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New Diagnostic and Therapeutic Approaches in Adrenocortical Cancer

BY

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Abstract

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Adrenocortical cancer (ACC) is a rare disease that is often difficult to diagnose, and therefore often presents at an advanced stage. Various cytotoxic treatments have been tried with little success. Evaluation of new diagnostic methods and improvement of medical therapies are therefore crucial.

The diagnostic potential of 11C-metomidate positron emission tomography (PET) was evaluated in eleven ACC patients. PET visualized all viable tumors with high tracer uptake, including two lesions that CT failed to detect. Necrotic or fibrotic tumors were PET negative. Medication with adrenal steroid inhibitors and chemotherapy may decrease the tracer uptake.

We performed a phase-II study with streptozocin and o,p'-DDD (SO) combination therapy in 40 ACC patients. The SO therapy was found to have impact on the disease-free interval (P = 0.02) as well as on survival (P = 0.01) in patients who received adjuvant therapy after curative resection. Complete or partial response was obtained in 36.4% of patients with measurable disease.

The efficacy and tolerability of combination therapy with vincristine, cisplatin, teniposide, and cyclophosphamide (OPEC) were evaluated in eleven patients with advanced ACC after failure of SO therapy. The median survival was 21 months from the start of treatment. A partial response was achieved in two patients. Adverse events were mainly restricted to grade 1-2 toxicities, and grade 3 toxicities were observed in only two cycles.

We tested 21 ACC tumors to analyze the expression of receptor tyrosine kinases and 15 ACC for mutation analysis of c-Kit exon 11, which can be targeted by antagonists such as imatinib. All ACCs expressed one or more kinases: c-Kit in 19 ACC and phospho-c-Kit in three while 14 ACCs expressed PDGFR-beta, suggesting the potential usefulness of tyrosine kinase inhibitors. No c-Kit mutations were detected in exon 11. Further evaluation of other mutations targeted by this drug may be needed.

Keywords: Adrenocortical cancer, Positron Emission Tomography, Metomidate, Combination chemotherapy, Streptozocin, op'-DDD, OPEC, Disease-free Interval, Survival, Responses, Side effects, Receptor protein-tyrosine kinases, c-Kit, Phospho-c-Kit, PDGFRβ, Mutation, Imatinib

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To my beloved family
Munna, Israa and Shahir

List of Papers

I Khan TS, Sundin A, Juhlin C, Långström B, Bergström M, Eriksson B. 11C-metomidate PET imaging of adrenocortical cancer . *European Journal of Nuclear Medicine and Molecular Imaging* 30(3): 403 - 410. (2003)

II Khan TS, Imam H, Juhlin C, Skogseid B, Gröndal S, Tibblin S, Wilander E, Öberg K, Eriksson B. Streptozocin and o,p'DDD in the treatment of adrenocortical cancer patients: long-term survival in its adjuvant use. *Annals of Oncology* 11(10): 1281-1287. (2000)

III Khan TS, Sundin A, Juhlin C, Wilander E, Öberg K, Eriksson B. Vincristine, Cisplatin, Teniposide and Cyclophosphamide Combination in the Treatment of Recurrent or Metastatic Adrenocortical Cancer. *Medical Oncology* 21(2): 000-000. (2004) (Accepted)

IV Khan TS, Gobl A, Imam H, Juhlin C, Wilander E, Öberg K, Eriksson B. Expression of c-Kit, phospho-c-Kit, PDGFRβ and absence of c-Kit mutation in exon 11 in adrenocortical cancer. (Manuscript)

Contents

Abbreviations

ACC	Adrenocortical cancer
ACTH	Adrenocorticotropic hormone
AML	Acute myeloid leukemia
Bq	Becquerel
^{11}C	Carbon-11
CAP	Cyclophosphamide, adriamycin, cisplatin
CML	Chronic myeloid leukemia
CR	Complete response
CT	Computed tomography
DDT	Dichlorodiphenyltrichloroethane
DFI	Disease-free interval
DHEA-S	Dehydroepiandrosterone sulfate
EDP	Etoposide, doxorubicin, cisplatin
EP	Etoposide, cisplatin
^{18}F	Fluorine-18
FDG	Fluoro-deoxy-glucose
5-FU	5-fluorouracil
FNA	Fine-needle aspiration
GCT	Germ cell tumor
GIST	Gastrointestinal stromal tumor
Grb2	Growth factor receptor bound protein 2
Hs	Hot spot
IGF	Insulin-like growth factor
JM	Juxtamembrane
MDR	Multidrug resistance
MPD	Myeloproliferative disease
MRI	Magnetic resonance imaging
NP-59	^{131}I-6β-iodomethyl-19-norcholesterol
o,p'-DDD	1,1-dichloro-diphenyl-dichloroethane
OPEC	Vincristine, cyclophosphamide, teniposide, cisplatin
PDGFR	Platelet-derived growth factor receptor
PET	Positron Emission Tomography
PFI	Progression-free interval
PR	Partial response
PD	Progressive disease
RTK	Receptor tyrosine kinase

ROI	Region of interest
SCF	Stem cell factor
SD	Stable disease
SNL	Sinonasal lymphoma
SO	Streptozocin, o,p'-DDD
SUV	Standardized uptake value
US	Ultrasound
VOI	Volume of interest

Introduction

Basic Considerations

The adrenal glands are located retroperitoneally above the kidneys. The normal adrenal gland weighs about 3-6 g. In high power cross section, the gland comprises three outer golden-yellow cortical layers (85%) and an inner reddish-brown medullary layer. The three distinct cortical layers are the outer zona glomerulosa (15%), the middle zona fasciculata (75%), and the inner zona reticularis (10%). The adrenal cortex is a mesodermal derivative and involved in the production of numerous hormones (corticosteroids) in each of the three zones where the three major classes of steroids are: (1) glucocorticoids (cortisol), (2) mineralocorticoids (aldosterone), and (3) adrenal androgens (dehydroepiandrosterone, DHEA, and its sulfate ester).[1] Cholesterol is the substrate for steroidogenesis (Figure 1). Uptake of cholesterol by the adrenal cortex is mediated by low-density lipoprotein (LDL) receptors present in the cell membrane. The number of LDL receptors increases with long-term stimulation of adrenal cortex by adrenocorticotropic hormone (ACTH).[1] The function of the adrenal cortex is dependent on ACTH throughout the life. Of the endogenous corticosteroids, secretion of cortisol is controlled almost entirely by ACTH that also enhances the production of adrenal androgens.

Pregnenolone is the precursor of all three steroids (Figure 1). Specific enzymes required for the formation of each type of steroid accompany different zones of the adrenal cortex. Aldosterone synthase is normally expressed only in the outer glomerulosa cell layer; whereas 17α-hydroxylase is expressed in the inner fasciculata-reticularis cell layers.[1] 11β-hydroxylase and 21β-hydroxylase are the essential enzymes in cortisol- and aldosterone synthesis.[2] The basic structure of a steroid is a cyclopentenoperhydro-phenanthrane nucleus consisting of three 6-carbon hexane rings and a single 5-carbon pentane ring. Adrenal steroids contain either 19 or 21 carbon atoms: C21 (aldosterone, cortisol and corticosterone) and C19 (androgens). C19 steroids with a ketone group at C-17 are termed 17-ketosteroids. C21 steroids with a hydroxyl group at position 17 are termed 17-hydroxycorticosteroids.[1,3]

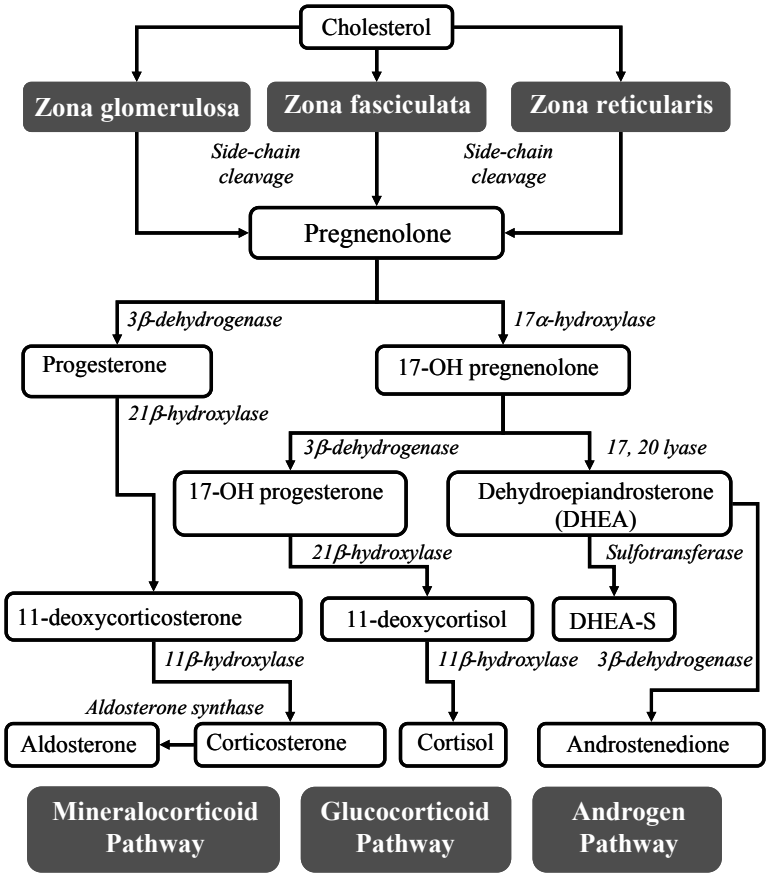

Figure 1. Biosynthesis of Adrenal steroids; major pathways to mineralocorticoids, glucocorticoids, and androgens

Adrenocortical Cancer

Adrenocortical cancer (ACC) is a rare, highly malignant tumor derived from the adrenal cortical cells, and is usually diagnosed at an advanced stage. These tumors are often characterized by overproduction of adrenal hormones and present with a variety of symptoms that may simulate other conditions. In United States, ACC affects two inhabitants per 1 million per year, accounting for an approximately 0.05-0.2% of all malignancies,[4] whereas in Sweden, 16 new cases are diagnosed each year.[5-7] The prevalence of non-functioning tumors has been estimated to be 0.35-5% among incidentally discovered adrenal masses,[8] that are found on abdominal imaging, performed for reasons other than suspected adrenal disease.[9] Autopsy studies show that approximately 5-15% of the general adult population may have adrenal incidentalomas. Approximately one per 1500 adrenal tumors is malignant.[10]

