117] A. C. Kim, F. M. Barlaskar, J. H. Heaton et al., "In search of adrenocortical stem and progenitor cells," *Endocrine Reviews*, vol. 30, no. 3, pp. 241–263, 2009.

[118] S. Gaujoux, S. Grabar, M. Fassnacht et al., "β-catenin activation is associated with specific clinical and pathologic characteristics and a poor outcome in adrenocortical carcinoma," *Clinical Cancer Research*, vol. 17, no. 2, pp. 328–336, 2011.

[119] M. Doghman, J. Cazareth, and E. Lalli, "The T cell factor/β-catenin antagonist PKF115-584 inhibits proliferation of adrenocortical carcinoma cells," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 8, pp. 3222–3225, 2008.

[120] M. J. Demeure, K. J. Bussey, and L. S. Kirschner, "Targeted therapies for adrenocortical carcinoma: IGF and beyond," *Hormone Cancer*, vol. 2, no. 6, pp. 385–392, 2011.

[121] K. Garber, "Drugging the Wnt pathway: problems and progress," *Journal of the National Cancer Institute*, vol. 101, no. 8, pp. 548–550, 2009.

[122] M. Q. Almeida, I. C. Soares, T. C. Ribeiro et al., "Steroidogenic factor 1 overexpression and gene amplification are more frequent in adrenocortical tumors from children than from adults," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 3, pp. 1458–1462, 2010.

[123] S. Sbiera, S. Schmull, G. Assie et al., "High diagnostic and prognostic value of steroidogenic factor-1 expression in adrenal tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 10, pp. E161–E171, 2010.

[124] M. Doghman, T. Karpova, G. A. Rodrigues et al., "Increased steroidogenic factor-1 dosage triggers adrenocortical cell proliferation and cancer," *Molecular Endocrinology*, vol. 21, no. 12, pp. 2968–2987, 2007.

[125] A. Ehrlund, P. Jonsson, L. L. Vedin et al., "Knockdown of SF-1 and RNF31 affects components of steroidogenesis, TGFâ, and Wnt/â-catenin signaling in adrenocortical carcinoma cells," *PLoS One*, vol. 7, no. 3, Article ID e32080, 2012.

[126] M. Doghman, J. Cazareth, D. Douguet, F. Madoux, P. Hodder, and E. Lalli, "Inhibition of adrenocortical carcinoma cell proliferation by steroidogenic factor-1 inverse agonists," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 6, pp. 2178–2183, 2009.

[127] Y. Chuman, Z. Zhan, and T. Fojo, "Construction of gene therapy vectors targeting adrenocortical cells: Enhancement of activity and specificity with agents modulating the cyclic adenosine 3′,5′-monophosphate pathway," *Journal of Clinical Endocrinology and Metabolism*, vol. 85, no. 1, pp. 253–262, 2000.

[128] S. Sbiera, S. Wortmann, and M. Fassnacht, "Dendritic cell based immunotherapy—a promising therapeutic approach for endocrine malignancies," *Hormone and Metabolic Research*, vol. 40, no. 2, pp. 89–98, 2008.

[129] C. Papewalis, M. Fassnacht, H. S. Willenberg et al., "Dendritic cells as potential adjuvant for immunotherapy in adrenocortical carcinoma," *Clinical Endocrinology*, vol. 65, no. 2, pp. 215–222, 2006.

[130] D. Ortmann, J. Hausmann, F. Beuschlein et al., "Steroidogenic acute regulatory (StaR)-directed immunotherapy protects against tumor growth of StaR-expressing Sp2-0 cells in a rodent adrenocortical carcinoma model," *Endocrinology*, vol. 145, no. 4, pp. 1760–1766, 2004.

[131] L. Barzon, G. Masi, M. Pacenti et al., "Expression of aromatase and estrogen receptors in human adrenocortical tumors," *Virchows Archiv*, vol. 452, no. 2, pp. 181–191, 2008.

[132] J. Matthews and J. A. Gustafsson, "Estrogen signaling: a subtle balance between ER alpha and ER beta," *Mol Interv*, vol. 3, no. 5, pp. 281–292, 2003.

[133] P. de Cremoux, D. Rosenberg, J. Goussard et al., "Expression of progesterone and estradiol receptors in normal adrenal cortex, adrenocortical tumors, and primary pigmented nodular adrenocortical disease," *Endocrine-Related Cancer*, vol. 15, no. 2, pp. 465–474, 2008.

[134] D. Montanaro, M. Maggiolini, A. G. Recchia et al., "Antiestrogens upregulate estrogen receptor β expression and inhibit adrenocortical H295R cell proliferation," *Journal of Molecular Endocrinology*, vol. 35, no. 2, pp. 245–256, 2005.

[135] R. Sirianni, F. Zolea, A. Chimento et al., "Targeting estrogen receptor áreduces adrenocortical cancer (ACC) cell growth in vitro and in vivo: potential therapeutic role of Selective Estrogen Receptor modulators (SERMs) for ACC treatment," submitted to Journal Clinical Endocrinology and Metabolism.

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 685213, 9 pages
doi:10.1155/2012/685213

Review Article

Management Strategies for Aggressive Cushing's Syndrome: From Macroadenomas to Ectopics

Carlotta Pozza, Chiara Graziadio, Elisa Giannetta, Andrea Lenzi, and Andrea M. Isidori

Pathophysiology Section, Department of Experimental Medicine, Sapienza University of Rome, Viale del Policlinico, 155-00161 Rome, Italy

Correspondence should be addressed to Andrea M. Isidori, andrea.isidori@uniroma1.it

Received 27 April 2012; Accepted 13 June 2012

Academic Editor: Marialuisa Appetecchia

Cushing's syndrome (CS) is a rare but severe clinical condition represented by an excessive endogenous cortisol secretion and hence excess circulating free cortisol, characterized by loss of the normal feedback regulation and circadian rhythm of the hypothalamic-pituitary axis due to inappropriate secretion of ACTH from a pituitary tumor (Cushing's disease, CD) or an ectopic source (ectopic ACTH secretion, EAS). The remaining causes (20%) are ACTH independent. As soon as the diagnosis is established, the therapeutic goal is the removal of the tumor. Whenever surgery is not curative, management of patients with CS requires a major effort to control hypercortisolemia and associated symptoms. A multidisciplinary approach that includes endocrinologists, neurosurgeons, oncologists, and radiotherapists should be adopted. This paper will focus on traditional and novel medical therapy for aggressive ACTH-dependent CS. Several drugs are able to reduce cortisol levels. Their mechanism of action involves blocking adrenal steroidogenesis (ketoconazole, metyrapone, aminoglutethimide, mitotane, etomidate) or inhibiting the peripheral action of cortisol through blocking its receptors (mifepristone "RU-486"). Other drugs include centrally acting agents (dopamine agonists, somatostatin receptor agonists, retinoic acid, peroxisome proliferator-activated receptor γ "PPAR-γ" ligands) and novel chemotherapeutic agents (temozolomide and tyrosine kinase inhibitors) which have a significant activity against aggressive pituitary or ectopic tumors.

1. Introduction

Cushing's syndrome (CS) is a rare but severe clinical condition caused by cortisol excess of various etiologies. It is associated with significant morbidity and mortality and leads to metabolic, cardiovascular, infectious, psychiatric, and gonadal complications (Table 1). This complex endocrine disorder is a challenge in terms of efficient treatment. This paper will focus on traditional and novel medical therapy for hypercortisolism secondary to ACTH-secreting pituitary macroadenoma or carcinoma (Cushing's disease, CD) or to ectopic ACTH secretion.

The natural history of *pituitary adenomas* varies widely. In the majority of cases, ACTH-secreting pituitary adenomas are small (<1 cm in diameter) and confined within the sella turcica. Pituitary microadenomas have a typically indolent growth rate, and clinically significant invasion and malignant transformation remain uncommon. However, 4–10% of patients present with larger tumors (>1 cm in diameter). These can cause symptoms due to mass effect before any full endocrine manifestations. Moreover, they are more refractory to surgical treatment and show a more unfavorable prognosis than microadenomas. For their behavior, presentation, and outcome, ACTH secreting macroadenomas present a distinct profile compared with microadenomas, although they probably represent one end of a spectrum of tumor autonomy, with specific growth and biochemical characteristics [1]. Morbidity and mortality are high with aggressive tumor behavior [2]. The 2004 WHO classification of pituitary adenomas now includes an "atypical" variant, defined as an MIB-1 proliferative index greater than 3%, excessive p53 immunoreactivity and increased mitotic activity. In the absence of metastases, however, invasive or aggressive pituitary tumors are not considered malignant. Pituitary carcinomas, defined as primary tumors with intra- or extracranial metastases, are rare, encountered in less than

Table 1

Clinical features of hypercortisolism
Weight gain
Central obesity
Moon face
Purple stretch marks
Plethora
Easy bruising
Hirsutism
Acne
Severe fatigue and muscle weakness
High blood pressure
Depression
Cognitive impairment
Diabetes
Loss of libido
Menstrual disorders
Osteoporosis
Psychosis

1% of all hypophyseal tumors. They generally secrete ACTH or Prolactin.

Ectopic ACTH Secretion (EAS) accounts for 15–20% of cases of Cushing's syndrome and covers a spectrum of tumors from undetectable isolated lesions to extensive metastatic and aggressive malignancies. EAS is often associated with severe hypercortisolemia causing hypokalemia, diabetes, generalized infections, hypertension, and psychotic reactions. Isidori et al. [3] proposed a classification based on the detection of the source of ectopic secretion. EAS is defined as *overt* when the tumor source is easily detected during the initial endocrine and radiological investigations, *covert* in patients presenting with hypercortisolemia where the ectopic source is not detected during initial tests but is discovered on subsequent evaluation or during prolonged followup, and *occult* when the patient's clinical features suggest CS and all tests indicate an ectopic source, but the primary lesion is not identified even after prolonged and repeated followup. Occult EAS is one of the most intriguing challenges for the clinical endocrinologist, as in some cases no tumor is found even after long-term followup or on autopsy [3]. The overall prognosis of patients with ectopic ACTH secretion is primarily determined by the nature of the underlying malignancy and the tumor stage on diagnosis.

2. Management of Cushing's Syndrome

Management of patients with CS requires a major effort to understand the etiology and to control hypercortisolemia as soon as the diagnosis is established. The most appropriate management of ACTH-dependent CS derives from a multidisciplinary approach that includes endocrinologists, neurosurgeons, oncologists, and radiotherapists.

The definitive treatment of CS consists in surgical resection of the tumor secreting ACTH. When the source of the excessive secretion *is the pituitary gland,* the standard approach is to perform an endoscopic endonasal transsphenoidal exploration, with excision of the tumor, if found. This surgical procedure is demanding and should only be performed in centers with extensive experience, to minimize operative risks, reduce the possibility of remission, and maintain other pituitary functions. It is successful in about 70% of cases (defined by suppressed plasma cortisol levels and normal 24 h urinary free cortisol) [4]. Success rates can reach 90% in selective adenectomy of microadenomas (<10 mm in diameter), but decrease to 65% for macroadenomas [5]. About 20% of tumors recur, and recurrence is more likely (and quicker) in larger than in smaller tumors.

Pituitary irradiation achieves eucortisolism in 50–60% of cases, albeit after 3–5 years [4], and patients can develop pituitary insufficiency, brain vascular morbidity or secondary neoplasms. Stereotactic radiosurgery (RS) proved less effective results in macroadenomas, especially if they had already infiltrate the cavernous sinus. To obtain optimal efficacy, RS should thus be reserved to small well-defined lesions. The management of aggressive adenomas invading adjacent structures is a real challenge, as they rarely respond to any treatment.

In the presence of *ectopic secretion of ACTH*, surgical resection of the primary tumor is recommended. This results in the complete remission, especially in cases of benign tumor. Often, however, the tumor may already have metastasized, it may not be resectable, or it may not be identified despite extensive investigation (occult).

Bilateral adrenalectomy can be chosen as a final approach, reserved for patients who do not respond to surgical exploration of the hypophysis or radiation therapy, or when the source of ectopic ACTH is not found.

Adrenalectomy necessarily requires steroid replacement therapy for the rest of the patient's life, as with primary adrenocortical insufficiency. There is also a significant risk of developing Nelson's syndrome, which occurs in 5–10% of the patients, likely a subset with an aggressive phenotype, after adrenalectomy for Cushing's syndrome [4, 6]. It has been demonstrated that patients with invasive corticotrophinomas have a greater risk of subsequent (and earlier) development of Nelson's syndrome compared with less aggressive forms [7]. Prophylactic, conventional 3-field radiotherapy can be used to reduce the incidence of subsequent Nelson's and it should always be considered in the management of these patients [8]. When these approaches cannot be applied, a treatment is needed that has fewer side effects and can quickly reduce symptoms, and severe complications of hypercortisolism, aiming for the normalization of ACTH and serum cortisol values [9].

3. Medical Treatments

The therapeutic goal in the treatment of patients with ACTH-dependent Cushing's syndrome is normalization of plasma ACTH and serum cortisol values, tumor shrinkage and preservation of anterior pituitary function, in cases of pituitary ACTH-secreting tumor. Medical treatment can improve the clinical condition of patients with severe hypercortisolism pending surgery, during acute diseases

(infections, psychosis, etc.), or in patients undergoing radiotherapy while awaiting the effects of the radiotherapy itself. In addition, patients with ectopic secretion of ACTH may be treated while expecting confirmation of the source, in the presence of metastatic cancer, or in patients who are not candidates for surgery for some reason.

Current drug-based therapy for CS includes drugs that act on the adrenal glands to reduce steroid synthesis, which therefore do not treat the underlying cause of the disease, and neuromodulators acting at the hypothalamic-pituitary level [10]. The existing treatments can be divided according to the site of action into adrenal acting drugs and in centrally acting drugs (Table 2).

3.1. Adrenal-Acting Drugs. Adrenal function must be carefully monitored, as excessive inhibition of steroidogenesis may cause adrenal insufficiency and may require the administration of small doses of glucocorticoids.

3.1.1. Ketoconazole. This is the most currently used drug in patients with hypercortisolism. It is a synthetic antifungal drug that works principally by inhibiting the cytochrome P450 system and 17,20-lyase, which are involved in the synthesis and degradation of steroids. It has also been suggested that this drug may directly inhibit the pituitary corticotroph function, inhibiting ACTH secretion [11–13]. This is a fast-acting drug that quickly reduces urinary free cortisol (UFC) levels [14]. Its use has been reported as effective in 50% of patients with ectopic ACTH secretion. The most common side effects include gynecomastia, hypogonadism, gastrointestinal symptoms and reversible increases in liver enzymes. Severe liver toxicity is rare and liver function is usually restored after discontinuation. The drug does not inhibit the growth of the ACTH-secreting tumor.

3.1.2. Metyrapone and LCI699. Metyrapone predominantly inhibits 11β hydroxylase and has been used either as a monotherapy, leading to a normalization of cortisol levels in 75–80% of patients, or in combination with other steroidogenesis inhibitors or with radiation therapy, achieving even higher efficacy [15, 16]. It is able to reduce cortisol production in patients with ectopic ACTH production and Cushing's disease. Side effects are dose-dependent, with the most common being hypertension, edema, increased acne and hirsutism in women due to its ability to inhibit the synthesis of aldosterone, resulting in an accumulation of its precursors with mineralocorticoid and weak androgen activity. However, when combined with ketoconazole, it offers a valuable and safe adjunct to control hypercortisolism. Recently, LCI699 [17], a novel orally active drug that inhibits at high doses the 11-beta hydroxylase activity (as well as aldosterone synthase) is under phase 2 evaluation for the management of hypercortisolism (http://clinicaltrial.gov/ identifier NCT01331239).

3.1.3. Aminoglutethimide. Aminoglutethimide is a potent reversible inhibitor of adrenal mineralocorticoid and glucocorticoid synthesis. It blocks cholesterol side-chain cleavage to pregnenolone, by inhibiting P450 enzymes. Side effects are skin rash, headache, a generalized pruritic rash, hypothyroidism, and goiter, and because of its toxicity is reserved for adrenal cancer.

3.1.4. Mitotane (o,p'-DDD). It is a DDD (dichlorodiphenyldichloroethane) isomer and a derivative of DDT. A study of 177 patients showed a significant increase in the recurrence-free interval after radical surgery followed by mitotane when compared to surgery alone [18]. Mitotane blocks several steroidogenic enzymes, thus altering peripheral steroid metabolism, directly suppressing the adrenal cortex and altering cortisone metabolism. Its adrenolytic function appears at high doses (>4 g/day). It is effective in reducing UFC levels in 83% of treated patients [19, 20]. A 2006 study confirmed that most patients under mitotane treatment in a dose ranging from 4 to 6.5 g daily had dramatic increase in CBG levels, and serum cortisol levels can be elevated even when the circulating free cortisol level is not, thus making difficult to control its biochemical effect [21, 22]. It is commonly used in patients with adrenal carcinoma. Its main use is in patients with persistent disease despite surgical resection, those who are not candidates for surgery, and patients with metastatic disease.

Serum levels should be monitored to optimize therapy. The compound is distributed in the adipose tissue and has a long half-life. Gastrointestinal and neurologic symptoms are the most common side effects.

3.1.5. Etomidate. Etomidate, an imidazole derivative, is an i.v. nonopioid anesthetic used for both induction and maintenance of anesthesia. It suppresses corticosteroid synthesis in the adrenal cortex by reversibly inhibiting 11-β-hydroxylase and 17,20 lyase at non-hypnotic doses. It has a very rapid onset of action and can be used in acute settings in patients with CS [23]. In addition, its intravenous administration makes it easily used in patients with no oral or enteral access. Studies and case reports support its use in patients with Cushing's syndrome. Chronic therapeutic use of ethyl-alcohol-containing Etomidate was effective for 8 weeks in a patient with ectopic CS and peritonitis [24]. In a 2001 case report, Etomidate was administered over 5.5 months, with daily dose modulation on the basis of serum cortisol levels. Suppression of steroidogenesis persisted for at least 14 days after cessation of the medication [25].

3.1.6. Mifepristone (RU486). Mifepristone is a synthetic steroid. It is a progesterone receptor antagonist and a powerful type-2 glucocorticoid receptor (GR) antagonist. It binds to human GR with an affinity three to four times higher than that of dexamethasone and about 18 times higher than that of cortisol. Its antiglucocorticoid effects are dose dependent. Mifepristone affects both the central actions of cortisol (negative feedback on CRH/ACTH secretion) and its peripheral actions and increases plasma ACTH and cortisol levels due to the loss of negative feedback of cortisol. This drug, currently used in the interruption of early pregnancy, was recently approved in patients with hyperglycemia induced by CS who are not candidates for surgery or where surgery has failed [26]. Medical literature

Table 2: Medical treatments for Cushing's syndrome (in clinical use or investigational).

Drug	Mechanism of action	Dose (range)	Side effects	Safety monitoring
Ketoconazole	Inhibits steroidogenesis via inhibition of cytochrome P450 function	200–1800 mg per os (in divided doses, b.i.d.-t.i.d.)	Reversible liver dysfunction, severe liver toxicity, GI disorders, skin rash, loss of libido, impotence	Transaminase, testosterone, and SHBG in men
Metyrapone	Inhibits 11-β hydroxylase in the adrenal gland	750–6000 mg per os (in divided doses, t.i.d.-q.i.d.)	Hirsutism, acne, GI disorders, dizziness, hypertension, edema, hypokalemia	Androgens, mineralocorticoid, electrolytes
Aminoglutethimide	Prevents conversion of cholesterol to pregnenolone	250–750 mg per os (in divided doses, b.i.d.-t.i.d.)	Generalized, self-limiting itchy rash, nausea, dizziness, blurred vision, cholestasis, bone marrow suppression	Blood count, thyroid hormones, hepatic function, abdominal US
Mitotane	Inhibits steroidogenesis via inhibition of cytochrome P450; adrenolytic (high doses)	500 mg–12 g per os (daily)	Severe nausea, vomiting, diarrhea, rash, somnolence, ataxia, vertigo, dyslipidemia	Plasma mitotane, blood count, electrolytes, liver function, cholesterol
Etomidate	Inhibits 11-β hydroxylase and 17–20 lyase	<0.1 mg/kg/hr i.v.	Sedative effects, anesthesia	Monitoring by anesthesiologists
Mifepristone (RU-486)	Glucocorticoid, androgen, and progesterone receptor antagonist	300–1200 mg per os, daily dose	Hypoadrenalism, hypokalemia, hypertension, irregular menses, endometrial hyperplasia	Blood count, electrolytes, pelvic US
Cabergoline	D2 receptor agonist	1–7 mg per os, weekly dose	Nausea, vomiting, dizziness, valvulopathy	Echocardiogram
Octreotide	Somatostatin receptor agonist (isoform 2)	200–1000 mcg s.c. t.i.d., or LAR formulation 10–30 mg i.m. every 4 weeks	GI disorders, gallstones or biliary sludge, hyperglycemia, sinus bradycardia	Glycaemia, HbA1c, ECG, abdominal US
Pasireotide (SOM 230)	Somatostatin receptor agonist (isoforms 1, 2, 3, 5)	600–900 mcg s.c. b.i.d., LAR formulation under investigation	GI disorders, gallstones or biliary sludge, hyperglycemia or diabetes mellitus, sinus bradycardia	Glycaemia, HbA1c, Q-T interval, abdominal US
Retinoic acid	Inhibits POMC transcription and cell-cycle progression	No data *in vivo* in humans in Cushing's syndrome	Anaemia, mucocutaneous and ocular symptoms	Toxic effects of vitamin A, liver function, blood count
Rosiglitazone	PPAR-γ agonist	4–16 mg per os, daily doses	Weight increase, edema, somnolence, hirsutism	Blood count, transaminase, ECG, echocardiogram
Temozolomide	Alkylating agent	150–200 mg/m^2 per os for 5 days once every 28 days, or 75 mg/m^2 daily for 21 days with 7 day break	Bone marrow suppression, nausea, vomiting, dizziness diarrhea, rash	Blood count, liver and renal function, electrolytes
Gefitinib	Tyrosine kinase inhibitor	No data *in vivo* in humans in Cushing's disease	Fatigue, nausea, vomiting, stomatitis, bone pain, dyspnea, interstitial lung disease	Transaminase, pulmonary toxicity
Everolimus	mTOR inhibitor	5 mg/day	Bone marrow suppression, nausea, angioedema, GI disorders, extremity pain	Liver and renal function, blood count, glycaemia, HbA1c, lipid profile

b.i.d.: twice daily; t.i.d.: three times daily; q.i.d.: four times daily; i.v.: intravenous; i.m.: intramuscular; s.c.: subcutaneous; POMC: proopiomelanocortin; US: ultrasound; HbA1c: glycated hemoglobin; GI: gastrointestinal.

suggests that mifepristone can improve clinical symptoms in 73–80% of patients [27] within one month after starting treatment. Castinetti et al. [28] reviewed the data of 37 treated CS patients (12 with EAS, 5 with Cushing's disease, the others affected by other causes of CS). A third of these developed hypokalemia. It was suggested that this resulted from cortisol stimulation of the mineralocorticoid receptor, while GRs were blocked by mifepristone. Spironolactone and potassium chloride replacement therapy can readily restore hypokalemia and blood pressure. Followup of efficacy and the onset of adrenal insufficiency (reported in 16% of 37 patients treated with Mifepristone) should only be clinical (weight, blood pressure, skin lesions) and biological (regular blood potassium sampling). The therapeutic dose

adjustments should be based on these parameters. Mifepristone is often associated with the development of endometrial hyperplasia, so regular vaginal ultrasound is recommended in long-term treatment.

3.2. Centrally Acting Drugs . In the last years several novel therapies have been studied with a view to the potential biochemical control and inhibition of pituitary tumor growth [29].

3.2.1. Dopamine Agonists. Dopamine (DA) is a catecholamine hormone with a wide range of functions. DA receptors have been found in a variety of organs (pituitary, adrenals, brain, kidney, gastrointestinal tract, cardiovascular system), and possibly exert an inhibitory effect when activated. D2-receptor agonists inhibit pituitary hormone secretion, particularly PRL and proopiomelanocortin-derived hormones, and drugs such as cabergoline and bromocriptine effectively inhibit PRL secretion in prolactinomas. Studies on corticotroph adenomas have shown that 80% of these tumors express D2 receptors [30, 31]. In recent decades, published case reports and case series have demonstrated the effective use of DA agonists in persistent or recurrent Cushing's disease.

The efficacy of bromocriptine in shrinking pituitary tumors was first reported in Nelson's syndrome and in the short-term treatment of CD [32–34]. However, the effect was not very strong, and response to long-term treatment was <30%. Cabergoline has a higher affinity for D2 receptors and a longer half-life compared to bromocriptine. In the short term [31] UFC levels normalized (40%) or decreased (20%) in a total cohort of 20 patients, 10 of whom underwent remission during long-term treatment (12–24 months) [35]. More recently a study demonstrated a 25% complete response to cabergoline in 12 patients with a followup of 6 months [36, 37] and confirmed that short-term treatment of CD with cabergoline improves cortisol secretion in half the cohort studied (30 patients), while long-term followup (37 months) demonstrated sustained effectiveness of cabergoline in 30% of subjects.

There are a few documented cases of use of DA agonists in ectopic ACTH secretion. A study [38] describes 6 cases of ectopic tumors, three of which were not cured by surgery. UFC was normalized in two of these patients, although one exhibited treatment escape. A prospective study [39] evaluated the efficacy of cabergoline in monotherapy in patients with uncured CD, using sleeping midnight serum cortisol and the standard Low Dose Dexamethasone Suppression Test (LDDST) cut-off value as the response criteria. Cabergoline was effective and safe in 28% of 20 treated patients. This drug is generally well tolerated by most patients, and none of the subjects treated in these clinical trials showed signs of secondary heart dysfunction or valvulopathy, except a patient with a history of tricuspid regurgitation [40]. Cabergoline has also been described as having potential positive metabolic effects (pressure lowering, improvement of glucose tolerance), independently of its cortisol lowering effect. These findings renew interest in the potential use of dopamine agonists in Cushing's disease.

3.2.2. PPAR-γ Ligands. Peroxisome proliferative-activated receptor-γ (PPAR-γ), a member of the nuclear receptor superfamily, functions as a transcription factor mediating ligand-dependent transcriptional regulation [41]. PPAR-γ is expressed in several organs, and its administration is reported to inhibit tumor cell growth in the prostate and colon [42, 43]. Heaney et al. [41] documented the abundant expression of PPAR-γ in a series of ACTH-secreting tumor samples compared with minimal expression in normal pituitary tissues, suggesting that thiazolidinediones, that activate PPAR-γ receptors, might be effective as a treatment for Cushing's disease. The literature evidence [44, 45] does not support this treatment, due to the lack of long-term benefit. Despite the finding of an initial reduction of ACTH and cortisol levels in a subset of patients with CD, clinical symptoms and biochemical parameters subsequently relapsed in this group of subjects. The administration of thiazolidinediones does not seem to be more effective than other currently available neuromodulators [45].

3.2.3. Pasireotide (SOM230). It is a somatostatin receptor (SSR) ligand with high binding affinity for multiple receptor isoforms (SST1-3 and SST5). SST5 and SST2 are highly expressed in ACTH pituitary adenomas, and animal studies documented that SSR mediates inhibition of cAMP and regulation of ACTH secretion [46]. A phase 2 trial [47] suggested that administration of Pasireotide for a 2-week period provoked a reduction in UFC in 76% of 29 patients affected by newly diagnosed, persistent or recurrent ACTH-dependent Cushing's disease. In a double blind, phase 3 study [48], 162 patients were randomly assigned to receive 600 mcg or 900 mcg subcutaneously twice daily. At 12 months, 26% and 15% of patients receiving, respectively, the higher and lower Pasireotide dose showed normalization of UFC levels. Serum and salivary cortisol and plasma ACTH decreased, and clinical features of hypercortisolism diminished. Side effects of this therapy included hyperglycemia (73%) and diabetes in 34% of patients, requiring treatment with glucose lowering medications in 45%. The other common symptoms were gastrointestinal disorders (diarrhea, abdominal pain, vomiting).

The significant results described in this 12-month phase 3 study support the use of Pasireotide as a targeted therapy for ACTH-secreting tumors. It is still not known if this treatment could act on pituitary tumor size. Octreotide, which acts predominantly on SSTR2 receptors, has not proven effective in inhibiting ACTH secretion in patients with Cushing's disease.

3.2.4. Chemotherapy. In most cases, pituitary adenomas are benign slow-growing tumors. However, their rate of growth can be fast and they can be resistant to standard medical, surgical and radiation treatment [49], especially ACTH macroadenomas. The Crooke's cell variant of corticotroph adenoma has been described to be more aggressive and refractory to therapy, with a predisposition to malignant transformation [50–52]. When invasive tumors recur repeatedly despite radical surgery and postoperative radiotherapy, with widespread extrasellar extension, proximity to cranial

nerves and critical blood vessels [2], combined cytotoxic therapy may be useful. It has also been suggested that early application of chemotherapy may be useful in patients who have already exhausted all surgical and radiotherapy options and are at high risk of malignant transformation [53, 54]. Kaiser et al. [55] reported a good response to cyclophosphamide, doxorubicin and 5-fluorouracil (5FU) in a patient with adrenocorticotroph tumor, with regression of the metastases. Kaltsas et al. [53] recommended the use of CCNU/5FU for relatively indolent tumor in the first instance. There have been partial and short-lasting responses to other combinations of chemotherapy agents [2], such as paclitaxel and etoposide in ectopic Cushing's syndrome [56]. In animal studies, cytotoxic hybrid compounds between the somatostatin analog vapreotide (no longer commercially available) and doxorubicin increased the effects of doxorubicin without increasing its toxicity [57].

3.2.5. Temozolomide. Temozolomide (TMZ) is a second-generation alkylating cytostatic agent. Combined with radiotherapy, it is known to be effective in some patients with glioblastoma multiforme and cerebral metastases of malignant melanoma. It is administered orally, does not require hepatic metabolism for activation, and is able to cross the blood-brain barrier. TMZ promotes apoptosis of target cells and induces massive cell shrinkage and necrosis, depleting the DNA repair enzyme O6-methylguanine-DNA-methyl transferase (MGMT) in various cell types. Multiple studies suggest that reduced intratumor levels of MGMT predict responsiveness to TMZ. TMZ may also inhibit angiogenesis. Its use was firstly described in 2006 for the treatment of a pituitary carcinoma, and the first corticotroph adenoma was treated in 2007 [58]. Since then, more than 30 case reports on its use in ACTH-secreting pituitary tumors have been published, and on the whole described some type of positive response. Recently Raverot et al. [59] described four patients with ACTH tumors with 50% positive response after only four cycles, in terms of marked shrinkage of the pituitary tumor together with a markedly reduced extension of the vertebral metastases, and a drop in ACTH levels with clinical improvement. Curtò et al. [60] published a case report of a patient with a corticotroph carcinoma in whom a 90% reduction in the size of the tumor, and a stabilization of the metastases volume was documented after four cycles of TMZ. Dillard et al. [61] described a case of an aggressive 3 cm corticotroph adenoma refractory to multiple surgery and radiotherapy which showed a 60% regression in size after TMZ administration. TMZ treatment was generally well tolerated. It has been reported [59] that the initial response does not always correlate with long-term control of the disease and that the absence of MGMT expression may be associated with a better response. Tumor stabilization or reduction of tumor size can improve clinical outcomes, and it remains a last-line defense for life-threatening pituitary tumors.

3.2.6. Retinoic Acid. Retinoids are a family of signaling molecules that are related to vitamin A (retinol) in terms of their chemical structure. The cell cycle is driven by complexes of cyclin-dependent kinases (CDKs) and cyclins. There is abundant evidence that retinoids, via various signaling pathways, inhibit cell-cycle progression in a variety of human cancer cells by directly or indirectly modulating cyclins, CDKs, and cell-cycle inhibitors.

Retinoic acid (RA) has been studied in various types of tumor. Páez-Pereda et al. [62] examined its effects on human *in vitro* and mouse *in vivo* pituitary cells. RA inhibited ACTH biosynthesis only in tumorous corticotroph cells, while normal cells were unaffected. The authors concluded that RA inhibits ACTH synthesis by inhibiting POMC transcription through its activity on AP-1 and Nur77/Nurr1 and reduces the proliferation and survival of the corticotroph adenoma. It is thus of potential therapeutic use in CD [62, 63]. Castillo et al. [63] published an *in vivo* animal study in which retinoic acid or ketoconazole was administered to 42 dogs with Cushing's canine syndrome. A reduction in ACTH and alpha-MSH levels and pituitary adenoma volume was noted after 180 days of therapy with retinoic acid or ketoconazole, with similar results for both treatments.

3.2.7. mTOR Inhibitors. Mammalian target of rapamycin (mTOR) functions as a central element in a signaling pathway involved in the control of cell growth and proliferation. Everolimus is an mTOR inhibitor, and recent studies [64] have demonstrated its antineoplastic activity in several human cancers, mostly when associated with the long-acting repeatable (LAR) formulation of Octreotide in neuroendocrine tumors. Jouanneau et al. [65] hypothesized its use in pituitary aggressive adenomas and carcinomas. The authors described the effects of a combination therapy with everolimus (5 mg/day) and octreotide (30 mg/months) and studied mTOR expression in 1 pituitary carcinoma against 17 ACTH adenomas. Combined therapy did not control pituitary tumor growth or ACTH secretion, but the authors are waiting for more clinical cases before drawing any conclusions on this combined treatment.

3.2.8. Tyrosine Kinase Inhibitors. Epidermal growth factor receptor (EGFR) activation, due to either mutation or ligand or receptor overexpression, is associated with a variety of human cancers. Approximately 60% of pituitary tumors, including ACTH-secreting adenomas, express EGFR. In pituitary corticotroph tumors expressing EGFR, $p27^{Kip1}$, a cyclin-dependent kinase inhibitor, is down regulated. In a recent study [66], the authors hypothesized that the receptor could be a novel target for treatment of Cushing's disease, suppressing ACTH in corticotroph adenomas. In human ACTH-secreting tumors [67] gefitinib (a tyrosine kinase inhibitor targeting EGFR) was found to suppress *in vitro* POMC expression by approximately 95%. This effect was confirmed in canine corticotroph adenoma cells. Gefitinib effectively suppressed ACTH secretion and inhibited tumor growth in EGFR-expressing tumors *in vivo* (mouse model), in support of the *in vitro* results.

3.2.9. Combined Therapy. Since corticotroph adenomas express DA and SST receptors simultaneously, some authors hypothesized the use of DA agonists with SS analogues

to reach a synergic effect in the treatment of ACTH-dependent CS. Recent studies [37] evaluated the association of cabergoline and ketoconazole, which normalized UFC in approximately 2/3 of patients not achieving a full response to cabergoline alone. In a prospective open-label, multicenter trial, Feelders et al. [68] administered Pasireotide in monotherapy followed by sequential addition of cabergoline and ketoconazole if UFC remained high after 28 and 60 days of treatment, respectively. At the end of the study 68% of the 17 treated patients showed complete biochemical control.

An innovative chimeric molecule that acts simultaneously on SST and DA receptors was created with a view to treatment of pituitary tumors. This compound (BIM-23A760) has been tested in a phase 1 and phase 2A study in 11 patients affected by acromegaly from GH-secreting pituitary adenoma, but the weak evidence of somatostatinergic activity led to the discontinuation of its development [69].

4. Conclusions

Management of persistent or recurrent CS is a challenge and medical therapy plays a critical role in the control of hypercortisolemia and associated symptoms. Unfortunately, this approach is not always successful. A multidisciplinary approach should thus be adopted, including chemotherapy, radiotherapy, neuromodulatory drugs, and hormone analogs to control tumor growth and associated symptoms. Ideally, management should be commenced in centers with appropriate experience and knowledge and involve a multidisciplinary team, including endocrinologists, neuroradiologists, dedicated neurosurgeons with expertise in pituitary tumor surgery (or general surgeons in cases of ectopic tumors), nuclear medicine physicians and oncologists.

Several studies have demonstrated that early application of medical treatment, possibly incorporating new therapeutic developments, may have improved effectiveness and led to more acceptable side effects. Nowadays, combination of tumor debulking, radiotherapy, medical treatment, and chemotherapy, appropriately and timely used, can avoid progression of an otherwise lethal condition. A better understanding of the pathogenesis of tumors underlying this puzzling syndrome is needed in order to help identify more effective and safe medical therapies. Control of hypercortisolemia should be obtained whenever possible, even in rapidly progressive disease, as small cell lung carcinomas, to reduce the associated complications (generalized infections, hypokalemia, diabetes, hypertension, psychotic reactions, and reduction of quality of life). Total bilateral adrenalectomy induces a rapid resolution of the clinical features. With low morbidity associated with laparoscopic adrenal surgery, this approach has been considered more frequently, and possibly even as main treatment in some individuals with Cushing's disease, especially when disease is severe or because of patient preference. Unfortunately the poor prognosis of this subgroup of EAS often makes the physician give up any drug control of the disease. We hope that the several offered noninvasive medical strategies can serve as a guide for the oncologists to improve the quality of life of their patients.

References

[1] Y. S. Woo, A. M. Isidori, W. Z. Wat et al., "Clinical and biochemical characteristics of adrenocorticotropin-secreting macroadenomas," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 8, pp. 4963–4969, 2005.

[2] Z. M. Bush, J. A. Longtine, T. Cunningham et al., "Temozolomide treatment for aggressive pituitary tumors: correlation of clinical outcome with O6-methylguanine methyltransferase (MGMT) promoter methylation and expression," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 11, pp. E280–E290, 2010.

[3] A. M. Isidori, G. A. Kaltsas, C. Pozza et al., "The ectopic adrenocorticotropin syndrome: clinical features, diagnosis, management, and long-term follow-up," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 2, pp. 371–377, 2006.

[4] B. M. K. Biller, A. B. Grossman, P. M. Stewart et al., "Treatment of adrenocorticotropin-dependent cushing's syndrome: a consensus statement," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 7, pp. 2454–2462, 2008.

[5] G. Arnaldi, A. Angeli, A. B. Atkinson et al., "Diagnosis and complications of Cushing's syndrome: a consensus statement," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 12, pp. 5593–5602, 2003.

[6] G. Assié, H. Bahurel, J. Bertherat, M. Kujas, P. Legmann, and X. Bertagna, "The Nelson's syndrome... revisited," *Pituitary*, vol. 7, no. 4, pp. 209–215, 2004.

[7] T. M. Barber, E. Adams, O. Ansorge, J. V. Byrne, N. Karavitaki, and J. A. H. Wass, "Nelson's syndrome," *European Journal of Endocrinology*, vol. 163, no. 4, pp. 495–507, 2010.

[8] P. J. Jenkins, P. J. Trainer, P. N. Plowman et al., "The long term outcome after adrenalectomy and prophylactic pituitary radiotherapy in adrenocorticotropin-dependent Cushing's syndrome," *Journal of Clinical Endocrinology and Metabolism*, vol. 80, no. 1, pp. 165–171, 1995.

[9] A. P. Heaney, "PPAR-γ in Cushing's disease," *Pituitary*, vol. 7, no. 4, pp. 265–269, 2004.

[10] J. Newell-Price, X. Bertagna, A. B. Grossman, and L. K. Nieman, "Cushing's syndrome," *Lancet*, vol. 367, no. 9522, pp. 1605–1617, 2006.

[11] M. Deuschle, O. Lecei, G. K. Stalla et al., "Steroid synthesis inhibition with ketoconazole and its effect upon the regulation of the hypothalamus-pituitary-adrenal system in healthy humans," *Neuropsychopharmacology*, vol. 28, no. 2, pp. 379–383, 2003.

[12] L. Jimenez Reina, A. Leal-Cerro, J. Garcia, P. P. Garcia-Luna, R. Astorga, and G. Bernal, "In vitro effects of ketoconazole on corticotrope cell morphology and ACTH secretion of two pituitary adnomas removed from patients with Nelson's syndrome," *Acta Endocrinologica*, vol. 121, no. 2, pp. 185–190, 1989.

[13] G. K. Stalla, J. Stalla, K. Von Werder et al., "Nitroimidazole derivatives inhibit anterior pituitary cell function apparently by a direct effect on the catalytic subunit of the adenylate cyclase holoenzyme," *Endocrinology*, vol. 125, no. 2, pp. 699–706, 1989.

[14] D. Engelhardt and M. M. Weber, "Therapy of Cushing's syndrome with steroid biosynthesis inhibitors," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 49, no. 4–6, pp. 261–267, 1994.

[15] M. Thoren, U. Adamson, and H. E. Sjoberg, "Aminoglutethimide and metyrapone in the management of Cushing's

syndrome," *Acta Endocrinologica*, vol. 109, no. 4, pp. 451–457, 1985.

[16] J. A. Verhelst, P. J. Trainer, T. A. Howlett et al., "Short and long-term responses to metyrapone in the medical management of 91 patients with Cushing's syndrome," *Clinical Endocrinology*, vol. 35, no. 2, pp. 169–178, 1991.

[17] D. A. Calhoun, W. B. White, H. Krum et al., "Effects of a novel aldosterone synthase inhibitor for treatment of primary hypertension: results of a randomized, double-blind, placebo- and active-controlled phase 2 trial," *Circulation*, vol. 124, pp. 1945–1955, 2011.

[18] M. Terzolo, A. Angeli, M. Fassnacht et al., "Adjuvant mitotane treatment for adrenocortical carcinoma," *New England Journal of Medicine*, vol. 356, no. 23, pp. 2372–2380, 2007.

[19] S. Leiba, R. Weinstein, B. Shindel et al., "The protracted effect of o,p'-DDD in Cushing's disease and its impact on adrenal morphogenesis of young human embryo," *Annales d'Endocrinologie*, vol. 50, no. 1, pp. 49–53, 1989.

[20] J. P. Luton, J. A. Mahoudeau, P. Bouchard et al., "Treatment of Cushing's disease by O,p'DDD. Survey of 62 cases," *New England Journal of Medicine*, vol. 300, no. 9, pp. 459–464, 1979.

[21] A. P. Van Seters and A. J. Moolenaar, "Mitotane increases the blood levels of hormone-binding proteins," *Acta Endocrinologica*, vol. 124, no. 5, pp. 526–533, 1991.

[22] N. Nader, G. Raverot, A. Emptoz-Bonneton et al., "Mitotane has an estrogenic effect on sex hormone-binding globulin and corticosteroid-binding globulin in humans," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 6, pp. 2165–2170, 2006.

[23] V. A. Preda, J. Sen, N. Karavitaki, and A. Grossman, "THERAPY IN ENDOCRINE DISEASE: Etomidate in the management of hypercortisolaemia in Cushing's syndrome," *European Journal of Endocrinology*, vol. 167, no. 2, pp. 137–143, 2012.

[24] W. M. Drake, L. A. Perry, C. J. Hinds, D. G. Lowe, R. H. Reznek, and G. M. Besser, "Emergency and prolonged use of intravenous etomidate to control hypercortisolemia in a patient with Cushing's syndrome and peritonitis," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 10, pp. 3542–3544, 1998.

[25] J. Krakoff, C. A. Koch, K. A. Calis, R. H. Alexander, and L. K. Nieman, "Use of a parenteral propylene glycol-containing etomidate preparation for the long-term management of ectopic Cushing's syndrome," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 9, pp. 4104–4108, 2001.

[26] N. A. Tritos and B. M. Biller, "Advances in medical therapies for Cushing's syndrome," *Discovery Medicine*, vol. 13, pp. 171–179, 2012.

[27] F. Castinetti, M. Fassnacht, S. Johanssen et al., "Merits and pitfalls of mifepristone in Cushing's syndrome," *European Journal of Endocrinology*, vol. 160, no. 6, pp. 1003–1010, 2009.

[28] F. Castinetti, B. Conte-Devolx, and T. Brue, "Medical treatment of Cushing's syndrome: glucocorticoid receptor antagonists and mifepristone," *Neuroendocrinology*, vol. 92, no. 1, supplement, pp. 125–130, 2010.

[29] S. S. Praw and A. P. Heaney, "Medical treatment of Cushing's disease: overview and recent findings," *International Journal of General Medicine*, vol. 2, pp. 209–217, 2009.

[30] P. Petrossians, A. S. Thonnard, and A. Beckers, "Medical treatment in Cushing's syndrome: dopamine agonists and cabergoline," *Neuroendocrinology*, vol. 92, no. 1, pp. 116–119, 2010.

[31] R. Pivonello, D. Ferone, W. W. De Herder et al., "Dopamine receptor expression and function in corticotroph pituitary tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 5, pp. 2452–2462, 2004.

[32] C. Invitti, M. De Martin, L. Danesi, and F. Cavagnini, "Effect of injectable bromocriptine in patients with Cushing's disease," *Experimental and Clinical Endocrinology and Diabetes*, vol. 103, no. 4, pp. 266–271, 1995.

[33] S. W. J. Lamberts and J. C. Birkenhaeger, "Bromocriptine in Nelson's syndrome and Cushing's disease," *Lancet*, vol. 2, no. 7989, p. 811, 1976.

[34] S. W. J. Lamberts, J. G. M. Klijn, and M. De Quijada, "The mechanism of the suppressive action of bromocriptine on adrenocorticotropin secretion in patients with Cushing's disease and Nelson's syndrome," *Journal of Clinical Endocrinology and Metabolism*, vol. 51, no. 2, pp. 307–311, 1980.

[35] R. Pivonello, M. C. De Martino, P. Cappabianca et al., "The medical treatment of Cushing's disease: effectiveness of chronic treatment with the dopamine agonist cabergoline in patients unsuccessfully treated by surgery," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 1, pp. 223–230, 2009.

[36] A. Godbout, M. Manavela, K. Danilowicz, H. Beauregard, O. D. Bruno, and A. Lacroix, "Cabergoline monotherapy in the long-term treatment of Cushing's disease," *European Journal of Endocrinology*, vol. 163, no. 5, pp. 709–716, 2010.

[37] L. Vilar, L. A. Naves, M. F. Azevedo et al., "Effectiveness of cabergoline in monotherapy and combined with ketoconazole in the management of Cushing's disease," *Pituitary*, vol. 13, no. 2, pp. 123–129, 2010.

[38] R. Pivonello, D. Ferone, W. W. De Herder et al., "Dopamine receptor expression and function in corticotroph ectopic tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 1, pp. 65–69, 2007.

[39] A. R. Lila, R. A. Gopal, S. V. Acharya et al., "Efficacy of cabergoline in uncured (persistent or recurrent) cushing disease after pituitary surgical treatment with or without radiotherapy," *Endocrine Practice*, vol. 16, no. 6, pp. 968–976, 2010.

[40] D. Cheung and A. Heaney, "Dopamine agonists and valvular heart disease," *Current Opinion in Endocrinology, Diabetes and Obesity*, vol. 16, no. 4, pp. 316–320, 2009.

[41] A. P. Heaney, M. Fernando, W. H. Yong, and S. Melmed, "Functional PPAR-γ receptor is a novel therapeutic target for ACTH-secreting pituitary adenomas," *Nature Medicine*, vol. 8, no. 11, pp. 1281–1287, 2002.

[42] E. Elstner, E. A. Williamson, C. Zang et al., "Novel therapeutic approach: ligands for PPARγ and retinoid receptors induce apoptosis in bcl-2-positive human breast cancer cells," *Breast Cancer Research and Treatment*, vol. 74, no. 2, pp. 155–165, 2002.

[43] T. Kubota, K. Koshizuka, E. A. Williamson et al., "Ligand for peroxisome proliferator-activated receptor γ (Troglitazone) has potent antitumor effect against human prostate cancer both in vitro and in vivo," *Cancer Research*, vol. 58, no. 15, pp. 3344–3352, 1998.

[44] B. Ambrosi, C. Dall'Asta, S. Cannavò et al., "Effects of chronic administration of PPAR-γ ligand rosiglitazone in Cushing's disease," *European Journal of Endocrinology*, vol. 151, no. 2, pp. 173–178, 2004.

[45] G. F. Pecori, C. Scaroni, E. Arvat et al., "Effect of protracted treatment with rosiglitazone, a PPARγ agonist, in patients with Cushing's disease," *Clinical Endocrinology*, vol. 64, no. 2, pp. 219–224, 2006.

[46] L. J. Hofland, J. van der Hoek, R. Feelders et al., "The multi-ligand somatostatin analogue SOM230 inhibits ACTH

secretion by cultured human corticotroph adenomas via somatostatin receptor type 5," *European Journal of Endocrinology*, vol. 152, no. 4, pp. 645–654, 2005.

[47] M. Boscaro, W. H. Ludlam, B. Atkinson et al., "Treatment of pituitary-dependent Cushing's disease with the multireceptor ligand somatostatin analog pasireotide (SOM230): a multicenter, phase II trial," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 1, pp. 115–122, 2009.

[48] A. Colao, S. Petersenn, J. Newell-Price et al., "A 12-month phase 3 study of pasireotide in cushing's disease," *New England Journal of Medicine*, vol. 366, no. 10, pp. 914–924, 2012.

[49] M. Losa, E. Mazza, M. R. Terreni et al., "Salvage therapy with temozolomide in patients with aggressive or metastatic pituitary adenomas: experience in six cases," *European Journal of Endocrinology*, vol. 163, no. 6, pp. 843–851, 2010.

[50] D. H. George, B. W. Scheithauer, K. Kovacs et al., "Crooke's cell adenoma of the pituitary: an aggressive variant of corticotroph adenoma," *American Journal of Surgical Pathology*, vol. 27, no. 10, pp. 1330–1336, 2003.

[51] D. J. Holthouse, P. D. Robbins, R. Kahler, N. Knuckey, and P. Pullan, "Corticotroph pituitary carcinoma: case report and literature review," *Endocrine Pathology*, vol. 12, no. 3, pp. 329–341, 2001.

[52] F. Roncaroli, M. Faustini-Fustini, F. Mauri, S. Asioli, and G. Frank, "Crooke's hyalinization in silent corticotroph adenoma: report of two cases," *Endocrine Pathology*, vol. 13, no. 3, pp. 245–249, 2002.

[53] G. A. Kaltsas, J. J. Mukherjee, P. N. Plowman, J. P. Monson, A. B. Grossman, and G. M. Besser, "The role of cytotoxic chemotherapy in the management of aggressive and malignant pituitary tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 12, pp. 4233–4238, 1998.

[54] G. A. Kaltsas, P. Nomikos, G. Kontogeorgos, M. Buchfelder, and A. B. Grossman, "Clinical review: diagnosis and management of pituitary carcinomas," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 5, pp. 3089–3099, 2005.

[55] F. E. Kaiser, D. N. Orth, K. Mukai, and J. H. Oppenheimer, "A pituitary parasellar tumor with extracranial metastases and high, partially suppressible levels of adrenocorticotropin and related peptides," *Journal of Clinical Endocrinology and Metabolism*, vol. 57, no. 3, pp. 649–653, 1983.

[56] S. Y. Park, Y. Rhee, J. C. Youn et al., "Ectopic Cushing's syndrome due to concurrent corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) secreted by malignant gastrinoma," *Experimental and Clinical Endocrinology and Diabetes*, vol. 115, no. 1, pp. 13–16, 2007.

[57] A. V. Schally, J. B. Engel, G. Emons, N. L. Block, and J. Pinski, "Use of analogs of peptide hormones conjugated to cytotoxic radicals for chemotherapy targeted to receptors on tumors," *Current Drug Delivery*, vol. 8, no. 1, pp. 11–25, 2011.

[58] K. Kovacs, B. W. Scheithauer, M. Lombardero et al., "MGMT immunoexpression predicts responsiveness of pituitary tumors to temozolomide therapy," *Acta Neuropathologica*, vol. 115, no. 2, pp. 261–262, 2008.

[59] G. Raverot, N. Sturm, F. De Fraipont et al., "Temozolomide treatment in aggressive pituitary tumors and pituitary carcinomas: a French multicenter experience," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 10, pp. 4592–4599, 2010.

[60] L. Curtò, M. L. Torre, F. Ferraù et al., "Temozolomide-induced shrinkage of a pituitary carcinoma causing cushing's disease - Report of a case and literature review," *TheScientificWorldJournal*, vol. 10, pp. 2132–2138, 2010.

[61] T. H. Dillard, S. H. Gultekin, J. B. Delashaw, C. G. Yedinak, E. A. Neuwelt, and M. Fleseriu, "Temozolomide for corticotroph pituitary adenomas refractory to standard therapy," *Pituitary*, vol. 14, no. 1, pp. 80–91, 2011.

[62] M. Páez-Pereda, D. Kovalovsky, U. Hopfner et al., "Retinoic acid prevents experimental Cushing syndrome," *Journal of Clinical Investigation*, vol. 108, no. 8, pp. 1123–1131, 2001.

[63] V. A. Castillo, M. F. Cabrera Blatter, N. V. Gómez, V. Sinatra, M. F. Gallelli, and M. C. Ghersevich, "Diurnal ACTH and plasma cortisol variations in healthy dogs and in those with pituitary-dependent Cushing's syndrome before and after treatment with retinoic acid," *Research in Veterinary Science*, vol. 86, no. 2, pp. 223–229, 2009.

[64] J. C. Yao, M. H. Shah, T. Ito et al., "Everolimus for advanced pancreatic neuroendocrine tumors," *New England Journal of Medicine*, vol. 364, no. 6, pp. 514–523, 2011.

[65] E. Jouanneau, A. Wierinckx, F. Ducray et al., "New targeted therapies in pituitary carcinoma resistant to temozolomide," *Pituitary*, vol. 15, no. 1, pp. 37–43, 2012.

[66] F. E. Wondisford, "A new medical therapy for cushing disease?" *Journal of Clinical Investigation*, vol. 121, no. 12, pp. 4621–4623, 2011.

[67] H. Fukuoka, O. Cooper, A. Ben-Shlomo et al., "EGFR as a therapeutic target for human, canine, and mouse ACTH-secreting pituitary adenomas," *Journal of Clinical Investigation*, vol. 121, no. 12, pp. 4712–4721, 2011.

[68] R. A. Feelders, C. De Bruin, A. M. Pereira et al., "Pasireotide alone or with cabergoline and ketoconazole in Cushing's disease," *New England Journal of Medicine*, vol. 362, no. 19, pp. 1846–1848, 2010.

[69] M. Gueorguiev and A. B. Grossman, "Pituitary tumors in 2010: a new therapeutic era for pituitary tumors," *Nature Reviews Endocrinology*, vol. 7, no. 2, pp. 71–73, 2011.

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 320198, 10 pages
doi:10.1155/2012/320198

Clinical Study

Radiolabeled Somatostatin Analogues Therapy in Advanced Neuroendocrine Tumors: A Single Centre Experience

A. Filice,[1] A. Fraternali,[1] A. Frasoldati,[2] M. Asti,[1] E. Grassi,[3] L. Massi,[1] M. Sollini,[1,4] A. Froio,[1] P. A. Erba,[1,4] and A. Versari[1]

[1] *Department of Nuclear Medicine, Azienda Ospedaliera Santa Maria Nuova, IRCCS Reggio Emilia, Via Risorgimento 80, 42100 Reggio Emilia, Italy*
[2] *Department of Endocrinology, Azienda Ospedaliera Santa Maria Nuova, IRCCS Reggio Emilia, Via Risorgimento 80, 42100 Reggio Emilia, Italy*
[3] *Department of Medical Physics, Azienda Ospedaliera Santa Maria Nuova, IRCCS Reggio Emilia, Via Risorgimento 80, 42100 Reggio Emilia, Italy*
[4] *Nuclear Medicine Unit, University of Pisa, Via Roma 55, 56125 Pisa, Italy*

Correspondence should be addressed to A. Versari, versari.annibale@asmn.re.it

Received 30 April 2012; Revised 25 June 2012; Accepted 26 June 2012

Academic Editor: Marialuisa Appetecchia

The aim of this study was to assess the efficacy of PRRT in patients with advanced neuroendocrine tumors (NETs). *Patients and Methods*. From January 2007 to August 2011, we enrolled 65 patients (m/f 38/27; mean age 65 years, range 33–83) with advanced NETs having enhanced SSTR expression, treated with PRRT. The enhanced expression of SSTR was assessed using ^{68}Ga-DOTATOC/DOTATATE PET/CT. Among all the enrolled patients, 6 of them were excluded from the present analysis since they voluntarily interrupted treatment. Mean activity/cycle of 2.6 GBq (^{90}Y-DOTATOC/DOTATATE) or 6.0 GBq (^{177}Lu-DOTATOC/DOTATATE) was administrated intravenously (max 9 cycles). *Results*. Complete response (CR) was found in 1/59 (2%) patients, partial remission (PR) in 24/59 (40.5%) patients, stable disease (SD) in 24/59 (40.5%), and progression (PD) in 10/59 (17%) patients. The overall tumor response rate (CR + PR) was 42.5%. In 40.5% of patients, the disease could be stabilized. Overall, 49 out of 59 patients had no tumor progression (83%). Twelve patients out of 59 (20%) had grade 2-3 hematological side effects including anemia, thrombocytopenia, and leukopenia. Long-term nephrotoxicity was observed in 3 patients (2 moderate, 1 severe). *Conclusions*. PRRT is a promising perspective for patients with advanced NETs.

1. Introduction

Neuroendocrine tumors (NETs) are considered a class of rare neoplasms accounting <5% of all tumors. However, diagnosis of NETs has increased substantially over the last decades and prevalence is now greater than that of any other upper gastrointestinal tumor [1]. These tumors originate from dispersed neuroendocrine cells, distributed almost ubiquitously in the body [2], and occur in 5/100,000 people per year [1].

The most frequent sites of NETs are gastroenteropancreatic tract (GEP NETs), followed by lungs; less frequently skin, adrenal glands, thyroid, and genital tracts may be affected. Different nomenclature systems and classifications have been used for NETs.

Current pathological staging and grading differ between Europe and USA; however, both classification systems are centered on the primary site of the tumor and histological grade. In Europe, the Ki-67 proliferative index is used to differentiate tumors of low (<2%), intermediate (2–20%) and high (>20%) grade, whereas in the USA, tumors are graded as "well-" and "poorly-" differentiated where "well" equates to low-intermediate grade and "poorly" equates to high-grade tumors [3, 4].

Up to 80% of GEP NETs express somatostatin receptors (SSTR2 and SSTR5 primarily). Therefore, somatostatin

analogues have been used for both diagnosis and treatment of NETs. ^{111}In-labeled SST-analogues SPECT and ^{68}Ga SST-analogues PET/CT represent an accurate methods for NETs diagnosis peptide radioreceptor therapy (PRRT) indication and patients management [5–8].

When beta-emitters isotopes as ^{90}Y ($T_{1/2}$ of 2.67 days, maximum range of tissue irradiation of 12 mm) or ^{177}Lu ($T_{1/2}$ of 6.73 days, maximum range of irradiation of 1.5 mm) are used to label SST-analogues linked to a chelator, PRRT may be performed. After the i.v. injection, the radiopharmaceutical will distribute in the body, selectively bind to SSTRs, and actively be taken up by the cells through a process called receptor-ligand internalization [9, 10]. The internalization will ultimately lead to a selective accumulation of radioactivity in the tumor, thus determining cell death. The majority of clinical trials data available is from non-randomized retrospective case series. Due to variation in patients selection, dosing, scheduling, and total number of treatments it can be challenging to draw firm conclusions from the literature. However, it seems to be a benefit for selected patients with response rates in the range of 40% [11–14].

Here we present the results of a phase II study designed to treat disseminated or nonoperable NETs patients with PRRT. Patients demonstrated enhanced SSTR expression at PET/CT with ^{68}Ga-peptide (DOTATOC/DOTATATE).

2. Materials and Methods

2.1. Study Design. This was a prospective nonrandomized single-arm clinical trial performed at the Department of Nuclear Medicine, Santa Maria Nuova Hospital, Reggio Emilia (Italy). All patients with advanced, progressive NET fulfilling the study inclusion criteria were first evaluated with ^{68}Ga-peptide PET/CT followed by ^{111}In-peptide dosimetric evaluation to determine both the presence of SSTR expression as a target for the following treatment and eligibility to PRRT, that is in presence of provisional adsorbed doses: (a) >10 Gy to tumor, (b) <10 Gy to the kidneys, (c) <6 Gy for the liver, (d) <1.5 Gy for red marrow, (e) <3 Gy for lung, and (f) <8 Gy for whole body. In case of ^{177}Lu-PRRT (^{177}Lu-DOTATOC/DOTATATE), dosimetric evaluation was performed acquiring images during the first cycle of therapy. A fractionated treatment protocol was followed with the intravenous administration of an average activity of 2.6 GBq/cycle for ^{90}Y-PRRT and 6.0 GBq/cycle for ^{177}Lu-PRRT, respectively, with an interval of about 2 months.

Toxicity and tolerability were recorded through all the study and for additionally 6 months after the study completion. Serial follow-up ^{68}Ga-peptide PET/CT imaging was repeated after each PRRT cycle during the first part of the study as required by our ethic committee. The clinical trial was subsequently amended and the number of PET/CT examinations reduced to baseline, intermediate (after 2-3 PRRT cycles), and end-treatment (3–6 months after the last PRRT) scans. In order to homogenize data analysis, treatment response was assessed comparing PET/CT studies performed at baseline and at the end of treatment as well as patient's clinical response. The intermediate PET/CT evaluation was used only to assess the early progressive disease (PD).

The study was conducted in accordance with International Conference on Harmonization Good Clinical Practice guidelines, the Declaration of Helsinki and it was approved by local and national authorities (EudraCT numbers 2006-000897-65 and 2008-000983-17).

2.2. Patients. From January 2007 to August 2011, we enrolled 65 patients (38 men and 27 females; mean age = 65 years, range 33–83). All patients presented progressive disease and fulfilled the following inclusion/exclusion criteria.

2.2.1. Inclusion Criteria. The inclusion criteria were as follows:

(i) Age > 18 years;

(ii) histological confirmation of NET; inoperable or metastatic disease;

(iii) presence of at least one measurable lesion;

(iv) positive 68Ga-peptide PET/CT defined as radiopharmaceutical uptake in tumor and/or metastasis higher than liver, evaluated within 3 months before PRRT (qualitative analysis);

(v) adequate hematological parameter: hemoglobin level (Hb) $\geq$ 10 g/dL; leucocytes (WBC) $\geq 2.5 \times 10^3$/mL; platelets (PLT) $\geq 100 \times 10^3$/mL;

(vi) adequate liver and renal function: bilirubin levels <2.5 mg/dL; creatinine levels <2 mg/dL;

(vii) ECOG performance status <2;

(viii) Signed informed consent;

(ix) discontinuation of cold SST-analogues treatment at least 4 weeks before PRRT;

(x) Life expectancy of at least 6 months.

2.2.2. Exclusion Criteria. The exclusion criteria were as follows:

(i) other treatment (such as chemotherapy or radiotherapy) or participation in any investigational drug trial within 1 month of PRRT and for the following 2 months;

(ii) Pregnancy or lactation;

(iii) Bone marrow involvement >25%;

(iv) other concomitant tumors, except "in situ" basal cell carcinoma and tumors of the uterine cervix treated with radical surgery.

Additionally, before each PRRT cycle the following parameters should be maintained: Hb $\geq$ 10 g/dL, WBC $\geq 2.5 \times 10^3$/mL; PLT $\geq 100 \times 10^3$/mL, creatinine levels <2 mg/dL; bilirubin levels <2.5 mg/dL.

The final analysis was based on a total of 59 patients (m/f 33/26) since 6 patients (2 with GI tumor, 1 with

carcinoid tumor of the lung, and 3 with pancreatic tumor) voluntarily interrupted the treatment. Tumor was localized in the gastrointestinal tract in 19/59 cases (32%), followed by pancreas in 16/59 cases (27%) and lung in 13/59 cases (22%). In 11/59 patients (19%), the origin was unknown.

All patients at enrollment had metastatic (stage IV) PD (Table 1). Histopathological findings including grading were not reported for patients since histological diagnosis was performed in different centers thus features were reported in different not comparable modalities. Previous treatments are reported in Table 2. Diabetes was present in 11/59 cases and 16/59 patients suffered from blood hypertension. Additionally, 9/59 had previous tumors (3/9 prostate cancers, 3/9 breast cancers, 2/9 large-bowel cancers and 1/59 stomach cancer) with a minimal time of free disease of 5 years. Main baseline clinical signs and symptoms were diarrhea (18/59), pain (12/59), weight loss (7/59), flush (5/59), cough (4/59), constipation (3/59), nausea (2/59), and carcinoid syndrome (1/59). Additionally, 32/59 patients presented at enrolment a variable grade of asthenia. Twenty-seven patients were asymptomatic at baseline. Serum baseline CgA levels were normal in 19/59 patients.

Table 1: Site and number of metastasis in the 59 evaluated patients at the enrollment in the clinical trial.

Site of metastasis	Number of metastasis	
	≤5	>5
Bone (21/59)	0/21	21/21
Liver (42/59)	2/42	40/42
Lung (4/59)	0/4	4/4
Lymph nodes (34/59)	3/34	31/34
Other (6/59)	1/6	5/6

Table 2: List of previous treatments in the 59 evaluated patients order on the basis of their frequency.

Previous treatment	Number of patients
Surgery	39/59
"Cold" SST analogues	25/59
Chemotherapy	13/59
TACE or RFTA	7/59
External beam radiotherapy	5/59

TACE: intra-arterial hepatic chemoembolization; RFTA: radiofrequency thermoablation.

2.3. Radiopharmaceuticals Preparation. ^{111}In-, ^{90}Y-, and ^{177}Lu-peptide (DOTATOC or DOTATATE) were synthesized by following internal protocol [15]. Every preparation was obtained by carrying out the following steps: (a) a 3 mL syringe was filled with a 1 mL solution containing 30 μg of sodium ascorbate and an amount of a 4 mg/mL peptide solution proportional to the ^{90}Y-, and ^{177}Lu- or ^{111}In- activity in order to achieve a radiolabeling specific activity of 106 MBq/nmol, 48 MBq/nmol, and 6 MBq/nmol, respectively (b) this solution was added to a 3 mL Schott vial containing an activity ranging between 7.4 to 30 GBq of ^{90}Y chloride solution, between 15 to 60 GBq of ^{177}Lu chloride solution or between 222 to 444 MBq of ^{111}In- chloride solution (Perkin Elmer, Boston, MA, United States) in 0.05 M hydrochloric acid obtaining a 4.6 pH solution; (c) the Schott vial was heated for 30 minutes at 90°C in a heating block; (d) a 5 μL aliquot of the solution was withdrawn for carrying out the quality controls by using solid phase extraction or chromatographic methods [16]; (e) only for ^{90}Y and ^{177}Lu-peptide: the preparation was transferred to a bigger vial containing 0.5 mL of 1 mM DTPA solution and diluted with 20 mL of 0.9% sodium chloride solution [17]; (f) single doses for the patients were obtained by fractioning the mother solution in vials containing 2 mL of an ascorbic acid/sodium ascorbate buffer solution in order to decrease the effects of radiolysis. The radiochemical purity of the ^{111}In-, ^{177}Lu-, and ^{90}Y-peptide preparations was always >99.8%.

The radiolabeling of ^{68}Ga-peptide was performed by means of a modular lab synthesizer (Eckert & Ziegler, Berlin, Germany) as already described [18]. Briefly, the fraction of about 2 mL of the ^{68}Ge/^{68}Ga-generator eluate containing about 80% of the ^{68}Ga activity in 0.1 M hydrochloric acid was selected and directed to a reactor vial containing a 20 μL of peptide solution (1 mg/mL) and 200 μL of a 1.5 M sodium formate solution or 140 μL of a 1.5 M sodium acetate solution in order to obtain a pH ranging between 3.2 and 3.5. The mixture was heated at 100°C for 5 minutes and, then, passed through a light C-18 cartridge. ^{68}Ga-peptide was eluted with 0.5–1 mL of a 50% ethanol solution and diluted with 8 mL of 0.9% sodium chloride solution. The synthesis was carried out in 14 minutes with a mean yield of 63 ± 3% (not corrected for decay). Quality controls were performed by chromatographic methods as already described, obtaining a radiochemical purity always >95% [19].

2.3.1. Pretherapeutic Somatostatin Receptor Imaging. Pretherapeutic imaging was performed by ^{68}Ga-peptide PET/CT. For this study, PET/CT scans were acquired on a GE Discovery at 60 min after injection of about 120 MBq of ^{68}Ga-peptide. Seven or eight bed positions with 5 slices overlap were acquired for 4 min emission time in 3D. The CT-exposure factors for all examinations were 120 kVp and 80 mA in 0.8 seconds. PET images were reconstructed using CT-attenuation correction (OSEM). All studies were visually and semiquantitatively assessed. SUV calculations were performed on a Xeleris workstation. Mean and maximum SUV (activity concentration corrected for patient weight and total injected dose) was determined in all lesions and recorded.

2.3.2. Selection of Patients Eligible for PRRT. ^{68}Ga-peptide PET/CT was considered positive in patients who showed uptake in the tumor lesions at least two-times higher than the liver; thus they were considered eligible for PRRT and, therefore, admitted to dosimetric evaluation.

2.4. Dosimetry. Planar imaging was initially performed after the i.v. injection of 185 MBq of ^{111}In-peptide with a dual-head gamma camera (Genesys, Philips, The Netherlands)

using parallel-hole, medium-energy, general-purpose collimators. The windows were centered over both ^{111}In- photon peaks (247 and 172 keV with a window width of 20%), whereas scatter fraction was evaluated at 140 keV (width 20%).