Classification

Tumors are classified as functioning and non-functioning. Functioning tumors are associated with the clinical signs and symptoms developed by elevation of corticosteroids (glucocorticoids, mineralocorticoids, androgens, and rarely a group of biosynthetic precursors such as progesterone, 11-deoxycorticosterone, and 11-deoxycortisol). Non-functioning tumors, not associated with any clinical evidence of hormonal excess, may be more common than the functioning ones.[11]

Clinical Features

Functioning tumors of the adrenal cortex may display various signs and symptoms of hormonal overproduction. Those with an excess of cortisol may present with the Cushing's syndrome that causes obesity, moon face, hypertension, and osteoporosis.[12] Signs of sex hormone overproduction may include menstrual cycle alterations, a deepening of the voice and hirsuitism in women (virilization syndromes, associated with excess androgen) and breast development in men (feminization, associated with excess estrogen). Conn's syndrome (excess aldosterone, causing hypertension and hypokalemia) is rare in ACC. Mixed endocrine syndromes occur in approximately 35% of patients. Women have functioning tumors more often than men and present with manifestations of excess steroid production, such as Cushing's syndrome and virilization.[13,14] All male patients with both adrenal mass and feminizing symptoms have a malignant tumor. Moreover, approximately 40% of adrenal tumors with Cushing's syndrome are malignant. The non-functioning tumors may present in a variety of ways, the majority being diagnosed by a palpable mass and/or abdominal pain, by the presence of metastases, or are found incidentally in association with radiological examination of the abdomen. These tumors usually present in older patients of either sex.[3,12,13] Malaise, weakness, weight loss, or other symptoms of malignant disease can be seen. Signs of adrenal insufficiency rarely occur.

Diagnosis of ACC

It is important to evaluate all adrenal masses including incidentalomas, focusing on the characterization of functioning masses and early diagnosis of ACCs. Even patients without signs of hormone overproduction or malignancy require subsequent follow-up.

Screening Tests

Urine and blood are analyzed to detect high levels of hormones secreted by the tumor. These include cortisol, aldosterone, estradiol, testosterone, an-

drostenedione, DHEA-S, and 17-OH-progesterone. In addition to these, urinary catecholamines are measured by radioimmunoassay to exclude possible adrenomedullary tumors (pheochromocytoma). The urinary steroid profiles can be monitored routinely to detect steroid precursors.[15]

Imaging Techniques

An ACC is typically visualized as a large unilateral adrenal mass with an irregular margin. Various imaging modalities may be used to identify and characterize adrenal lesion, as well as to evaluate the extent of disease. Radiological procedures include magnetic resonance imaging (MRI), computed tomography (CT) and ultrasound (US). The increasing utilization of these techniques has led to increasing incidental discovery of adrenal masses. CT and MRI can accurately provide anatomic details of adrenal tumors, and in some patients may characterize some of these as benign adenomas because of their fat content, however, none of the parameters to evaluate an adrenal lesion investigated with these procedures has been proven sensitive and specific enough.[16-18] US has lower sensitivity for detecting adrenal tumors, but is of particular value in the follow-up of previously detected incidentalomas.[19]

Scintigraphy using NP-59 (^{131}I-6β-iodomethyl-19-norcholesterol) and ^{75}Se-selenomethyl-norcholesterol is rarely indicated in suspected ACC, but it may allow the discrimination of a non-functioning adenoma from a possible ACC, thus complementing the morphological imaging techniques.[16] Tumors with discordant patterns in NP-59 scintigraphy show a significant risk of ACC,[16] in which case, the authors suggest fine-needle aspiration (FNA) cytology.[16] However, FNA is not recommended since the procedure is not a preferable method for characterization.

US- or CT-guided core biopsies are sometimes required when morphological imaging criteria are unable to distinguish between benign and malignant lesions before definitive therapy is initiated. Currently, these are useful only in the evaluation of patients with a known malignancy in order to exclude adrenal metastases.

Positron Emission Tomography (PET)

PET is a noninvasive method allowing measurement and imaging of physiological and pathophysiological processes. It has recently become one of the most effective nuclear medicine imaging modalities in oncology. It aids in the initial preoperative staging by helping in the diagnostic evaluation of suspected lesions and identification of metastatic or recurrent lesions, and facilitates decisions on treatment strategy and predictions of response to therapy.

Molecules such as agonists or antagonists binding to a receptor or an enzyme, labeled with positron emitters (e.g. ^{18}F, ^{11}C, ^{15}O) may be used as PET tracers. Other PET tracers, such as ^{18}F-fluoro-deoxy-glucose (FDG) and ^{11}C-methionine, binds through various metabolic pathways. After administering the tracer to the patient, usually intravenously, the uptake of these tracers in various tissues in the body may be assessed *in vivo*. Each positron emission gives rise to two photons by annihilation (Figure 2). These photons are detected by the gamma detector rings in the PET camera where tomographic images of radioactivity concentration in the body may be reconstructed (Figure 2).

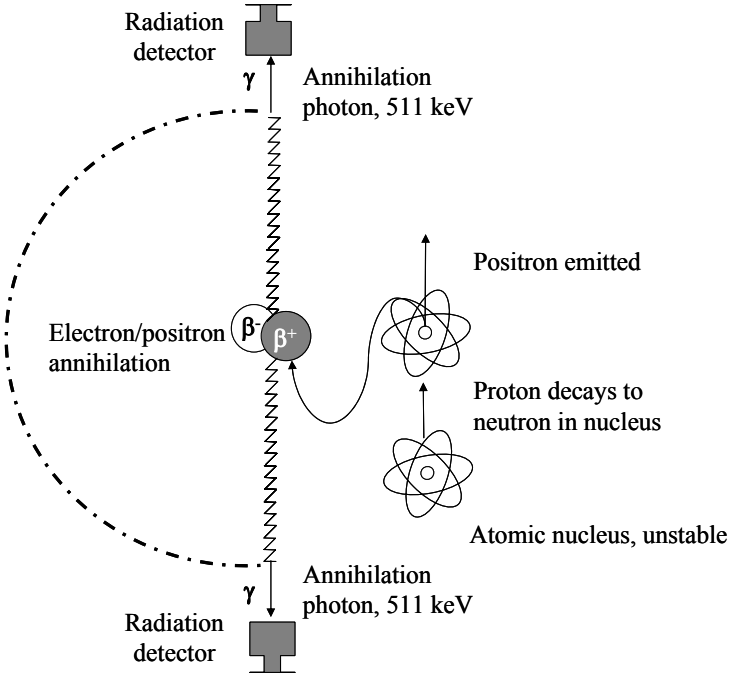

Figure 2 Positron emission and annihilation

Particularly, ^{18}F-FDG has been useful for PET to visualize various cancers. Usually, high glucose metabolism in most malignant tumors accounts for an increased FDG-uptake.[20] The presence of increased FDG uptake in cancer cells may be related to proliferative tissue activity and the number of viable cells.[21] Conversely, the metabolic cellular activity can be only slightly increased or even normal in well-differentiated and slow-growing tumors where FDG uptake may remain normal.[20] Although FDG-PET appears effective in distinguishing adrenal adenoma from adrenal carcinoma,[17,22-24] it does not discriminate adrenocortical tumors from nonadrenocortical tumors. New emerging PET-tracers such as ^{11}C-hydroxyephedrine and ^{11}C-metomidate

may be useful to specifically characterize pheochromocytoma and adrenocortical neoplasms, respectively.[25,26]

^{11}C-Metomidate PET

Recently, ^{11}C-etomidate and ^{11}C-metomidate, the inhibitors of 11β-hydroxylase, have been developed as potential PET tracers. Metomidate (Figure 3) is a methyl ester of etomidate that has been used as an anesthetic agent. ^{11}C can be incorporated into a ligand without changing its molecular structure or chemical characterization. However, it must be synthesized and administered quickly since it has a half-life of only 20 min. *In vitro* frozen section autoradiography with ^{11}C-etomidate and ^{11}C-metomidate showed a very high uptake in normal adrenal cortex from rat, pig and man.[2] *In vivo* biodistribution studies in the rhesus monkey demonstrated a very high uptake with excellent visualization of the normal adrenal cortex, adrenocortical tumors and normal liver.[2] Moreover, ^{11}C-metomidate-PET could differentiate adrenocortical from adrenomedullary tumors *in vivo*.[26-28]

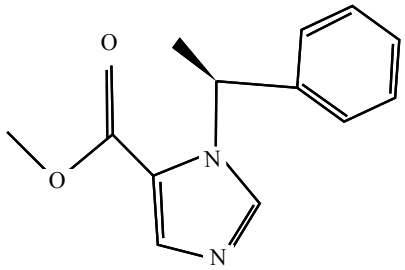

Figure 3. The chemical structure of metomidate.

Histopathology

Our knowledge of neoplasms arising from the adrenal cortex has greatly expanded in the past decade. Histological criteria have been developed to distinguish benign from malignant adrenal cortical neoplasms. The histopathologic diagnosis may be difficult if clinical evidence of metastasis is lacking. Table 1 shows the clinical and pathological diagnostic criteria of ACC.

Immunohistochemistry

Adrenocortical and adrenomedullary tumors can easily be distinguished because of their respective distinctive histologic appearance and immunohistochemical-staining pattern. Adrenocortical cells stain positive for D11 and inhibin while adrenomedullary tumors stain positive for neuroendocrine markers (e.g. chromogranin A), with very little overlap.[29,30]

Table 1: *Diagnosis of ACC*

Reliability	Clinical Criteria	Pathologic and Genetic Criteria
Diagnostic of malignancy	Weight loss, feminization, nodal or distant metastases	Tumor weight > 100 g, tumor necrosis, fibrous bands, vascular invasion, number of mitoses per high-power field
Consistent with malignancy	Virilism, Cushing's virilism, no hormone production	Nuclear pleomorphism, aneuploidy
Suggestive of malignancy	Elevated urinary 17-ketosteroids	Capsular invasion, inhibin, 21-hydroxylase deficiency
Unreliable	Hypercortisolism, hyperaldosteronism	Tumor giant cells, cytoplasmic size variation, ratio between compact and clear cells

Adapted with permission from Cancer: Principles and Practice of Oncology, 6th Edition, Norton and Le 2000.