In all the patients, whole-body scan and, in selected cases, spot images of the abdomen were obtained after 1, 4, 20, 48, and 72 hours for control of biodistribution. To determine blood clearance, we drew blood samples at 30 and 60 minutes and at 4, 20, and 48 hours after injection. Radioactivity in blood was measured with a HPGe spectrometer (DSPEC jr 2.0—Ortec). For dosimetric calculations, regions of interest were drawn manually on the whole-body scans from anterior and posterior projections and ULMDOS software (University of Ulm, Germany) was used. Background regions were placed on the abdomen or on the thigh for background correction. Scans were corrected for background, self-absorption, patient thickness attenuation, and organ overlapping. Whole-body activity acquired immediately after injection was defined as 100% of the injected activity. Data were expressed as percentage injected activity as a function of time. The resulting time-activity points were fitted to a monoexponential or multiexponential curve for whole-body, kidneys, liver, spleen, and red marrow to calculate residence time. Patient-specific organ masses were also considered. The estimated doses delivered to critical organs and to the tumor were obtained by the software OLINDA/EXM [20]. The activity in blood was fitted to a biexponential curve to determine the residence time in blood. The dose to the red marrow was calculated from the residence time in blood, assuming no specific uptake, a uniform distribution of activity, and clearance from red marrow equal to that from blood. A correction factor of 1 was used as described by Cremonesi et al. [21].

In case of ^{177}Lu-PRRT the dosimetric evaluation was performed acquiring images during the first cycle of therapy, thanks to the low gamma emission of this isotope.

2.5. Therapy (Administration Protocol). A fractionated treatment protocol was followed with the intravenous administration of an average activity of 2.6 GBq and 6.0 GBq per cycle for ^{90}Y-PRRT and for ^{177}Lu-PRRT, respectively, with an interval of about 2 months. For each cycle, patients were hospitalized for 3 days in accordance with local requirements. Thirty minutes before administration of the radiopeptide 2 L of amino acid solution of Hartmann-Hepa 8 (Ringer's Lactate Hartmann, Proteinsteril Hepa 8%, Mg 5-sulfat) were infused, which were continued up to 3 hours after injection to inhibit tubular reabsorption of the radioactive tracer. Repeated treatments were performed in case of response and significant improvement in symptoms and quality of life, except in cases of renal toxicity and rejection by the patient for further treatment within 3 months. Additional cycles were suspended in case of PD.

2.6. Biodistribution of the Radiotracer. In order to evaluate the biodistribution of therapeutic activity, after each treatment, planar imaging was performed with a dual head SPECT gamma camera (Genesys, Philips, The Netherlands) or with a dual-head SPECT/CT gamma camera (Symbia-T, Siemens, Germany) using parallel-hole, medium-energy, general-purpose collimators. The windows were centered over ^{177}Lu-PRRT photon peaks (208 keV and 110 keV width 20% in both cases; scatter window at 160 keV) in case of treatment with ^{177}Lu-PRRT; while at 170 keV (20%) and 80 keV (55%) in case of treatment with ^{90}Y-PRRT, as Bremsstrahlung planar scan. Whole-body scans (acquisition time: 25 minutes) and spot images (acquisition time: 10 minutes) were obtained.

2.7. Assessment of Clinical Benefit and Evaluation of PRRT Response. Clinical benefit was assessed comparing baseline clinical conditions with end-treatment parameters. In the clinical benefit evaluation the worsening of clinical conditions (i.e., appearance of new sign(s)/symptom(s)) were considered as PD. Indeed any significant variations in baseline clinical conditions was defined as stable disease (SD). Clinical benefit was defined as non-PD/SD. For the follow-up blood tests were evaluated, as described in the clinical protocol, repeated before and after each treatment cycle and every two weeks. Blood tests included hematological parameters, liver and renal function. Baseline and end-treatment serum CgA values were compared and the trend was defined as increased, stable (variation over time $\leq$10%) or decreased. All patients were followed for an additional 6 months after the last radiopharmaceutical administration. Acute and long-term adverse events were graded according to the Common Terminology Criteria for Adverse Events, version 3.0 of the National Cancer Institute [22]. To assess response to treatment PET/CT studies performed at baseline and at the end of treatment were considered.

Treatment responses assessed by PET/CT scan were defined as follows:

(i) complete response (CR): disappearance of radiopharmaceutical uptake in all detectable lesions;

(ii) partial response (PR): reduction of radiopharmaceutical uptake (>50%) in all detectable lesions in absence of appearance of new lesion(s);

(iii) stable disease (SD): no variation or reduction of radiopharmaceutical uptake (<50%) in some detectable lesions in absence of appearance of new lesion(s);

(iv) progressive disease (PD): increase >25% of radiopharmaceutical uptake in one or more lesions or appearance of new lesions and/or >10% increasing of tumor marker.

In this series of patients, we did not assess treatment response based on the size of lesions using the CT component of PET/CT images or CT scan, but as described above we evaluate only the functional response.

2.8. Statistical Analysis. All values are expressed as median and range, as customary for nonparametric data.Correlation analysis was performed using the Mann-Whitney test.

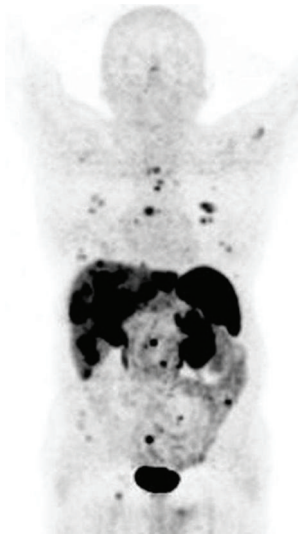


Figure 1: ^{68}Ga-DOTATOC PET/CT: liver, lung, lymph node, and bone metastases from NET of unknown origin.

3. Results

^{68}Ga-DOTATOC/DOTATATE PET/CT was performed in all the patients to evaluate the eligibility. Baseline ^{68}Ga-DOTATOC/DOTATATE PET/CT demonstrated at least one site of radiopharmaceutical uptake.

Figure 1 shows an example of ^{68}Ga-DOTATOC accumulation in tumor lesions. ^{68}Ga-DOTATOC positive lesions were preferentially localized at liver, lymph nodes, lung, and skeleton.

An end-treatment ^{68}Ga-peptide PET/CT was performed in all treated patients about 3–6 months after the last PRRT administration except for 6 patients in which PD was determined on the basis of worsening of clinical conditions.

Figures 2 and 3 represent examples of pre- and posttherapeutic ^{68}Ga-DOTATOC PET/CT.

Dosimetric estimates for kidney and bone marrow are summarized in Table 3. No toxicities were recorded after radiopharmaceutical injection administered for dosimetric purpose.

PRRT cycles were administered at 70 ± 24.6 days apart (range 35–140) with a median cumulative activity of 5.5 GBq (range 3.6–7.4 GBq). Thirty-five patients received 4 or 5 PRRT cycles, 10/59 more than 5 cycles while 14/59 patients had <4 PRRT cycles. ^{90}Y-PRRT was administered in 33/59 patients (56%), ^{177}Lu-PRRT in 9/59 patients (15%) while 17/59 patients (29%) received both ^{90}Y-PRRT and ^{177}Lu-PRRT in different cycles. Posttherapeutic scintigraphy confirmed a correct distribution of the radiopharmaceutical in all patients.

In 18/59 (30%) patients no adverse effects after administration of the radiopharmaceuticals were observed. Hematological toxicity including grade 2-3 anemia, thrombocytopenia and leukopenia occur in 12/59 patients (20%). Two of the 12 patients who had hematological toxicity presented baseline grade 1 anemia and thrombocytopenia resulting from previous chemotherapies. Asthenia (grade 2-3, 28/59) nausea (grade 1-2, 14/59), vomiting (grade 2-3, 5/59), were frequently observed. Stomatitis (grade 2) and gastritis (grade 1) were also reported in 1 case each. Long-term nephrotoxicity was observed in 3 patients (2 moderate; 1 severe requiring dialysis). Patients who developed nephrotoxicity were treated with ^{90}Y-PRRT receiving 5 (2/3) and 6 (1/3) cycles. One of them suffered from both diabetes and blood hypertension. Patient who required dialysis had only one kidney. Clinical benefit was recorded in 21/59 patients while a worsening of clinical conditions was observed in 9/59 patients. All patients which were asymptomatic at baseline and not present modifications of their clinical conditions.

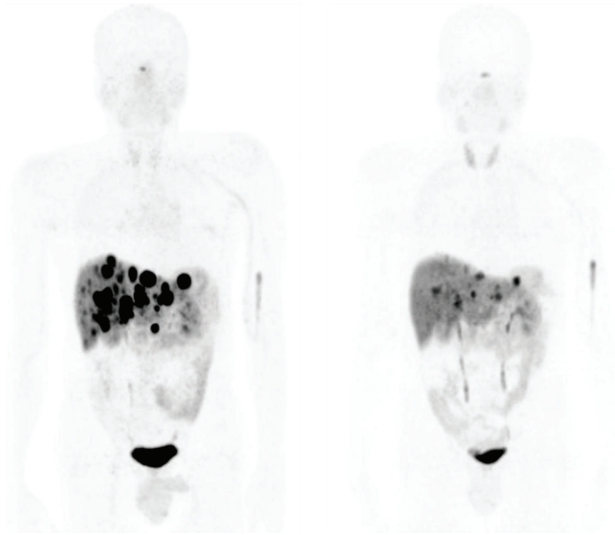
Best objective response was CR in 1/59 patient (2%), PR in 24/59 (40.5%), SD in 24/59 patients (40.5%) while PD was demonstrated in 10/59 (17%) of patients. The overall tumor response rate considering both CR and PR was 42.5%. SUVmax values in the main lesion for both baseline and end-treatment ^{68}Ga-peptide PET/CT scans were reported in Table 4 based on functional response. A significant difference in cumulated administered activity between PD and non-PD patients was found as shown by Figure 4.

Table 5 shows treatment responses based on primary tumor site. Table 6 shows results of treatment responses based on the type of treatment (^{90}Y-PRRT, ^{177}Lu-PRRT, or combined ^{90}Y-PRRT and ^{177}Lu-PRRT) while treatment responses based on the numbers of PRRT cycles are reported in Table 7. In Table 8 functional response was tabulated on the basis of clinical benefit assessment. In the eleven patients with both normal baseline and end-treatment serum CgA levels functional response assessed by ^{68}Ga-peptide PET/CT resulted in 1/11 CR, 5/11 PR, 4/11 SD, and 1/11 PD. Discordant results between serum CgA levels trend over time and ^{68}Ga-peptide results were found in 23/59 patients. Despite the increase of CgA values ^{68}Ga-peptide PET/CT documented a PR in 7 patients and a SD in 6 cases, respectively (Table 9). In one patient classified as SD by ^{68}Ga-peptide PET/CT, serum CgA levels completely normalized after PRRT.

4. Discussion

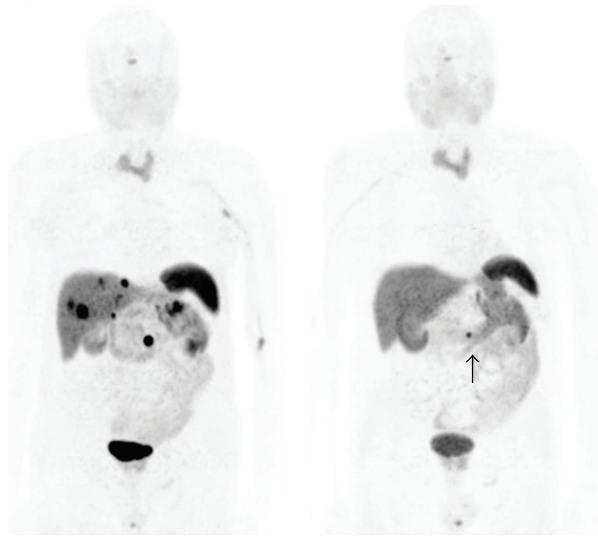
The development of imaging agents specifically designed to target tumor metabolic pathways and associated antigens including membrane receptors opens new horizon both for the selection of patients candidate to target treatment by the *in vivo* detection of enhanced target expressions as well as for the development of new multimodality treatment strategies.

The expression of SSTRs by NETs made molecular imaging with specific SST-analogues for specific SSTR subtypes the method of choice for their diagnostic workup. In fact ^{111}In-labeled SST analogues scintigraphy and more recently ^{68}Ga-DOTA-peptides significantly change the diagnostic approach to neuroendocrine tumors. In our study, ^{68}Ga-peptide PET/CT as first-selection procedure to determine the presence of high SSTR expression and a tumor uptake at least two times higher than the liver were considered the criteria to be eligible for dosimetric evaluation with ^{111}In-peptide.



(a) (b)

Figure 2: Male, 56 years old, with pancreatic NET and multiple liver metastases. ^{68}Ga-DOTATOC PET/CT before therapy (a) and after PRRT (b). The result was a partial response.



(a) (b)

Figure 3: Male, 73 year old, pancreas NET with liver metastases. ^{68}Ga-DOTATOC PET/CT before (left) and after therapy (right). PRRT with ^{90}Y-DOTATOC (2 cycles) and ^{177}Lu-DOTATOC (4 cycles) was administered at interval of 2 months. The response was complete in the liver but partial in the pancreatics region (arrow).

Table 3: Dosimetric estimates for kidney and bone marrow.

	Mean	Median	SD	Range
^{90}Y-kidney dose (Gy/GBq)	$2.4E+00$	$1.5E+00$	1.9	0.32–8.90
^{90}Y-bone marrow dose (Gy/GBq)	$9.7E-02$	$5.8E-02$	0.1	0.0047–0.51
^{177}Lu-kidney dose (Gy/GBq)	$3.9\,E-01$	$2.5E-01$	0.3	0.05–1.47
^{177}Lu-bone marrow dose (Gy/GBq)	$2.81E-02$	$1.29E-02$	0.04	0.0163–0.256

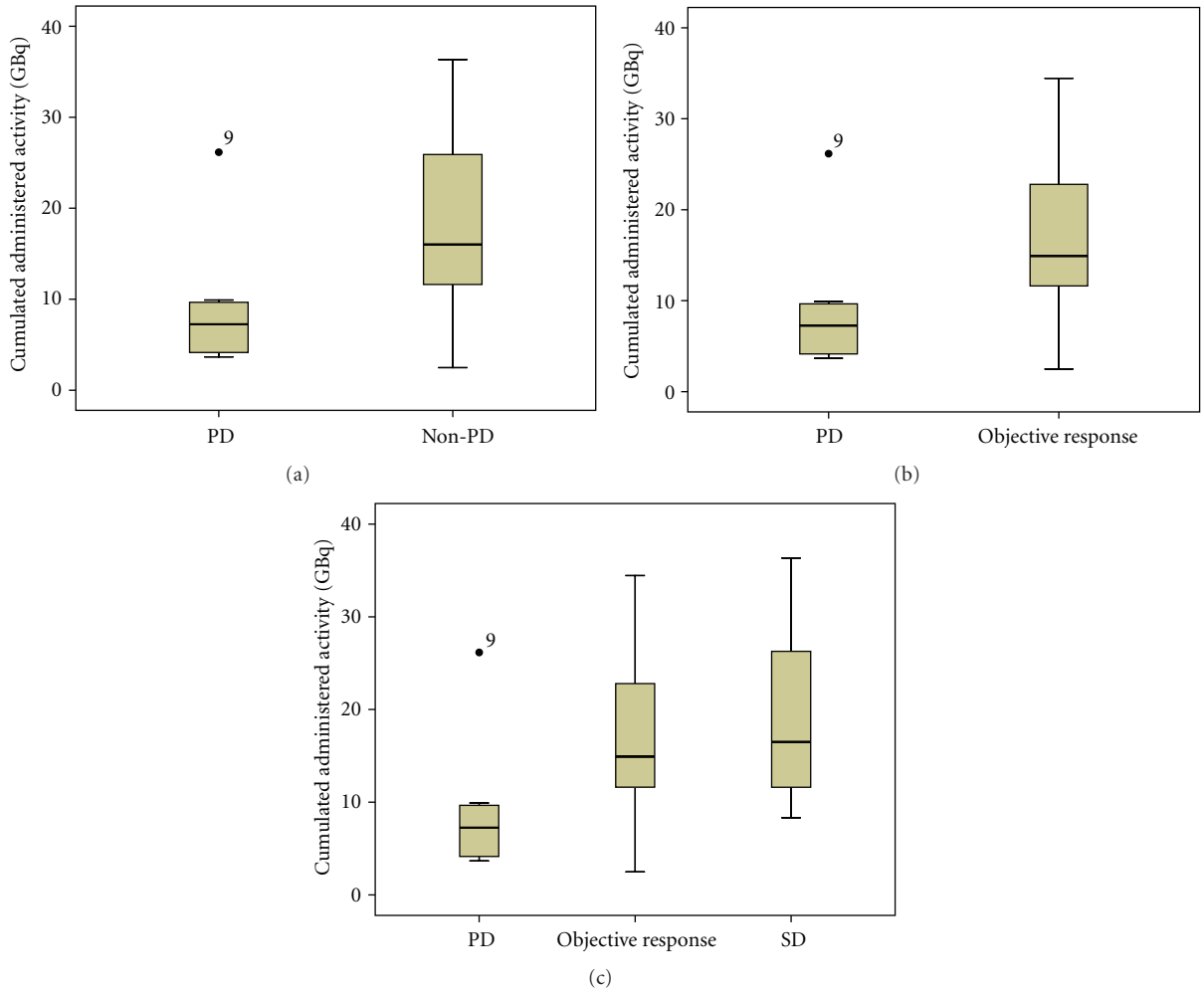


Figure 4: (a) Mann-Whitney test plot shows a significant difference in cumulated administered activity between PD and non-PD patients ($P < 0.001$). Similar results (b) have been obtained by using Mann-Whitney test excluding patients who had SD and thus considering only patients who had objective response ($P = 0.002$). When all subgroups of patients are considered (c), the cumulated administered activity remains significantly different in PD group *versus* both objective response and SD groups while no significant differences have been found in cumulated administered activity comparing patients who presented objective response and stable disease ($P = 0.435$). PD = patients who presented progressive disease; non-PD = patients who presented complete or partial response or disease stabilization; objective response = patients who presented complete or partial response; SD = patients who presented stable disease.

Originally, the study was designed for radiolabelled DOTA-TOC but because of problem of commercial availability we were obliged to amend the study protocol and substitute DOTATOC with DOTATATE. Using ^{68}Ga-peptide PET/CT high SSTR expression to be eligible for subsequent PRRT was found in our study in all patients. Dosimetric estimates confirmed the eligibility of all patients and demonstrated that a fractionated treatment protocol with the intravenous administration of an average activity of 2.6 GBq/cycle for ^{90}Y-PRRT and 6.0 GBq/cycle for ^{177}Lu-PRRT, respectively, with an interval about 2 months was within the safety threshold of toxicity, particularly for the kidney (the critical organ for PRRT) and the bone marrow. Initially for PRRT, we used ^{90}Y since in our department ^{177}Lu was allowed from 2009. Subsequently, the criteria of choice of radionuclide used for PRRT was mainly based on tumor size [23] (reserving ^{90}Y for lesion(s) >2 cm, ^{177}Lu for lesion(s) <2 cm, and ^{90}Y/^{177}Lu in presence of both conditions) and on dosimetric estimates.

Administration of ^{90}Y-PRRT (average activity of about 2.6 GBq/cycle) and ^{177}Lu-PRRT (average activity of about 6.0 GBq/cycle) induced disease control in 83% of patients (1 CR, 24 PR, and 24 SD) with a duration of response of at least 6 months. In the majority of cases, objective response was associated to symptomatic response with an improvement of quality of life.

These responses rates are comparable with data from literature [11, 24, 25] demonstrating radiological response of 34.1% and clinical response in 29.7% for ^{90}Y-PRRT with longer median survival in responders compared to nonresponders (44.7 versus 18.3 months) and response rates of up to 30% with median time to progression of 40 months for ^{177}Lu-PRRT [26]. Interestingly, in these

Table 4: Baseline and end-treatment SUV max values recorder for all patients in the main lesion assessed by ^{68}Ga-peptide PET/CT tabulated on the basis of patients' functional response.

	Main lesion					
	Baseline SUV max value			End-treatment SUV max value		
Functional response	Mean	Median	Range	Mean	Median	Range
CR	45.3	45.3	—	0.9	0.9	—
PR	79.7	29.2	6.3–119.9	39.2	17.8	3.2–49.1
SD	31.1	20.9	6.6–82.0	31.2	28.2	11.2–61.3
PD[ϛ]	27.9	22	11.7–55.8	35.7	26	15.7–74.7

[ϛ] Data from the 4 patients in which PD was assessed using ^{68}Ga-peptide PET/CT.

Table 5: Results of treatment responses tabulated on the basis of primary tumor site.

Site of primitive tumor	CR	PR	SD	PD
GI (19/59)	—	8/19 (42%)	9/19 (47%)	2/19 (11%)
Pancreas (16/19)	1/16 (6%)	5/16 (31%)	6/16 (38%)	4/16 (25%)
Lung (13/59)	—	8/13 (62%)	3/13 (23%)	2/13 (15%)
Unknown origin (11/59)	—	3/11 (27%)	6/11 (55%)	2/11 (18%)

studies the degree of uptake on the pretreatment ^{111}In-peptide was found to be predictive of response to treatment and overall survival. Despite the small number of patients, combined treatments with labeled peptide using both ^{90}Y and ^{177}Lu seem to perform better (no evidence of PD) when compared to "single radionuclide" PRRT (8 and 2 cases of PD administering only ^{90}Y-peptide and ^{177}Lu-peptide, resp.). In our patients population, the SUVmax value in tha main lesion at baseline ^{68}Ga-peptide PET/CT examination compared to end-treatment scan was in line with the functional response evaluation. Additionally, in our series of patients, cumulated administered activity was significantly different in responders and no-responders. In agreement with previous reports in literature [25] our data supported the hypothesis that progression at baseline could be a prognostic factor of objective response to PRRT. Indeed previous reports showed also that both SD and objective response (CR+PR) in previously progressive patients showed the same favorable trend [25]. Finally, PRRT showed beneficial effect on symptoms in the majority of patients (36%) and all asymptomatic patients (46%) remained stable over the time. In 39% of our patients discordant results between serum CgA trend and ^{68}Ga-peptide findings were observed. Particularly, CgA values increased (with variation up to +263%) in 68% of patients in which PRRT determined PR or SD. Our results on CgA trend and antitumor activity are in contrast with previous reported ones in the literature [25]. However, the majority of our patients assumed proton-pump inhibitor as prophylactic therapy and as well known these drugs may cause substantial increase of blood CgA levels [27] underling the need of a more reliable biomarker to monitoring NETs [28].

The prevalence of partial responses and stable disease obtained is mainly related to the advanced stage of the disease when patients are referred to PRRT. In fact, the majority of patients have entered the protocol after the failure of multiple types of treatment situation when more affords are needed to avoid toxicity (i.e., accurate dosimetric evaluation). In this setting of patients an individualized dosimetry has been demonstrated as the ideal method to plan PRRT in order to combine the highest possible dose of radiation to the tumor with the maximum tolerated dose by the dose-limiting organ [29]. In fact, since the absorbed dose to the kidneys and bone marrow might vary considerably between patients, using fixed dose regime side effects may be reduced but at the cost of undertreatment for certain patients, thus, reducing the potential effectiveness of the PRRT.

The fractionated schedule that we apply in the protocol was selected based on literature data and personal experience. Treatment with this schedule was generally well tolerated with the most common side effects of nausea and vomiting being caused by the administration of amino acid solutions. Reversible bone marrow suppression was seen in 20% of patients. However, in 3 patients we also observed delayed nephrotoxicity requiring in one case dialysis. According to literature data [30] we observed nephrotoxicity in case of ^{90}Y-PRRT; however, the presence of high-risk comorbidities in 2/3 cases (only one kidney and blood hypertension plus diabetes each) is not negligible especially if we consider that a low BED threshold (<28 Gy) have been maintained in these specific patients. In all these patients PRRT was administered before the availability of Lu-177. No myelodysplastic syndrome or acute leukemia occurred.

Despite these interesting results, our study presents some limitations. First the choice of radionuclide used for PRRT was based in some cases on radiopharmaceutical availability at our center. Secondly we evaluate only the functional response. The reason for this choice lies in the fact that the majority of patients (42/59) had liver lesions and their sizes were difficult to accurately be measured using the CT component of PET/CT images without contrast medium. Finally, as previously described, histopathological features of NETs including grading were not comparable avoiding the possibility to further speculate on results.

Table 6: Results of treatment responses tabulated on the basis of the type of treatment.

Type of treatment	CR	PR	SD	PD
^{90}Y-PRRT (33/59)	1/33 (3%)	13/33 (40%)	11/33 (33%)	8/33 (24%)
^{177}Lu-PRRT (10/59)	—	2/10 (20%)	6/10 (60%)	2/10 (20%)
Both ^{90}Y-PRRT and ^{177}Lu-PRRT (16/59)	—	9/16 (56%)	7/16 (44%)	—

Table 7: Results of treatment responses tabulated on the basis of numbers of PRRT cycles.

Numbers of cycles	CR	PR	SD	PD[#]
2-3 (11/59)	1/11 (9%)	5/11 (46%)	2/11 (18%)	3/11 (27%)
4-5 (35/59)	—	14/35 (40%)	17/35 (49%)	4/35 (11%)
>5 (10/59)	—	5/10 (50%)	5/10 (50%)	—

[#]Three patients were treated with only one cycle.

Table 8: Results of functional response to PRRT assessed by ^{68}Ga-peptide PET/CT based on clinical benefit evaluation.

	Functional response			
Clinical response	CR	PR	SD	PD
Clinical benefit	—	5	16	—
SD	—	—	1	1
PD	—	—	—	9
Asymptomatic patients	1	19	7	—

Table 9: Trend of serum CgA values comparing baseline and end-treatment levels are tabulated on the basis of patients' functional response in the 23/59 cases in which discordant results between CgA trend and ^{68}Ga-peptide PET/CT results were observed.

Functional response	CgA values		
	Increased	Stable[$]	Decreased
PR (9/23)	7*	2	—
SD (10/23)	6^	—	4[§]
PD (4/23)	—	4	—

[$]Variation $\leq 10\%$; *mean variation = 271 ± 220 (range 15–682); ^mean variation = 104 ± 119 (range 3–305); [§]mean variation = 669 ± 554 (range 41–1343).

5. Conclusions

Our study demonstrated that PRRT using a fractionated treatment protocol with the intravenous administration of an average activity of 2.6 GBq/cycle for ^{90}Y-PRRT and 6.0 GBq/cycle for ^{177}Lu-PRRT, respectively, and with an interval of about 2 months is a feasible therapeutic option for patients with neuroendocrine tumors able to induce disease control in up to 83% of patients, associated with significant clinical response. The use of ^{68}Ga-peptide PET/CT as first-selection procedure to determine the presence of high SSTR expression followed by standard dosimetric estimates may be used for patients selection.

However, there is the clinical need of randomized clinical trials to determine what is optimal treatment schedule based on the specific biological and molecular features of the tumor. Future therapeutic trials should also aim to include patients at earlier stage of disease and to investigate the best setting where to introduce radioreceptor therapy in combination, rather than an alternative, to other treatment options.

Conflict of Interests

The authors report there is no conflict of interests.

References

[1] J. C. Yao, M. Hassan, A. Phan et al., "One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States," *Journal of Clinical Oncology*, vol. 26, no. 18, pp. 3063–3072, 2008.

[2] L. Bodei, D. Ferone, C. M. Grana et al., "Peptide receptor therapies in neuroendocrine tumors," *Journal of Endocrinological Investigation*, vol. 32, no. 4, pp. 360–369, 2009.

[3] G. Rindi, G. Klöppel, H. Alhman et al., "TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system," *Virchows Archiv*, vol. 449, no. 4, pp. 395–401, 2006.

[4] M. H. Kulke, L. L. Siu, J. E. Tepper et al., "Future directions in the treatment of neuroendocrine tumors: consensus report of the National Cancer Institute Neuroendocrine Tumor clinical trials planning meeting," *Journal of Clinical Oncology*, vol. 29, no. 7, pp. 934–943, 2011.

[5] G. A. Kaltsas, A. Rockall, D. Papadogias, R. Reznek, and A. B. Grossman, "Recent advances in radiological and radionuclide imaging and therapy of neuroendocrine tumours," *European Journal of Endocrinology*, vol. 151, no. 1, pp. 15–27, 2004.

[6] A. Versari, L. Camellini, G. Carlinfante et al., "Ga-68 dOTA-TOC PET, endoscopic ultrasonography, and multidetector CT in the diagnosis of duodenopancreatic neuroendocrine tumors: a single-centre retrospective study," *Clinical Nuclear Medicine*, vol. 35, no. 5, pp. 321–328, 2010.

[7] R. P. Baum, I. Virgolini, V. Ambrosini et al., "Procedure guidelines for PET/CT tumour imaging with ^{68}Ga-DOTA-conjugated peptides: ^{68}Ga-DOTA-TOC, ^{68}Ga-DOTA-NOC, ^{68}Ga-DOTA-TATE," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 37, no. 10, pp. 2004–2010, 2010.

[8] N. Naswa, P. Sharma, A. Kumar et al., "Gallium-68-DOTA-NOC PET/CT of patients with gastroenteropancreatic neuroendocrine tumors: a prospective single-center study," *American Journal of Roentgenology*, vol. 197, no. 5, pp. 1221–1228, 2011.

[9] M. de Jong, B. F. Bernard, E. De Bruin et al., "Internalization of radiolabelled [DTPA^0]octreotide and [DOTA^0, Tyr^3]octreotide: peptides for somatostatin receptor-targeted scintigraphy and radionuclide therapy," *Nuclear Medicine Communications*, vol. 19, no. 3, pp. 283–288, 1998.

[10] G. D. Slooter, W. A. Breeman, R. L. Marquet et al., "Antiproliferative effect of radiolabeled octreotide in a metastases model in rat liver," *International Journal of Cancer*, vol. 81, pp. 767–771, 1999.

[11] I. J. Virgolini, M. Gabriel, E. von Guggenberg, D. Putzer, D. Kendler, and C. Decristoforo, "Role of radiopharmaceuticals in the diagnosis and treatment of neuroendocrine tumours," *European Journal of Cancer*, vol. 45, supplement 1, pp. 274–279, 2009.

[12] E. Bombardieri and I. Virgolini, "Diagnosis and therapy of neuroendocrine tumours," *Quarterly Journal of Nuclear Medicine and Molecular Imaging*, vol. 54, no. 1, pp. 1–2, 2010.

[13] J. Kunikowska, L. Królicki, A. Hubalewska-Dydejczyk, R. Mikołajczak, A. Sowa-Staszczak, and D. Pawlak, "Clinical results of radionuclide therapy of neuroendocrine tumours with ^{90}Y-DOTATATE and tandem ^{90}Y/^{177}Lu-DOTATATE: which is a better therapy option?" *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 38, no. 10, pp. 1788–1797, 2011.

[14] V. Ambrosini, M. Fani, S. Fanti et al., "Radiopeptide imaging and therapy in Europe," *Journal of Nuclear Medicine*, vol. 52, supplement 2, pp. 42S–55S, 2011.

[15] E. Grassi, R. Sghedoni, M. Asti, F. Fioroni, D. Salvo, and G. Borasi, "Radiation protection in 90Y-labelled DOTA-D-Phe1-Tyr3-octreotide preparations," *Nuclear Medicine Communications*, vol. 30, no. 2, pp. 176–182, 2009.

[16] C. Wehrmann, S. Senftleben, C. Zachert, D. Müller, and R. P. Baum, "Results of individual patient dosimetry in peptide receptor radionuclide therapy with ^{177}Lu DOTA-TATE and ^{177}Lu DOTA-NOC," *Cancer Biotherapy and Radiopharmaceuticals*, vol. 22, no. 3, pp. 406–416, 2007.

[17] W. A. Breeman, M. T. De Jong, E. De Blois, B. F. Bernard, M. De Jong, and E. P. Krenning, "Reduction of skeletal accumulation of radioactivity by co-injection of DTPA in [^{90}Y-DOTA^0,Tyr^3]octreotide solutions containing free ^{90}Y^{3+}," *Nuclear Medicine and Biology*, vol. 31, no. 6, pp. 821–824, 2004.

[18] C. Decristoforo, R. Knopp, E. von Guggenberg et al., "A fully automated synthesis for the preparation of 68Ga-labelled peptides," *Nuclear Medicine Communications*, vol. 28, no. 11, pp. 870–875, 2007.

[19] M. Asti, G. De Pietri, A. Fraternali et al., "Validation of ^{68}Ge/^{68}Ga generator processing by chemical purification for routine clinical application of ^{68}Ga-DOTATOC," *Nuclear Medicine and Biology*, vol. 35, no. 6, pp. 721–724, 2008.

[20] M. G. Stabin, R. B. Sparks, and E. Crowe, "OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine," *Journal of Nuclear Medicine*, vol. 46, no. 6, pp. 1023–1027, 2005.

[21] M. Cremonesi, M. Ferrari, S. Zoboli et al., "Biokinetics and dosimetry in patients administered with ^{111}In-DOTA-Tyr^3-octreotide: implications for internal radiotherapy with ^{90}Y-DOTATOC," *European Journal of Nuclear Medicine*, vol. 26, no. 8, pp. 877–886, 1999.

[22] National Cancer Institute, *CTEP: NCI Guidance on CTC Terminology Applications*, National Cancer Institute, Bethesda, Md, USA, 2006.

[23] M. de Jong, W. A. Breeman, R. Valkema, B. F. Bernard, and E. P. Krenning, "Combination radionuclide therapy using ^{177}Lu and ^{90}Y-labeled somatostatin analogs," *Journal of Nuclear Medicine*, vol. 46, supplement 1, pp. 13S–17S, 2005.

[24] M. Gabriel, U. Andergassen, D. Putzer et al., "Individualized peptide-related-radionuclide-therapy concept using different radiolabelled somatostatin analogs in advanced cancer patients," *Quarterly Journal of Nuclear Medicine and Molecular Imaging*, vol. 54, no. 1, pp. 92–99, 2010.

[25] L. Bodei, M. Cremonesi, C. M. Grana et al., "Peptide receptor radionuclide therapy with ^{177}Lu-DOTATATE: the IEO phase I-II study," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 38, no. 12, pp. 2125–2135, 2011.

[26] D. J. Kwekkeboom, W. W. de Herder, B. L. Kam et al., "Treatment with the radiolabeled somatostatin analog [^{177}Lu-DOTA0,Tyr^3]octreotate: toxicity, efficacy, and survival," *Journal of Clinical Oncology*, vol. 26, no. 13, pp. 2124–2130, 2008.

[27] P. Glinicki and W. Jeske, "Chromogranin A (CgA)—the influence of various factors in vivo and in vitro, and existing disorders on it's concentration in blood," *Endokrynologia Polska*, vol. 61, no. 4, pp. 384–387, 2010.

[28] T. Ito, H. Igarashi, and R. T. Jensen, "Serum pancreastatin: the long sought universal, sensitive, specific tumor marker for neuroendocrine tumors?" *Pancreas*, vol. 41, no. 4, pp. 505–507, 2012.

[29] L. Bodei, M. Cremonesi, M. Ferrari et al., "Long-term evaluation of renal toxicity after peptide receptor radionuclide therapy with ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE: the role of associated risk factors," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 35, no. 10, pp. 1847–1856, 2008.

[30] R. Barone, F. Borson-Chazot, R. Valkema et al., "Patient-specific dosimetry in predicting renal toxicity with ^{90}Y-DOTATOC: relevance of kidney volume and dose rate in finding a dose-effect relationship," *Journal of Nuclear Medicine*, vol. 46, supplement 1, pp. 99S–106S, 2005.

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 635614, 19 pages
doi:10.1155/2012/635614

Review Article

The Insulin and IGF-I Pathway in Endocrine Glands Carcinogenesis

Roberta Malaguarnera, Alaide Morcavallo, and Antonino Belfiore

Endocrinology, Department of Health Sciences, Magna Græcia University of Catanzaro, Campus Universitario, Località Germaneto, 88100 Catanzaro, Italy

Correspondence should be addressed to Antonino Belfiore, belfiore@unicz.it

Received 3 May 2012; Accepted 20 June 2012

Academic Editor: Marialuisa Appetecchia

Endocrine cancers are a heterogeneous group of diseases that may arise from endocrine cells in any gland of the endocrine system. These malignancies may show an aggressive behavior and resistance to the common anticancer therapies. The etiopathogenesis of these tumors remains mostly unknown. The normal embryological development and differentiation of several endocrine glands are regulated by specific pituitary tropins, which, in adult life, control the function and trophism of the endocrine gland. Pituitary tropins act in concert with peptide growth factors, including the insulin-like growth factors (IGFs), which are considered key regulators of cell growth, proliferation, and apoptosis. While pituitary TSH is regarded as tumor-promoting factor for metastatic thyroid cancer, the role of other pituitary hormones in endocrine cancers is uncertain. However, multiple molecular abnormalities of the IGF system frequently occur in endocrine cancers and may have a role in tumorigenesis as well as in tumor progression and resistance to therapies. Herein, we will review studies indicating a role of IGF system dysregulation in endocrine cancers and will discuss the possible implications of these findings for tumor prevention and treatment, with a major focus on cancers from the thyroid, adrenal, and ovary, which are the most extensively studied.

1. Introduction

Endocrine malignancies, including carcinomas of the thyroid, adrenal, and ovary, are relatively rare tumors deriving from cells present in endocrine glands. Surgery is currently the treatment of choice for these tumors and is often successful in early stages of disease. However, this therapeutic approach for the advanced tumors remains unsatisfactory and is associated with poor prognosis. Thus, a better understanding of the molecular mechanisms and the critical intracellular networks underlying endocrine oncogenesis may help in discovering new targets that could represent promising therapeutic options for these malignancies. As specific pituitary tropins control the trophism and function of specific endocrine glands, it is tempting to speculate about a possible role for these pituitary hormones in endocrine glands tumorigenesis. However, this assumption is controversial as other signaling effectors, including the IGF system, have often a major role in endocrine tumorigenesis.

This paper covers the recent molecular advances in this field focusing on the role of the IGF system in endocrine tumorigenesis with particular attention on the endocrine cancers best characterized until now (i.e., thyroid, adrenal, and ovarian tumors).

2. Regulation of Thyroid, Adrenocortical, and Ovarian Tumor Growth: The Role of Pituitary Hormones

Thyroid cancer growth regulation has been extensively characterized. Several molecular alterations associated with thyroid tumorigenesis have been identified and often converge into the activation of MAPK (mitogen-activated protein

kinase) and PI3K (phosphatidylinositol-3-kinase) signaling pathways [1].

Thyroid gland function and trophism is mainly regulated by thyrotropin hormone (TSH). TSH is considered the key player of thyrocyte differentiation and proliferation. Its mitogenic actions are mainly mediated by cAMP, which in turn activates protein kinase A (PKA) dependent and independent pathways. Activating mutations of the TSH receptor (TSHR) or of the gene encoding the $G_s\alpha$ subunit of the heterotrimeric G protein that couples TSHR to adenylyl cyclase (GSP) have been described in 30% of autonomously functioning thyroid adenomas while they are rare in thyroid carcinomas [2, 3]. TSH, however, has a well-known promoting role for thyroid cancer metastases, and TSH suppressive therapy with L-thyroxine is a well-established therapy in the postoperative management of differentiated thyroid cancer [4]. To exert its maximal mitogenic effects, TSH requires concomitant ligand-activated tyrosine kinase receptor (RTK) signaling. Studies carried out in thyroid cell cultures have especially highlighted the importance of the IGF system in regulating thyroid cell growth in response to TSH [5, 6].

TSH makes the cells competent to progress into the G1 phase in response to insulin or IGF-I, which can thus be qualified as the only genuine mitogens [7]. In fact, the protumorigenic effects of TSH are irrelevant in the absence of growth factors, but they are greatly potentiated by the presence of insulin or IGF-I at physiological concentrations [5, 8]. Furthermore, we have recently reported a key role of the IGF system in the biology of follicular thyroid progenitor/stem cells [9]. Insulin/IGF-I signaling pathways are important also in the regulation of thyroid-specific genes transcription, including the TSH receptor [10], thyroglobulin (Tg), and thyroperoxidase (TPO) [11, 12]. Gene expression of both Tg [13] and TPO [14] is mediated predominantly by thyroid transcription factor-2 (TTF-2), a thyroid-specific transcription factor that binds to the promoter of both genes [15, 16] and is stimulated by both the cAMP and the insulin/IGF-I pathways, which may have additive effects [17].

Regarding the adrenocortical cancers, their molecular pathogenesis is still incompletely understood. In contrast to thyroid carcinomas, the cAMP/PKA pathway seems to be less involved in the development of these tumors. Although pituitary adrenocorticotropic hormone (ACTH) stimulates adrenal function by inducing steroidogenic enzymes and increases adrenal gland weight, the proliferative action of ACTH for adrenal tumors has been questioned, and opposite effects, under defined cell culture conditions, have been reported. *In vitro* inhibition of adrenal cell proliferation by physiological ACTH concentrations has been reported by several groups [18–21]. In support of the growth-inhibiting effect of ACTH, no activating mutations of the ACTH receptor have been found in benign or malignant adrenocortical tumors [22, 23]. Conversely, allelic loss of the ACTH receptor gene has been reported in a subset of sporadic benign and malignant adrenocortical tumors where it was associated with undifferentiated phenotype and worse prognosis [24]. These data tend to exclude a role of ACTH receptor as putative oncogene in adrenal oncogenesis while supporting its role as tumor suppressor. In summary, in the adrenal cortex, the ACTH/PKA signaling is mainly involved in regulating steroid hormone synthesis and cellular differentiation rather than in controlling cellular proliferation and tumor growth. Similarly to thyroid cancer, molecular alterations frequently observed in adrenocortical carcinoma include deregulation of the IGF system as well as mutations in p53 and RAS [25]. In addition to IGF-II overexpression, increased levels of the IGF-IR and IGFBP-2 have been found in advanced human adrenal carcinomas, resulting in increased IGF-dependent cell proliferation and inhibition of the ACTH antiproliferative effect. Although the functional significance of the strong and specific overexpression of IGFs in adrenocortical carcinomas remains still unknown, these factors may regulate both steroidogenic and mitogenic effects and, similarly to what is seen in thyroid cancer, establish autocrine positive loops that promote growth advantage and transformation toward a more malignant phenotype.

In ovarian cancers, the role of the pituitary tropins is still controversial. Pituitary LH and FSH lead to increased sex steroids secretion which may favor ovarian cancer development [26, 27]. A role for gonadotropins in ovarian tumorigenesis is also supported by the observation that ovarian cancer incidence reaches a peak in the postmenopausal period, during which FSH levels are particularly high [28]. Yet, a study on normal rabbit ovarian surface epithelium showed that FSH and LH/hCG stimulate growth *in vitro* [29]. However, controversial results have been obtained by different research groups. For instance, a more recent study has reported no increase in cell proliferation with LH [30]. Although the mechanisms by which FSH and LH stimulate or inhibit the proliferation of ovarian epithelium remain still unknown, these hormones exert their effects interacting with their specific receptors. Some ovarian cancers, especially those poorly differentiated, lose FSH receptor (FSHR) expression [30]. This observation suggests that FSH may be a growth-promoting factor important at early stages of ovarian epithelial tumorigenesis, with some ovarian tumors losing their requirement for FSH later in tumor development. Like adrenal and thyroid cancers, also in ovarian tumors, IGF system components are often overexpressed. IGF-II appears to increase proliferation and induce differentiation in granulosa cells via the IGF-IR and synergizes with FSH to induce steroidogenesis as well as mitogenesis [31]. This synergism likely involves IGF-induced upregulation of the FSHR [31, 32] and/or the involvement of intracellular mechanisms leading to the activation of intracellular pathways (i.e., protein kinase C, cAMP, MAPK, and PI3K).

A schematic representation of the interplay between pituitary hormones and main signaling pathways of the IGF system in thyroid, adrenal, and ovary cancers is shown in Figure 1.

Altogether, the lines of evidence reported in these three tumor histotypes suggest that, although the specific pituitary tropin exerts an important role in regulating the growth, differentiation, and function of the target endocrine gland, the interplay of pituitary hormones with other factors, such as the IGF system, is crucial for deregulated cell proliferation

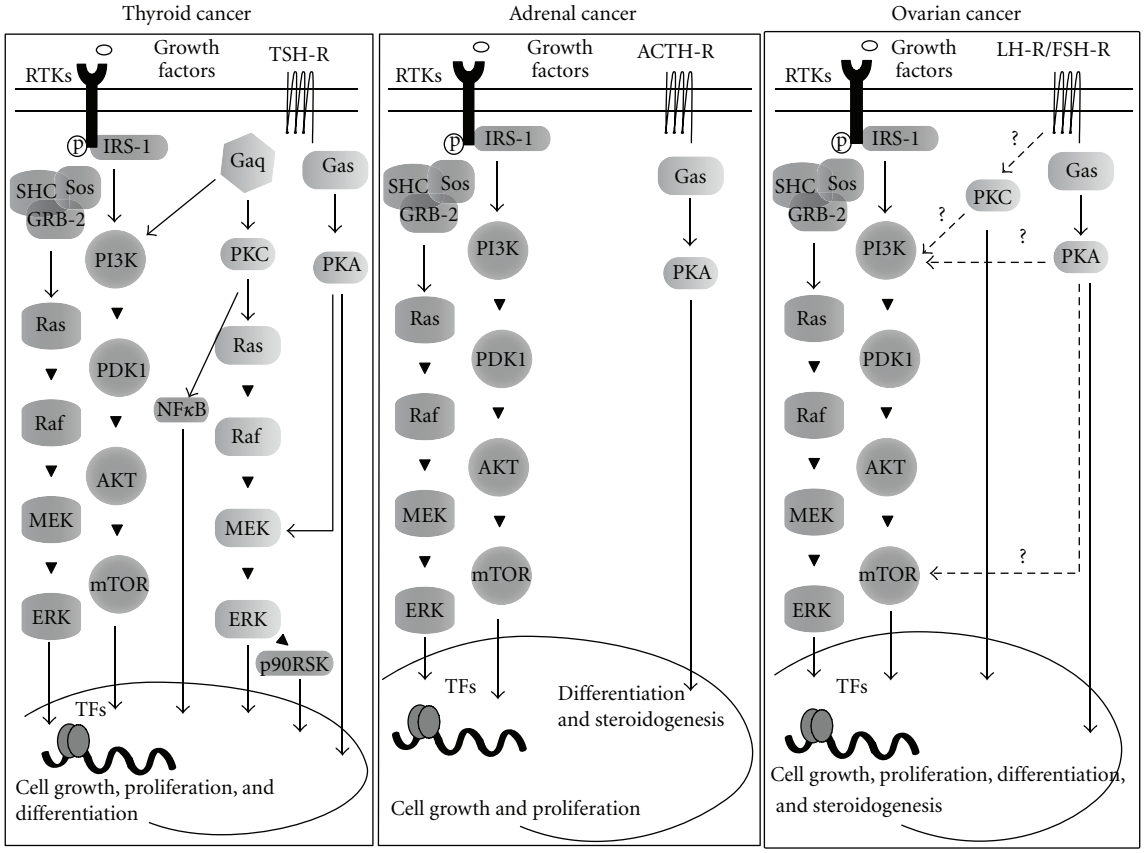


Figure 1: Schematic representation of the interplay between pituitary hormones and main signaling pathways of the IGF system in thyroid, adrenal and ovary cancers. Dot lines: proposed signaling pathways.

and transformation. The IGF system may represent, therefore, a promising therapeutical target for these tumors.

3. The IGF System and Its Involvement in Cancer

In mammals the IGF system includes four receptors (the insulin receptor (IR), the IGF-I receptor (IGF-IR), the insulin-receptor-related receptor (IRR), and the Mannose-6-phosphate/IGF-II receptor (IGF-IIR)), four ligands (proinsulin, insulin, IGF-I, and IGF-II), and six high-affinity binding proteins (IGFP-1 to 6). The human IR exists in two isoforms (IR-A and IR-B) generated by alternative splicing of the IR gene with the exclusion (IR-A) or inclusion (IR-B) of 12 amino acids encoded by exon 11. The IR and the IGF-IR have highly homologous structure, but different functions. Given the high degree of homology, IR and IGF-IR can heterodimerize leading to the formation of insulin/IGF-I hybrid receptors (HRs) [33, 34]. The IGF-IIR is a structurally distinct cell surface receptor whose major function is to induce internalization and degradation of IGF-II, thus modulating its extracellular levels [34].

With regard to the ligands, insulin and IGFs are related peptides, involved in metabolism as well as in growth and reproduction. Insulin largely circulates in free form while more than 90% of IGFs circulates bound to a complex family of IGF-binding proteins (IGFBPs), which regulate both the half-life and the biological effects of IGFs [35]. Insulin and IGFs bind with different affinity IR isoforms and IGF-IR (for more details see [36–38]).

Recently, it has been reported that proinsulin, the insulin prohormone, which is characterized by low metabolic activity compared to mature insulin, is a selective IR-A ligand and may exert a putative role on growth and cell proliferation [39, 40].

After ligand binding, phosphorylated receptors activate two main signaling pathways, the PI3K and the MAPK cascade, involved in the regulation of cell metabolism, proliferation, and survival. Although both the IR and IGF-IR similarly activate these signaling networks, subtle differences exist in the recruitment of certain intracellular mediators and substrates between the two receptors, leading to the specific biological effects of each hormone. Details regarding the IGF system have been previously covered by several reviews to which we refer for more information [34, 36, 41].

Since the IGF system exerts a pivotal role in cell growth and homeostasis, it is not surprising that aberrant expression of receptors belonging to this system might be involved in cancer development, progression, and metastasis. The key role of IGF-IR in oncogenic transformation derives from the

studies showing that IGF-IR null cells cannot be transformed by several cellular or viral oncogenes, whereas they become susceptible to the oncogenic mediated transformation after the reintroduction of a functional IGF-IR [42, 43]. However, increased levels of IGF-IR do not result in autonomous receptor signaling in the absence of IGF ligand, while it can induce malignant transformation in presence of its specific ligands [44]. Similarly, in estrogen responsive breast cancer cell lines, growth response to insulin could be specifically inhibited by anti-IR but not anti-IGF-IR blocking antibodies while it can be mimicked by an anti-IR stimulating antibody [45]. These data are in agreement with studies indicating that IR-transfected cells acquire insulin-dependent malignant changes [46, 47] and support the notion that IR may elicit mitogenic and antiapoptotic effects similar to IGF-IR, contributing to cancer development and progression. The first direct evidence that IR may be overexpressed in cancer cells was reported by Papa et al. in breast tumors [37]. Subsequent studies demonstrated that IR is also overexpressed in other human malignancies, including endocrine tumors, such as cancer of the thyroid, ovary, and adrenal glands [38, 48–50]. In most of these tumors, cell growth is dependent on IR activation by insulin, suggesting a mitogenic role of this hormone [51], although IR, isoform A, may also be activated by IGF-II [46, 52, 53]. Both IR isoforms may be overexpressed in cancer, but usually IR-A is predominant, representing 60–100% of total IR. Data showing an increased relative abundance of IR-A are also available for certain endocrine cancers [38, 48, 54]. This observation is particularly interesting, as IR-A is mainly expressed in fetal life, while IR-B predominates in differentiated tissues [38, 55]. Furthermore, at variance with IR-B, which is a highly specific receptor for insulin, IR-A is a high-affinity receptor for insulin; it shows intermediate affinity for IGF-II and low affinity for IGF-I [38]. Although IGF-II is able to bind both to IGF-IR and IR-A with similar affinity, its binding to IR-A has important implications. Indeed, IR-A overexpression amplifies IGF-II effects in cancer cells and serves as a signaling diversification factor, as IR-A and IGF-IR activate different downstream signals.

Because of the high homology between the IR and the IGF-IR [56], in cells coexpressing IRs and IGF-IR [57] hybrid IR/IGF-IR receptors (HRs) may form [58–60]. Functionally, HRs are considered high-affinity IGF-I-binding sites as they bind insulin with much lower affinity [60]. In thyroid cancers large amounts of HRs have been measured both in well-differentiated papillary carcinomas and in poorly differentiated/undifferentiated carcinomas, probably as a consequence of increased IR expression [33]. In these tumors, HRs account for 50–75% of the total IGF-I binding sites and mediate IGF-I mitogenic signaling. No data are available regarding HRs expression in other endocrine malignancies.

3.1. IR and IGF-IR Signaling Pathways: Relevance to Endocrine Malignancies. IR and IGF-IR share many similarities not only in their structures but also in their downstream signaling pathways. Upon ligand binding, the intrinsic tyrosine kinases of both IR and IGF-IR are activated and this results in the phosphorylation of several receptor substrates including the components of the IRS family and Shc. These substrates, in turn, act as multisite "docking" proteins for kinases and adaptors, such as PI3K, Syp, Fyn, Nck, and Grb2, which trigger the activation of downstream kinase cascades [61]. IRS proteins are also involved in the crosstalk with other signaling pathways, including those coming from other growth factors [62], cytokines [63], and integrins [64].

The two main signaling pathways downstream to IR and IGF-IR include the mitogen-activated protein kinases cascade (MAPKs), which involves the sequential activation of a cascade of serine/threonine protein kinases with a key role in the regulation of cellular proliferation and gene expression and the PI3K signaling pathway, which mediates metabolic actions but also stimulates cell growth and survival. Both MAPK and PI3K pathways enhance protein synthesis through mTOR activation and trigger antiapoptotic effects through the phosphorylation and inactivation of Bad [65]. Molecular alterations (mutational and nonmutational) in both PI3K and MAPK have been reported in several malignancies including those from thyroid, ovary, and adrenal glands.

Conditional or constitutive deregulation of MAPK and PI3K cascades is a common event in thyroid cancer and may play a pathogenetic role in this tumor [1]. Indeed, deregulated activation of the MAPK cascade via mutations and/or rearrangements in RET, RAS, and BRAF genes occurs in ~70% of papillary thyroid carcinomas (the most common subtype of thyroid cancers) [66–68]. Thyroid carcinomas also show mutations in PI3K signaling effectors such as PTEN and phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA). PTEN is downregulated in ~37% of well-differentiated thyroid carcinomas and downregulated or lost in >50% of highly malignant thyroid cancers [69]; point mutations or copy number changes in PIK3CA are found in ~23% of anaplastic thyroid cancers where they can coexist with either RAS or BRAF mutations [70].

In adrenal cancer, pathway analysis for the genomic regions associated with poor prognosis has shown deletions of genes that negatively regulate the activation of ERK1/2 and loss of PTEN gene [71]. Yet, several reports have identified activating RAS mutations [72, 73], while only two papers have analyzed mutations in BRAF gene and found that their prevalence is low [74, 75]. Although functional studies are needed to better characterize the effect of these mutations in adrenocortical tumors, it is possible that deregulation in the MAPK pathway may significantly contribute to aggressive phenotypes.

In ovarian cancer, mutually exclusive mutations of KRAS and BRAF have been described in about 30–50% of low-grade tumors [76–79], while they are rare in high-grade tumors. RAS mutations may promote ovarian tumorigenesis not only through MAPK but also via the interaction with the PI3K/AKT pathway. In ovarian cancers PI3K activation, occurring via either PIK3CA gene amplification/mutations or PTEN protein loss, has been reported by several studies [80–83] with the highest frequency in most malignant histotypes [84].

In the context of the three endocrine tumors mentioned above, the dysregulation of the IGF system may represent one nonmutational mechanism activating MAPK and PI3K signaling cascades. The increased IGF-IR-mediated activation of MAPK/PI3K signaling may, in turn, induce IGF-IR and/or its ligands expression and reduce the expression of IGFBP-3 [85–87]. This close relationship between the IGF system and MAPK/PI3K-mediated signals may contribute to cancer development, progression, invasion, and aggressive behavior.

Indeed, via PI3K/AKT/PTEN and ERK dependent mechanisms [88–90], IGFs control several cycle checkpoints, in particular the G0-G1 transition, increasing cyclin D1 and CDK4 gene expression and down-regulating the cyclin-dependent kinase inhibitor (CDKI) p27. Moreover, through the same pathways, IGFs regulate cell invasion and tumor-dependent angiogenesis modulating the expression of molecular mediators of extracellular matrix remodeling and degradation including type IV collagenases, matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), and the membrane type 1 MMP (MMP-14) [91, 92]. These enzymes play an important role in malignant progression and metastatic spread of solid tumors, including endocrine ones. MMPs expression has been found to be elevated in papillary thyroid cancer as compared with normal thyroid tissue [93, 94]. A strong MMP2 expression has been also found in malignant adrenal tumors and considered an unfavorable prognostic factor [95]. In ovarian cancers, MMPs are frequently overexpressed and appear to be an early event of ovarian tumorigenesis suggesting a role of these enzymes in ovarian tumor initiation and not only in tumor progression and invasion [96].

Relevant crosstalks between the IGF system and other signaling pathways also include the involvement of the janus kinase (JAK)-1/2 mediated signaling and the activation of transcription proteins STAT. In particular, STAT-3 may be required for the maintenance of transforming activity of IGF-IR [97]. IGF-I is able to activate STAT-3, but not STAT-5, and this activation is probably mediated by JAK proteins [98]. These mechanisms have been demonstrated in several models, and they may also occur in endocrine cancers, where both STAT and IGF signaling play an important role in tumor invasion and metastasization. Indeed, the STAT-3 pathway is significantly upregulated in metastatic thyroid papillary cancers, suggesting a potential role for activated STAT-3 in lymphatic metastases [91]. Yet, in both ovarian and adrenal cancers a role for STAT signaling in invasion and cancer prognosis has been also identified [99, 100].

Other molecules interacting with the IGF system and involved in the pathogenesis of thyroid, adrenal, and ovarian cancers include the tumor suppressor p53. Inactivating mutations of p53 gene occur in 10%, 47%, and 25% of thyroid, ovary, and adrenal sporadic carcinomas [101, 102], respectively. However, in all these tumors, also when not mutated, p53 activity may be inhibited by other mechanisms among which are an unbalanced expression of isoforms with a dominant negative function, the interaction with Mdm2, and the cooperation with other members of the p53 family such as TAp63α, TAp73α, and their dominant negative variants (ΔNp63 and ΔNp73) [103]. The activity of wild-type p53 reduces IGF axis activity by multiple mechanisms which include inhibition of IGF-IR [104], IR [105], and IGF-II expression [106] with a concomitant increase of IGFBP-3 transcription [107]. Therefore, aberrant p53 (i.e., p53 lacking its suppressor function through point mutations or via other mechanisms) greatly enhances the activity of IGF axis at multiple levels [104]. In the three endocrine tumors mentioned above, the crosstalk between the IGF system and p53 appears an important prerequisite for oncogene-driven tumor cell trasformation, cancer progression, and resistance to anticancer therapies.

3.2. Circulating Levels of Insulin and IGFs and Endocrine Cancers. Epidemiological studies have shown that elevated plasma concentrations of IGFs are associated to increased risk for the development of several human malignancies including cancers of the breast, colon, and prostate as well as sarcomas [61, 108–113]. For instance, several studies have provided strong evidence that premenopausal, but not postmenopausal, women in the highest tertile of serum IGF-I levels had an increased risk of developing breast cancer [114], and that a high IGF-I : IGFBP-3 ratio may be associated with greater breast density and increased breast cancer risk [108, 115].

High circulating IGFs concentrations may exert biological effects in malignant cells not only through IGF-IR but also via IR-A and HRs. As described in more detail below, all these receptors are overexpressed in endocrine cancers [31, 48] as well as in thyroid cancer stem-like cells [9].

A possible role for serum IGF-I in thyroid cancerogenesis has been suggested by the observation that acromegalic patients, who are exposed to sustained high serum IGF-I levels, show an increased frequency of thyroid cancer [116, 117]. In adrenocortical tumors high serum IGFs and low IGFBP3 levels are correlated with cancer risk and are predictive of metastases development [111, 118].

Finally, some haplotypes and SNPs in the IGF components may influence ovarian risk, either directly or by increasing the IGF-I plasma levels. In particular, the following SNPs in the IGFBPs (rs10228265, rs4988515, rs2270628, rs2854746, and rs2854744), in IGF-I (rs11111285, rs1996656 and rs1019731), and in IGF-II (rs4320932, rs4244809, rs680, rs1003483, and rs7924316) have been associated with increased ovarian cancer risk [119, 120].

Not only IGFs but also circulating insulin has been suggested to be involved in the tumorigenesis process. Indeed, a number of population studies have provided substantial and circumstantial lines of evidence that insulin resistance and hyperinsulinemia, common factors underlying obesity and type 2 diabetes mellitus (T2DM), are strong candidates for the increased cancer risk associated with these disorders [121–123]. Although insulin is considered a hormone regulating energy metabolism, it also exerts proliferative, antiapoptotic, and migratory actions, collectively indicated as "mitogenic effects," via its own receptor (IR). This observation is known from long time and helps in understanding

the link between insulin resistance/hyperinsulinemia and cancer.

The involvement of insulin in cell trasformation and cancer development was firstly suggested by *in vivo* evidence that administration of insulin induced growth of mammary tumor in mice [124] and promoted aberrant crypt foci in the colon of rats [125–127], while insulin deficiency or calorie restriction exerted a protective role [124]. Similarly, in obese mice, insulin levels were positively associated with the proliferation of transplanted lung and colon cancer cells [127].

In light of these experimental lines of evidence, clinical studies have been conducted to investigate the possible role of hyperinsulinemia and insulin resistance in endocrine tumors. At this regard, several case-control and prospective studies have found a strong positive association between overweight/obesity and thyroid cancer risk [128–137], although the data are not entirely consistent [138–145].

The exact nature of the relationship between body mass index (BMI) and thyroid cancer incidence remains still unclear. Besides the high circulating levels of insulin present in overweight/obese patients, other potential mechanisms may include increased levels of inflammatory adipokines [146]. In addition, although obesity is associated with poor prognosis for several malignancies, this relationship has not been reported for thyroid cancer [142].

In partial support with the finding that hyperinsulinemia and insulin resistance are risk factors for thyroid cancer, studies conducted in T2DM patients have shown that higher fasting glucose levels are associated with increased thyroid cancer risk [144, 147]. However, also for this association conflicting results have been obtained [134].

Concerning the association between hyperinsulinemia/insulin resistance and ovarian cancer risk, several lines of evidence suggest that women affected by polycystic ovary syndrome (PCOS), a condition associated with insulin-resistance, are more likely to develop ovarian cancer (OR, 2.5; 95% CI 1.08–5.89) [148]. Furthermore, a meta-analysis of ten cohort studies has shown that overweight and obesity are associated with higher ovarian cancer mortality (OR, 1.6; 95% CI 1.1–2.34) and that, among patients with advanced ovarian cancer, premorbid obesity is associated with worse prognosis (OR, 1.5; 95% CI 1.09–1.93) [149]. However, other studies have not supported these results [150], suggesting that further investigation is needed to firmly establish the association between ovarian cancer and insulin resistance [151].

Finally, it has been suggested that adrenal incidentalomas, usually benign tumors, might be related to hyperinsulinemia and insulin resistance. This hypothesis was postulated for the first time by Reincke et al. [152], who observed a proliferative effect of insulin on adrenal cancer cells without effect on cortisol synthesis [152]. However, a causative role of adrenal incidentalomas for metabolic syndrome cannot be excluded, as some patients show a slight hypercortisolism that may contribute to the insulin resistance. In fact, surgical tumor resection may revert or ameliorate these metabolic alterations [153]. Regarding the link between hyperinsulinemia and malignant adrenal tumors, scanty data are present in the literature so far.

4. IGF System Abnormalities in Specific Endocrine Cancer and Possible Therapeutical Implications

4.1. Thyroid Cancer. Human thyroid carcinomas derived from the thyroid follicular cells (TFCs) include a variety of histotypes ranging from well-differentiated (papillary and follicular) to undifferentiated (anaplastic) cancers. Altogether, they represent approximately 1% (3% in women) of all human cancers [154, 155].

Well-differentiated thyroid carcinomas account for approximately 90% of all thyroid cancers. They retain a variable degree of TSH responsiveness and have a mortality rate of approximately 10%. Poorly differentiated and undifferentiated carcinomas account for only approximately 10% of all thyroid cancers; they have weak or no TSH responsiveness and have a mortality rate ranging from 50% to 100% [48, 156].

As previously mentioned, the IGF-I system plays an important role in regulating normal growth and development in the thyroid [6, 9] and appears also to be involved in thyroid tumorigenesis.

The coexpression of IGF-I and its cognate receptor, IGF-IR, has been documented by various studies in both cultured thyroid cells and tissue specimens. In particular, cultured human and ovine thyrocytes are able to release IGF-I in the culture media [157, 158]. Also, thyroid adenoma cell lines synthesize IGF-I, which stimulates cell growth by autocrine mechanisms [159]. Immunoreactive IGF-I and IGFBPs were also found in the extracts of normal and nodular thyroid tissue specimens obtained at surgery from patients with nontoxic goiter [160–162] (Table 1).

Functional IGF-IR is usually expressed at high levels in thyroid cancer cells. In SW579 thyroid carcinoma cells IGF-I induced angiogenic activity via increased synthesis of HIF-1α transcription factor and consequent stimulation of vascular endothelial growth factor (VEGF) expression [163]. Belfiore et al. measured IGFs and cognate receptors in both thyroid cell lines and tissue specimens. IGF-I content ranged from 104 to 2566 nM/g in cancer tissue and 69 to 680 nM/g in normal thyroid tissue. By using a specific ELISA, they also found that IGF-IR is overexpressed in both thyroid cancer cell lines and specimens as compared to the normal tissue [33] (Table 1).

IGF-IR overexpression in thyroid cancer specimens has been also found by using immunohistochemistry and in situ hybridization, and IGF-I was found to be produced in either paracrine or autocrine manner [8, 164]. IGF-I and IGF-IR immunoreactivity was found to be increased both in adenomas and carcinomas compared with normal thyroid. IGF-I overexpression was more marked in the undifferentiated and poorly differentiated histotypes of thyroid cancer [165].

The above-mentioned study of Vella et al. demonstrated that thyroid cancers overexpress not only IGF-I and IGF-IR, but also IGF-II and IR. In particular, the IGF-II/IR-A autocrine loop is especially activated in poorly differentiated and anaplastic cancers. The relative abundance of IR-A also increases in dedifferentiated cancers. In this context, the

IGF-IR seems less important than IR-A in mediating IGF-II mitogenic effects, and blocking antibodies to IR markedly reduced the effects of IGF-II [48].

The concomitant high expression of both IGF-IR and IR-A in thyroid cancer cells causes overexpression of IR/IGF-IR hybrid receptors, which, in most cases, exceed the IGF-IR content. In cells with a high IR/IGF-IR content, blocking antibodies specific to these receptors substantially inhibited IGF-I-induced cell growth. These data indicate that, in addition to IGF-IR and IR-A, also IR/IGF-IR hybrids may be a target in thyroid cancers [33] (Table 1).

Progenitor/stem cells are increasingly considered to be at the origin of most malignancies [166]. Therefore, we recently isolated progenitor/stem cells from both normal and cancer specimens and cultured them as thyrospheres, in order to study the IGF system in this model [9]. We found that IGF-I and IGF-II are produced at high levels by all thyrospheres. However, the IGF-I : IGF-II ratio was approximately 5 : 1 in normal thyrospheres whereas it was 1 : 1 in cancer thyrospheres. IR and IGF-IR in human thyrospheres were markedly overexpressed and with a higher IR : IGF-IR ratio as compared to primary cultures. The IR : IGF-IR ratio was also higher in cancer than in normal thyrospheres. Receptors (IR and IGF-IR) and ligands (IGF-I and IGF-II), all expressed at high levels in thyrospheres, markedly decreased in differentiating cells. IR-A was the predominant isoform in thyrospheres, especially from cancer, while IR-B was predominant in differentiating cells. IR-A relative abundance was associated with characteristics of stemness and with cancer: it ranged from 65 to 86% in cancer thyrospheres, from 50 to 65% in normal thyrospheres, and from 40 to 45% in normal thyroid primary cultures or differentiated sphere-derived thyrocytes [9]. The expression of IR, IGF-IR, and their ligands was evaluated by quantitative real-time PCR. Western blot analysis for IR and IGF-IR confirmed PCR data. Cancer thyrosphere growth was stimulated by insulin and IGFs, while IGF-II was most potent in inducing cell renewal [9] (Table 1).

Considering the involvement of IGF-I system in thyroid cancer [9, 55, 156], Wang et al. studied the potential therapeutic role of anti-IGF-IR humanized monoclonal antibody A12 both *in vitro* and *in vivo*. In accordance to other studies, they found that IGF-IR is expressed in various human thyroid cancer cell lines and in normal and neoplastic human thyroid tissues, including surgical specimens of papillary and anaplastic carcinomas. IGF-IR antibody A12 was able to significantly inhibit the proliferation of cultured anaplastic cancer cells by downregulating the IGF-IR signaling pathway. Moreover, administration of A12 also reduced tumor volume in an orthotopic anaplastic cancer nude mouse model and prolonged survival [167] (Table 2).

The PPARγ agonists thiazolidinediones and biguanides (metformin) are used as antidiabetic drugs for their insulin-sensitizing effect achieved by different mechanisms [168]. Because of these effects, both these classes of drugs lower circulating insulin levels, and, in principle, they may have favorable effects in patients with IR-overexpressing tumors. Moreover, both thiazolidinediones and metformin have direct and pleiotropic anti-IGF effects in cultured cancer cells. In particular, in anaplastic thyroid cancer (ATC) cells, rosiglitazone antagonized the biological effects of IGF-I by upregulating phosphatase and tensin homolog deleted from chromosome 10 (PTEN) and consequently inhibiting the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway. As a consequence, it reduced anchorage-dependent and -independent growth and migration, increased apoptosis rate, and induced partial redifferentiation in these cancer cells [169]. Rosiglitazone also potentiated the antitumor effect of doxorubicin (Table 2).

Recently, Chen et al. evaluated the effects of metformin, in ATC cell lines and in thyroid cancer stem cells. They found that metformin antagonized the growth-stimulatory effect of insulin in thyroid cancer cell lines. Specifically, metformin inhibited cell cycle progression, inhibited clonal cell growth, and reduced thyroid cancer sphere formation. Moreover, the metformin potentiated the antimitogenic effect of chemotherapeutic agents, such as doxorubicin and cisplatin, in ATC cells [170] (Table 2).