Staging

The MacFarlane system later modified by Sullivan is the basis for the staging of ACC.[3,31] Stage I-II tumors are confined to the adrenal gland and stage III-IV tumors are characterized by local invasion and/or lymph node or distant metastasis (Table 2).[14]

Table 2: *The surgical staging of ACC*

Stages	Extent of disease
I	Tumor less than 5 cm without local invasion, nodal, or distant metastases
II	Same as stage I except tumor more than 5 cm
III	Tumor with local invasion or positive lymph nodes
IV	Tumor with local invasion and positive lymph nodes or distant metastases

Adapted with permission from Cancer: Principles and Practice of Oncology, 6th Ed., Norton and Le 2000.

Treatment Strategies

Surgery

Surgical removal of all gross tumors can be curative in stage I-III disease.[32-34] Subtotal resection of advanced ACC may be helpful by reducing the amount of hormone-secreting tissue.[6] All non-functioning adrenal tumors larger than or equal to 6 cm should be removed because of the significant potential cancer risk. A standardized diagnostic program suggested operation if the size of the incidentaloma is more than 3 cm or if it exhibits endocrine

activity, since an ACC can not be ruled out.[19] Despite operative intervention, most patients with adrenal carcinoma die within 2 years of diagnosis. Recurrent local and metastatic disease is common and reoperation should be attempted.[34] Metastases occur most often in liver, lung, lymph nodes and bone. Therefore, cytotoxic treatment is necessary for these patients.

Medical Treatment

o,p'-DDD

o,p'-DDD (1,1-dichloro-diphenyl-dichloroethane, Figure 4), also called mitotane, is an isomer of the insecticide DDT,[35] the only drug so far known to have adrenolytic action. It alters mitochondrial function, inhibits cholesterol side-chain cleavage, blocks 11β-hydroxylation leading to suppression of steroid production, and thus decreases plasma as well as urinary steroid levels.[7] Mitotane increases extra-adrenal metabolism of cortisol leading to a reduction in urinary 17-OH-corticosteroids and increased formation of 6β-hydroxycortisol, without changing the plasma levels of corticosteroids. The drug is usually given orally 2-6 g/day, with a gradual increase to 9-10 g/day to tolerability. The maximum tolerated dose varies from 2 to 16 g/day.[36] Small proportions are metabolized to inactive metabolites by both the liver and kidney. About 60% of the drug is excreted unchanged in the feces, 10-25% as metabolites in urine and small amounts are excreted in bile.

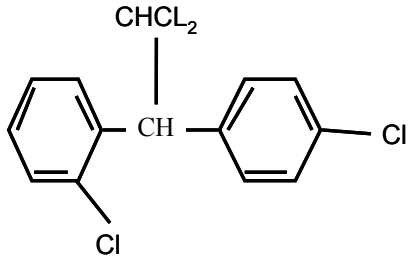

Figure 4. The chemical structure of o,p'-DDD

It takes weeks of treatment for mitotane to reach its therapeutic effect. Tumor response has been reported to correlate with serum levels and hence, therapy may be required for at least 3 months before deciding whether the drug has efficacy in the management of a patient.[37] At higher doses, almost all patients experience side effects, which may be gastrointestinal (anorexia, diarrhea, vomiting) or neuromuscular (lethargy, somnolence, dizziness). Some experts therefore do not recommend o,p'-DDD treatment due to its toxicity in dose ranges considered therapeutic.[38-40] Monitoring of serum mitotane levels may be helpful during therapy.[7,37,41] However, lack of an asso-

ciation has been observed between mitotane concentrations and the response that occurred before mitotane levels reached the therapeutic range.[6,42] Some studies suggest that mitotane should be kept at a critical threshold plasma level to prolong survival.[7,37,39] Conversely, higher serum levels may produce severe toxic side effects, underscoring the need for controlled studies to confirm the appropriate therapeutic levels.

o,p'-DDD is used in the treatment of inoperable ACC.[34] In a series of studies, 13.5-35% of patients have shown regression of both primary tumor and metastases (Table 3),[13,36,37,39,41,43-45] although there is no evidence that this improves survival.[38] The role of mitotane as an adjuvant agent after surgical resection is still not known even though some experts recommend its use.[6,36,44,46-49] Moreover, multidrug resistance (MDR) mediated by *MDR1* gene/P-glycoprotein (Pgp) can be reverted by mitotane since it interferes with Pgp function and the high levels of Pgp have been found in ACC,[50-52] opening for the exploration of mitotane use in combination with chemotherapeutic agents. All patients treated with o,p'-DDD should receive long-term glucocorticoid maintenance therapy as there is a risk of adrenal insufficiency. Some patients may in addition need mineralocorticoid replacement. The elimination half-life of the parent compounds ranges between 18 and 159 days, although the blood levels become undetectable after 6 to 9 weeks after discontinuation of therapy in most patients.[53]

Table 3: *Responses to o,p'-DDD treatment in different studies*

Study	Year	No. of Patients	Responses (%)
van Slooten et al[41]	1984	34	PR, 8 (23.5%)
Luton et al[36]	1990	59	PR, 8 (13.5%)
Decker et al[43]	1991	36	CR, 2, PR, 6 (22%)
Pommier et al[44]	1992	29	PR, 7 (24%)
Wooten and King et al[13]	1992	551	CR, PR (35%)
Haak et al[39]	1994	55	CR, 8, PR 7 (27%)
Barzon et al[45]	1997	11	PR, 2 (18%)
Baudin et al[37]	2001	13	CR, 1, PR, 3 (31%)

CR, complete response; PR, partial response

Streptozocin

Streptozocin is a member of a group of alkylating antineoplastic agents known as alkylnitrosoureas (Figure 5). It acts by inhibiting DNA synthesis and RNA transcription, thus preventing cell division. Severe DNA damage from this drug results in cell death by apoptosis or necrosis. Moreover, streptozocin is cell cycle phase nonspecific and non-cross-resistant with other nitrosoureas.[54] The plasma half-life is only 35-40 minutes, and <10% of the drug is excreted by the kidneys. Nausea and vomiting are common side ef-

fects. Elevated liver enzymes and renal toxicity may occur with prolonged treatment and with higher doses.[55]

Streptozocin is employed in the treatment of gastrointestinal endocrine tumors. It is often used to induce diabetes mellitus in experimental animals because of its toxic effects on pancreatic β cells. Moreover, it has been shown to concentrate in the adrenal cortex in mice.[56] This interesting observation led to a clinical trial with the combination of streptozocin and o,p'-DDD (SO) therapy that has shown a beneficial effect in two out of three patients with advanced ACC.[57] By using this combination therapy, the dosages of both drugs can be decreased to more tolerable levels.[57]

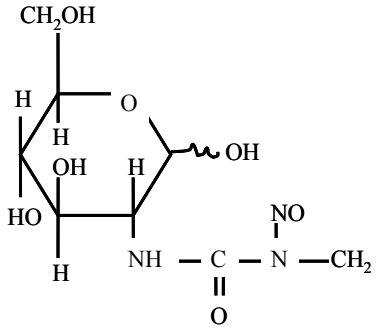

Figure 5. The chemical structure of streptozocin

Cisplatin-based Chemotherapy

Since there is no evidence of a long-term benefit from o,p'-DDD treatment alone, alternative chemotherapeutic approaches have been tried. Chemotherapy using single agents has not been effective in the treatment of ACC.[13,14,51] Moreover, a combination of cytotoxic drugs such as doxorubicin, vincristine, and etoposide together with oral mitotane has been used in patients with metastatic ACC with a response rate of 22% where mitotane does not appear to act as an effective Pgp antagonist.[42] Therefore, more effective combinations of chemotherapeutic agents may be considered.[51,58]

The preferred second-line chemotherapy in locally recurrent or metastatic ACC is platinum-based therapy.[14,34,59,60] The responses obtained in different studies using most commonly used cisplatin-based chemotherapy regimens with or without o,p'-DDD are shown in Table 4. Other combinations including OED (vincristine, etoposide, doxorubicin) or OC (vincristine, cyclophosphamide) regimens have also shown partial responses.[42,61] Doxorubicin has been ineffective as second-line chemotherapy for patients with well-differentiated or functioning tumors in which mitotane has been ineffective.[43] One child with ACC who received combination therapy including OPC (vincristine, cisplatin, cyclophosphamide) as first-line therapy had a complete response after the fifth cycle.[62] Moreover, regression of metastatic

ACC has been reported where all steroid levels returned to normal after the sixth cycle with the OPEC (vincristine, cisplatin, teniposide, cyclophosphamide) combination therapy.[63]

Table 4: *Responses to cisplatin-based chemotherapy with or without o,p'-DDD in different studies including more than 10 patients*

Study	Year	Drugs	No. of Patients	Responses (%)
van Slooten et at[64]	1983	CAP	11	PR, 2 (18%)
Schlumberger et al[65]	1991	FDP	13	CR, 1, PR, 2 (23%)
Bukowski et al[51]	1993	P	37	CR, 1, PR, 10 (30%)
Berruti et al[58]	1998	EDP	28	CR, 2, PR, 13 (53.5%)
Bonacci et al[66]	1998	EP	18	CR, 3, PR, 3 (33%)
Williamson et al[67]	2000	EP	45	PR, 5 (11%)

CAP, cyclophosphamide + doxorubicin + cisplatin; FDP, 5-fluorouracil (5-FU) + doxorubicin + cisplatin; P, cisplatin; EDP, etoposide (VP-16) + doxorubicin + cisplatin; EP, etoposide + cisplatin; CR, complete response; PR, partial response

The common side effects of different cytotoxic chemotherapies include anemia, loss of appetite, nausea, vomiting, diarrhea, risk for bleeding, risk for infection and hair loss. The major adverse events by individual cytotoxic drugs may also occur as shown in Table 5.