4.2. Adrenal Gland Cancer. Adrenal tumors are classified into benign and malignant groups. Tumor histotypes can be either hormonally silent or hormone secreting. In this case tumors may produce glucocorticoids, androgens, mineralocorticoids, estrogens, and combinations thereof [171]. The vast majority of adrenocortical tumors are benign, while adrenocortical carcinomas (ACCs) are relatively rare; they presents with extremely poor prognosis as a consequence of metastases or local invasion [172]. The frequency of small benign adrenocortical tumors increases with age, ranging between 3 and 7% of all adrenal carcinomas in adults over 50 years. However, ACCs account for only 0.05–0.2% of all cancers [173], with an estimated incidence between 1 and 2 per million and per year in adults in North America and Europe [174, 175]. In children, the incidence is approximately 10-fold lower except in South Brazil where there is a high incidence of pediatric ACC [176].

As previously mentioned, the IGF system has a physiological role in normal adrenal growth and development [177] and is also involved in ACC proliferation and progression [178]. An increased expression of the IGF-IR was demonstrated in SW13 and in H295R human adrenocortical carcinoma cell lines [179]. Both IGF-I and IGF-II are produced by H295R cells [180], and IGF-II increases during proliferation. In the same cell model an IGF-IR blocking antibody (α-IR3) was able to hamper cell growth, demonstrating that autocrine IGF-II production may stimulate cell growth through the IGF-IR [181] (Table 1).

In the reticularis layer of normal adrenal tissues a large number of IGF-I-positive cells with granular cytoplasmic (GC) staining pattern are present [182]. The proportion of these cells increases with the tumorigenesis process; hyperplastic glands show 10–50% of IGF-I-positive cells, while adenomas and carcinomas have over 50% of IGF-I positive cells in 64% and 83% of cases, respectively. Similarly, the IGF-IR is more expressed in adenomatous adrenal tissues than in nontumoral tissues [179]. IGF-II is one of the most expressed genes in adrenocortical carcinomas [183, 184]. The IGF-II gene is located at locus 11p15, which is

Table 1: Main molecular alterations involving the IGF system components in thyroid, adrenal, and ovarian cancer.

Molecular alteration	Thyroid cancer	Adrenal cancer	Ovarian cancer
IGF-IR overexpression	+	+	+
IR overexpression	+ (IR-A)	?	+ (IR-A)
HRs overexpression	+	?	?
IGFs overexpression	+ (IGF-I and IGF-II)	+ (IGF-II > IGF-I)	+ (IGF-II > IGF-I)
IGFs autocrine production	+ (IGF-I and IGF-II)	+ (IGF-II > IGF-I)	+ (IGF-I and IGF-II)
IGF-II/IR-A loop activation	+	?	+
IGFBPs overexpression	+	+ (IGFBP-2, IGFBP-3, IGFBP-6)	+ (IGFBP-2 > IGFBP-3 > IGFBP-4)
Relationship with elevated levels of insulin	+	+	+

Table 2: Preclinical and clinical studies.

	In vitro	*In vivo*	Trials	References
Thyroid cancer	Ab, IS	Ab, IS	—	Wang et al. 2006 [167]; Aiello et al. 2006 [169]; Chen et al. 2012 [170]
Adrenal cancer	TKI, Ab, IS	TKI, Ab	Ab, TKI	Barlaskar et al., 2009 [209]; Shen et al. 2007 [210]; Almeida et al., 2008 [177]; Ferruzzi et al., 2005 [213]; Cantini et al. 2008 [179]
Ovarian cancer	Ab, TKI, IS	IS	Ab, IS	Chakrabarty and Kondratick 2006 [223]; Gotlieb et al., 2006 [236]; Liao et al. 2012 [238]; Li et al., 2012 [237]; Romero et al., 2012 [239]

TKI: tyrosine kinase inhibitor targeting the IGF system; Ab: antibody; IS: insulin sensitizer.

maternally imprinted and consequently expressed only from the paternal allele. Structural abnormalities, characterized by the loss of maternal allele with the duplication of paternal allele, lead to biallelic expression of IGF-II gene. These alterations are frequently observed in sporadic adult ACCs, but only rarely in adenomas [118, 185, 186]. High IGF-II mRNA levels are associated with a more aggressive phenotype of ACC and a 5-fold increased risk of recurrence [180, 187] (Table 1).

In phosphoenolpyruvate carboxykinase (PEPCK) promoter human IGF-II transgenic mice, postnatal overexpression of IGF-II induced significantly increased adrenal weights, mainly caused by hyperplasia of the zona fasciculate [188]. This is in accordance with elevated serum corticosterone levels in IGF-II transgenic animals [189]. However, the observation that transgenic mice overexpressing IGFs or IGFBP-2 do not develop adrenal tumors indicates that IGF-II alone is not a tumor initiator for adrenal cells but rather a tumor progression factor that requires additional effectors for triggering adrenal tumorigenesis [189]. This notion is also supported by the clinical observation that deregulation of the IGF system is a late event often associated with advanced stage of the disease and poor clinical prognosis [118, 180, 190].

Indeed, in a cohort of pediatric and adult patients with adrenocortical tumors, IGF-II transcripts were mainly overexpressed in adult ACCs compared to adenomas [177]. Yet, a microarray analysis of 24 pediatric adrenocortical tumors (5 adenomas, 18 carcinomas, and 1 undetermined) demonstrated that the median expression of IGF-II in adrenocortical tumors was 18 times higher than in normal adrenal glands [191].

IGF-IR [192] and the IGF-binding protein-2 (IGFBP-2) [180] are also specifically overexpressed in ACCs. These molecular alterations may trigger a cascade of molecular events that can ultimately lead to malignancy in adrenocortical tumor progression [193]. This notion is confirmed by studies in adrenocortical tumor mouse cell line Y1, which have shown that stable transfection with human IGF-IR cDNA results in increased mitogenic response (+140%) to IGF-I as compared with nontransfected Y1 cells. In IGF-IR transfected cells the antiproliferative effect of ACTH was blunted and could be further antagonized by exogenous IGF-I [194] (Table 1).

In order to further clarify the significance of the IGF-IR in tumorigenesis of the human adrenal gland, Weber et al. examined the binding characteristics and concentrations of IGF-IR in normal adult human adrenocortical glands and in adrenocortical tumours of various origin. IGF IR binding in adrenocortical hyperplasias and adenomas was similar than in normal adrenocortical tissue. In contrast, three out of four hormonally active ACCs showed strongly elevated specific IGF-I binding with a 3-4-fold increase in IGF-IR concentration, as compared with normal adrenocortical tissue [194]. H295R cells overexpress also IGFBP-2 [195], which accounts for only 12% of the IGFBP activity in normal adrenocortical cells, but seems to play a specific role in the progression of ACCs by modulating IGF-II activity

[196]. In support of the hypothesis of a tumor-growth-promoting effect of IGFBP-2 is the observation that Y-1 mouse adrenocortical tumor cells overexpressing IGFBP-2 show increased tumorigenic potential and cell proliferation [197]. However, the mechanisms of the IGFBP-2-associated increase in adrenal tumorigenesis remain largely unclear (see below).

Although the regulation of IGFBP production by IGFs is highly cell and species specific, a stimulatory effect of IGFs on IGFBP-3 has been reported in a large variety of cell systems [168, 198–204]. Treatment of adult human adrenocortical cells with ACTH predominantly stimulated the abundance of IGFBP-1 and to a lesser extent that of IGFBP-3, while IGF-I and IGF-II selectively induced the accumulation of IGFBP-3 and IGFBP-5 in the medium [205, 206]. Quantification of the specific bands by γ counting revealed that IGFBP-3 accounts for more than half of the detected IGFBP activity, followed by IGFBP-1 with 20% and IGFBP-4 with approximately 10% [196].

The gene expression profiles of IGFs system component may even distinguish malignant and benign tumours [207]. By analyzing the transcriptional profiles in 7 patients with ACCs and 13 with adenomas, Velázquez-Fernández et al. showed that in ACCs several IGF-related genes as IGF-II, IGF-IR, IGFBP3, and IGFBP6 were most significantly upregulated [207].

Recently, miRNAs able to regulate the IGF expression pattern in childhood adrenocortical tumors have been identified. Functional analysis of these miRNAs showed miR-99a and miR-100 regulate expression of IGF-IR, mTOR, and rictor in adrenocortical cancer cells, acting on target sites in their 3′-UTR regions. Downregulation of endogenous miR-100 in H295R and SW-13 cells increased protein expression of mTOR, raptor, and IGF-IR [208].

In order to evaluate the functional consequences of IGF-IR inhibition in adrenal carcinomas, Barlaskar et al. analyzed a large series of benign and malignant human adrenal tumors and a panel of ACC cell lines using a tyrosine kinase inhibitor, NVP-AEW541, and a fully human monoclonal antibody anti-IGF-IR, IMC-A12, both specifically targeting IGF-IR. Treatments with both NVP-AEW541 and IMC-A12 resulted in inhibition of growth of ACC cells *in vitro*. In xenograft tumors, IGF-IR blockade was more potent than mitotane, the first-line adrenolytic drug used in patients with ACC, and significantly enhanced mitotane response [209]. *In vitro* (in H295 and in SW-13 cells) efficacy of NVP-AEW541 treatment was confirmed by other studies [177, 210].

Thiazolidinediones (TZDs), a class of antidiabetic drugs, have also been investigated as potential therapeutic agents for ACC. TZDs are ligands for the peroxisome-proliferator-activated receptor (PPAR)-γ, a member of the nuclear receptor superfamily of ligand-dependent transcription factors, that is expressed predominantly in the adipose tissue but also in other tissues, although at much lower levels. PPAR-γ exerts a critical role in several biological processes such as adipogenesis, glucose metabolism, inflammation, cell growth, and differentiation [211]. Nowadays, the molecular basis for the antitumor action of PPAR-γ agonists remains incompletely elucidated. However, numerous studies support the notion that PPAR-γ activation induces apoptosis and thus exerts anticancer effects [212]. Although no differences in the expression of PPAR-γ are seen in normal and tumor tissue, the PPAR-γ agonist rosiglitazone inhibited growth and invasiveness of H295R cells [213]. Indeed, both in SW-13 and H295 ACC cells, rosiglitazone inhibited the signaling pathways downstream IGF-IR, but not the receptor itself [179] (Table 2).

Clinical trials are currently investigating the efficacy of monoclonal antibody IMC-A12, either used as monotherapy or in combination with mitotane (trials NCT00831844 and NCT00778817, resp.). The dual kinase inhibitor of both IGF-IR and IR, OSI-906, is currently being evaluated in ACC patients (trial NCT00924989) (Table 2).

4.3. Ovarian Cancer. Epithelial ovarian cancer (EOC) constitutes 90% of ovarian malignancies [214] and is the most common cause of gynecological cancer-related mortality [215]. It is fairly common in Scandinavia, less common in western Europe and North America, and infrequent in the developing countries and in Japan [216]. A first-degree family history of EOC is associated with approximately 3-fold increased risk [217]. EOCs are subdivided into four major categories: high-grade serous (70%), endometrioid (10%), clear cell (10%), mucinous (3%), and low-grade serous carcinomas (<5%) [214]. The marked clinical differences in ovarian cancer stage at presentation, response to therapy, and survival are manifestations of a complex underlying molecular heterogeneity of ovarian cancers [215]. Relatively little is known about the basic molecular and cellular mechanism that modulates growth of epithelial ovarian cancer and, presently, there are no available treatments capable of curing recurrent ovarian carcinomas due to their rapid evolution into a chemoresistant disease [218].

In physiology, as previously mentioned, regulation of ovarian activity requires a functional IGF axis. Moreover, over the last decade, accumulating data suggest that the insulin/IGF pathway might be a promising therapeutic target in ovarian cancer [219] (Table 1).

In 1991, Yee et al. examined the possibility that the IGF system could be important in regulating the autocrine growth of EOC cells [220]. The expressions of IGF-I-, IGF-IR-, and IGF-binding proteins were studied in ovarian cancer cell lines and tissues. IGF-IR mRNA was found in ovarian cancer cell lines and the primary or metastatic ovarian cancer tissues. In OVCAR-3 cell line, IGF-binding proteins, including IGFBP-2, IGFBP-3 and IGFBP-4, were expressed. In epithelial cells derived from untreated, ovarian cancer specimens, exogenous IGF-I induces cell proliferation. These cells secrete IGFs and IGF-binding proteins and express IGF-IR [221]. Ovary cancer cell lines also express IR; elevated levels of IR and insulin binding capacity were present in six cancer cell lines as compared to normal ovarian epithelium cell lines and were associated with mitogenic signaling in response to low doses of insulin. IR isoform analysis has shown preferential expression of IR-A, suggesting the ability of exogenous IGF-II to stimulate EOC cell proliferation through IR-A [54] (Table 1).

Moreover, studies in NIH-OVCAR3 cells have shown that IGF-I and IGFBP-2 promote ovarian cancer cell growth and invasiveness. IGFBP-2 is dramatically increased in the serum and ovarian cyst fluid of women with epithelial ovarian cancer [222, 223] and is involved in stimulation of cell growth [223]. In agreement with these data, elevated serum levels of IGF-I and IGFBP-2 have been associated with an increased risk of ovarian cancer [224, 225] (Table 1).

Other studies have shown that IGFBP-2 expression level in epithelial ovarian cancers is up to 38-fold higher than in normal ovarian epithelium [226]. Moreover, serum IGFBP-2 levels are elevated in women with early- and advanced-stage ovarian cancer as compared to controls and to patients with benign gynecological conditions, indicating that IGFBP-2 may be useful as a serum biomarker for detection and monitoring of epithelial ovarian cancer. Although the cellular mechanisms through which IGFBP-2 exerts a role in ovarian tumorigenesis are not completely elucidated, experimental data demonstrate that the growth-modulating effects of IGFBP-2 in ovarian cancer cells may be mediated by the activation of three specific cascades controlling cell growth, proliferation, and differentiation, that is, extracellular signal-regulated protein kinases (ERKs), stress-activated protein kinases (SAPKs) or c-Jun N-terminal protein kinases (JNKs) and p38 kinases. Furthermore, it has been seen that IGFBP-2 may regulate the expression of several potential cancer-promoting cytokines including fibroblast growth factors 6 and 7 (FGF-6 and -7), neurotrophin-4 (NT-4), and placental growth factor (PIGF) [223]. Thus, although IGFBPs are potent modulators of the mitogenic effects of IGFs, IGF-independent actions have also been recognized suggesting that IGFBPs are a separate class of growth modulators.

IGF-II is also considered a molecular marker and potential therapeutic target for the most aggressive EOCs. Indeed, when compared with normal ovarian surface epithelium samples, ovarian cancers show approximately 300-fold higher expression of the IGF-II gene. High IGF-II and lower IGFBP-3 expression are associated with high-grade, poorly differentiated, and advanced-stage disease [227, 228].

The association between IGF-II expression and ovarian cancer survival is driven by two specific promoters of IGF-II gene [229]. The IGF-II gene has four promoters, and each initiates a promoter-specific transcript which is expressed in a temporal and spatial-dependent manner. The transcription of three of the four IGF-II promoters, promoters 2, 3, and 4 (P2, P3 and P4), is regulated by DNA methylation [230]. DNA methylation alterations have been identified as being involved in tumorigenesis and disease progression [231, 232].

Using methylation-specific polymerase chain reaction (MSP) assay [233] it has been found that the methylation pattern of P2 and P3 IGF-II promoters as well as the levels of IGF-II mRNA and peptide was significantly different among patients with distinct tumor grade, residual tumor size, and treatment response. Patients with methylated P2 and unmethylated P3 (P2M/P3U) had 5 times higher mRNA expression and nearly 2-fold higher peptide levels compared to those with opposite pattern of methylation (P2U/P3M) [232].

Recently, genetic variations across the IGF components have been correlated with ovarian cancer risk [119, 120]. In primary ovarian cancer tissue, using microarray technology, Spentzos et al. have analyzed the expression patterns of gene families and pathways of IGF axis. Studying sixty-four patients with advanced stages of EOC, they found that expression patterns of IGF axis genes have prognostic significance in this highly lethal disease [234].

However, components of the IGF-I pathway were found overexpressed also in low-grade tumors, which respond to treatment with exogenous IGF-I with increased proliferation and migration [219].

Early data have shown that phosphorothioate antisense oligodeoxynucleotides (S-ODNs) [235] inhibit the function of the IGF-IR in NIH-OVCAR3 ovarian cancer cells and suppress cancer cell growth *in vitro*, but have small effects *in vivo* [235]. In the same cell model, a neutralizing antibody to IGFBP-2 also inhibits cell growth and downregulates the expression of a number of potential cancer-promoting cytokines [223].

More recently, it has been shown that NVP-AEW541, an IGF-IR tyrosine kinase inhibitor, is able to inhibit growth in EOC cell lines, OVCAR-3 and OVCAR-4, and to sensitize cells to cisplatin [236] (Table 2).

Today, at least five ongoing clinical trials aim to target the IGF-I axis in EOC patients. A phase II trial is currently investigating the fully human anti-IGF-IR monoclonal antibody AMG 479 in combination with paclitaxel and carboplatin (NCT00718523). Another phase II trial is examining AMG 479 in recurrent platinum-sensitive ovarian cancer (NCT00719212) (Table 2).

The combination between AMG 479 and AMG 655 (an human anti-DR5 monoclonal antibody) is the object of a study (phase I/II study) in patients with EOC and other advanced, refractory solid tumors (NCT00819169). A phase I/II trial is currently evaluating intermittent and continuous OSI-906, in combination with weekly paclitaxel, in patients with recurrent EOCs or other solid tumors (NCT00889382). Another phase I trial (NCT01322802) is testing the safety and immunogenicity of a DNA-plasmid-based vaccine encoding the amino acids 1–163 of IGFBP-2 in patients with advanced EOC.

Recently, an alternative therapeutical approach using insulin sensitizers, such as metformin, has been suggested against ovarian cancer. The rationale for using these drugs comes from evidence that a combination therapy with metformin and LY294002, an inhibitor of PI3K, reduces growth and induces apoptosis in ovarian cancer cells [237] by inhibiting PI3K/AKT and mTOR [238] while activating the AMPK/ACC pathway. In agreement with these preclinical data, an epidemiologic study conducted in 341 patients with EOC has shown that patients with T2DM who used metformin had longer progression-free survival than nonusers, despite receiving similar treatment for ovarian cancer [239]. A close relationship is now established between the use of metformin and progression, survival, and chemosensitivity of EOC [237–240] (Table 2).

Recently, a phase II clinical trial (NCT01579812) started to establish the potential role of metformin as anticancer stem cell agent in EOC patients. The primary objective of this study is to determine if metformin, administered as the time of traditional adjuvant chemotherapy to women with advanced EOC, will improve recurrence-free survival at 18 months compared to controls (Table 2).

5. Conclusions and Perspectives

Advanced endocrine tumors are characterized by poor prognosis and resistance to the common DNA-damaging chemotherapies or radiotherapy. Most extensively characterized endocrine malignancies include thyroid, adrenal, and ovarian cancers. In these tumors, a crosstalk between the IGF system and the pituitary hormones specific for each endocrine gland has been recognized and seems to exert a role in the tumorigenesis process. Recently, the risk of certain malignancies, including endocrine related cancers, has been found 2-3-fold increased in obese and T2DM patients. Insulin resistance and compensatory hyperinsulinemia, typical features of both obesity and diabetes, are the major candidates for cancer risk and are also associated with poor cancer prognosis and resistance to conventional and targeted anticancer therapies. Multiple alterations in the IGF system as well as association with high circulating levels of insulin/IGFs have been reported by several studies for these three endocrine cancer histotypes. This scenario may have important implications for endocrine cancer prevention and treatment. However, the potential role of the IGF system as therapeutical target in these tumors has being only recently evaluated and few clinical trials are currently ongoing.

Today the therapeutical strategies proposed to overcome IGF axis alterations in these malignancies include IGF-IR blocking antibodies, IGF-IR/IR tyrosine-kinase inhibitors, and insulin sensitizers. So far, preclinical results obtained with the first two classes of drugs mentioned above have shown promising hopes although the results are not conclusive and no complete responses have been reported. Furthermore, like in other malignancies, the development of intrinsic and adaptative resistance to IGF axis blockage could occur. Aberrant IR expression, particularly IR-A isoform, as well as HR-A formation and enhanced IGF-II autocrine production are very common alterations in endocrine cancers and could mediate the resistance to IGF-IR blocking drugs. Furthermore, insulin resistance and hyperinsulinemia are side effects of these drugs and may contribute to IR-A overactivation. New approaches aimed at specifically and safely targeting IR-A activation and/or disrupting the autocrine IGF-II/IR-A loop are urgently needed. In light of lines of evidence of association of endocrine cancers risk and hyperinsulinemia, insulin-sensitizers, such as metformin, hold promise as measures useful in cancer prevention.

Abbreviations

RTKs: Receptors tyrosine kinase
SHC: Src homology domain C-terminal
Sos: Son of sevenless
Grb-2: Growth factor receptor-bound protein 2
PKA: Protein kinase A
PKC: Protein kinase C
Gαs: G-protein (s) subunit alpha
Gaq: G-protein (q) subunit alpha
IRS-1: Insulin receptor substrate-1
PI3K: Phosphoinositide 3-kinase
PDK1: Phosphorylated 3-phosphoinoside-dependent protein kinase
Akt: Protein kinase B
mTOR: Mammalian target of rapamycin
Ras/Raf/MEK: Mitogen-activated protein kinase
ERK kinase: Extracellular-signal-regulated kinase
NFκB: Nuclear factor kappa-B
p90RSK: p90 ribosomal S6 kinase
TFs: Transcription factors.

Author's Contributions

R. Malaguarnera, A. Morcavallo equally contributed to the paper.

Acknowledgments

This workwas supported in part by grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC) to A. Belfiore (Grant no. 10625/12), AIRC Project Calabria 2012 and Fondazione Cassa di Risparmio di Calabria e Lucania to A. Belfiore, from PON (Programma Operativo Nazionale Ricerca e Competitività, Grant 01_01078), and from PRIN-MIUR, Grant 2008BKRFBH_005, to A. Belfiore.

References

[1] Y. E. Nikiforov and M. N. Nikiforova, "Molecular genetics and diagnosis of thyroid cancer," *Nature Reviews Endocrinology*, vol. 7, pp. 569–580.

[2] D. Russo, F. Arturi, H. G. Suarez et al., "Thyrotropin receptor gene alterations in thyroid hyperfunctioning adenomas," *Journal of Clinical Endocrinology and Metabolism*, vol. 81, no. 4, pp. 1548–1551, 1996.

[3] J. Parma, L. Duprez, J. van Sande et al., "Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas," *Nature*, vol. 365, no. 6447, pp. 649–651, 1993.

[4] B. Biondi, S. Filetti, and M. Schlumberger, "Thyroid-hormone therapy and thyroid cancer: a reassessment," *Nature clinical practice. Endocrinology & metabolism*, vol. 1, no. 1, pp. 32–40, 2005.

[5] G. Milazzo, G. L. La Rosa, R. Catalfamo, R. Vigneri, and A. Belfiore, "Effect of TSH in human thyroid cells: evidence for both mitogenic and antimitogenic effects," *Journal of Cellular Biochemistry*, vol. 49, no. 3, pp. 231–238, 1992.

[6] J. E. Dumont, F. Lamy, P. Roger, and C. Maenhaut, "Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors," *Physiological Reviews*, vol. 72, no. 3, pp. 667–697, 1992.

[7] A. Ciampolillo, C. De Tullio, and F. Giorgino, "The IGF-I/IGF-I receptor pathway: implications in the pathophysiology of thyroid cancer," *Current Medicinal Chemistry*, vol. 12, no. 24, pp. 2881–2891, 2005.

[8] B. F. A. M. van der Laan, J. L. Freeman, and S. L. Asa, "Expression of growth factors and growth factor receptors in normal and tumorous human thyroid tissues," *Thyroid*, vol. 5, no. 1, pp. 67–73, 1995.

[9] R. Malaguarnera, F. Frasca, A. Garozzo et al., "Insulin receptor isoforms and insulin-like growth factor receptor in human follicular cell precursors from papillary thyroid cancer and normal thyroid," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 3, pp. 766–774, 2011.

[10] M. Saji and L. D. Kohn, "Insulin and insulin-like growth factor-I inhibit thyrotropin-increased iodide transport in serum-depleted FRTL-5 rat thyroid cells: modulation of adenosine 3',5'-monophosphate signal action," *Endocrinology*, vol. 128, no. 2, pp. 1136–1143, 1991.

[11] P. Santisteban, L. D. Kohn, and R. di Lauro, "Thyroglobulin gene expression is regulated by insulin and insulin-like growth factor I, as well as thyrotropin, in FRTL-5 thyroid cells," *Journal of Biological Chemistry*, vol. 262, no. 9, pp. 4048–4052, 1987.

[12] R. Zarrilli, S. Formisano, and B. Di Jeso, "Hormonal regulation of thyroid peroxidase in normal and transformed rat thyroid cells," *Molecular Endocrinology*, vol. 4, no. 1, pp. 39–45, 1990.

[13] P. Santisteban, A. Acebron, M. Polycarpou-Schwarz, and R. Di Lauro, "Insulin and insulin-like growth factor I regulate a thyroid-specific nuclear protein that binds to the thyroglobulin promoter," *Molecular Endocrinology*, vol. 6, no. 8, pp. 1310–1317, 1992.

[14] P. Aza-Blanc, R. Di Lauro, and P. Santisteban, "Identification of a cis-regulatory element and a thyroid-specific nuclear factor mediating the hormonal regulation of rat thyroid peroxidase promoter activity," *Molecular Endocrinology*, vol. 7, no. 10, pp. 1297–1306, 1993.

[15] D. Civitareale, R. Lonigro, A. J. Sinclair, and R. Di Lauro, "A thyroid-specific nuclear protein essential for tissue-specific expression of the thyroglobulin promoter," *EMBO Journal*, vol. 8, no. 9, pp. 2537–2542, 1989.

[16] H. Francis-Lang, M. Zannini, M. de Felice, M. T. Berlingieri, A. Fusco, and R. Di Lauro, "Multiple mechanisms of interference between transformation and differentiation in thyroid cells," *Molecular and Cellular Biology*, vol. 12, no. 12, pp. 5793–5800, 1992.

[17] L. Ortiz, M. Zannini, R. D. Lauro, and P. Santisteban, "Transcriptional control of the forkhead thyroid transcription factor TTF-2 by thyrotropin, insulin, and insulin-like growth factor I," *Journal of Biological Chemistry*, vol. 272, no. 37, pp. 23334–23339, 1997.

[18] H. Masui and L. D. Garren, "Inhibition of replication in functional mouse adrenal tumor cells by adrenocorticotropic hormone mediated by adenosine 3':5'-cyclic monophosphate," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 68, no. 12, pp. 3206–3210, 1971.

[19] E. R. Weidman and G. N. Gill, "Differential effects of ACTH or 8 Br cAMP on growth and replication in a functional adrenal tumor cell line," *Journal of Cellular Physiology*, vol. 90, no. 1, pp. 91–104, 1977.

[20] A. M. Morera and J. M. Saez, "In vitro mitogenic and steroidogenic effects of ACTH analogues on an adrenal tumor cell line (Y-1)," *Experimental Cell Research*, vol. 127, no. 2, pp. 446–451, 1980.

[21] J. Ramachandran and A. T. Suyama, "Inhibition of replication of normal adrenocortical cells in culture by adrenocorticotropin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 72, no. 1, pp. 113–117, 1975.

[22] A. C. Latronico, M. Reincke, B. B. Mendonca et al., "No evidence for oncogenic mutations in the adrenocorticotropin receptor gene in human adrenocortical neoplasms," *Journal of Clinical Endocrinology and Metabolism*, vol. 80, no. 3, pp. 875–877, 1995.

[23] K. Light, P. J. Jenkins, A. Weber et al., "Are activating mutations of the adrenocorticotropin receptor involved in adrenal cortical neoplasia?" *Life Sciences*, vol. 56, no. 18, pp. 1523–1527, 1995.

[24] M. Reincke, P. Mora, F. Beuschlein, W. Arlt, G. P. Chrousos, and B. Allolio, "Deletion of the adrenocorticotropin receptor gene in human adrenocortical tumors: implications for tumorigenesis," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 9, pp. 3054–3058, 1997.

[25] L. S. Kirschner, "Signaling pathways in adrenocortical cancer," *Annals of the New York Academy of Sciences*, vol. 968, pp. 222–239, 2002.

[26] D. W. Cramer, G. B. Hutchison, and W. R. Welch, "Determinants of ovarian cancer risk. I. Reproductive experiences and family history," *Journal of the National Cancer Institute*, vol. 71, no. 4, pp. 711–716, 1983.

[27] D. W. Cramer and W. R. Welch, "Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis," *Journal of the National Cancer Institute*, vol. 71, no. 4, pp. 717–721, 1983.

[28] B. V. Stadel, "The etiology and prevention of ovarian cancer," *American Journal of Obstetrics and Gynecology*, vol. 123, no. 7, pp. 772–774, 1975.

[29] S. V. Nicosia, J. H. Johnson, and E. J. Streibel, "Growth characteristics of rabbit ovarian mesothelial (surface epithelial) cells," *International Journal of Gynecological Pathology*, vol. 4, pp. 58–74, 1985.

[30] W. Zheng, J. J. Lu, F. Luo et al., "Ovarian epithelial tumor growth promotion by follicle-stimulating hormone and inhibition of the effect by luteinizing hormone," *Gynecologic Oncology*, vol. 76, no. 1, pp. 80–88, 2000.

[31] L. J. Spicer and P. Y. Aad, "Insulin-like growth factor (IGF) 2 stimulates steroidogenesis and mitosis of bovine granulosa cells through the IGF1 receptor: role of follicle-stimulating hormone and IGF2 receptor," *Biology of Reproduction*, vol. 77, pp. 18–27, 2007.

[32] J. Zhou, T. R. Kumar, M. M. Matzuk, and C. Bondy, "Insulin-like growth factor I regulates gonadotropin responsiveness in the murine ovary," *Molecular Endocrinology*, vol. 11, no. 13, pp. 1924–1933, 1997.

[33] A. Belfiore, G. Pandini, V. Vella, S. Squatrito, and R. Vigneri, "Insulin/IGF-I hybrid receptors play a major role in IGF-I signaling in thyroid cancer," *Biochimie*, vol. 81, no. 4, pp. 403–407, 1999.

[34] A. Belfiore, F. Frasca, G. Pandini, L. Sciacca, and R. Vigneri, "Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease," *Endocrine Reviews*, vol. 30, no. 6, pp. 586–623, 2009.

[35] S. Rajaram, D. J. Baylink, and S. Mohan, "Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions," *Endocrine Reviews*, vol. 18, no. 6, pp. 801–831, 1997.

[36] E. J. Gallagher and D. LeRoith, "Minireview: IGF, insulin, and cancer," *Endocrinology*, vol. 152, no. 7, pp. 2546–2551, 2011.

[37] V. Papa, V. Pezzino, A. Costantino et al., "Elevated insulin receptor content in human breast cancer," *Journal of Clinical Investigation*, vol. 86, no. 5, pp. 1503–1510, 1990.

[38] F. Frasca, G. Pandini, P. Scalia et al., "Insulin receptor isoform A, a newly recognized, high-affinity insulin- like growth factor II receptor in fetal and cancer cells," *Molecular and Cellular Biology*, vol. 19, no. 5, pp. 3278–3288, 1999.

[39] R. Malaguarnera, A. Sacco, C. Voci, G. Pandini, R. Vigneri, and A. Belfiore, "Proinsulin binds with high affinity the insulin receptor isoform A and predominantly activates the mitogenic pathway," *Endocrinology*, vol. 153, no. 5, pp. 2152–2163, 2012.

[40] C. Hernández-Sánchez, A. Mansilla, E. J. de la Rosa, and F. de Pablo, "Proinsulin in development: new roles for an ancient prohormone," *Diabetologia*, vol. 49, no. 6, pp. 1142–1150, 2006.

[41] D. LeRoith and C. T. Roberts Jr., "The insulin-like growth factor system and cancer," *Cancer Letters*, vol. 195, no. 2, pp. 127–137, 2003.

[42] A. Morrione, T. DeAngelis, and R. Baserga, "Failure of the bovine papillomavirus to transform mouse embryo fibroblasts with a targeted disruption of the insulin-like growth factor I receptor genes," *Journal of Virology*, vol. 69, no. 9, pp. 5300–5303, 1995.

[43] C. Sell, G. Dumenil, C. Deveaud et al., "Effect of a null mutation of the insulin-like growth factor I receptor gene on growth and transformation of mouse embryo fibroblasts," *Molecular and Cellular Biology*, vol. 14, no. 6, pp. 3604–3612, 1994.

[44] M. Kaleko, W. J. Rutter, and A. D. Miller, "Overexpression of the human insulinlike growth factor I receptor promotes ligand-dependent neoplastic transformation," *Molecular and Cellular Biology*, vol. 10, no. 2, pp. 464–473, 1990.

[45] G. Milazzo, F. Giorgino, G. Damante et al., "Insulin receptor expression and function in human breast cancer cell lines," *Cancer Research*, vol. 52, no. 14, pp. 3924–3930, 1992.

[46] F. Giorgino, A. Belfiore, G. Milazzo et al., "Overexpression of insulin receptors in fibroblast and ovary cells induces a ligand-mediated transformed phenotype," *Molecular Endocrinology*, vol. 5, no. 3, pp. 452–459, 1991.

[47] C. C. Mastick, H. Kato, C. T. Roberts Jr., D. LeRoith, and A. R. Saltiel, "Insulin and insulin-like growth factor-I receptors similarly stimulate deoxyribonucleic acid synthesis despite differences in cellular protein tyrosine phosphorylation," *Endocrinology*, vol. 135, no. 1, pp. 214–222, 1994.

[48] V. Vella, G. Pandini, L. Sciacca et al., "A novel autocrine loop involving IGF-II and the insulin receptor isoform-A stimulates growth of thyroid cancer," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 1, pp. 245–254, 2002.

[49] T. Kamio, K. Shigematsu, K. Kawai, and H. Tsuchiyama, "Immunoreactivity and receptor expression of insulinlike growth factor I and insulin in human adrenal tumors. An immunohistochemical study of 94 cases," *American Journal of Pathology*, vol. 138, no. 1, pp. 83–91, 1991.

[50] E. P. Beck, P. Russo, B. Gliozzo et al., "Identification of insulin and insulin-like growth factor I, (IGF I) receptors in ovarian cancer tissue," *Gynecologic Oncology*, vol. 53, pp. 196–201, 1994.

[51] G. Pillemer, H. Lugasi-Evgi, G. Scharovsky, and D. Naor, "Insulin dependence of murine lymphoid T-cell leukemia," *International Journal of Cancer*, vol. 50, no. 1, pp. 80–85, 1992.