Table 5: *Possible Adverse events by individual cytotoxic drugs*

Cytotoxic drugs	Toxicity
Cisplatin	Renal toxicity, impaired hearing, tingling and numbness (neuropathy)
Doxorubicin	Congestive heart failure
Etoposide	Liver toxicity, skin rash, hypersensitivity
Cyclophosphamide	Skin rash, hypersensitivity
Vincristine	Constipation, abdominal pain, bone pain, peripheral neuropathy
Fluorouracil	Mucositis, arrythmia, cerebral ataxia

Symptomatic Treatment

Adrenal steroid inhibitors may be indicated for symptomatic relief of functioning and metastatic or inoperable disease, although they have no antitumor effects.[67] Inhibition of steroidogenesis in severely cushingoid subjects before surgical intervention by steroid synthesis blockers such as ketoconazole, aminoglutethimide, metyrapone, etomidate and/or o,p'-DDD may be effective.[34] Ketoconazole is an imidazole-derivative antifungal agent that blocks 11β-hydroxylase and other enzymes in the biosynthetic pathway of corticosteroid production. Metyrapone also inhibits cortisol production by inhibiting the 11β-hydroxylase. Aminoglutethimide blocks adrenal steroido-

genesis by preventing the conversion of cholesterol to pregnenolone. Spironolactone, amiloride, and various antihypertensive drugs are used in hyperaldosteronism. Spironolactone usually corrects hypokalemia but is frequently inadequate in controlling hypertension. Ketoconazole and spironolactone also have specific antiandrogenic effects. Adrenal insufficiency is a risk when using all these agents, and replacement steroids such as hydrocortisone and fludrocortisone may therefore be required.[48]

Radiotherapy

There is no evidence suggesting that radiation therapy has any role in the management of primary ACC. However, local radiotherapy may be helpful for palliative treatment of bone metastases.[14,34,68]

Molecular Biology

Despite the significant improvements in diagnostic imaging and the extensive research performed on the molecular mechanisms involved in adrenal carcinogenesis, the results from trials on therapy of advanced ACC are still discouraging. Thus, further investigation of the genetic and molecular mechanisms involved in the pathogenesis of ACC is essential in the development of new treatment strategies for this disease.

ACCs are generally monoclonal because of oncogenic mutations of single cells with transformation and expansion into one malignant clone.[69,70] Molecular genetic analyses suggest that one or several tumor suppressor genes may be involved in the pathogenesis of adrenal cortical neoplasms.[69] The genetic alterations frequently observed, such as upregulation of the insulin-like growth factor II (IGF-II) as well as mutations in the p53 gene occur during the late stages of adrenocortical tumorigenesis.[69] While the mutation-induced inactivation of tumor suppressor genes appears to be a probable mechanism for ACC development, efforts to identify and characterize other events such as activation of various proto-oncogenes required for neoplastic transformation have met with limited success. Table 6 shows genetic alterations in oncogenes and tumor-suppressor genes in ACC in different studies.

Deregulation of signal transduction pathways are frequent during malignant cellular transformation.[71] Critical molecules involved in signal transduction constitute proto-oncogenes; some tyrosine kinases are very potent oncogenes that are susceptible to mutations and can activate multiple downstream signaling pathways thereby altering cell phenotypes. Although several receptor tyrosine kinases (RTKs) with oncogenic capabilities, including vascular endothelial growth factor (VEGF), IGF-I, epidermal growth factor receptor (EGFR) are expressed in ACC,[72-74] the use of inhibitors against these RTKs has not been reported in ACC.

Table 6. *Genetic alterations in oncogenes and tumor-suppressor genes in ACC*

Study	Year	Gene	Genetic alterations	Prevalence
Skogseid et al[75]	1992	MEN I	LOH 11q13	1/1
Ilvesmäki *et al*[76]	1993	IGF II	Overexpression	4/4
Ohgaki *et al*. [77]	1993	p53	Exon 5-8 point mutations	3/15
Reincke *et al*[78]	1994	p53	Exon 5-8 point mutations	5/13
Yashiro *et al*[79]	1994	N-ras	Point mutations	3/24
Reincke *et al*[80]	1997	ACTH-R	Deletions	2/4
Gicquel *et al*[81]	1997	IGF II/H19	Overexpression/LOH 11p15	27/29
Liu *et al*[82]	1997	p57/H19	Low expression	6/6
		IGF II	Overexpression	6/6
Kjellman *et al*[83]	1999	MEN 1	LOH at 11	11/13
Heppner et al [84]	1999	MEN 1	LOH 11q13	5/5
Pilon et al[85]	1999	p16	No expression/LOH 9p21	3/7
Zhao et al [86]	1999	p53	Gain at 17	3/12
		p53	LOH 17p13	1/12
		p57/H19	LOH 11p15	3/12
		MEN 1	LOH 11q13	4/12
Zwermann et al[87]	2000	MEN 1	LOH 11q13	5/6
Dohna et al [88]	2000	p53	Gain at 17	3/14
Barzon et al [89]	2001	p53	Point mutations	8/14
		p57KIP2	Low expression	6/7
Wachenfeld et al [90]	2001	p53	LOH 17p13	6/6
		MEN 1	LOH 11q13	6/8
Gicquel et al [91]	2001	p53	LOH 17p13	11/13
		IGF II	Overexpression/LOH 11p15	15/18
Stojadinovic et al [92]	2002	p21	Overexpression	25/36
		p27	Overexpression	34/36

MEN I, multiple endocrine neoplasia I; ACTH-R, adrenocorticotropin receptor; LOH, loss of heterozygosity; p57KIP2, cyclin-dependent kinase inhibitor gene

Receptor Tyrosine Kinases (RTKs)

RTKs are transmembrane enzymes possessing an extracellular ligand-binding region, a transmembrane domain and an intracellular region. The latter is made up of a juxtamembrane (JM) domain, a kinase domain with kinase insert, and a C-terminal domain (Figure 6A).[93] Based on the sequence of the kinase domain and the type of domains in the extracellular parts, the RTK family can be subdivided in 20 classes. Five immunoglobulin-like motifs are present in the extracellular ligand-binding region in class III RTKs

such as c-Kit and platelet-growth factor receptors (PDGFR-α, -β) (Figure 6A).[93,94] These RTKs are responsible for transducing extracellular signals from peptide growth factors across the cell membrane, involved in the functions of many cellular behaviors that are modified by neoplastic transformation of cells.[71] Immunodetection of c-Kit and platelet growth factor receptors (PDGF-α, -β) has been recently used for diagnosis. c-Kit and/or PDGFR-positive tumors can potentially benefit from tyrosine kinase inhibitor treatment.

c-Kit

c-Kit, a class III transmembrane RTK of 145 kDa, is the cellular counterpart of v-kit derived from the Hardy-Zuckerman 4 feline sarcoma virus.[95] It encodes a glycoprotein receptor that binds c-Kit-ligand, also known as mast cell growth factor or stem cell factor (SCF), and is encoded by the steel locus.[96] c-Kit plays an important role in tyrosine phosphorylation of protein substrate, and through subsequent activation of intracellular signaling cascades, it controls cell proliferation, apoptosis, migration and differentiation.[97]

Under normal circumstances, Kit activity is modulated by SCF, a bivalent dimer that binds to the extracellular domain of two proximal Kit receptors, leads to their dimerization and concomitant activation of their tyrosine kinase by autophosphorylation of intracellular tyrosine residues. This activated Kit then transfers phosphate groups from ATP to the tyrosine residues that initiate signaling cascade activation in turn involving several proteins such as mitogen-activated protein (MAP) kinase.[98] Phosphorylated tyrosine residues act as specific binding sites for downstream signaling proteins containing *Src* homology 2 (SH2) domains.[99] The Src tyrosine kinases containing SH2 domain may be involved in the adrenal cell steroidogenesis.[100] Moreover, the autophosphoylation site of Tyr-703 in the c-Kit/SCF receptor in the kinase insert has been demonstrated to interact with the SH2 domain-containing adaptor molecule growth factor receptor-bound protein 2 (Grb2, Figure 6B), whereby the MAP kinase signaling pathway is activated.[101]

c-Kit is expressed in a variety of normal human tissue such as hematopoeitic stem cells, mast cells, melanocytes, primordial germ cells, Leydig cell of Sertoli, interstitial cells of Cajal, breast and ovarian epithelial cells.[102-104] Kit activation seems to be an early tumor-promoting event in pathogenesis. Dysregulation of Kit has been implicated in the etiology of a number of tumors including acute myelogenous leukemia (AML), germ cell tumors (GCTs), mast cell tumors, gastrointestinal stromal tumors (GISTs) and sinonasal lymphoma (SNL).[105-109] In addition, it has been seen in some endocrine tumors, such as thyroid cancers, malignant endocrine pancreatic tumors, testicular and ovarian cancers.[110-113] However, their role in the pathogenesis of these malignancies has not been defined.