[52] M. Mamounas, D. Gervin, and E. Englesberg, "The insulin receptor as a transmitter of a mitogenic signal in Chinese hamster ovary CHO-K1 cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 23, pp. 9294–9298, 1989.

[53] A. Morrione, B. Valentinis, S. Q. Xu et al., "Insulin-like growth factor II stimulates cell proliferation through the insulin receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 8, pp. 3777–3782, 1997.

[54] K. R. Kalli, O. I. Falowo, L. K. Bale, M. A. Zschunke, P. C. Roche, and C. A. Conover, "Functional insulin receptors on human epithelial ovarian carcinoma cells: implications for IGF-II mitogenic signaling," *Endocrinology*, vol. 143, no. 9, pp. 3259–3267, 2002.

[55] A. Belfiore, "The role of insulin receptor isoforms and hybrid insulin/IGF-I receptors in human cancer," *Current Pharmaceutical Design*, vol. 13, no. 7, pp. 671–686, 2007.

[56] A. Ullrich, A. Gray, A. W. Tam et al., "Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity," *EMBO Journal*, vol. 5, no. 10, pp. 2503–2512, 1986.

[57] G. Pandini, R. Vigneri, A. Costantino et al., "Insulin and insulin-like growth factor-I (IGF-I) receptor overexpression in breast cancers leads to insulin/IGF-I hybrid receptor overexpression: evidence for a second mechanism of IGF-I signaling," *Clinical Cancer Research*, vol. 5, no. 7, pp. 1935–1944, 1999.

[58] C. P. Moxham and S. Jacobs, "Insulin/IGF-I receptor hybrids: a mechanism for increasing receptor diversity," *Journal of Cellular Biochemistry*, vol. 48, no. 2, pp. 136–140, 1992.

[59] E. M. Bailyes, B. T. Nave, M. A. Soos, S. R. Orr, A. C. Hayward, and K. Siddle, "Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting," *Biochemical Journal*, vol. 327, part 1, pp. 209–215, 1997.

[60] M. A. Soos, B. T. Nave, and K. Siddle, "Immunological studies of type I IGF receptors and insulin receptors: characterisation of hybrid and atypical receptor subtypes," *Advances in Experimental Medicine and Biology*, vol. 343, pp. 145–157, 1993.

[61] A. A. Samani and P. Brodt, "The receptor for the type I insulin-like growth factor and its ligands regulate multiple cellular functions that impact on metastasis," *Surgical Oncology Clinics of North America*, vol. 10, no. 2, pp. 289–312, 2001.

[62] L. S. Argetsinger, G. W. Hsu, M. G. Myers Jr., N. Billestrup, M. F. White, and C. Carter- Su, "Growth hormone, interferon-γ, and leukemia inhibitory factor promoted tyrosyl phosphorylation of insulin receptor substrate-1," *Journal of Biological Chemistry*, vol. 270, no. 24, pp. 14685–14692, 1995.

[63] M. G. Myers Jr., T. C. Grammer, L. M. Wang et al., "Insulin receptor substrate-1 mediates phosphatidylinositol 3'-kinase and p70(S6k) signaling during insulin, insulin-like growth factor-1, and interleukin-4 stimulation," *Journal of Biological Chemistry*, vol. 269, no. 46, pp. 28783–28789, 1994.

[64] K. Vuori and E. Ruoslahti, "Association of insulin receptor substrate-1 with integrins," *Science*, vol. 266, no. 5190, pp. 1576–1578, 1994.

[65] T. Petley, K. Graff, W. Jiang, H. Yang, and J. R. Florini, "Variation among cell types in the signaling pathways by which IGF-I stimulates specific cellular responses," *Hormone and Metabolic Research*, vol. 31, no. 2-3, pp. 70–76, 1999.

[66] A. V. Espinosa, L. Porchia, and M. D. Ringel, "Targeting BRAF in thyroid cancer," *British Journal of Cancer*, vol. 96, no. 1, pp. 16–20, 2007.

[67] L. Fugazzola, E. Puxeddu, N. Avenia et al., "Correlation between B-RAFV600E mutation and clinico-pathologic parameters in papillary thyroid carcinoma: data from a multicentric Italian study and review of the literature," *Endocrine-Related Cancer*, vol. 13, no. 2, pp. 455–464, 2006.

[68] A. Chiloeches and R. Marais, "Is BRAF the Achilles' heel of thyroid cancer?" *Clinical Cancer Research*, vol. 12, no. 6, pp. 1661–1664, 2006.

[69] N. Halachmi, S. Halachmi, E. Evron et al., "Somatic mutations of the PTEN tumor suppressor gene in sporadic follicular thyroid tumors," *Genes Chromosomes Cancer*, vol. 23, pp. 239–243, 1998.

[70] P. Hou, D. Liu, Y. Shan et al., "Genetic alterations and their relationship in the phosphatidylinositol 3-kinase/Akt pathway in thyroid cancer," *Clinical Cancer Research*, vol. 13, no. 4, pp. 1161–1170, 2007.

[71] K. J. Bussey and M. J. Demeure, "Genomic and expression profiling of adrenocortical carcinoma: application to diagnosis, prognosis and treatment," *Future Oncology*, vol. 5, no. 5, pp. 641–655, 2009.

[72] T. Yashiro, H. Hara, N. C. Fulton et al., "Point mutations of ras genes in human adrenal cortical tumors: absence in adrenocortical hyperplasia," *World Journal of Surgery*, vol. 18, no. 4, pp. 455–461, 1994.

[73] S. R. Lin, J. H. Tsai, Y. C. Yang, and S. C. Lee, "Mutations of K-ras oncogene in human adrenal tumours in Taiwan," *British Journal of Cancer*, vol. 77, no. 7, pp. 1060–1065, 1998.

[74] V. Kotoula, E. Sozopoulos, H. Litsiou et al., "Mutational analysis of the BRAF, RAS and EGFR genes in human adrenocortical carcinomas," *Endocrine-Related Cancer*, vol. 16, no. 2, pp. 565–572, 2009.

[75] G. Masi, E. Lavezzo, M. Iacobone, G. Favia, G. Palù, and L. Barzon, "Investigation of BRAF and CTNNB1 activating mutations in adrenocortical tumors," *Journal of Endocrinological Investigation*, vol. 32, no. 7, pp. 597–600, 2009.

[76] G. Singer, R. J. Kurman, H. W. Chang, S. K. R. Cho, and I. M. Shih, "Diverse tumorigenic pathways in ovarian serous carcinoma," *American Journal of Pathology*, vol. 160, no. 4, pp. 1223–1228, 2002.

[77] G. Singer, R. Oldt, Y. Cohen et al., "Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma," *Journal of the National Cancer Institute*, vol. 95, no. 6, pp. 484–486, 2003.

[78] J. Willner, K. Wurz, K. H. Allison et al., "Alternate molecular genetic pathways in ovarian carcinomas of common histological types," *Human Pathology*, vol. 38, no. 4, pp. 607–613, 2007.

[79] Y. H. Cho, D. Y. Kim, J. H. Kim et al., "Mutational analysis of KRAS, BRAF, and TP53 genes of ovarian serous carcinomas in Korean women," *Yonsei Medical Journal*, vol. 50, no. 2, pp. 266–272, 2009.

[80] J. Abubaker, P. Bavi, W. Al-Haqawi et al., "PIK3CA alterations in Middle Eastern ovarian cancers," *Molecular Cancer*, vol. 8, article 51, 2009.

[81] L. Shayesteh, Y. Lu, W. L. Kuo et al., "PIK3CA is implicated as an oncogene in ovarian cancer," *Nature Genetics*, vol. 21, no. 1, pp. 99–102, 1999.

[82] I. G. Campbell, S. E. Russell, D. Y. H. Choong et al., "Mutation of the PIK3CA gene in ovarian and breast cancer," *Cancer Research*, vol. 64, no. 21, pp. 7678–7681, 2004.

[83] J. Woenckhaus, K. Steger, K. Sturm, K. Münstedt, F. E. Franke, and I. Fenic, "Prognostic value of PIK3CA and phosphorylated AKT expression in ovarian cancer," *Virchows Archiv*, vol. 450, no. 4, pp. 387–395, 2007.

[84] K. T. Kuo, T. L. Mao, S. Jones et al., "Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma," *American Journal of Pathology*, vol. 174, no. 5, pp. 1597–1601, 2009.

[85] P. H. Hwang, S. Y. Kim, J. C. Lee, S. J. Kim, H. K. Yi, and D. Y. Lee, "PTEN/MMAC1 enhances the growth inhibition by anticancer drugs with downregulation of IGF-II expression in gastric cancer cells," *Experimental and Molecular Medicine*, vol. 37, no. 5, pp. 391–398, 2005.

[86] H. K. Yi, S. Y. Kim, P. H. Hwang et al., "Impact of PTEN on the expression of insulin-like growth factors (IGFs) and IGF-binding proteins in human gastric adenocarcinoma cells," *Biochemical and Biophysical Research Communications*, vol. 330, no. 3, pp. 760–767, 2005.

[87] H. Zhao, J. Dupont, S. Yakar, M. Karas, and D. LeRoith, "PTEN inhibits cell proliferation and induces apoptosis by downregulating cell surface IGF-IR expression in prostate cancer cells," *Oncogene*, vol. 23, no. 3, pp. 786–794, 2004.

[88] J. Dupont, M. Karas, and D. LeRoith, "The potentiation of estrogen on insulin-like growth factor I action in MCF-7 human breast cancer cells includes cell cycle components," *Journal of Biological Chemistry*, vol. 275, no. 46, pp. 35893–35901, 2000.

[89] S. M. Rosenthal and Z. Q. Cheng, "Opposing early and late effects of insulin-like growth factor I on differentiation and the cell cycle regulatory retinoblastoma protein in skeletal myoblasts," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 22, pp. 10307–10311, 1995.

[90] S. Coats, W. M. Flanagan, J. Nourse, and J. M. Roberts, "Requirement of p27Kip1 for restriction point control of the fibroblast cell cycle," *Science*, vol. 272, no. 5263, pp. 877–880, 1996.

[91] D. Zhang and P. Brodt, "Type 1 insulin-like growth factor regulates MT1-MMP synthesis and tumor invasion via PI 3-kinase/Akt signaling," *Oncogene*, vol. 22, no. 7, pp. 974–982, 2003.

[92] E. Mira, S. Mañes, R. A. Lacalle, G. Márquez, and C. Martínez-A, "Insulin-like growth factor I-triggered cell migration and invasion are mediated by matrix metalloproteinase-9," *Endocrinology*, vol. 140, no. 4, pp. 1657–1664, 1999.

[93] H. Maeta, S. Ohgi, and T. Terada, "Protein expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinase 1 and 2 in papillary thyroid carcinomas," *Virchows Archiv*, vol. 438, no. 2, pp. 121–128, 2001.

[94] E. Baldini, M. Toller, F. M. Graziano et al., "Expression of matrix metalloproteinases and their specific inhibitors in normal and different human thyroid tumor cell lines," *Thyroid*, vol. 14, no. 11, pp. 881–888, 2004.

[95] M. Volante, P. Sperone, E. Bollito et al., "Matrix metalloproteinase type 2 expression in malignant adrenocortical tumors: diagnostic and prognostic significance in a series of 50 adrenocortical carcinomas," *Modern Pathology*, vol. 19, no. 12, pp. 1563–1569, 2006.

[96] K. Q. Cai, W. L. Yang, C. D. Capo-Chichi et al., "Prominent expression of metalloproteinases in early stages of ovarian tumorigenesis," *Molecular Carcinogenesis*, vol. 46, no. 2, pp. 130–143, 2007.

[97] C. S. Zong, L. Zeng, Y. Jiang, H. B. Sadowski, and L. H. Wang, "Stat3 plays an important role in oncogenic Ros-

and insulin-like growth factor I receptor-induced anchorage-independent growth," *Journal of Biological Chemistry*, vol. 273, no. 43, pp. 28065–28072, 1998.

[98] C. S. Zong, J. Chan, D. E. Levy, C. Horvath, H. B. Sadowski, and L. H. Wang, "Mechanism of STAT3 activation by insulin-like growth factor I receptor," *Journal of Biological Chemistry*, vol. 275, no. 20, pp. 15099–15105, 2000.

[99] H. Min and Z. Wei-Hong, "Constitutive activation of signal transducer and activator of transcription 3 in epithelial ovarian carcinoma," *Journal of Obstetrics and Gynaecology Research*, vol. 35, no. 5, pp. 918–925, 2009.

[100] M. Doghman, T. Karpova, G. A. Rodrigues et al., "Increased steroidogenic factor-1 dosage triggers adrenocortical cell proliferation and cancer," *Molecular Endocrinology*, vol. 21, no. 12, pp. 2968–2987, 2007.

[101] M. Olivier, R. Eeles, M. Hollstein, M. A. Khan, C. C. Harris, and P. Hainaut, "The IARC TP53 database: new online mutation analysis and recommendations to users," *Human Mutation*, vol. 19, no. 6, pp. 607–614, 2002.

[102] M. Reincke, F. Beuschlein, M. Slawik, and K. Borm, "Molecular adrenocortical tumourigenesis," *European Journal of Clinical Investigation*, vol. 30, supplement 3, pp. 63–68, 2000.

[103] R. Malaguarnera, V. Vella, R. Vigneri, and F. Frasca, "p53 family proteins in thyroid cancer," *Endocrine-Related Cancer*, vol. 14, no. 1, pp. 43–60, 2007.

[104] H. Werner, E. Karnieli, F. J. Rauscher, and D. LeRoith, "Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 16, pp. 8318–8323, 1996.

[105] N. J. G. Webster, J. L. Resnik, D. B. Reichart, B. Strauss, M. Haas, and B. L. Seely, "Repression of the insulin receptor promoter by the tumor suppressor gene product p53: a possible mechanism for receptor overexpression in breast cancer," *Cancer Research*, vol. 56, no. 12, pp. 2781–2788, 1996.

[106] L. Zhang, F. Kashanchi, Q. Zhan et al., "Regulation of insulin-like growth factor II P3 promoter by p53: a potential mechanism for tumorigenesis," *Cancer Research*, vol. 56, no. 6, pp. 1367–1373, 1996.

[107] L. Buckbinder, R. Talbott, S. Velasco-Miguel et al., "Induction of the growth inhibitor IGF-binding protein 3 by p53," *Nature*, vol. 377, no. 6550, pp. 646–649, 1995.

[108] M. K. Tennant, J. B. Thrasher, P. A. Twomey, R. S. Birnbaum, and S. R. Plymate, "Insulin-like growth factor-binding protein-2 and -3 expression in benign human prostate epithelium, prostate intraepithelial neoplasia, and adenocarcinoma of the prostate," *Journal of Clinical Endocrinology and Metabolism*, vol. 81, no. 1, pp. 411–420, 1996.

[109] A. A. Rasmussen and K. J. Cullen, "Paracrine/autocrine regulation of breast cancer by the insulin-like growth factors," *Breast Cancer Research and Treatment*, vol. 47, no. 3, pp. 219–233, 1998.

[110] N. P. Michell, M. J. S. Langman, and M. C. Eggo, "Insulin-like growth factors and their binding proteins in human colonocytes: preferential degradation of insulin-like growth factor binding protein 2 in colonic cancers," *British Journal of Cancer*, vol. 76, no. 1, pp. 60–66, 1997.

[111] A. Hakam, T. J. Yeatman, L. Lu et al., "Expression of insulin-like growth factor-1 receptor in human colorectal cancer," *Human Pathology*, vol. 30, no. 10, pp. 1128–1133, 1999.

[112] J. DiGiovanni, K. Kiguchi, A. Frijhoff et al., "Deregulated expression of insulin-like growth factor 1 in prostate epithelium leads to neoplasia in transgenic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 7, pp. 3455–3460, 2000.

[113] K. Scotlandi, M. C. Manara, M. Serra et al., "Expression of insulin-like growth factor system components in Ewing's sarcoma and their association with survival," *European Journal of Cancer*, vol. 47, no. 8, pp. 1258–1266, 2011.

[114] S. E. Hankinson, W. C. Willett, G. A. Colditz et al., "Circulating concentrations of insulin-like growth factor-I and risk of breast cancer," *The Lancet*, vol. 351, no. 9113, pp. 1393–1396, 1998.

[115] P. Cohen, D. M. Peehl, B. Baker, F. Liu, R. L. Hintz, and R. G. Rosenfeld, "Insulin-like growth factor axis abnormalities in prostatic stromal cells from patients with benign prostatic hyperplasia," *Journal of Clinical Endocrinology and Metabolism*, vol. 79, no. 5, pp. 1410–1415, 1994.

[116] C. Balkany and G. W. Cushing, "An association between acromegaly and thyroid carcinoma," *Thyroid*, vol. 5, no. 1, pp. 47–50, 1995.

[117] P. Tita, M. R. Ambrosio, C. Scollo et al., "High prevalence of differentiated thyroid carcinoma in acromegaly," *Clinical Endocrinology*, vol. 63, no. 2, pp. 161–167, 2005.

[118] C. Gicquel, X. Bertagna, V. Gaston et al., "Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors," *Cancer Research*, vol. 61, no. 18, pp. 6762–6767, 2001.

[119] K. L. Terry, S. S. Tworoger, M. A. Gates, D. W. Cramer, and S. E. Hankinson, "Common genetic variation in IGF1, IGFBP1 and IGFBP3 and ovarian cancer risk," *Carcinogenesis*, vol. 30, no. 12, pp. 2042–2046, 2009.

[120] C. L. Pearce, J. A. Doherty, D. J. van den Berg et al., "Genetic variation in insulin-like growth factor 2 may play a role in ovarian cancer risk," *Human Molecular Genetics*, vol. 20, no. 11, pp. 2263–2272, 2011.

[121] A. M. Fair, Q. Dai, X. O. Shu et al., "Energy balance, insulin resistance biomarkers, and breast cancer risk," *Cancer Detection and Prevention*, vol. 31, no. 3, pp. 214–219, 2007.

[122] P. Pisani, "Hyper-insulinaemia and cancer, meta-analyses of epidemiological studies," *Archives of Physiology and Biochemistry*, vol. 114, no. 1, pp. 63–70, 2008.

[123] P. Vigneri, F. Frasca, L. Sciacca, G. Pandini, and R. Vigneri, "Diabetes and cancer," *Endocrine-Related Cancer*, vol. 16, no. 4, pp. 1103–1123, 2009.

[124] J. C. Heuson and N. Legros, "Influence of insulin deprivation on growth of the 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma in rats subjected to alloxan diabetes and food restriction," *Cancer Research*, vol. 32, no. 2, pp. 226–232, 1972.

[125] G. Steinbach, S. P. Kumar, B. S. Reddy, M. Lipkin, and P. R. Holt, "Effects of caloric restriction and dietary fat on epithelial cell proliferation in rat colon," *Cancer Research*, vol. 53, no. 12, pp. 2745–2749, 1993.

[126] D. E. Corpet, C. Jacquinet, G. Peiffer, and S. Taché, "Insulin injections promote the growth of aberrant crypt foci in the colon of rats," *Nutrition and Cancer*, vol. 27, no. 3, pp. 316–320, 1997.

[127] T. T. Tran, A. Medline, and W. R. Bruce, "Insulin promotion of colon tumors in rats," *Cancer Epidemiology Biomarkers and Prevention*, vol. 5, no. 12, pp. 1013–1015, 1996.

[128] J. N. Rezzónico, M. Rezzónico, E. Pusiol, F. Pitoia, and H. Niepomniszcze, "Increased prevalence of insulin resistance in patients with differentiated thyroid carcinoma," *Metabolic Syndrome and Related Disorders*, vol. 7, no. 4, pp. 375–380, 2009.

[129] J. Rezzonico, M. Rezzonico, E. Pusiol, F. Pitoia, and H. Niepomniszcze, "Introducing the thyroid gland as another victim of the insulin resistance syndrome," *Thyroid*, vol. 18, no. 4, pp. 461–464, 2008.

[130] A. Engeland, S. Tretli, L. A. Akslen, and T. Bjørge, "Body size and thyroid cancer in two million Norwegian men and women," *British Journal of Cancer*, vol. 95, no. 3, pp. 366–370, 2006.

[131] S. W. Oh, Y. S. Yoon, and S. A. Shin, "Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation study," *Journal of Clinical Oncology*, vol. 23, no. 21, pp. 4742–4754, 2005.

[132] T. Suzuki, K. Matsuo, Y. Hasegawa et al., "Anthropometric factors at age 20 years and risk of thyroid cancer," *Cancer Causes and Control*, vol. 19, no. 10, pp. 1233–1242, 2008.

[133] P. Brindel, F. Doyon, F. Rachédi et al., "Anthropometric factors in differentiated thyroid cancer in French Polynesia: a case-control study," *Cancer Causes and Control*, vol. 20, no. 5, pp. 581–590, 2009.

[134] C. M. Kitahara, E. A. Platz, L. E. B. Freeman et al., "Obesity and thyroid cancer risk among U.S. men and women: a pooled analysis of five prospective studies," *Cancer Epidemiology Biomarkers and Prevention*, vol. 20, no. 3, pp. 464–472, 2011.

[135] F. Clavel-Chapelon, G. Guillas, L. Tondeur, C. Kernaleguen, and M. C. Boutron-Ruault, "Risk of differentiated thyroid cancer in relation to adult weight, height and body shape over life: the French E3N cohort," *International Journal of Cancer*, vol. 126, no. 12, pp. 2984–2990, 2010.

[136] L. D. Maso, C. L. Vecchia, S. Franceschi et al., "A pooled analysis of thyroid cancer studies. V. Anthropometric factors," *Cancer Causes and Control*, vol. 11, no. 2, pp. 137–144, 2000.

[137] A. G. Renehan, M. Tyson, M. Egger, R. F. Heller, and M. Zwahlen, "Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies," *The Lancet*, vol. 371, no. 9612, pp. 569–578, 2008.

[138] K. Rapp, J. Schroeder, J. Klenk et al., "Obesity and incidence of cancer: a large cohort study of over 145000 adults in Austria," *British Journal of Cancer*, vol. 93, no. 9, pp. 1062–1067, 2005.

[139] C. Samanic, W. H. Chow, G. Gridley, B. Jarvholm, and J. F. Fraumeni Jr., "Relation of body mass index to cancer risk in 362,552 Swedish men," *Cancer Causes and Control*, vol. 17, no. 7, pp. 901–909, 2006.

[140] T. Mijović, J. How, M. Pakdaman et al., "Body mass index in the evaluation of thyroid cancer risk," *Thyroid*, vol. 19, no. 5, pp. 467–472, 2009.

[141] C. Iribarren, T. Haselkorn, I. S. Tekawa, and G. D. Friedman, "Cohort study of thyroid cancer in a San Francisco bay area population," *International Journal of Cancer*, vol. 93, no. 5, pp. 745–750, 2001.

[142] J. E. Paes, K. Hua, R. Nagy, R. T. Kloos, D. Jarjoura, and M. D. Ringel, "The relationship between body mass index and thyroid cancer pathology features and outcomes: a clinicopathological cohort study," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 9, pp. 4244–4250, 2010.

[143] M. Almquist, D. Johansen, T. Björge et al., "Metabolic factors and risk of thyroid cancer in the Metabolic syndrome and Cancer project (Me-Can)," *Cancer Causes and Control*, vol. 22, no. 5, pp. 743–751, 2011.

[144] B. Aschebrook-Kilfoy, M. M. Sabra, A. Brenner et al., "Diabetes and thyroid cancer risk in the National Institutes of Health-AARP Diet and Health Study," *Thyroid*, vol. 21, pp. 957–963.

[145] A. Gursoy, "Rising thyroid cancer incidence in the world might be related to insulin resistance," *Medical Hypotheses*, vol. 74, no. 1, pp. 35–36, 2010.

[146] L. Vona-Davis and D. P. Rose, "Adipokines as endocrine, paracrine, and autocrine factors in breast cancer risk and progression," *Endocrine-Related Cancer*, vol. 14, no. 2, pp. 189–206, 2007.

[147] K. Rapp, J. Schroeder, J. Klenk et al., "Fasting blood glucose and cancer risk in a cohort of more than 140,000 adults in Austria," *Diabetologia*, vol. 49, no. 5, pp. 945–952, 2006.

[148] B. G. Chittenden, G. Fullerton, A. Maheshwari, and S. Bhattacharya, "Polycystic ovary syndrome and the risk of gynaecological cancer: a systematic review," *Reproductive Biomedicine Online*, vol. 19, no. 3, pp. 398–405, 2009.

[149] H. S. Yang, C. Yoon, S. K. Myung, and S. M. Park, "Effect of obesity on survival of women with epithelial ovarian cancer: a systematic review and meta-analysis of observational studies," *International Journal of Gynecological Cancer*, vol. 21, no. 9, pp. 1525–1532, 2011.

[150] T. Pierpoint, P. M. McKeigue, A. J. Isaacs, S. H. Wild, and H. S. Jacobs, "Mortality of women with polycystic ovary syndrome at long-term follow-up," *Journal of Clinical Epidemiology*, vol. 51, no. 7, pp. 581–586, 1998.

[151] A. Gadducci, A. Gargini, E. Palla, A. Fanucchi, and A. R. Genazzani, "Polycystic ovary syndrome and gynecological cancers: is there a link?" *Gynecological Endocrinology*, vol. 20, no. 4, pp. 200–208, 2005.

[152] M. Reincke, M. Fassnacht, S. Väth, P. Mora, and B. Allolio, "Adrenal incidentalomas: a manifestation of the metabolic syndrome?" *Endocrine Research*, vol. 22, no. 4, pp. 757–761, 1996.

[153] S. Midorikawa, H. Sanada, S. Hashimoto, T. Suzuki, and T. Watanabe, "The improvement of insulin resistance in patients with adrenal incidentaloma by surgical resection," *Clinical Endocrinology*, vol. 54, no. 6, pp. 797–804, 2001.

[154] S. J. Chen, S. N. Yu, J. E. Tzeng et al., "Characterization of the major histopathological components of thyroid nodules using sonographic textural features for clinical diagnosis and management," *Ultrasound in Medicine and Biology*, vol. 35, no. 2, pp. 201–208, 2009.

[155] K. Wojciechowska and A. Lewinski, "BRAF mutations in papillary thyroid carcinoma," *Endocrine Regulations*, vol. 40, no. 4, pp. 129–138, 2006.

[156] V. Vella, L. Sciacca, G. Pandini et al., "The IGF system in thyroid cancer: new concepts," *Journal of Clinical Pathology*, vol. 54, no. 3, pp. 121–124, 2001.

[157] B. Tode, M. Serio, C. M. Rotella et al., "Insulin-like growth factor-I: autocrine secretion by human thyroid follicular cells in primary culture," *Journal of Clinical Endocrinology and Metabolism*, vol. 69, no. 3, pp. 639–647, 1989.

[158] L. K. Bachrach, F. R. Liu, G. N. Burrow, and M. C. Eggo, "Characterization of insulin-like growth factor-binding proteins from sheep thyroid cells," *Endocrinology*, vol. 125, no. 6, pp. 2831–2838, 1989.

[159] D. W. Williams, E. D. Williams, and D. Wynford-Thomas, "Loss of dependence on IGF-1 for proliferation of human thyroid adenoma cells," *British Journal of Cancer*, vol. 57, no. 6, pp. 535–539, 1988.

[160] N. Onoda, E. Ohmura, T. Tsushima et al., "Autocrine role on insulin-like growth factor (IGF)-I in a human thyroid cancer cell line," *European Journal of Cancer A*, vol. 28, no. 11, pp. 1904–1909, 1992.

[161] S. Masood, L. J. Auguste, A. Westerband, C. Belluco, E. Valderama, and J. Attie, "Differential oncogenic expression in thyroid follicular and Hurthle cell carcinomas," *American Journal of Surgery*, vol. 166, no. 4, pp. 366–368, 1993.

[162] F. Minuto, A. Barreca, P. del Monte, G. Cariola, G. C. Torre, and G. Giordano, "Immunoreactive insulin-like growth factor I (IGF-I) and IGF-I-binding protein content in human thyroid tissue," *Journal of Clinical Endocrinology and Metabolism*, vol. 68, no. 3, pp. 621–626, 1989.

[163] V. Poulaki, C. S. Mitsiades, C. McMullan et al., "Regulation of vascular endothelial growth factor expression by onsulin-like growth factor I in thyroid carcinomas," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 11, pp. 5392–5398, 2003.

[164] H. Gydee, J. T. O'Neill, A. Patel, A. J. Bauer, R. M. Tuttle, and G. L. Francis, "Differentiated thyroid crcinomas from children and adolescents express IGF-I and the IGF-I Receptor (IGF-I-R). Cancers with the most intense IGF-I-R expression may be more aggressive," *Pediatric Research*, vol. 55, no. 4, pp. 709–715, 2004.

[165] E. Maiorano, A. Ciampolillo, G. Viale et al., "Insulin-like growth factor I expression in thyroid tumors," *Applied Immunohistochemistry and Molecular Morphology*, vol. 8, no. 2, pp. 110–119, 2000.

[166] M. S. Wicha, S. Liu, and G. Dontu, "Cancer stem cells: an old idea—a paradigm shift," *Cancer Research*, vol. 66, no. 4, pp. 1883–1890, 2006.

[167] Z. Wang, G. Chakravarty, S. Kim et al., "Growth-inhibitory effects of human anti-insulin-like growth factor-1 receptor antibody (A12) in an orthotopic nude mouse model of anaplastic thyroid carcinoma," *Clinical Cancer Research*, vol. 12, no. 15, pp. 4755–4765, 2006.

[168] E. Phielix, J. Szendroedi, and M. Roden, "The role of metformin and thiazolidinediones in the regulation of hepatic glucose metabolism and its clinical impact," *Trends in Pharmacological Sciences*, vol. 32, pp. 607–616, 2011.

[169] A. Aiello, G. Pandini, F. Frasca et al., "Peroxisomal proliferator-activated receptor-γ agonists induce partial reversion of epithelial-mesenchymal transition in anaplastic thyroid cancer cells," *Endocrinology*, vol. 147, no. 9, pp. 4463–4475, 2006.

[170] G. Chen, S. Xu, K. Renko, and M. Derwahl, "Metformin inhibits growth of thyroid carcinoma cells, suppresses self-renewal of derived cancer stem cells, and potentiates the effect of chemotherapeutic agents," *Journal of Clinical Endocrinology and Metabolism*, vol. 97, pp. E510–E520, 2012.

[171] A. C. Latronico and G. P. Chrousos, "Extensive personal, experience: adrenocortical tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 5, pp. 1317–1324, 1997.

[172] C. Fottner, A. Hoeflich, E. Wolf, and M. M. Weber, "Role of the insulin-like growth factor system in adrenocortical growth control and carcinogenesis," *Hormone and Metabolic Research*, vol. 36, no. 6, pp. 397–405, 2004.

[173] C. Shon, V. Mladenovski, R. Petkov et al., "Diagnosis and surgical treatment of adrenocortical carcinoma. A review of the literature and report of two cases," *Khirurgiia*, vol. 56, no. 2, pp. 45–49, 2000.

[174] J. A. Soreide, K. Brabrand, and S. O. Thoresen, "Adrenal cortical carcinoma in Norway, 1970–1984," *World Journal of Surgery*, vol. 16, no. 4, pp. 663–667, 1992.

[175] J. Lindholm, S. Juul, J. O. L. Jørgensen et al., "Incidence and late prognosis of Cushing's syndrome: a population-based study," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 1, pp. 117–123, 2001.

[176] R. C. Ribeiro, E. M. Pinto, G. P. Zambetti, and C. Rodriguez-Galindo, "The international pediatric adrenocortical tumor registry initiative: contributions to clinical, biological, and treatment advances in pediatric adrenocortical tumors," *Molecular and Cellular Endocrinology*, vol. 351, pp. 37–43, 2012.

[177] M. Q. Almeida, M. C. B. V. Fragoso, C. F. P. Lotfi et al., "Expression of insulin-like growth factor-II and its receptor in pediatric and adult adrenocortical tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 9, pp. 3524–3531, 2008.

[178] H. M. Khandwala, I. E. McCutcheon, A. Flyvbjerg, and K. E. Friend, "The effects of insulin-like growth factors on tumorigenesis and neoplastic growth," *Endocrine Reviews*, vol. 21, no. 3, pp. 215–244, 2000.

[179] G. Cantini, A. Lombardi, E. Piscitelli et al., "Rosiglitazone inhibits adrenocortical cancer cell proliferation by interfering with the IGF-IR intracellular signaling," *PPAR Research*, Article ID 904041, 2008.

[180] N. Boulle, A. Logié, C. Gicquel, L. Perin, and Y. L. Bouc, "Increased levels of insulin-like growth factor II (IGF-II) and IGF- binding protein-2 are associated with malignancy in sporadic adrenocortical tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 5, pp. 1713–1720, 1998.

[181] A. Logié, N. Boulle, V. Gaston et al., "Autocrine role of IGF-II in proliferation of human adrenocortical carcinoma NCI H295R cell line," *Journal of Molecular Endocrinology*, vol. 23, no. 1, pp. 23–32, 1999.

[182] S. Faiçal, R. M. B. Maciel, V. Nosé-Alberti, M. C. Santos, and C. E. Kater, "Immunodetection of insulin-like growth factor I (IGF-I) in normal and pathological adrenocortical tissue," *Endocrine Pathology*, vol. 9, no. 1, pp. 63–70, 1998.

[183] T. J. Giordano, "Gene expression profiling of endocrine tumors using DNA microarrays: progress and Promise," *Endocrine Pathology*, vol. 14, no. 2, pp. 107–116, 2003.

[184] F. de Fraipont, M. El Atifi, N. Cherradi et al., "Gene expression profiling of human adrenocortical tumors using complementary deoxyribonucleic acid microarrays identifies several candidate genes as markers of malignancy," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 3, pp. 1819–1829, 2005.

[185] C. Gicquel, X. Bertagna, H. Schneid et al., "Rearrangements at the 11p15 locus and overexpression of insulin-like growth factor-II gene in sporadic adrenocortical tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 78, no. 6, pp. 1444–1453, 1994.

[186] C. Gicquel, M. L. Raffin-Sanson, V. Gaston et al., "Structural and functional abnormalities at 11p15 are associated with the malignant phenotype in sporadic adrenocortical tumors: study on a series of 82 tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 8, pp. 2559–2565, 1997.

[187] C. Gicquel, N. Boulle, A. Logié, N. Bourcigaux, V. Gaston, and Y. Le Bouc, "Involvement of the IGF system in the pathogenesis of adrenocortical tumors," *Annales d'Endocrinologie*, vol. 62, no. 2, pp. 189–192, 2001.

[188] E. Wolf, A. Hoeflich, and H. Lahm, "What is the function of IGF-II in postnatal life? Answers from transgenic mouse models," *Growth Hormone and IGF Research*, vol. 8, no. 3, pp. 185–193, 1998.

[189] M. M. Weber, C. Fottner, P. Schmidt et al., "Postnatal overexpression of insulin-like growth factor II in transgenic mice is associated with adrenocortical hyperplasia and enhanced steroidogenesis," *Endocrinology*, vol. 140, no. 4, pp. 1537–1543, 1999.