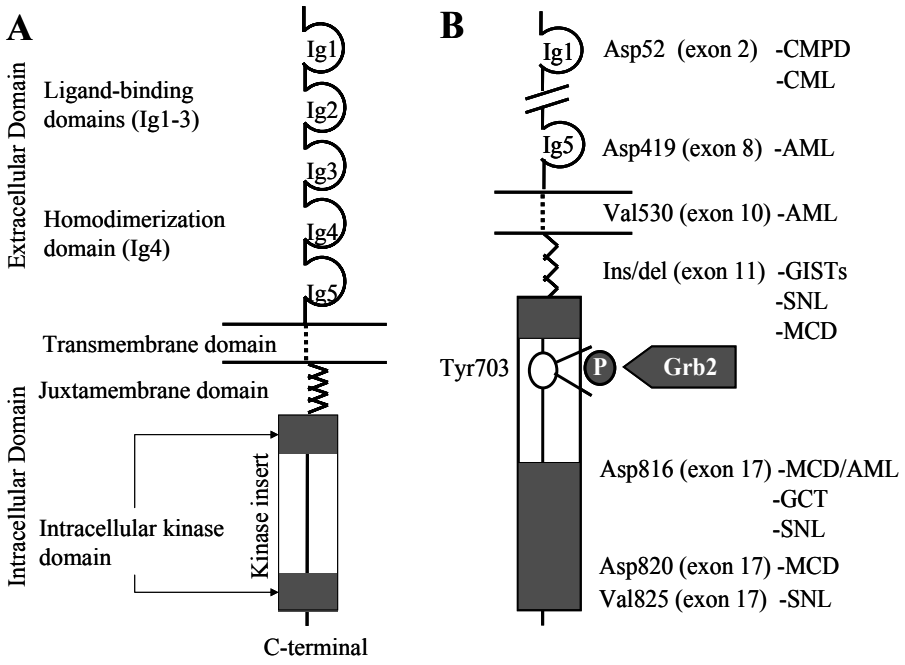

Figure 6. A. Structural domains of class III RTK demonstrating five immunoglobin-like motifs (Ig1-5) in the extracellular region. B. The position of Tyr703 and reported mutations as well as their disease associations for c-kit are highlighted. P, phosphorylation; Grb2, growth factor receptor bound protein 2; CMPD, chronic myeloproliferative diseases; AML, acute myeloid leukemia; GISTs, gastrointestinal stromal tumors; SNL, sinonasal lymphomas; MCD, mast cell disease; and GCT, germ cell tumor. *Modified with permission from Reilly JT 2002.*

c-Kit Mutation

Mutation in the c-Kit or SCF loci in the mouse have deleterious effects,[114] conferring proliferation and/or anti-apoptotic activity. Kit has 21 exons and mutation has been identified in different exons, mainly in AML. Some reported mutations and their disease associations for c-kit are highlighted in Figure 6B.[93] A linkage has been found between c-Kit mutations and the pathogenesis of some non-haemopoietic tumors such as GISTs and seminoma.[106,108] Most of the GISTs expressing the c-Kit protein have mutations leading to constitutive or higher activation of this kinase.[115] In 50-77% of sporadic GISTs, mutations have been identified in exon 11, which encodes the intracellular JM region (Figure 6B). Mutations in exon 9, encoding the extracellular domain, have been found in 3-18%.[115-117] Mutations have also been described in exons 13 and 17, which encodes the intracellular part of the receptor.[118]

PDGFRβ

PDGFRβ is a 170-190 kDa transmembrane class III glycoprotein RTK. Its ligand (PDGF AB or BB) is a potent stimulant of mesenchymal cell proliferation, differentiation and migration, and plays an important role in wound healing by matrix deposition.[119,120] PDGFRβ plays an essential role in haematopoiesis, probably in megakaryocytopoiesis, in the poiesis of B and T lymphocytes, natural killer (NK) cells and other haematopoietic cells.[93] The biological role of PDGF signaling can vary from autocrine stimulation of cell growth to more subtle paracrine interactions involving adjacent stroma and angiogenesis.[121] PDGFRβ is normally expressed in fibroblasts, smooth muscle cells, glial cells, chrondrocytes, multipotent stem cells, mast cells, myeloid progenitor cell lines, T lymphocytes and NK cells.[93,122,123] PDGFRβ has also been found to be expressed in glioblastoma, prostate carcinomas, gliomas, sarcomas, ovarian serous carcinoma, carcinoids and malignant endocrine pancreatic tumors.[112,113,122,124,125]

Receptor Tyrosine Kinase Inhibitors

Emerging new treatment modalities are targeted to specific tyrosine kinases of cancer cells in the signaling pathway of tumor cells.[126] Imatinib mesylate is a phenylamino-pyrimidine in which the introduction of a "flag-methyl group" increases its potency to inhibit c-Abl in chronic myeloid leukemia (CML), SCF-mediated c-Kit activation in GISTs as well as ligand-activated PDGFRβ in myeloproliferative diseases (MPDs) and chronic myelomonocytic leukemia (CMML).[127-131] It is the first tyrosine kinase inhibitor that has been approved by the FDA as an antitumor drug for CML and GISTs. Imatinib blocks the ATP-binding site of tyrosine kinases, thus preventing the kinase from transferring phosphate from ATP to tyrosine residues of its substrates. This leads to inhibition of downstream signaling causing a shift in the balance between cell survival and proliferation towards apoptosis.[132] This drug is metabolized mainly in the liver and excreted via bile into the stool. The half-life of imatinib in the circulation is 20 hours.[127,133]

Complete or partial responses have been reported in 53-54% of GISTs with imatinib treatment,[133,134] which has been linked to the presence of c-Kit mutation in exon 11.[111] PDGFRβ expression is associated with tumor neoangiogenesis that can be inhibited by imatinib.[135] Clinical responses in CMML with PDGFRβ fusion oncogene have been observed with imatinib treatment.[130] An effect of imatinib in MPDs with a translocation involving PDGFRβ has been described,[128] and it is suggested that any neoplasm arising from an abnormality of PDGFRβ should respond to this drug.[136] However, the Asp816 mutant isoform has been identified as resistant to imatinib.[137] Imatinib has shown a synergistic cell killing effect with cisplatin on non-small cell lung cancer cells.[97] This indicates that the utility of imatinib and/or

related compounds might extend well beyond CML, so that once again further studies of Kit and PDGFRβ promise to be powerful tools to help design potential new therapies.

Prognostic Features

ACC recurs frequently and usually progresses rapidly. The prognosis for ACC is poor with a 5-year survival rate of 26-38%.[32,33,49] The stage and aggressiveness of the disease determine the prognosis. Early diagnosis and aggressive surgical extirpation may lead to increased survival. Non-functioning tumors operated at an early stage show a better prognosis.[13] Regardless of treatment of metastatic ACC, 50% of patients die within a year of diagnosis. Most of the patients develop local recurrence or metastasis after an apparently radical resection of the tumor. ACC may recur more than 10 years after curative surgery and life-long follow-up is therefore necessary.

Aims of the Present Study

- To assess the diagnostic potential of PET using ^{11}C-metomidate in ACC for tumor staging purposes
- To analyze the outcome of streptozocin and o,p'-DDD (SO) combination therapy in ACC as an adjuvant therapy following curative surgery as well as in recurrent and/or metastatic ACC
- To evaluate the therapeutic effects and tolerability of OPEC combination therapy after failure of SO therapy in advanced ACC
- To find out the expression of receptor tyrosine kinase-targeted proteins such as c-Kit, phospho-c-Kit and PDGFRβ in ACC
- To detect c-Kit mutations in ACC that are common in other tumors targeted by receptor tyrosine kinase inhibitor therapy

Patients and Methods

Patients

Paper I

The study was performed at the Uppsala University PET Center from November 1996 through December 1998. Eleven patients with verified ACC or suspected malignancy based on CT were referred from the Department of Endocrine Oncology, Uppsala University Hospital. The median age was 52 years at diagnosis. Six patients presented with functioning tumors and five had non-functioning tumors. At study inclusion, four patients had primary ACC (stage I-II, *n* = 2; stage III-IV, *n* = 2) while seven patients had recurrent disease (local recurrence, *n* = 1; metastatic, *n* = 6). Moreover, one patient with stage I-II and another with stage III-IV were re-referred during follow-up at the time of relapse for a second PET examination. Thus, 13 PET studies were performed in eleven patients where 21 tumor lesions were detected or suspected at CT before referral, and in addition, patients in the seven studies were receiving cytotoxic treatment or adrenal steroid inhibitors (Table 7). The median time interval between CT and PET was 3.5 days.

Table 7: *Patients' status at study inclusion (n = 11)*

Extent of disease	No. of Patients	No. of Tumor lesions at CT	Patients on treatment
Stage I-II	2[a]	2	-
Stage III-IV	2[a]	4[b]	1[c]
Recurrent	7	15[b]	6[c]

[a]one patient was referred for second PET study at recurrence, [b]one of the lesions was suspected at CT, [c]One patient was on treatment on both occasions (prior surgery and follow-up at recurrence).

Paper II

Forty patients with histopathologically verified ACC were treated with a new SO combination therapy at the Department of Endocrine Oncology, Uppsala University Hospital, Karolinska Hospital, Stockholm, and Lund University Hospital during 1979-1999 (Table 8). At study entry, 29 patients

had primary tumors (stage I-II, *n* = 14, stage III-IV, *n* = 15), and eleven had recurrent ACC. The latter patients were referred from other centers at their first relapse. They had previously undergone radical surgery for their primary tumor, but had not received any adjuvant treatment and had a median disease-free interval (DFI) of 12 months (range, 3-79 months). The median age of all patients at diagnosis was 44 years and the male to female ratio was 1:2. Twenty-two patients presented with functioning tumors whereas 18 patients had non-functioning tumors. Ten of 18 non-functioning patients showed positive biochemical evidence of disease. The median tumor size was 11 cm.

Table 8: *Patients' status at study inclusion (n = 40)*

	Uppsala	Stockholm	Lund
Stage I-II	14	-	-
Stage III-IV	13	2	-
Recurrent	7	3	1
Total no. of Patients	**34**	**5**	**1**

Paper III

Eleven patients (four males and seven females) with locally recurrent and/or metastatic ACC were included in this study. All patients had received SO therapy until progression of their disease before they were included in the present study. The median age was 45 years, seven patients had functioning and four had non-functioning tumors. At the time of initial diagnosis, five patients had localized disease (stage I-II) and six presented with distant metastases (stage III-IV). The median duration of disease from diagnosis until inclusion in the study was 16 months (range, 4 – 54.5 months). At study entry, eight patients had previously undergone radical surgery for the primary tumor with a median DFI of 20 months (range, 4 - 42 months). The metastatic sites before start of OPEC treatment included liver (n = 8), lymph nodes (n = 5), adrenal bed (n = 4), lung (n = 2), bone (n = 1), and bilateral mixed adrenal tumors (n = 1).

Sample Collections

Paper IV

Tumor tissue samples were collected from 21 patients with ACC (7 males, 14 females). After surgery, pieces of tissue from resected tumors were immediately frozen in liquid nitrogen and kept at -70°C. The rest of the tissue

was used to make formalin-fixed, paraffin-embedded sections. Paraffin blocks were retrieved from the Department of Pathology, Uppsala University Hospital. For DNA extraction, frozen surgical specimens of 15 of 21 ACC were retrieved from the Department of Surgery at Uppsala University Hospital. Normal adrenal cortex was collected from two patients who underwent nephrectomy for renal disease.

Diagnosis

Routine Investigations

Paper II-III

Routine hematology, serum electrolytes, liver enzymes, serum creatinine, creatinine-clearance, and urinary albumin were measured before and at regular intervals during treatment.

Biochemical Evaluation

Paper II-III

Serum levels of cortisol, estradiol, testosterone, DHEA-S, 17-OH-progesterone and androstenedione were assessed to evaluate the therapeutic response or to detect recurrence. In addition, urinary cortisol, aldosterone and catecholamines were measured by radioimmunoassay. Urinary steroid profiles were also assessed to detect steroid precursors.

Radiological Evaluation

Paper I-III

In paper I, the findings at PET were correlated to those at intravenously contrast-enhanced CT. For therapy monitoring, US and intravenously contrast-enhanced CT were utilized, using standard scanning protocols (paper II and III). A bone scan was performed to detect possible bone metastases, only when there was a suspicion. Chest X-ray was done every 3 months to detect possible lung metastases.

^{11}C-Metomidate PET

Paper I

PET studies were divided into two groups according to therapy that potentially could interfere with 11β-hydroxylase activity and thereby the tumor uptake of ^{11}C-metomidate. Six PET examinations made up group A, in which patients were free of medication, and the remaining seven studies comprised group B, where patients were monitored during treatment or had received treatment within 7 weeks prior to the study. A second study in one patient from group A was performed while the patient was on potentially interfering treatment and was therefore included in group B. Another patient in group B underwent PET twice while receiving therapy and both studies were thus included in this group.

PET

The synthesis of [O-methyl-^{11}C] metomidate was performed as previously described (Figure 7).[2,138] The patients were examined in a Scanditronix GE 4096 whole-body PET camera (GE Medical Systems, Milwaukee, Wis.). The camera simultaneously produced 15 contiguous 6.5-mm axial slices with an in-plane resolution of 5-6 mm.[139] CT was used as a means of positioning the tumor region in the PET camera and for anatomical correlation of the findings in the PET examinations. A 10-min transmission scan was generated with an external rotating germanium-68 pin to correct the ensuing emission scans for attenuation. After a rapid intravenous bolus of approximately 800 MBq of ^{11}C-metomidate, a 45-min dynamic examination sequence was started.

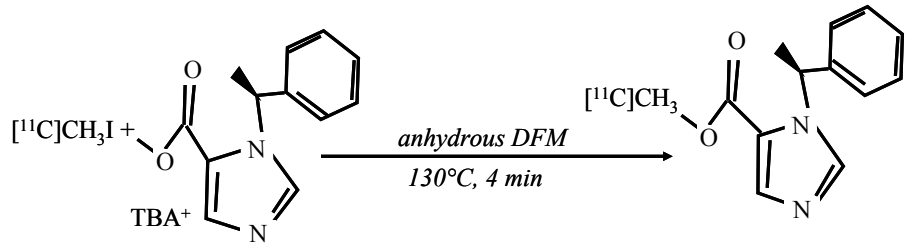

Figure 7. Synthesis of ^{11}C-metomidate from ^{11}C-methyliodide (TBA, tetrabutylammonium salt). *Adapted from Mitterhauser et al 2003*

Image reconstruction and data analysis

Images obtained 15-45 minutes post injection were summed to create an average image based on analysis of the tracer accumulation pattern over time for tumor and various normal tissues. The radioactivity concentrations in these images were recalculated to provide images of standardized uptake

values (SUV), whereby the radioactivity concentration (Bq/cc) was divided by the injected dose per gram body weight. Regions of interest (ROIs) were drawn manually in the summation images to include all tumors and various normal tissues. The tissues were delineated according to a standardized procedure whereby an isocontour (ROI_{mean}) was positioned halfway between the highest activity in the specified tissue and its immediate surroundings. In each tumor an additional region was drawn, designated the hot spot (hs), comprising four contiguous pixels at the site of the highest SUV (ROI_{hs}). ROIs for each tissue in at least three adjacent slices were combined to form volumes of interest (VOIs). Time-activity curves were generated for these VOIs and recalculated to represent SUV plotted over time. In some studies, PET was done at additional bed positions and SUV was calculated 50-60 min or 60-80 min post injection, however, these SUVs were not included in the calculations of mean SUV.

Detection Criteria

^{11}C-metomidate PET observations were defined as true positive for tumor when a high tracer uptake corresponded with CT findings, histopathological examination or when the corresponding lesion was later visualized at CT during the follow-up period. ^{11}C-metomidate PET was defined as false negative when it failed to detect a tumor diagnosed at CT and/or verified at surgery and/or histopathological examination. Non-tumor lesions on ^{11}C-metomidate PET verified as such by histopathological examination but diagnosed as tumor lesions at CT were considered as true negative observations.

Histopathology

Paper I-IV

Histopathologic examination including routine morphological examination and chromogranin A staining was performed on surgical specimens, US-guided core biopsies, or biopsies obtained at autopsy to establish the diagnosis of ACC.

Treatment

Surgery

Paper I

Five patients (primary tumor, *n* = 4, local recurrence, *n* = 1) underwent surgery after the metomidate-PET study. One patient was at metomidate-PET found to have a primary tumor that was resected but a second metomidate-PET examination showed recurrent and metastatic disease and the patient was therefore re-operated.

Paper II

An apparently radical operation was performed in 17 of 29 patients with a primary tumor and in one of eleven patients with recurrent ACC (adrenalectomy, *n* = 17, lymphadenectomy, *n* = 4, nephrectomy, *n* = 2, splenectomy, *n* = 2, and local recurrence, *n* = 1). Palliative surgery was tried in nine patients with recurrent and/or metastatic ACC (stage III-IV, *n* = 6, recurrent, *n* = 3), and tumor was removed partially in these subjects, including resection of part of all organs involved and removal of involved lymph nodes. The remaining 13 patients with recurrent and/or metastatic tumor were inoperable; however, soon after treatment with SO therapy (median duration, 8 months), radical surgery was achieved in four patients (adrenalectomy, *n* = 3, hemihepatectomy, *n* = 2, and local recurrence, *n* = 1). The surgical reports and the findings at histopathological examination in the surgical specimens were reviewed. Ten patients underwent re-operation for local recurrence or metastasis after SO therapy. The extent of disease prior to SO treatment initiation is shown in Table 9.

Table 9: *Extent of disease prior SO therapy*

Extent of disease at study entry	**Total no. of patients**	**Radical resection**	**Measurable disease**	
			Partial resection	*Inoperable*
Primary tumor and/or metastasis	29	17	6	6
Recurrence and/or metastasis[a]	11	1	3	7

[a]previously undergone radical resection of primary tumor had a median DFI of 12 months

Paper III

The subjects consisted of eight patients who had previously undergone radical surgery for primary disease, three of whom underwent a second surgical procedure because of local recurrence and/or metastases. A sub-total resection was performed in two patients who had primary tumors with lymph node metastases; however, in one patient, three operations were attempted and the third operation was considered radical. One patient who had bilateral mixed adrenal tumors with liver, lung, and lymph nodes metastases was judged inoperable at diagnosis. The surgical procedures prior to OPEC therapy included adrenalectomy (n = 10), nephrectomy (n = 4), lymphadenectomy (n = 3), splenectomy (n = 2), extirpation of soft tissue metastasis (n = 2), partial liver resection (n = 1), partial extirpation of the inferior vena caval wall (n = 1), extirpation of local recurrence (n = 1), resection of the pancreatic tail (n = 1), and partial gastrectomy (n = 1). All operative reports were reviewed. Re-operation was performed in two patients after OPEC therapy due to local recurrence or intramuscular metastasis.

Treatment Protocol of SO Therapy

Streptozocin (Zanosar, Pharmacia and Upjohn Co., NJ, USA) was given intravenously by a brief infusion with an induction course of 1 g/day for 5 days, and thereafter 2 g every 3 weeks. Pre-medication with 5HT3-receptor blocker was used before administering streptozocin. o,p'-DDD (Lysodren, Mead Johnson and Co Sub Bristol Myers Co, NY, USA) was given orally at a relatively low dose of 1-4 g daily (median, 3 g/d) in 2-3 divided doses according to the level of tolerance. Cortisone replacement (25-100 mg, hydrocortisone acetate) was given simultaneously with the o,p'-DDD to avoid Addisonian crisis. The SO therapy was given as adjuvant treatment following complete resection of a primary tumor, or in recurrent and/or metastatic ACC. Treatment was planned to continue for one year, until tumor recurrence or progression.

Paper I

Five patients in group B received chemotherapy comprising streptozocin and/or o,p'-DDD during the PET study.

Paper II

All patients were treated at Uppsala University Hospital except six patients: five of them were treated at Karolinska Hospital, Stockholm and one patient at Lund University Hospital using our national protocol. Seventeen patients received SO therapy as an adjuvant to radical resection of primary tumor.

Ten of 23 patients with recurrent and/or metastatic ACC received the combination treatment after resections were carried out (radical, *n* = 1, partial, *n* = 9). The SO therapy was also given to the remaining 13 patients with inoperable disease; however, four of those were able to undergo radical resection, and continued the treatment post-operatively. The median duration of treatment in all patients was 5 months. The total number of courses varied, ranging from 1 to 23. The median delivered dose of streptozocin was 17 g.

Paper III

All patients received SO therapy as a first-line medical treatment with a median duration of 5 months (range, 3 weeks–11.25 months). Table 10 illustrates the outcomes of SO therapy in these patients. Three patients (stage I-II) with radical adrenalectomy received the therapy as an adjuvant treatment and the remaining eight patients (stage III-IV, n = 3, recurrent, n = 5) received the regimen with advanced disease.