[190] N. Boulle, E. Baudin, C. Gicquel et al., "Evaluation of plasma insulin-like growth factor binding protein-2 as a marker for adrenocortical tumors," *European Journal of Endocrinology*, vol. 144, no. 1, pp. 29–36, 2001.

[191] A. N. West, G. A. Neale, S. Pounds et al., "Gene expression profiling of childhood adrenocortical tumors," *Cancer Research*, vol. 67, no. 2, pp. 600–608, 2007.

[192] M. M. Weber, C. J. Auernhammer, W. Kiess, and D. Engelhardt, "Insulin-like growth factor receptors in normal and tumorous adult human adrenocortical glands," *European Journal of Endocrinology*, vol. 136, no. 3, pp. 296–303, 1997.

[193] A. Blackburn, R. A. Dressendörfer, W. F. Blum et al., "Interactions of insulin-like growth factor (IGF)-II and growth hormone in vivo: circulating levels of IGF-I and IGF-binding proteins in transgenic mice," *European Journal of Endocrinology*, vol. 137, no. 6, pp. 701–708, 1997.

[194] M. M. Weber, C. Fottner, and E. Wolf, "The role of the insulin-like growth factor system in adrenocortical tumourigenesis," *European Journal of Clinical Investigation*, vol. 30, supplement 3, pp. 69–75, 2000.

[195] J. G. Reeve, L. B. Kirby, A. Brinkman, S. A. Hughes, J. Schwander, and N. M. Bleechen, "Insulin-like growth-factor-binding protein gene expression and protein production by human tumour cell lines," *International Journal of Cancer*, vol. 51, no. 5, pp. 818–821, 1992.

[196] C. Fottner, D. Engelhardt, M. W. Elmlinger, and M. M. Weber, "Identification and characterization of insulin-like growth factor (IGF)-binding protein expression and secretion by adult human adrenocortical cells: differential regulation by IGFs and adrenocorticotropin," *Journal of Endocrinology*, vol. 168, no. 3, pp. 465–474, 2001.

[197] A. Hoeflich, O. Fettscher, H. Lahm et al., "Overexpression of insulin-like growth factor-binding protein-2 results in increased tumorigenic potential in Y-1 adrenocortical tumor cells," *Cancer Research*, vol. 60, no. 4, pp. 834–838, 2000.

[198] L. K. Bale and C. A. Conover, "Regulation of insulin-like growth factor binding protein-3 messenger ribonucleic acid expression by insulin-like growth factor I," *Endocrinology*, vol. 131, no. 2, pp. 608–614, 1992.

[199] C. Camacho-Hubner, W. H. Busby Jr., R. H. McCusker, G. Wright, and D. R. Clemmons, "Identification of the forms of insulin-like growth factor-binding proteins produced by human fibroblasts and the mechanisms that regulate their secretion," *Journal of Biological Chemistry*, vol. 267, no. 17, pp. 11949–11956, 1992.

[200] J. L. Martin, M. Ballesteros, and R. C. Baxter, "Insulin-like growth factor-I (IGF-I) and transforming growth factor-β1 release IGF-binding protein-3 from human fibroblasts by different mechanisms," *Endocrinology*, vol. 131, no. 4, pp. 1703–1710, 1992.

[201] M. M. Rechler, "Insulin-like growthfactor binding proteins," *Vitamins and Hormones*, vol. 47, pp. 1–114, 1993.

[202] C. A. Conover and D. D. de Leon, "Acid-activated insulin-like growth factor-binding protein-3 proteolysis in normal and transformed cells. Role of cathepsin D," *Journal of Biological Chemistry*, vol. 269, no. 10, pp. 7076–7080, 1994.

[203] C. A. Conover, L. K. Bale, S. K. Durham, and D. R. Powell, "Insulin-like growth factor (IGF) binding protein-3 potentiation of IGF action is mediated through the phosphatidylinositol-3-kinase pathway and is associated with alteration in protein kinase B/AKT sensitivity," *Endocrinology*, vol. 141, no. 9, pp. 3098–3103, 2000.

[204] J. I. Jones and D. R. Clemmons, "Insulin-like growth factors and their binding proteins: biological actions," *Endocrine Reviews*, vol. 16, no. 1, pp. 3–34, 1995.

[205] M. M. Weber, P. Simmler, C. Fottner, and D. Engelhardt, "Insulin-like growth factor II (IGF-II) is more potent than IGF-I in stimulating cortisol secretion from cultured bovine adrenocortical cells: interaction with the IGF-I receptor and IGF-binding proteins," *Endocrinology*, vol. 136, no. 9, pp. 3714–3720, 1995.

[206] C. Fottner, D. Engelhardt, and M. M. Weber, "Characterization of insulin-like growth factor binding proteins (IGFBPs) secreted by bovine adrenocortical cells in primary culture: regulation by insulin-like growth factors (IGFs) and adrenocorticotropin (ACTH)," *Hormone and Metabolic Research*, vol. 31, no. 2-3, pp. 203–208, 1999.

[207] D. Velázquez-Fernández, C. Laurell, J. Geli et al., "Expression profiling of adrenocortical neoplasms suggests a molecular signature of malignancy," *Surgery*, vol. 138, no. 6, pp. 1087–1094, 2005.

[208] M. Doghman, A. El Wakil, B. Cardinaud et al., "Regulation of insulin-like growth factor-mammalian target of rapamycin signaling by MicroRNA in childhood adrenocortical tumors," *Cancer Research*, vol. 70, no. 11, pp. 4666–4675, 2010.

[209] F. M. Barlaskar, A. C. Spalding, J. H. Heaton et al., "Preclinical targeting of the type I insulin-like growth factor receptor in adrenocortical carcinoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 1, pp. 204–212, 2009.

[210] W. Shen, J. Xian, W. L. Hu, and J. Liu, "Effect of insulin-like growth factor and its receptor-I antibody on growth of human adrenocortical carcinoma SW-13 cell lines in vitro," *Nan Fang Yi Ke Da Xue Xue Bao*, vol. 27, no. 1, pp. 88–91, 2007.

[211] E. D. Rosen and B. M. Spiegelman, "PPARγ: a nuclear regulator of metabolism, differentiation, and cell growth," *Journal of Biological Chemistry*, vol. 276, no. 41, pp. 37731–37734, 2001.

[212] C. E. Clay, A. M. Namen, G. I. Atsumi et al., "Influence of J series prostaglandins on apoptosis and tumorigenesis of breast cancer cells," *Carcinogenesis*, vol. 20, no. 10, pp. 1905–1911, 1999.

[213] P. Ferruzzi, E. Ceni, M. Tarocchi et al., "Thiazolidinediones inhibit growth and invasiveness of the human adrenocortical cancer cell line H295R," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 3, pp. 1332–1339, 2005.

[214] J. R. Chien, G. Aletti, D. A. Bell, G. L. Keeney, V. Shridhar, and L. C. Hartmann, "Molecular pathogenesis and therapeutic targets in epithelial ovarian cancer," *Journal of Cellular Biochemistry*, vol. 102, no. 5, pp. 1117–1129, 2007.

[215] H. Kajihara, Y. Yamada, S. Kanayama et al., "Clear cell carcinoma of the ovary: potential pathogenic mechanisms (review)," *Oncology Reports*, vol. 23, no. 5, pp. 1193–1203, 2010.

[216] T. Riman, I. Persson, and S. Nilsson, "Hormonal aspects of epithelial ovarian cancer: review of epidemiological evidence," *Clinical Endocrinology*, vol. 49, no. 6, pp. 695–707, 1998.

[217] A. Auranen, S. Grénman, J. Mäkinen, E. Pukkala, R. Sankila, and T. Salmi, "Borderline ovarian tumors in Finland: epidemiology and familial occurrence," *American Journal of Epidemiology*, vol. 144, no. 6, pp. 548–553, 1996.

[218] M. C. Beauchamp, A. Yasmeen, A. Knafo, and W. H. Gotlieb, "Targeting insulin and insulin-like growth factor pathways

in epithelial ovarian cancer," *Journal of Oncology*, vol. 2010, Article ID 257058, 11 pages, 2010.

[219] E. R. King, Z. Zu, Y. T. M. Tsang et al., "The insulin-like growth factor 1 pathway is a potential therapeutic target for low-grade serous ovarian carcinoma," *Gynecologic Oncology*, vol. 123, no. 1, pp. 13–18, 2011.

[220] D. Yee, F. R. Morales, T. C. Hamilton, and D. D. Von Hoff, "Expression of insulin-like growth factor I, its binding proteins, and its receptor in ovarian cancer," *Cancer Research*, vol. 51, no. 19, pp. 5107–5112, 1991.

[221] C. A. Conover, L. C. Hartmann, S. Bradley et al., "Biological characterization of human epithelial ovarian carcinoma cells in primary culture: the insulin-like growth factor system," *Experimental Cell Research*, vol. 238, no. 2, pp. 439–449, 1998.

[222] K. R. Kalli and C. A. Conover, "The insulin-like growth factor/insulin system in epithelial ovarian cancer," *Frontiers in Bioscience*, vol. 8, pp. d714–d722, 2003.

[223] S. Chakrabarty and L. Kondratick, "Insulin-like growth factor binding protein-2 stimulates proliferation and activates multiple cascades of the mitogen-activated protein kinase pathways in NIH-OVCAR3 human epithelial ovarian cancer cells," *Cancer Biology and Therapy*, vol. 5, no. 2, pp. 189–197, 2006.

[224] A. Lukanova, E. Lundin, P. Toniolo et al., "Circulating levels of insulin-like growth factor-I and risk of ovarian cancer," *International Journal of Cancer*, vol. 101, no. 6, pp. 549–554, 2002.

[225] S. S. Tworoger, I. M. Lee, J. E. Buring, M. N. Pollak, and S. E. Hankinson, "Insulin-like growth factors and ovarian cancer risk: a nested case-control study in three cohorts," *Cancer Epidemiology Biomarkers and Prevention*, vol. 16, no. 8, pp. 1691–1695, 2007.

[226] J. M. Lancaster, R. A. Sayer, C. Blanchette et al., "High expression of insulin-like growth factor binding protein-2 messenger RNA in epithelial ovarian cancers produces elevated preoperative serum levels," *International Journal of Gynecological Cancer*, vol. 16, no. 4, pp. 1529–1535, 2006.

[227] R. A. Sayer, J. M. Lancaster, J. Pittman et al., "High insulin-like growth factor-2 (IGF-2) gene expression is an independent predictor of poor survival for patients with advanced stage serous epithelial ovarian cancer," *Gynecologic Oncology*, vol. 96, no. 2, pp. 355–361, 2005.

[228] L. Lu, D. Katsaros, A. Wiley et al., "The relationship of insulin-like growth factor-II, insulin-like growth factor binding protein-3, and estrogen receptor-α expression to disease progression in epithelial ovarian cancer," *Clinical Cancer Research*, vol. 12, no. 4, pp. 1208–1214, 2006.

[229] L. Lu, D. Katsaros, A. Wiley et al., "Promoter-specific transcription of insulin-like growth factor-II in epithelial ovarian cancer," *Gynecologic Oncology*, vol. 103, no. 3, pp. 990–995, 2006.

[230] J. S. Sussenbach, R. J. T. Rodenburg, W. Scheper, and P. Holthuizen, "Transcriptional and post-transcriptional regulation of the human IGF-II gene expression," *Advances in Experimental Medicine and Biology*, vol. 343, pp. 63–71, 1993.

[231] A. P. Wolffe and M. A. Matzke, "Epigenetics: regulation through repression," *Science*, vol. 286, no. 5439, pp. 481–486, 1999.

[232] B. Qian, D. Katsaros, L. Lu et al., "IGF-II promoter specific methylation and expression in epithelial ovarian cancer and their associations with disease characteristics," *Oncology Reports*, vol. 25, no. 1, pp. 203–213, 2011.

[233] A. C. Beeghly, D. Katsaros, A. L. Wiley et al., "IGF-II promoter methylation and ovarian cancer prognosis," *Journal of Cancer Research and Clinical Oncology*, vol. 133, no. 10, pp. 713–723, 2007.

[234] D. Spentzos, S. A. Cannistra, F. Grall et al., "IGF axis gene expression patterns are prognostic of survival in epithelial ovarian cancer," *Endocrine-Related Cancer*, vol. 14, no. 3, pp. 781–790, 2007.

[235] M. Muller, M. Dietel, A. Turzynski, and K. Wiechen, "Antisense phosphorothioate oligodeoxynucleotide down-regulation of the insulin-like growth factor I receptor in ovarian cancer cells," *International Journal of Cancer*, vol. 77, pp. 567–571, 1998.

[236] W. H. Gotlieb, I. Bruchim, J. Gu et al., "Insulin-like growth factor receptor I targeting in epithelial ovarian cancer," *Gynecologic Oncology*, vol. 100, no. 2, pp. 389–396, 2006.

[237] C. Li, V. W. Liu, D. W. Chan, K. M. Yao, and H. Y. Ngan, "LY294002 and metformin cooperatively enhance the inhibition of growth and the induction of apoptosis of ovarian cancer cells," *International Journal of Gynecological Cancer*, vol. 22, no. 1, pp. 15–22, 2012.

[238] H. Liao, Q. Zhou, Y. Gu, T. Duan, and Y. Feng, "Luteinizing hormone facilitates angiogenesis in ovarian epithelial tumor cells and metformin inhibits the effect through the mTOR signaling pathway," *Oncology Reports*, vol. 27, no. 6, pp. 1873–1878, 2012.

[239] I. L. Romero, A. McCormick, K. A. McEwen et al., "Relationship of type II diabetes and metformin use to ovarian cancer progression, survival, and chemosensitivity," *Obstet Gynecol*, vol. 119, no. 1, pp. 61–67, 2012.

[240] A. Bakhru, R. J. Buckanovich, and J. J. Griggs, "The impact of diabetes on survival in women with ovarian cancer," *Gynecologic Oncology*, vol. 121, no. 1, pp. 106–111, 2011.

Hindawi Publishing Corporation
Journal of Oncology
Volume 2012, Article ID 872713, 10 pages
doi:10.1155/2012/872713

Review Article

Updated and New Perspectives on Diagnosis, Prognosis, and Therapy of Malignant Pheochromocytoma/Paraganglioma

Gabriele Parenti,[1] Benedetta Zampetti,[2] Elena Rapizzi,[2,3] Tonino Ercolino,[1] Valentino Giachè,[2] and Massimo Mannelli[2,3]

[1] *Endocrinology Unit, Azienda Ospedaliero-Universitaria Careggi, Largo Brambilla 3, 50134 Florence, Italy*
[2] *Department of Clinical Pathophysiology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy*
[3] *Istituto Toscano Tumori, Via Taddeo Alderotti 26N, 50139 Florence, Italy*

Correspondence should be addressed to Massimo Mannelli, m.mannelli@dfc.unifi.it

Received 30 April 2012; Accepted 5 June 2012

Academic Editor: Marialuisa Appetecchia

Malignant pheochromocytomas/paragangliomas are rare tumors with a poor prognosis. Malignancy is diagnosed by the development of metastases as evidenced by recurrences in sites normally devoid of chromaffin tissue. Histopathological, biochemical, molecular and genetic markers offer only information on potential risk of metastatic spread. Large size, extraadrenal location, dopamine secretion, *SDHB* mutations, a PASS score higher than 6, a high Ki-67 index are indexes for potential malignancy. Metastases can be present at first diagnosis or occur years after primary surgery. Measurement of plasma and/or urinary metanephrine, normetanephrine and metoxytyramine are recommended for biochemical diagnosis. Anatomical and functional imaging using different radionuclides are necessary for localization of tumor and metastases. Metastatic pheochromocytomas/paragangliomas is incurable. When possible, surgical debulking of primary tumor is recommended as well as surgical or radiosurgical removal of metastases. I-131-MIBG radiotherapy is the treatment of choice although results are limited. Chemotherapy is reserved to more advanced disease stages. Recent genetic studies have highlighted the main pathways involved in pheochromocytomas/paragangliomas pathogenesis thus suggesting the use of targeted therapy which, nevertheless, has still to be validated. Large cooperative studies on tissue specimens and clinical trials in large cohorts of patients are necessary to achieve better therapeutic tools and improve patient prognosis.

1. Introduction

Paragangliomas (PGLs) are rare neuroendocrine tumors that arise in sympathetic and parasympathetic paraganglia and derive from neural crest cells. Approximately 80–85% of these tumour arise from the adrenal medulla and are named pheochromocytomas (PCCs), whereas 15–20% are located in extra-adrenal chromaffin tissue and are referred to as secreting paragangliomas (sPGLs). The latter term is also used to describe tumors derived from parasympathetic tissue in the head and neck (HNPGLs).

PCCs and abdominal sPGLs are usually catecholamine-producing tumours, whereas most of the HNPGLs are non-functioning [1].

The majority of PGLs are sporadic, but recent data have demonstrated a high prevalence of hereditary forms (approximately 35%) [2]. Sporadic PGLs are usually diagnosed in patients older than 40–50 years, whereas hereditary forms are diagnosed in younger patients.

Malignancy is defined by presence of metastases, tumor spread in sites where chromaffin tissue is normally absent such as lymph nodes, liver, lungs, and bones. Malignant PGLs are extremely rare. An estimated incidence in USA in 2002 was 93 cases per 400 million persons [3].

Nearly 10% and 20% of PCCs and abdominal sPGLs, respectively, are malignant [4], whereas HNPGLs are usually benign [5].

2. Clinical Feature

The high variability in clinical presentation of PGLs is well known [6]. It depends on the variability in the biology of

these tumours which can express different catecholamine biosynthetic enzymes, secrete different vasoactive peptide (i.e., neuropeptide Y, adrenomedullin, or atrial natriuretic peptide) [7], present different symptoms related to tumour mass or present symptoms related to other organs involvement in syndromic forms.

Hypertension is the most common feature of PCCs and sPGL: it can be continuous, intermittent, and often paroxysmal in nature. Hypertensive crises are frequently associated with the classic triad of severe headache, palpitations, and diaphoresis. Other signs or symptoms such as dyspnoea, weakness, arrhythmias, visual disturbances and metabolic effect such as glucose intolerance and weight loss are reported [1]. The cardiovascular complications (sudden death, myocardial infarction, hearth failure, and cerebrovascular accidents) represent the most frequent causes of morbidity and mortality in these patients.

HNPGLs are usually clinically silent, but they can determine manifestations related to mass effect or infiltration of the adjacent structures. In such situations the presence of a palpable neck mass as well as pain, dysphagia, tinnitus, or cranial nerve palsies has been reported [8].

In addition to the abovementioned symptoms and signs, malignant PGLs may also present "systemic" symptoms (anorexia, fatigue, and weight loss) or clinical manifestations related to the metastatic disease such as pain in bones affected by metastatic spread. Malignant PGLs, being less-differentiated tumours with a less differentiated biosynthetic pathway, generally secrete noradrenaline and/or dopamine causing even milder cardiovascular symptoms and a subclinical picture [9]. Metastatic spread may occur at presentation or even after many years from primary surgery.

3. Diagnosis: Biochemistry

The recommended screening test for initial assessment of PGLs is the measurement of plasma free-metanephrines or urine-deconjugated differential metanephrines [10]. In fact in comparison to plasma or urine catecohlamines and vanilmandelic acid, metanephrines show higher sensitivity, ranging around 98-99% [11, 12]. This is mainly related to their longer half-life and to their continuous production by the tumour where catecholamines are converted to metanephrines by the high methyltransferase activity of the chromaffin tissue [13].

The biochemical phenotype does not permit to differentiate malignant from benign PGLs.

PGLs exhibit different biochemical properties as PCCs mainly produce adrenaline, while sPGLs secrete noradrenaline. Malignant PCCs secrete predominantly noradrenaline [14], but due to an even less-differentiated catecholamine biosynthetic pathway, they may often produce mainly or exclusively dopamine [15]. Therefore, the presence of large predominantly noradrenaline-producing PGLs and increased levels of plasma dopamine or its metabolite methoxytyramine may suggest malignancy [11, 16].

Plasma chromogranin A (CgA), a protein stored and cosecreted with catecholamines, is often increased in functioning and nonfunctioning PGLs [17]. CgA shows a sensitivity of 83–89% for identifying PGLs, but it often shows false positive results because of liver or kidney failure or proton pump inhibitor therapy [18].

Malignancy is generally associated to very high plasma levels of CgA [11].

High plasma levels of neuron-specific enolase are sometimes found in patients with malignant PGLs [19, 20], while overexpression of secretogranin II and prohormone convertases I and II suggests a benign lesion [21].

4. Diagnosis: Anatomical and Functional Imaging

Anatomical imaging such as computed tomography (CT) or magnetic resonance imaging (MRI) is useful as the first radiological approach in patients with PGLs. CT shows a sensitivity of 77–98% and a specificity of 29–92% in the localization of adrenal or extra-adrenal tumors. A slightly better accuracy (sensitivity 90–100% and specificity 50–100%) has been reported for MRI, especially for the detection of extra-adrenal disease [22].

PGLs are highly vascular tumors with a high intracellular water content and frequent intratumoral cystic lesions, which show a typical, but not diagnostic, high signal on T2-weighted imaging, and strong enhancement after contrast-agent administration. Nevertheless, in large tumors with haemorrhagic and/or necrotic areas (features often detected in malignant lesions), the signal intensity on T2-weighted images may be low [23].

Ultrasound imaging is of limited diagnostic yield but can be useful for the detection of HNPGLs [22, 24].

After "anatomical" imaging, a "functional" imaging is generally recommended. ^{131}I or ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy has been used extensively as a first-line nuclear medicine technique in evaluation of patients with PGLs. MIBG has chemical similarities to norepinephrine and is concentrated in chromaffin tissue, via the human norepinephrine transporter (hNET), that is expressed in most of chromaffin cells and it is normally responsible for catecholamines uptake [25].

^{123}I-MIBG is superior to ^{131}I-MIBG in terms of physical properties, quality of images, and sensitivity. ^{123}I-MIBG scanning shows a sensitivity of 83–100% and a specificity of 95–100% [22]. The possibility to perform a whole-body study, may permit a better evaluation of extra-adrenal localization of the disease as well as of multiple tumors and/or metastatic sites [25]. The sensitivity of this technique in malignant PGLs may be lower as evidenced in situation highly associated with malignancy as in *SDHB* mutation carriers (see later) or patients with dopamine-secreting tumours which usually do not uptake MIBG [26].

In patients with negative MIBG scintigraphy, other tracers may be used. The expression of somatostatin receptors (SSTRs), especially SSTR 2, 3, and 5 on chromaffin cells, represents the rationale for the use of radiolabelled somatostatin analogues in localization of these tumors. Indium-11-DTPA-octreotide (^{111}In-penteotride) is the tracer most commonly used; it is of limited value in benign PCCs, but it may be

useful in detecting extra-adrenal disease as well as MIBG-negative metastases. In fact a sensitivity near to 90% has been reported for localizing sPGLs, HNPGLs, or malignant PCCs [22, 27].

Somatostatin analogues labelled with gallium-68 can be used in PET imaging; ^{68}Ga-DOTATOC (DOTA^0-D-Phe^1-Tyr^3-octreotide) has shown a better sensitivity than ^{111}In-penteotride in the detection of neuroendocrine tumors especially in detecting small lesions or neoplasms bearing only a low density of SSTR. Moreover, it permits a better identification of metastases located in the lung or in the skeleton. In relation to PGLs, this tracer seems superior to ^{18}F-labelled fluoro-deoxy-glucose (^{18}F-FDG) in detecting malignant PCCs and sPGLs [28–30].

Radiolabelled dopamine (DA) or dihydroxypheylalanine (DOPA) which are transported in to chromaffin cells by hNET, may be used as tracers in positron emission tomography (PET) imaging. PET with 6-[^{18}F]-fluoro-DA can detect metastatic PCCs with better sensitivity than ^{131}I-MIBG [31], whereas PET with 6-[^{18}F]-fluoroDOPA is superior in imaging sPGLs and HNPGLs [32]. However, as for MIBG, these tracers show a relative low sensitivity (70–88%) in PGLs associated with *SDHB* gene mutations. In such conditions PET with ^{18}F-FDG shows a higher sensitivity (97–100%) [33]; this PET scanning is useful in identifying glucose-avid metastatic lesions, particularly if they are MIBG-negative [34].

Finally PET imaging with ^{11}C-hydroxyephedrine has provided high sensitivity and specificity (92 and 100%, resp.) in the detection of PGLs, but the small number of patients studied makes not possible to draw conclusions on its utility [35].

5. Diagnosis: Histopathologic and Molecular Markers

Despite the increasing availability of molecular diagnostic and prognostic markers, it remains difficult to predict, on the basis of histological findings, whether an apparently benign PGL will develop in a malignant tumor. From a prognostic point of view, only relative risk factors can be taken into account. In general PGLs larger than 5 cm with necrotic areas as well as extra-adrenal tumors carry a higher risk of malignancy than neoplasms that are small or located in the adrenal. Several scoring systems considering invasion, histologic growth patterns, cytologic features, or mitotic activity have been proposed to calculate the risk of malignancy [36–38]. One of the most utilized score is "Pheochromocytoma of the Adrenal gland Scales Score (PASS)," proposed by Thompson on 2002. Table 1 reports the items and their values which are necessary to calculate the PASS. A PASS score ≥4 was at first considered suggestive for a biological aggressive behaviour, but a later study revealed that all malignant PCCs had a PASS >6 [39]. On the basis of these results a PASS score <4 or >6 suggest benign and malignant lesions respectively, whereas a value between 4 and 6 suggests an intermediate risk. In any event, as none of the available scores predicts malignant development unequivocally, after the removal of an isolated primary PGL, a followup of the patient is recommended in order to reveal early disease recurrence. Between histological features, high cellularity and particularly the presence of tumor necrosis are considered potential indicators of malignancy.

Table 1: Pheochromocytoma of the adrenal gland scoring scale (PASS) [38].

Items	Value
Nuclear hyperchromasia	1
Profound nuclear pleomorphism	1
Capsular invasion	1
Vascular invasion	1
Extension into adipose tissue	2
Atypical mitotic figures	2
Greater than 3 of 10 mitotic figures high-power field	2
Tumor cell spindling	2
Cellular monotony	2
High cellularity	2
Central or confluent tumor necrosis	2
Large nests or diffuse growth (>10% of tumor volume)	2
Total	20

Further information can derive from the evaluation of specific molecular markers. Several malignancy tissue markers such as cyclooxygenase-2, secretogranin II-derived peptide, *N*-cadherin, vascular endothelial growth factor (VEGF), endothelin receptor type A (ETA), and type B (ETB) and telomerase have been identified. In particular telomerase, which is a ribonucleoprotein complex that includes the telomerase RNA component, the telomerase-associated protein (TP1), the telomerase catalytic subunit (hTERT), and the heat shock protein 90 (HSP90) seem to be closely related to the malignant potential of PGLs. In fact an upregulation of hTERT, HSP90, and telomerase activity has been evidenced in malignant cells of PCCs [40].

The Ki-67 nuclear antigen represents another potential molecular marker which has been associated with more aggressive cancers. A Ki-67 index >3% is considered a useful parameter predicting malignant potential [41].

Another promising marker predicting metastatic potential seems the transcription factor SNAIL. Positive immunostaining has been found significantly higher in metastatic than benign PGLs [42, 43].

Novel biomarkers are recently being identified by micro-RNA expression profiling studies. Micro-RNA is small single-strand (~22 bp), nonprotein coding RNA fragments, which are able to negatively regulate protein expression by either cleavage or translational repression of mRNA [44]. Recently, Meyer-Rochow, and colleagues [45] investigated 12 malignant, 12 benign tumors, and 5 healthy adrenal medulla samples. They found that miR-483-5p was overexpressed, while miR15a and miR-16, which are involved in proliferation and apoptosis, were downregulated in malignant compared to benign tumors. MicroRNA expression is tissue specific, and it has been demonstrated to be altered in several other human tumors, For these reasons, they can be of great

Table 2: Correlations between gene mutations and clinical phenotype.

Syndrome	Gene	PCCs (%)	Sympathetic PGL	Parasympathetic PGL	Bilateral/multifocal neoplasia	Malignancy (%)
MEN 2A	*RET*	~50	Very rare	Extremely rare	+	<3
MEN 2B	*RET*	~50	Very rare	Extremely rare	+	<3
VHL	*VHL*	10–20	+	Rare	+	5
NF1	*NF1*	5	–	–	–	11
PGL1	*SDHD*	+	+	+	+	~5
PGL2	*SDHAF2*	–	–	+	+	Not known
PGL3	*SDHC*	–	Rare	+	–	Not known
PGL4	*SDHB*	Rare	+	Rare	+	~40
PGL5	*SDHA*	–	+	–	Not known	Not known
TMEM127 mutation carriers	*TMEM127*	100	–	–	+	~5
MAX mutation carriers	*MAX*	100	+	Extremely rare	+	~10

PCCs: pheochromocytomas; PGL: paragangliomas; +: present; –: absent.

relevance for the establishment of malignancy, but further investigations in larger cohorts of patients are necessary to confirm these encouraging results.

6. Diagnosis: Genetic Aspects

Until 2000, only 10% of PGLs were considered of genetic origin and linked to hereditary syndromes: von Hippel Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN2) and neurofibromatosis type 1 (NF1), due respectively to a germ line mutation in tumor-suppressor gene *VHL* [46, 47], protooncogene *RET* [48–52] and tumor-suppressor gene *NF1* [53].

In the last years it has been demonstrated that about 30% of the apparently sporadic PGLs are due to a germ-line mutation in one of the susceptibility genes [54]. This group of genes includes those encoding the four subunits (A, B, C, and D) of the succinate dehydrogenase (SDH) [55–58], the recently identified gene *SDHAF2*, which is responsible for the flavination of the SDHA subunit [59], and the very recently discovered *TMEM127* [60] and *MAX* [61], both mainly related to bilateral PCCs. Germ line mutations in *SDHA, SDHB, SDHC*, *SDHD*, and *SDHAF2* genes are responsible for the occurrence of syndromes named PGL5, PGL4, PGL3, PGL1, and PGL2, respectively; to note, *SDHB*-mutations are generally associated with higher morbidity and mortality than mutations in the other SDHx genes [62]. A recent meta-analisis of some studies involving *SDHB* mutated patients has highlighted that 31% of their tumors were malignant [3].

Overall, to date, 10 susceptibility PGLs genes have been identified, so that the initial 10% of cases classified as genetically determined has increased to 30%. Nevertheless, the number of the susceptibility genes is likely to increase. In fact, many young PGL patients, where the mutation frequency is higher, are still classified as sporadic, and some PGLs patients with a positive family history do not show any mutation in the so far known susceptibility genes.

Extensive genetic screening in PGLs has highlighted the correlation between genotype and phenotype thus facilitating a genetic testing algorithm based on clinical features as a guide for a more quick and cost-effective genetic screening (Table 2) [63].

Genetic analysis has also permitted to predict the malignancy risk which is higher for SDHB mutation carriers.

Furthermore, by studying tumor transcription profile, sporadic as well as hereditary PGLs have been divided in two main clusters linked to two different signalling pathways [64]: the first cluster contains all *VHL*- and *SDHx*-mutated tumors and is associated with angiogenesis, hipoxia, and reduced oxidative response [65], while the second cluster contains all *RET*- and *NF1*-mutated tumors and is associated with abnormal activation of kinase-signaling pathways, such as RAS/RAF/MAPK and PI3K/AKT/mTOR [66–69]; also *TMEM127* [60] and *MAX* [61] mutated tumors have been associated to the activation of mTOR-signaling pathway. These data have increased overall knowledge on molecular defects in PGLs and could be used for development of new effective molecular-targeted therapies.

7. Therapy: Surgery

The main goal of surgical treatment is represented by the removal of primary tumor and, when possible, the resection of local and distant metastases. The overall 5-year survival rate of patients with malignant PGLs varies between 34% and 60%. The survival rate may depend upon sites of metastatic lesions. In fact, patients with liver or lung metastases tend to have a worse prognosis (<5 years) than patients with isolated bone lesions [2].

The preoperative management with alpha blockade and fluid administration, essential in order to avoid surgical (i.e., hypertensive crisis arrhythmias) and/or postsurgical complications (i.e., hypotension), has to be performed in all patients [1].

Laparoscopic removal of intra-adrenal and extra-adrenal PGLs is the preferred surgical technique, but, in case of large tumors with a high risk of malignancy, a transabdominal approach should be considered. In such circumstances total adrenalectomy with resection of locoregional lymph nodes or complete excision of PGLs together with the removal of

distant metastases is recommended [70]. In case of malignant disease surgery alone is seldom curative, but surgical debulking of the tumors is regarded as a mainstay of palliative therapy. In fact, it permits to reduce local or systemic symptoms related to catecholamine secretion, it improves response to other therapeutic approaches and it may prevent further diffusion of the tumors. Pre-operative injection of ^{123}I-MIBG and intraoperative application of a γ-probe may permit the localization of lesions that are not evidenced by other imaging techniques [71].

In the presence of liver metastases arterial embolisation or chemoembolisation may provide transient response, but in such circumstances radiofrequency ablation has become the preferred choice [72]. In the next future, the rapidly evolving stereotaxic radiotherapic techniques will probably represent valid alternative tools for metastasis removal.

8. Therapy: Radiometabolic Treatment and External Radiotherapy

Radionuclide treatment may be considered in patients with metastatic disease and no resectable lesions. It can be performed using beta-emitting isotopes coupled with MIBG or somatostatine analogue.

^{131}I-MIBG was used in the treatment of malignant PCC for the first time in 1984 [73]. Patients are selected by the evidence of significant radioisotope uptake on diagnostic scintigraphy with ^{123}I-MIBG or ^{131}I-MIBG (>1% uptake of the injected dose). Single or fractionated doses as well as variable dosage (200–1400 mCi) have been proposed [74, 75]. About 60% of metastatic sites are ^{131}I-MIBG avid [76]. In general better responses are seen in patients with limited disease and in patients with soft-tissue metastases than in patients with bone metastases [77]. This treatment is well tolerated, and the main side effects are represented by transient leucopenia and thrombocytopenia, whereas severe bone marrow toxicity (associated with high-dose regimen) is rarely seen.

However treatment with ^{131}I-MIBG is not curative in most patients; therefore, other forms of therapy need to be considered.

The presence of SSTR in PGLs has allowed treatment with radiolabelled somatostatin analogues. The most commonly used are Yttrium-90-DOTATOC (^{90}Y-DOTA-TOC) and Lutetium-177-DOTA^0-Tyr^3-octreotate (^{177}Lu-DOTA-TATE) [75, 78, 79]. As for ^{131}I-MIBG, patients are selected by the demonstration of high tumor uptake at scintigraphy. The latter is usually performed with ^{111}In-pentetreotide, but it seems that PET using ^{68}Ga-DOTA-TOC provides higher accuracy in selecting patients [34]. This kind of therapy has a low toxicity, mainly leucopenia and thrombocytopenia, and it can be effective in order to reduce hormone secretion and determine tumor shrinkage. Therefore, even if its efficacy seems lower in PGLs than in gastroenteropancreatic neuroendocrine tumors, it represents an alternative option for the treatment of surgically incurable PGLs [80]. In the future, the development of new somatostatine analogues with higher affinity for the different SSTR subtypes will provide a further possibility in the treatment of these neoplasms.