Table 10: *Patients' status during treatment with SO therapy prior to OPEC (n = 11)*

Extent of disease	**No. of patients**	**Median duration of treatment**	**Therapeutic effects**	
			DFI	*Responses*
		(months)		
Stage I-II	3	11	28	-
Stage III-IV	3	2	-	1 SD (5.5)
Recurrent	5[a]	2.25	-	1 CR (9), 2 SD (3,7)

DFI, disease-free interval; SD, stable disease; CR, complete response; [a]previously undergone radical resection of primary tumor had a median DFI of 12 months

OPEC Regimen

The OPEC regimen was started as second-line treatment when the disease progressed during SO therapy. OPEC combination chemotherapy was administered as an intravenous infusion over four days according to our schedule (Table 11). Antiemetics such as tropisetron (Navoban®, Novartis Pharma AG, Basel, Switzerland), betametasone (Betapred™, Swedish Orphan AB, Stockholm, Sweden) were injected daily together with the cytotoxic drugs for four days; thereafter, were changed in tablet or capsule forms on day 5–9. Daily rehydration was maintained during the cycle. Diuretics like furosemide were given intravenously if the volume of urine was less than 400 mL/4 hours on day 2. Filgrastim (Neupogen®, Amgen Inc, CA, USA) was used if the neutrophil count was below 2.0×10^9/L (normal value, 2.5 - 6.0 $\times 10^9$/L). Metyrapone or ketoconazole was allowed for symptomatic relief of symptoms.

Table 11: *OPEC regimen*

Day	Drugs	Doses	Sources
Day 1	Cyclophosphamide	600 mg/m^2 mixed with distilled water (20 mg/mL), 3–5 min brief infusion	Sendoxan, Baxter Oncology GmbH, Halle, Germany
	Vincristine	1.5 mg/m^2, maximum dose 2.0 mg, 3–5 min brief infusion	Oncovin, Eli Lilly and Company Ltd., Indianapolis, USA
Day 2	Cisplatin	100 mg/m^2 mixed with 1000 mL NaCl over 24 hours as a continuous infusion	Platinol®, Bristol-Myers Squibb, Princeton, NJ, USA
Day 4	Teniposide (VM-26)	150 mg/m^2 mixed with NaCl, 30–60 min infusion	Vumon, Bristol-Myers Squibb, Princeton, NJ, USA

Paper II

The OPEC combination was tried in ten patients after discontinuation of SO therapy when the disease progressed.

Paper III

All patients received the OPEC regimen after treatment failure on the SO combination. The median interval between discontinuation of SO therapy and start of OPEC treatment was 1.8 months (range, 1 week–30.75 months). Before treatment, all patients were required to have an adequate hematological status (leukocyte count ≥3.5 x 10^9/L and platelet count ≥150 x 10^9/L), normal serum creatinine level, and adequate renal function (normal urinary albumin, calculated creatinine clearance ≥75 mL/min). This regimen was planned to be given a maximum of eight cycles and was changed to other forms of therapy if tumor progression was detected or the patient experienced intolerable side effects. The number of OPEC cycles varied from one to eight cycles (median, six cycles) in each patient. The median duration of treatment was 6 months (range, 0.5–11 months). Along with OPEC therapy, six patients were treated with filgrastim due to neutropenia, and o,p′-DDD was continued in one patient. Ketoconazole (n = 2), and/or metyrapone (n = 1) was given when needed in three patients for symptomatic relief together with the OPEC regimen.

Other Chemotherapies/Symptomatic Treatment

At the time of PET imaging in paper I, two patients in group B were with therapies other than streptozocin and/or SO combination therapy. One patient received adrenal steroid inhibitors (ketoconazole and metyrapone) and 5-fluorouracil (5-FU) while the other received only 5-FU. Prior to OPEC

therapy in paper III, four patients received other forms of treatments after discontinuation of SO therapy including liver embolization (n = 1), metalloproteinase-inhibitor (n = 1), suramin (n = 1), and ketoconazole (n = 1). In one patient ethanol was injected locally into liver metastases five times between the cycles.

Response Criteria

Paper II-III

The biochemical and radiological evaluations were performed according to WHO criteria.[43] Classification as a "complete response (CR)" required normalization of hormonal levels together with disappearance of all measurable tumors with no new lesions was defined as normalization of hormonal levels together with disappearance of all measurable tumors and no new lesions detected for at least 4 weeks. "Partial response (PR)" was defined as a ≥50% reduction of hormonal levels and/or all measurable lesions for a minimum of 4 weeks. The disease was considered as "stable disease (SD)" when hormonal levels and/or lesions decreased <50% or did not increase >25% from original measurements and when there was no appearance of new metastases for at least 4 weeks. "Progressive disease (PD)" was recorded in patients with an increase in hormonal levels/tumor mass ≥25%, or the occurrence of new lesions. Biochemical and radiological responses were summarized as "overall response", from the start of therapy until the disease progression either biochemically or radiologically.

Toxicity Criteria

Paper II

All patients were advised to take o,p'-DDD continuously and increase their dose gradually to the highest tolerable level (maximum 4 g/day). In some patients, a reduction of dose or temporary withdrawal of streptozocin or o,p'-DDD was required because of adverse reactions. If the disease progressed, streptozocin was withdrawn permanently.

Paper III

Dose modification of OPEC therapy was made based on the grading of adverse reactions. Grade, referring to the severity of the adverse effects, was measured according to National Cancer Institute (NCI)'s Common Toxicity Criteria, Version 2.0 (CTC v2.0). The Common Terminology Criteria for

Adverse Events (CTCAE) v3.0 displays Grades 1 through 5 with unique clinical descriptions of severity for each adverse event based on the general guideline (Table 12). In the newer version, some changes to adverse events are made from CTC v2.0 e.g., creatinine clearance is not included in CTCAE v3.0. Alternatively, it includes two options for laboratory evaluation of renal function: glomerular filtration rate and creatinine. Other criteria of possible adverse events for OPEC are not changed in v3.0. Vincristine dosages were reduced or excluded mainly if there were signs and symptoms of peripheral neuropathy. The reduction of cisplatin dose was needed when there was impairment of renal function. Teniposide was withdrawn from the next cycle if patient experience allergic reactions.

Table 12: *General guideline for grading of adverse events by CTCAE v3.0.*

Grading	Severity of Adverse Events
Grade 1	Mild adverse events
Grade 2	Moderate adverse events
Grade 3	Severe adverse events
Grade 4	Life-threatening or disabling adverse events
Grade 5	Death related to adverse events

Treatment Evaluation

Paper II-III

The evaluation of the combination therapy was based on the following variables: duration of treatment, DFI, survival, therapeutic responses, and progression-free interval (PFI) as well as side effects caused by the drugs. DFI was determined in radically operated patients from surgery until recurrence of the disease. Overall survival was calculated from the date of diagnosis of the disease or from the start date of the first course until the date of death or last follow-up. Therapeutic responses were determined in patients with measurable disease from the start date of therapy, or PFI from operation until the date of change of therapy, disease progression, or death of the patient, as appropriate. Possible adverse events due to the drugs in the combination were observed clinically and their severity graded routinely throughout the therapy.

In paper II, we divided the 28 patients with radically operated primary tumor in two groups: an adjuvant and a nonadjuvant group to compare the therapeutic effects of SO therapy on DFI and on survival. Seventeen patients who received SO therapy as adjuvant to radical resection of primary tumor constituted the adjuvant group. Eleven patients who were referred for ther-

apy due to their first recurrence, however, had not received any chemotherapy following radical resection of their primary tumor constituted the non-adjuvant group.

Immunohistochemical Analysis

Paper IV

Primary antibodies against proto-oncogene c-Kit, phospho-c-Kit (phosphorylated c-Kit specific for phosphotyrosine 703 residue of c-Kit) and PDGFRβ were used (Table 13).

Formalin-fixed, paraffin-embedded tissue sections were examined by immunohistochemistry using the avidin-biotin peroxidase complex method after antigen retrieval. Briefly, 4-μm sections were cut from the blocks, deparaffinized in xylene, and rehydrated. Incubation was performed at room temperature. Antigen retrieval was performed in a microwave oven (700 W) at full power with 0.01M citrate buffer, pH 6.0, for 15 minutes. Endogenous peroxidase activity was blocked by 0.3% H_2O_2. After rinsing in PBS, the sections were incubated with normal goat serum for 30 min to reduce the nonspecific binding. Thereafter, the sections were incubated with the primary antibodies overnight. After rinsing with PBS, tissues were incubated with secondary biotinylated goat antibody to rabbit IgG (DakoCytomation, Norden AB, Sweden) for 30 minutes. The sections were rinsed in PBS and incubated with Elite complex (*Elite* ABC Kit, Vector Laboratories, CA, U.S.A) for 30 minutes. After another wash with PBS, staining was performed by exposure to a solution of aminoethylcarbazole (chromogen) in acetate buffer, pH 5.0 and H_2O_2 as substrate, for 5 minutes. Finally, the sections were rinsed in distilled water and then counterstained with Mayer's hematoxylin for 30 sec, rinsed in tap water, and mounted.

Table 13: *Primary antibodies used in paper IV*

Antibody	Type	Working dilution	Source
c-Kit	rabbit polyclonal	1:150	sc-168, Santa Cruz Biotechnology, CA, U.S.A
Phospho-c-Kit (Tyr-703)	rabbit polyclonal	1:200	Zymed Laboratories, CA, USA
PDGFRβ	rabbit polyclonal	1:200	sc-19, Santa Cruz Biotechnology, CA, U.S.A

The intensity of immunoreactivities of adrenocortical cells was evaluated under light microscope and scored as *negative* (-), *weak* (+), *moderate* (++), or *strong* (+++). Each slide was reviewed by three of the authors independ-

ently, and the scores represent a concurrence. In parallel with all of the experiments, control sections were incubated with normal rabbit immunoglobulin fraction (DakoCytomation, Norden AB, Sweden) diluted to the same protein concentration as the antibodies, or without antibody (instead of primary antibody replaced by buffer) as negative controls; GISTs for c-Kit and phospho-c-Kit, carcinoid for PDGFRβ were used as positive controls.

DNA Extraction

Paper IV

Tumor DNA was isolated using the Qiagen DNeasy® tissue Kit (Qiagen, Hildesheim, Germany) as described by the manufacturer.