Finally a combined treatment with radiolabelled MIBG and radiolabelled somatostatin analogues might have a synergistic effect, and therefore it might be considered. Moreover, the combination treatment could permit the use of lower doses of both radionuclides, limiting side effects, particularly bone marrow toxicity.

External radiotherapy may be considered for treatment of inoperable PGLs and especially for palliation of painful bone metastases. During this procedure the patients need to be monitored because the radio-induced inflammation of the lesion can induce massive catecholamine secretion, thus inducing hypertensive crises [81].

9. Therapy: Antineoplastic Agents

The aim of chemotherapy is tumor size reduction and control of symptoms due to catecholamine secretion; it is usually reserved to patients with local advanced and/or metastatic disease, with unresectable lesions, resistant to treatment with radionuclide therapy [82]. Up to now, the most used and effective chemotherapy regimen is a combination of cyclophosphamide, vincristine, and daecarbazine (CVD), chosen for its use in treating another neuroendocrine tumor, neuroblastoma. It has been used for the first time in 1980s in a trial including 14 malignant PCCs cases [83], updated recently by NIH [84] in a 22-year followup, with demonstration of a tumor regression and symptom relief in up to 50% of patients treated and no significant change of survival. Once CVD is stopped, PCCs often recur, becoming unresponsive to the same treatment. For these reasons, CVD may have a role as a neoadjuvant therapy in few cases, to make tumors surgically resectable and to control symptoms. CVD plus anthracyclines has been tested in one case with quite a good result [85]. Other chemotherapic regimens have been tested in other trials, but currently none has demonstrated effectiveness in malignant PCCs treatment [86].

10. Therapy: Targeted Approach

Up to now, treatment options for malignant PGLs are limited to chemotherapy and radionuclide therapy. These often provide symptomatic and biochemical control but are less effective in causing survival increase. Understanding specific molecular pathways alteration responsible for malignant PGLs development might hopefully in the future lead to multiple molecular-targeted therapy for a successful treatment. Effectiveness of these therapies is due to a cytostatic effect, as they interfere with specific molecular targets found along the oncogenic signaling pathways responsible for carcinogenesis and tumor growth. As stated above, both benign and malignant PGLs gene mutations are part of two distinct molecular pathways leading to tumorigenesis: cluster 1 includes mutations of *VHL*, *SDHB*, and *SDHD* and is associated to pseudohypoxia and aberrant VEGF signaling, leading to abnormal hypoxia inducible factor (HIF) activation and overexpression of angiogenic factors, while cluster 2 includes mutations of *RET, NF1*, *TMEM127*, and

MAX and is associated with abnormal activation of kinase-signaling pathways such as PI3kinase/AKT, RAS/RAF/ERK, and mTOR1/p70s6K, leading to abnormal cell growth and lack of apoptosis capacity. In addition, malignant PCCs seem to overexpress HSP90, a molecular chaperone that assists in folding proteins and stabilizes various oncoproteins that play a role in malignant phenotype [87, 88].

Thus, HIF1a inihbitors are molecular targeted drugs interfering with HIF hypoxia-driven transcription pathway, decreasing HIF activity directly, PX-478 (S-2-amino-3-[4′-N,N,-bis (2-chloroethyl)amino]-phenyl propionic acid N-oxided ihydrochloride), and indirectly, PX-12 (1-methylpropyl 2-imidazolyl disulfide). These agents have shown marked antitumoral activity in human tumor xenografts in mice and seem to be promising also for malignant PGLs, but conclusive data are missing [89–91].

The mTOR inhibitor everolimus (RAD001) in combination with octreotide has been evaluated for low- and intermediate-grade neuroendocrine tumors [92], with good results. The efficacy of everolimus has been evaluated also in malignant PGLs, but all patients experienced disease progression [4, 93]. Maybe the low efficacy is due to a compensatory PI3K/AKT and ERK activation in response to mTOR inhibition, so a specific novel dual PI3k/mTOR inhibitor (NVP-BEZ235) might offer a novel therapeutic approach [94]. Further studies on the PI3K/AKT/mTOR pathway have to be conducted to find a more specific molecular target in its signalling.

Due to overexpression of HSP90 in malignant PCCs [40, 95], inhibition of its pathway could represent a future therapeutic challenge for the treatment of malignant PCCs, but at present current specific drug trials are missing.

Several studies have demonstrated overexpression in malignant PCCs of angiogenic molecules, such as VEGF, its receptor, angiopoietin-2, and the endothelin receptors ETA and ETB [96–100], leading a strong evidence that targeting this pathway with antiangiogenic therapies could represent a new promising treatment option. Accordingly, sunitinib, a receptor tyrosine kinase inhibitor acting on several targets (VEGF, PDGF, and c-KIT), with strong antiangiogenic and antitumor activity, has been used in the treatment of malignant PCCs, with promising results [101–104].

Imatinib, another tyrosine kinase inhibitor already used for hematologic and gastrointestinal stromal tumors, has not been found effective for malignant PCCs treatment [105].

Thalidomide, by targeting VEGF and basic fibroblast growth factor, is an antiangiogenic agent evaluated for treatment of metastatic renal cell cancer, multiple myeloma and nonsmall cell lung cancer [106, 107]. It has been used in combination with Temozolomide in neuroendocrine tumors [108] obtaining an objective biochemical response rate in about 40% and a radiologic response rate in 33% of malignant PCCs, but lymphopenia occurred in about 70% of treated patients.

Activators of prolyl hydroxylase (PHD) (such as ERBB2 inhibitors) are now on evaluation as promising antineoplastic therapies. These molecules decrease the expression levels of some angiogenic factors, such as VEGF, acting on HIF pathway, by activating the PHD, thus increasing HIF hydroxylation, and promoting its degradation [109, 110].

Treatment of malignant PGLs is up to now basically palliative. Molecular targeted therapies are promising strategies, but, due to the complexity of these tumors pathogenesis, further studies on tumor biology, discovery of novel targeted drugs, and new trials are needed to achieve more effective treatments.

11. Conclusions

Malignant PGLs, as defined by the presence of metastases, are very rare and aggressive tumors. Their study is made difficult by their rarity, and the consequent limited number of patients included in the series, by their biological variability, by their variable genetic background, and by the lack of specific and sensitive histopathological or biological markers proving malignancy. Therefore, as benign tumors are diagnosed by the lack of metastases, and as metastatic spread can occur also several years after surgical removal of primary tumor, studies comparing benign and malignant PGLs need a long clinical followup of patients.

Large collaborative international studies, as those presently conducted on behalf of the European Network for the Study of Adrenal Tumors (ENS@T), are needed to improve our knowledge on the pathogenesis of these malignant tumors and to achieve a satisfactory medical treatment for affected patients.

Acknowledgments

This work has been partly supported by Fondazione Cassa di Risparmio di Pistoia e Pescia Prot. 2010.0278). Gabriele Parenti, Benedetta Zampetti, Elena Rapizzi, Tonino Ercolino, and Massimo Mannelli are members of the European Network for the Study of Adrenal Tumors (ENS@T).

References

[1] J. W. M. Lenders, G. Eisenhofer, M. Mannelli, and K. Pacak, "Phaeochromocytoma," *Lancet*, vol. 366, no. 9486, pp. 665–675, 2005.

[2] K. Pacak, G. Eisenhofer, H. Ahlman et al., "Pheochromocytoma: recommendations for clinical practice from the First International Symposium," *Nature Clinical Practice Endocrinology and Metabolism*, vol. 3, no. 2, pp. 92–102, 2007.

[3] J. Welander, P. Söderkvist, and O. Gimm, "Genetics and clinical characteristics of hereditary pheochromocytomas and paragangliomas," *Endocrine-Related Cancer*, vol. 18, no. 6, pp. R253–R276, 2011.

[4] A. Chrisoulidou, G. Kaltsas, I. Ilias, and A. B. Grossman, "The diagnosis and management of malignant phaeochromocytoma and paraganglioma," *Endocrine-Related Cancer*, vol. 14, no. 3, pp. 569–585, 2007.

[5] J. H. Lee, F. Barich, L. H. Karnell et al., "National cancer data base report on malignant paragangliomas of the head and neck," *Cancer*, vol. 94, no. 3, pp. 730–737, 2002.

[6] M. Mannelli, L. Ianni, A. Cilotti, and A. Conti, "Pheochromocytoma in Italy: a multicentric retrospective study,"

European Journal of Endocrinology, vol. 141, no. 6, pp. 619–624, 1999.

[7] E. L. Bravo, "Evolving concepts in the pathophysiology, diagnosis, and treatment of pheochromocytoma," *Endocrine Reviews*, vol. 15, no. 3, pp. 356–368, 1994.

[8] D. Erickson, Y. C. Kudva, M. J. Ebersold et al., "Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 11, pp. 5210–5216, 2001.

[9] M. Mannelli, J. V. M. Lenders, K. Pacak et al., "Subclinical pheochromocytoma," *Best Practice & Research Clinical Endocrinology & Metabolism*. In press.

[10] J. W. M. Lenders, K. Pacak, M. M. Walther et al., "Biochemical diagnosis of pheochromocytoma: which test is best?" *Journal of the American Medical Association*, vol. 287, no. 11, pp. 1427–1434, 2002.

[11] A. Grossman, K. Pacak, A. Sawka et al., "Biochemical diagnosis and localization of pheochromocytoma: can we reach a consensus?" *Annals of the New York Academy of Sciences*, vol. 1073, pp. 332–347, 2006.

[12] F. D. Davidson, "Phaeochromocytoma with normal urinary catecholamines: the potential value of urinary free metadrenalines," *Annals of Clinical Biochemistry*, vol. 39, no. 6, pp. 557–566, 2002.

[13] G. Eisenhofer, H. Keiser, P. Friberg et al., "Plasma metanephrines are markers of pheochromocytoma produced by catechol-O-methyltransferase within tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 6, pp. 2175–2185, 1998.

[14] F. Rao, H. R. Keiser, and D. T. O'Connor, "Malignant pheochromocytoma: chromaffin granule transmitters and response to treatment," *Hypertension*, vol. 36, no. 6, pp. 1045–1052, 2000.

[15] F. M. Brouwers, G. Eisenhofer, J. J. Tao et al., "High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 11, pp. 4505–4509, 2006.

[16] E. van der Harst, W. W. de Herder, R. R. de Krijger et al., "The value of plasma markers for the clinical behaviour of phaeochromocytomas," *European Journal of Endocrinology*, vol. 147, no. 1, pp. 85–94, 2002.

[17] L. Guignat, J. M. Bidart, M. Nocera, E. Comoy, M. Schlumberger, and E. Baudin, "Chromogranin A and the α-subunit of glycoprotein hormones in medullary thyroid carcinoma and phaeochromocytoma," *British Journal of Cancer*, vol. 84, no. 6, pp. 808–812, 2001.

[18] A. Algeciras-Schimnich, C. M. Preissner, W. F. Young, R. J. Singh, and S. K. G. Grebe, "Plasma chromogranin A or urine fractionated metanephrines follow-up testing improves the diagnostic accuracy of plasma fractionated metanephrines for pheochromocytoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 1, pp. 91–95, 2008.

[19] A. M. Moreno, L. Castilla-Guerra, M. C. Martínez-Torres, F. Torres-Olivera, E. Fernández, and H. Galera-Davidson, "Expression of neuropeptides and other neuroendocrine markers in human phaeochromocytomas," *Neuropeptides*, vol. 33, no. 2, pp. 159–163, 1999.

[20] S. Oishi and T. Sato, "Elevated serum neuron-specific enolase in patients with malignant pheochromocytoma," *Cancer*, vol. 61, no. 6, pp. 1167–1170, 1988.

[21] J. Guillemot, L. Barbier, E. Thouennon et al., "Expression and processing of the neuroendocrine protein secretogranin II in benign and malignant pheochromocytomas," *Annals of the New York Academy of Sciences*, vol. 1073, pp. 527–532, 2006.

[22] I. Ilias and K. Pacak, "Current approaches and recommended algorithm for the diagnostic localization of pheochromocytoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 2, pp. 479–491, 2004.

[23] M. Mannelli, S. Colagrande, A. Valeri, and G. Parenti, "Incidental and metastatic adrenal masses," *Seminars in Oncology*, vol. 37, no. 6, pp. 649–661, 2010.

[24] M. A. Blake, M. K. Kalra, M. M. Maher et al., "Pheochromocytoma: an imaging chameleon," *Radiographics*, vol. 24, pp. S87–S99, 2004.

[25] B. L. Shulkin, I. Ilias, J. C. Sisson, and K. Pacak, "Current trends in functional imaging of pheochromocytomas and paragangliomas," *Annals of the New York Academy of Sciences*, vol. 1073, pp. 374–382, 2006.

[26] L. A. Dubois and D. K. Gray, "Dopamine-secreting pheochromocytomas: in search of a syndrome," *World Journal of Surgery*, vol. 29, no. 7, pp. 909–913, 2005.

[27] F. F. Telischi, R. Bustillo, M. L. H. Whiteman et al., "Octreotide scintigraphy for the detection of paragangliomas," *Otolaryngology, Head and Neck Surgery*, vol. 122, no. 3, pp. 358–362, 2000.

[28] I. Buchmann, M. Henze, S. Engelbrecht et al., "Comparison of ^{68}Ga-DOTATOC PET and ^{111}In-DTPAOC (Octreoscan) SPECT in patients with neuroendocrine tumours," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 34, no. 10, pp. 1617–1626, 2007.

[29] M. Hofmann, H. Maecke, A. R. Börner et al., "Biokinetics and imaging with the somatostatin receptor PET radioligand ^{68}Ga-DOTATOC: preliminary data," *European Journal of Nuclear Medicine*, vol. 28, no. 12, pp. 1751–1757, 2001.

[30] J. Kowalski, M. Henze, J. Schuhmacher, H. R. Mäcke, M. Hofmann, and U. Haberkorn, "Evaluation of positron emission tomography imaging using [^{68}Ga]-DOTA-D Phe1-Tyr3-octreotidein comparison to [^{111}In]-DTPAOC SPECT. First results in patients with neuroendocrine tumors," *Molecular Imaging and Biology*, vol. 5, no. 1, pp. 42–48, 2003.

[31] I. Ilias, J. Yu, J. A. Carrasquillo et al., "Superiority of 6-[^{18}F]-fluorodopamine positron emission tomography versus [^{131}I]-metaiodobenzylguanidine scintigraphy in the localization of metastatic pheochromocytoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 9, pp. 4083–4087, 2003.

[32] S. Hoegerle, E. Nitzsche, C. Altehoefer et al., "Pheochromocytomas: detection with ^{18}F-DOPA whole-body PET-initial results," *Radiology*, vol. 222, no. 2, pp. 507–512, 2002.

[33] H. J. L. M. Timmers, A. Kozupa, C. C. Chen et al., "Superiority of fluorodeoxyglucose positron emission tomography to other functional imaging techniques in the evaluation of metastatic SDHB-associated pheochromocytoma and paraganglioma," *Journal of Clinical Oncology*, vol. 25, no. 16, pp. 2262–2269, 2007.

[34] M. Mamede, J. A. Carrasquillo, C. C. Chen et al., "Discordant localization of 2-[^{18}F]-fluoro-2-deoxy-D-glucose in 6-[^{18}F]-fluorodopamine- and [^{123}I]-metaiodobenzylguanidine-negative metastatic pheochromocytoma sites," *Nuclear Medicine Communications*, vol. 27, no. 1, pp. 31–36, 2006.

[35] C. Trampal, H. Engler, C. Juhlin, M. Bergström, and B. Långström, "Pheochromocytomas: detection with ^{11}C Hydroxyephedrine PET," *Radiology*, vol. 230, no. 2, pp. 423–428, 2004.

[36] R. I. Linnoila, H. R. Keiser, S. M. Steinberg, and E. E. Lack, "Histopathology of benign versus malignant sympathoadrenal paragangliomas: clinicopathologic study of 120 cases including unusual histologic features," *Human Pathology*, vol. 21, no. 11, pp. 1168–1180, 1990.

[37] N. Kimura, T. Watanabe, T. Noshiro, S. Shizawa, and Y. Miura, "Histological grading of adrenal and extra-adrenal pheochromocytomas and relationship to prognosis: a clinicopathological analysis of 116 adrenal pheochromocytomas and 30 extra-adrenal sympathetic paragangliomas including 38 malignant tumors," *Endocrine Pathology*, vol. 16, no. 1, pp. 23–32, 2005.

[38] L. D. R. Thompson, "Pheochromocytoma of the adrenal gland scaled score (PASS) to separate benign from malignant neoplasms: a clinicopathologic and immunophenotypic study of 100 cases," *American Journal of Surgical Pathology*, vol. 26, no. 5, pp. 551–566, 2002.

[39] V. E. Strong, T. Kennedy, H. Al-Ahmadie et al., "Prognostic indicators of malignancy in adrenal pheochromocytomas: clinical, histopathologic, and cell cycle/apoptosis gene expression analysis," *Surgery*, vol. 143, no. 6, pp. 759–768, 2008.

[40] C. Boltze, J. Mundschenk, N. Unger et al., "Expression profile of the telomeric complex discriminates between benign and malignant pheochromocytoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 9, pp. 4280–4286, 2003.

[41] T. H. Liu, Y. J. Chen, S. F. Wu et al., "Distinction between benign and malignant pheochromocytomas," *Zhonghua Bing Li Xue Za Zhi*, vol. 33, no. 3, pp. 198–202, 2004.

[42] V. Häyry, K. Salmenkivi, J. Arola, P. Heikkilä, C. Haglund, and H. Sariola, "High frequency of SNAIL-expressing cells confirms and predicts metastatic potential of phaeochromocytoma," *Endocrine-Related Cancer*, vol. 16, no. 4, pp. 1211–1218, 2009.

[43] J. Waldmann, E. P. Slater, P. Langer et al., "Expression of the transcription factor Snail and its target gene Twist are associated with malignancy in pheochromocytomas," *Annals of Surgical Oncology*, vol. 16, no. 7, pp. 1997–2005, 2009.

[44] B. M. Engels and G. Hutvagner, "Principles and effects of microRNA-mediated post-transcriptional gene regulation," *Oncogene*, vol. 25, no. 46, pp. 6163–6169, 2006.

[45] G. Y. Meyer-Rochow, N. E. Jackson, J. V. Conaglen et al., "MicroRNA profiling of benign and malignant pheochromocytomas identifies novel diagnostic and therapeutic targets," *Endocrine-Related Cancer*, vol. 17, no. 3, pp. 835–846, 2010.

[46] F. Latif, K. Tory, J. Gnarra et al., "Identification of the von Hippel-Lindau disease tumor suppressor gene," *Science*, vol. 260, no. 5112, pp. 1317–1320, 1993.

[47] M. M. Walther, R. Reiter, H. R. Keiser et al., "Clinical and genetic characterization of pheochromocytoma in von Hippel- Lindau families: comparison with sporadic pheochromocytoma gives insight into natural history of pheochromocytoma," *Journal of Urology*, vol. 162, no. 3 I, pp. 659–664, 1999.

[48] H. P. H. Neumann, D. P. Berger, G. Sigmund et al., "pheochromocytomas, multiple endocrine neoplasia type 2, and von Hippel- Lindau disease," *New England Journal of Medicine*, vol. 329, no. 21, pp. 1531–1538, 1993.

[49] H. P. H. Neumann, D. P. Berger, G. Sigmund et al., "Erratum: pheochromocytomas, multiple endocrine neoplasia type 2, and von Hippel-Lindau disease," *New England Journal of Medicine*, vol. 331, no. 22, p. 1535, 1994.

[50] C. Eng, D. P. Smith, L. M. Mulligan et al., "Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours," *Human Molecular Genetics*, vol. 3, no. 2, pp. 237–241, 1994.

[51] C. Eng, D. P. Smith, L. M. Mulligan et al., "Erratum: point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours," *Human Molecular Genetics*, vol. 3, no. 4, p. 686, 1994.

[52] C. Eng, "Seminars in medicine of the Beth Israel Hospital, Boston: the RET proto- oncogene in multiple endocrine neoplasia type 2 and Hirschsprung's disease," *New England Journal of Medicine*, vol. 335, no. 13, pp. 943–951, 1996.

[53] R. White and P. O'Connell, "Identification and characterization of the gene for neurofibromatosis type 1," *Current Opinion in Genetics and Development*, vol. 1, no. 1, pp. 15–19, 1991.

[54] H. P. H. Neumann, B. Bausch, S. R. McWhinney et al., "Germ-line mutations in nonsyndromic pheochromocytoma," *New England Journal of Medicine*, vol. 346, no. 19, pp. 1459–1466, 2002.

[55] B. E. Baysal, R. E. Ferrell, J. E. Willett-Brozick et al., "Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma," *Science*, vol. 287, no. 5454, pp. 848–851, 2000.

[56] S. Niemann and U. Muller, "Mutations in SDHC cause autosomal dominant paraganglioma, type 3," *Nature Genetics*, vol. 26, no. 3, pp. 268–270, 2000.

[57] D. Astuti, F. Latif, A. Dallol et al., "Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma," *American Journal of Human Genetics*, vol. 69, no. 1, pp. 49–54, 2001.

[58] N. Burnichon, J. J. Brière, R. Libé et al., "SDHA is a tumor suppressor gene causing paraganglioma," *Human Molecular Genetics*, vol. 19, no. 15, pp. 3011–3020, 2010.

[59] H. X. Hao, O. Khalimonchuk, M. Schraders et al., "SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma," *Science*, vol. 325, no. 5944, pp. 1139–1142, 2009.

[60] Y. Qin, L. Yao, E. E. King et al., "Germline mutations in TMEM127 confer susceptibility to pheochromocytoma," *Nature Genetics*, vol. 42, no. 3, pp. 229–233, 2010.

[61] I. Comino-Méndez, F. J. Gracia-Aznárez, F. Schiavi et al., "Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma," *Nature Genetics*, vol. 43, no. 7, pp. 663–667, 2011.

[62] A. P. Gimenez-Roqueplo, J. Favier, P. Rustin et al., "Mutations in the SDHB gene are associated with extra-adrenal and/or malignant phaeochromocytomas," *Cancer Research*, vol. 63, no. 17, pp. 5615–5621, 2003.

[63] M. Mannelli, M. Castellano, F. Schiavi et al., "Clinically guided genetic screening in a large cohort of Italian patients with pheochromocytomas and/or functional or nonfunctional paragangliomas," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 5, pp. 1541–1547, 2009.

[64] P. L. M. Dahia, K. N. Ross, M. E. Wright et al., "A HIf1α regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas," *PLoS Genetics*, vol. 1, no. 1, pp. 72–80, 2005.

[65] J. Favier, J. J. Brière, N. Burnichon et al., "The Warburg effect is genetically determined in inherited pheochromocytomas," *PLoS One*, vol. 4, no. 9, Article ID e7094, 2009.

[66] D. Califano, C. Rizzo, A. D'Alessio et al., "Signaling through Ras is essential for ret oncogene-induced cell differentiation in PC12 cells," *Journal of Biological Chemistry*, vol. 275, no. 25, pp. 19297–19305, 2000.

[67] G. A. Martin, D. Viskochil, G. Bollag et al., "The GAP-related domain of the neurofibromatosis type 1 gene product interacts with ras p21," *Cell*, vol. 63, no. 4, pp. 843–849, 1990.

[68] C. M. Johannessen, E. E. Reczek, M. F. James, H. Brems, E. Legius, and K. Cichowski, "The NF1 tumor suppressor critically regulates TSC2 and mTOR," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 24, pp. 8573–8578, 2005.

[69] C. M. Johannessen, B. W. Johnson, S. G. Williams et al., "TORC1 Is Essential for NF1-Associated Malignancies," *Current Biology*, vol. 18, no. 1, pp. 56–62, 2008.

[70] M. Brauckhoff, O. Gimm, and H. Dralle, "Preoperative and surgical therapy in sporadic and familial phaeochromocytoma. H. Lehnert Eds.: Phaeochromocytoma, Pathophysiology and Clinical Management," *Frontiers of Hormone Research*, vol. 31, pp. 121–144, 2004.

[71] T. Buhl, J. Mortensen, and A. Kjaer, "I-123 MIBG imaging and intraoperative localization of metastatic pheochromocytoma a case report," *Clinical Nuclear Medicine*, vol. 27, no. 3, pp. 183–185, 2002.

[72] S. K. Maithel and Y. Fong, "Hepatic ablation for neuroendocrine tumor metastases," *Journal of Surgical Oncology*, vol. 100, no. 8, pp. 635–638, 2009.

[73] J. C. Sisson, B. Shapiro, and W. H. Beierwaltes, "Radiopharmaceutical treatment of malignant pheochromocytoma," *Journal of Nuclear Medicine*, vol. 25, no. 2, pp. 197–206, 1984.

[74] G. A. Kaltsas, J. J. Mukherjee, R. Foley, K. E. Britton, and A. B. Grossman, "Treatment of metastatic pheochromocytoma and paraganglioma with ^{131}I-meta-iodobenzylguanidine (MIBG)," *Endocrinologist*, vol. 13, no. 4, pp. 321–333, 2003.

[75] G. A. Kaltsas, D. Papadogias, P. Makras, and A. B. Grossman, "Treatment of advanced neuroendocrine tumours with radiolabelled somatostatin analogues," *Endocrine-Related Cancer*, vol. 12, no. 4, pp. 683–699, 2005.

[76] P. A. Fitzgerald, R. E. Goldsby, J. P. Huberty et al., "Malignant pheochromocytomas and paragangliomas: a phase II study of therapy with high-dose ^{131}I-metaiodobenzylguanidine (^{131}I-MIBG)," *Annals of the New York Academy of Sciences*, vol. 1073, pp. 465–470, 2006.

[77] K. C. Loh, P. A. Fitzgerald, K. K. Matthay, P. P. B. Yeo, and D. C. Price, "The treatment of malignant pheochromocytoma with iodine-131 metaiodobenzylguanidine (^{131}I-MIBG): a comprehensive review of 116 reported patients," *Journal of Endocrinological Investigation*, vol. 20, no. 11, pp. 648–658, 1997.

[78] D. J. Kwekkeboom, J. Mueller-Brand, G. Paganelli et al., "Overview of results of peptide receptor radionuclide therapy with 3 radiolabeled somatostatin analogs," *Journal of Nuclear Medicine*, vol. 46, no. 1, pp. S62–S66, 2005.

[79] D. J. Kwekkeboom, W. W. De Herder, B. L. Kam et al., "Treatment with the radiolabeled somatostatin analog [^{177}Lu-DOTA^0,Tyr^3]octreotate: toxicity, efficacy, and survival," *Journal of Clinical Oncology*, vol. 26, no. 13, pp. 2124–2130, 2008.

[80] F. Forrer, I. Riedweg, H. R. Maecke, and J. Mueller-Brand, "Radiolabeled DOTATOC in patients with advanced paraganglioma and pheochromocytoma," *Quarterly Journal of Nuclear Medicine and Molecular Imaging*, vol. 52, no. 4, pp. 334–340, 2008.

[81] S. Teno, A. Tanabe, K. Nomura, and H. Demura, "Acutely exacerbated hypertension and increased inflammatory signs due to radiation treatment for metastatic pheochromocytoma," *Endocrine Journal*, vol. 43, no. 5, pp. 511–516, 1996.

[82] K. F. Andersen, R. Altaf, A. Krarup-Hansen et al., "Malignant pheochromocytomas and paragangliomas—the importance of a multidisciplinary approach," *Cancer Treatment Reviews*, vol. 37, no. 2, pp. 111–119, 2011.

[83] S. D. Averbuch, C. S. Steakley, R. C. Young et al., "Malignant pheochromocytoma: effective treatment with a combination of cyclophosphamide, vincristine, and dacarbazine," *Annals of Internal Medicine*, vol. 109, no. 4, pp. 267–273, 1988.

[84] H. Huang, J. Abraham, E. Hung et al., "Treatment of malignant pheochromocytoma/paraganglioma with cyclophosphamide, vincristine, and dacarbazine: recommendation from a 22-year follow-up of 18 patients," *Cancer*, vol. 113, no. 8, pp. 2020–2028, 2008.

[85] M. Nakane, S. Takahashi, I. Sekine et al., "Successful treatment of malignant pheochromocytoma with combination chemotherapy containing anthracycline," *Annals of Oncology*, vol. 14, no. 9, pp. 1449–1451, 2003.

[86] R. H. Grogan, E. J. Mitmaker, and Q. Y. Duh, "Changing paradigms in the treatment of malignant pheochromocytoma," *Cancer Control*, vol. 18, no. 2, pp. 104–112, 2011.

[87] U. Banerji, "Heat shock protein 90 as a drug target: some like it hot," *Clinical Cancer Research*, vol. 15, no. 1, pp. 9–14, 2009.

[88] M. V. Powers and P. Workman, "Targeting of multiple signalling pathways by heat shock protein 90 molecular chaperone inhibitors," *Endocrine-Related Cancer*, vol. 13, no. 1, pp. S125–S135, 2006.

[89] G. L. Semenza, "Evaluation of HIF-1 inhibitors as anticancer agents," *Drug Discovery Today*, vol. 12, no. 19-20, pp. 853–859, 2007.

[90] S. Welsh, R. Williams, L. Kirkpatrick, G. Paine-Murrieta, and G. Powis, "Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1α," *Molecular Cancer Therapeutics*, vol. 3, no. 3, pp. 233–244, 2004.

[91] S. J. Welsh, R. R. Williams, A. Birmingham, D. J. Newman, D. L. Kirkpatrick, and G. Powis, "The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1alpha and vascular endothelial growth factor formation," *Molecular cancer therapeutics*, vol. 2, no. 3, pp. 235–243, 2003.

[92] J. C. Yao, A. T. Phan, D. Z. Chang et al., "Efficacy of RAD001 (everolimus) and octreotide LAR in advanced low- to intermediate-grade neuroendocrine tumors: results of a phase II study," *Journal of Clinical Oncology*, vol. 26, no. 26, pp. 4311–4318, 2008.

[93] M. R. Druce, G. A. Kaltsas, M. Fraenkel, D. J. Gross, and A. B. Grossman, "Novel and evolving therapies in the treatment of malignant phaeochromocytoma: experience with the mTOR inhibitor everolimus (RAD001)," *Hormone and Metabolic Research*, vol. 41, no. 9, pp. 697–702, 2009.

[94] S. Nölting and A. B. Grossman, "Signaling pathways in pheochromocytomas and paragangliomas: prospects for future therapies," *Endocrine Pathology*, vol. 23, no. 1, pp. 21–33, 2012.

[95] C. Boltze, H. Lehnert, R. Schneider-Stock, B. Peters, C. Hoang-Vu, and A. Roessner, "HSP90 is a key for telomerase activation and malignant transition in pheochromocytoma," *Endocrine*, vol. 22, no. 3, pp. 193–201, 2003.

[96] K. Salmenkivi, P. Heikkilä, J. Liu, C. Haglund, and J. Arola, "VEGF in 105 pheochromocytomas: enhanced expression correlates with malignant outcome," *APMIS*, vol. 111, no. 4, pp. 458–464, 2003.

[97] A. Zielke, M. Middeke, S. Hoffmann et al., "VEGF-mediated angiogenesis of human pheochromocytomas is associated to malignancy and inhibited by anti-VEGF antibodies in experimental tumors," *Surgery*, vol. 132, no. 6, pp. 1056–1063, 2002.

[98] J. Favier, P. F. Plouin, P. Corvol, and J. M. Gasc, "Angiogenesis and vascular architecture in pheochromocytomas: distinctive traits in malignant tumors," *American Journal of Pathology*, vol. 161, no. 4, pp. 1235–1246, 2002.

[99] K. Kolomecki, H. Stepien, M. Bartos, and K. Kuzdak, "Usefulness of VEGF, MMP-2, MMP-3 and TIMP-2 serum level evaluation in patients with adrenal tumours," *Endocrine Regulations*, vol. 35, no. 1, pp. 9–16, 2001.

[100] K. Takekoshi, K. Isobe, T. Yashiro et al., "Expression of vascular endothelial growth factor (VEGF) and its cognate receptors in human pheochromocytomas," *Life Sciences*, vol. 74, no. 7, pp. 863–871, 2004.

[101] C. Jimenez, M. E. Cabanillas, L. Santarpia et al., "Use of the tyrosine kinase inhibitor sunitinib in a patient with von Hippel-lindau disease: targeting angiogenic factors in pheochromocytoma and other von Hippel-Lindau disease-related tumors," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 2, pp. 386–391, 2009.

[102] A. M. Joshua, S. Ezzat, S. L. Asa et al., "Rationale and evidence for sunitinib in the treatment of malignant paraganglioma/pheochromocytoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 1, pp. 5–9, 2009.

[103] K. S. Park, J. L. Lee, H. Ahn et al., "Sunitinib, a novel therapy for anthracycline- and cisplatin-refractory malignant pheochromocytoma," *Japanese Journal of Clinical Oncology*, vol. 39, no. 5, pp. 327–331, 2009.

[104] N. M. Hahn, M. Reckova, L. Cheng, L. A. Baldridge, O. W. Cummings, and C. J. Sweeney, "Patient with malignant paraganglioma responding to the multikinase inhibitor sunitinib malate," *Journal of Clinical Oncology*, vol. 27, no. 3, pp. 460–463, 2009.

[105] D. J. Gross, G. Munter, M. Bitan et al., "The role of imatinib mesylate (Glivec) for treatment of patients with malignant endocrine tumors positive for c-kit or PDGF-R," *Endocrine-Related Cancer*, vol. 13, no. 2, pp. 535–540, 2006.

[106] S. Singhal, J. Mehta, R. Desikan et al., "Antitumor activity of thalidomide in refractory multiple myeloma," *New England Journal of Medicine*, vol. 341, no. 21, pp. 1565–1571, 1999.

[107] J. A. Sparano, R. Gray, B. Giantonio, P. O'Dwyer, and R. L. Comis, "Evaluating antiangiogenesis agents in the clinic: the Eastern Cooperative Oncology group portfolio of clinical trials," *Clinical Cancer Research*, vol. 10, no. 4, pp. 1206–1211, 2004.

[108] M. H. Kulke, K. Stuart, P. C. Enzinger et al., "Phase II study of temozolomide and thalidomide in patients with metastatic neuroendocrine tumors," *Journal of Clinical Oncology*, vol. 24, no. 3, pp. 401–406, 2006.

[109] H. J. Choi, B. J. Song, Y. D. Gong, W. J. Gwak, and Y. Soh, "Rapid degradation of hypoxia-inducible factor-1α by KRH102053, a new activator of prolyl hydroxylase 2," *British Journal of Pharmacology*, vol. 154, no. 1, pp. 114–125, 2008.

[110] E. Temes, S. Martín-Puig, B. Acosta-Iborra et al., "Activation of HIF-prolyl hydroxylases by R59949, an inhibitor of the diacylglycerol kinase," *Journal of Biological Chemistry*, vol. 280, no. 25, pp. 24238–24244, 2005.