PCR

Paper IV

The DNA was used as template for the amplification of exon 11 of the c-Kit gene by the polymerase chain reaction (PCR). The sequences of the primers used were forward primer 5'-GATCTATTTTTCCCTTTCTC-3'; and reverse primer 5' AGCCCCTGTTTCATACTGAC-3'. The PCR amplification reaction was carried out in a volume of 25 µl containing 0.125 µl *Taq* DNA polymerase and 3 mM $MgCl_2$ (Invitrogen, Carlsbad, CA). The PCR began with an initial 2 minute melting of the strands at 95°C, then 1 minute of denaturation at 95°C, 90 seconds of annealing at 49°C and 1 minute of extension at 72°C, for a totally of 40 cycles. The final extension was performed for 7 minutes at 72°C. Thereafter, 10 µl of the amplified product was examined by DNA gel-electrophoresis (2 % agarose gels). The size of the target band was 170 bp.

Direct Genomic DNA Sequencing

Paper IV

The amplified DNA fragment of exon 11 of the c-Kit gene was purified using QIAquick PCR purification Kit as recommended by the manufacturer. The DNA fragments were then sequenced using the same primers as for the PCR reaction using Big Dye terminator (ABI Prism, USA).

Statistics

Paper I-III

All statistical analyses were carried out using *Stat View, Version 4.0*. All values were expressed as median with range and/or mean with standard error of mean (mean ± SEM). *P*-values <0.05 were considered significant. In paper I, all comparative analyses were performed with unpaired or paired *t* test statistics using one-way ANOVA. Time-activity curves were generated for VOIs and recalculated to represent SUV plotted over time using cell line charts. Univariate scattergram was used to illustrate SUV in different tissues. Power of the test regarding the sample size was determined by using ANOVA, *samsci, Stata Version 6.0* (paper II). In paper II-III, statistical analyses of cumulative DFI or survival curves were plotted by the Kaplan–Meier method: overall survival, survival from the start date of therapy, or from the date of diagnosis of advanced disease, survival for adjuvant vs. non-adjuvant, responders vs. non-responders, or stage of tumor, as needed. Significance levels were calculated by Log rank (Mantel-Cox) test.

Results

PET as a Diagnostic Method (I)

^{11}C-Metomidate PET Imaging

PET visualized all viable tumors with high tracer uptake, as shown in Figure 8. Table 14 illustrates the correlation of PET findings with CT and histopathology. Most of the PET findings were correlated with CT and/or histopathological findings. Of them, 16 high metomidate uptake lesions were correlated well with findings at CT and were verified as tumor tissue by histopathological examination.

Table 14: *Tumor lesions correlating ^{11}C-metomidate PET with corresponding CT and/or histopathology*

Tumor lesions	PET	CT	Histopathology	PET diagnosis
16 tumor lesions[a]	Positive	Positive	Viable tumors	True +ve
1 suspected lymph node metastasis	Positive	Negative	Viable lymph node metastasis	True +ve
1 local recurrence	Positive	Negative[b]	Not done	True +ve
1 suspected liver metastasis	Negative	Positive	Fat vacuolation	True -ve
3 tumor lesions[c]	Negative	Positive	Necrotic/fibrotic tumors[d]	False -ve
1 lymph node metastasis (1cm)	Negative	Positive	Not done	Uncertain

[a](3 primary tumors, 6 liver metastases, 2 lung metastases, 2 lymph node metastases and 3 local recurrences); [b]found 1 month later at follow-up CT; [c](1 suspected local recurrence, a 5-cm primary tumor and a 4-cm mesenteric metastasis); [d]found few viable tumor cells

Two additional lesions (1 lymph node metastasis, 1 local recurrence) diagnosed at PET were, however, not visualized by CT. Of these lesions, the lymph node metastasis was missed during surgery but the diagnosis could later be established at autopsy and the lesion thus represented a true positive finding at PET (Figure 8). The second of these findings (the local recurrence) identified by PET was seen 1 month later on follow-up CT and therefore most likely represented a true positive lesion too. A true negative obser-

vation was obtained at PET in a patient with a suspected liver metastasis on CT that was found to have fat vacuolation at histopathological examination of an US-guided core biopsy specimen. One PET-negative enlarged lymph node (1 cm) detected on CT was not examined during autopsy, and therefore could not be accounted for (Table 14).

Conversely, one CT detected suspected recurrence; a 5-cm primary tumor (Figure 9), and a 4-cm mesenteric metastasis were devoid of tracer uptake on PET. All these three tumors were found to be predominantly necrotic and/or fibrotic at surgery or by core biopsy and were thus judged to be false negative PET findings (Table 14). However, additional tumors in these patients were clearly depicted by PET and correlated well with findings at CT (Figure 9) and surgery.

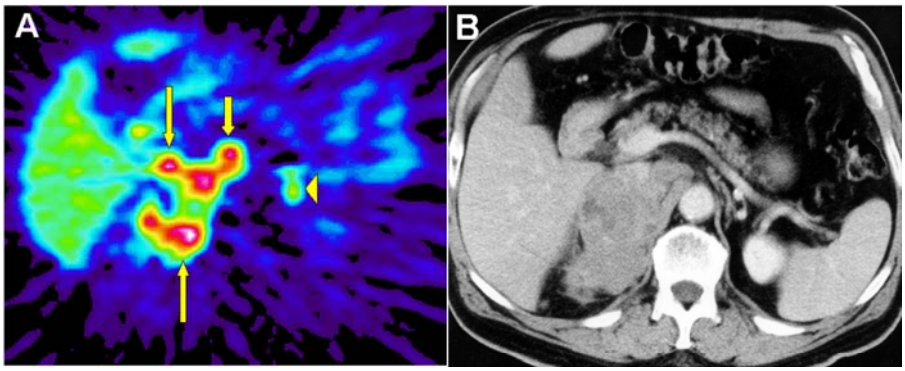

Figure 8. (A) ^{11}C-metomidate PET image; a patient with a right-sided primary tumor (*long arrows*), a lymph node metastasis (*short arrow*) and a normal left adrenal (*arrowhead*). (B) The corresponding CT image; the large heterogeneously contrasts-enhancing right adrenal tumor is seen to have a necrotic centre.

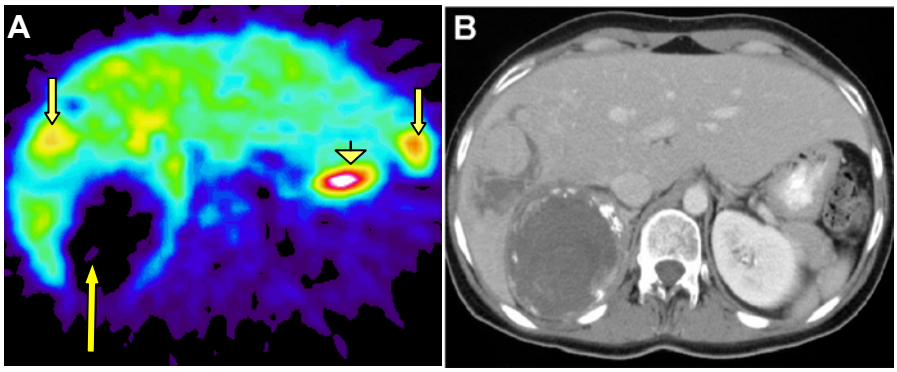

Figure *9.* (A) ^{11}C-metomidate PET image; a patient with a right-sided necrotic metomidate-negative primary tumor (*long arrow*), liver metastases (*short arrows)* and a high radioactivity concentration in gastric juice (*arrowhead*). (B) The corresponding CT image; a rim of calcifications surrounds the low attenuation adrenal tumor. Liver metastases are seen as areas of low attenuation.

PET Measurements and Pharmacokinetics

ROI_{mean} and ROI_{hs} SUVs were measured in all visible tumor lesions and normal adrenals, and ROI_{mean} SUV was calculated in the other normal tissues. Only the SUVs were calculated at 45 min post injection (tumor lesions, n = 14, normal adrenal, n = 8, and normal liver, n = 11) in 13 PET studies were included in calculations of mean SUV.

Tissue Specificity

Figure 10 shows a quantitative comparison of SUVs between tumor tissues, adrenals and liver. Tumor tissues showed a higher tracer uptake than the normal organs (adrenal, P = 0.02; liver, P = 0.005; and spleen, kidney, vertebral body, muscle, all P<0.001). The metomidate uptake was increased in primary ACC compared with normal adrenal (P = 0.05), liver and other normal tissues (all P <0.01). The liver metastases showed higher uptake than normal liver (P = 0.05). Normal adrenal had higher uptake than liver (P = 0.02) and all other normal tissues (P<0.001), while liver showed higher uptake than all other normal organs except adrenal (P<0.0001).

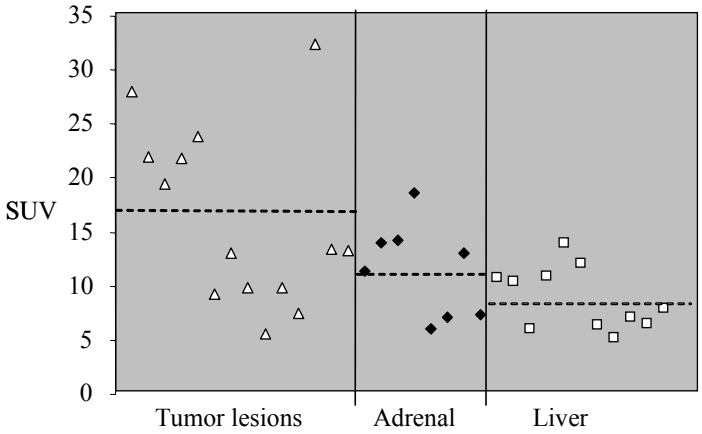

Figure 10. SUVs for ROI_{hs} in tumor tissues, adrenals and liver. Dotted lines represent the mean SUV

Treatment Effects

Figure 11 shows time-activity curves for the VOIs delineating tumor tissues and normal adrenal. All viable tumors showed higher tracer accumulation, based on the SUVs for ROI_{mean} and ROI_{hs}, in group A than in group B (P = 0.08, Figure 11A). The metomidate uptake in normal adrenal was markedly higher in group A than in group B (P = 0.03, Figure 11B). Liver also showed enhanced tracer uptake in group A compared to in group B (P = 0.01). In

other normal tissues, the tracer accumulation was similar in both groups. Table 15 demonstrates the treatment effects as well as the mean ROI_{hs} SUV of tumor lesions detected by PET in group B during monitoring.

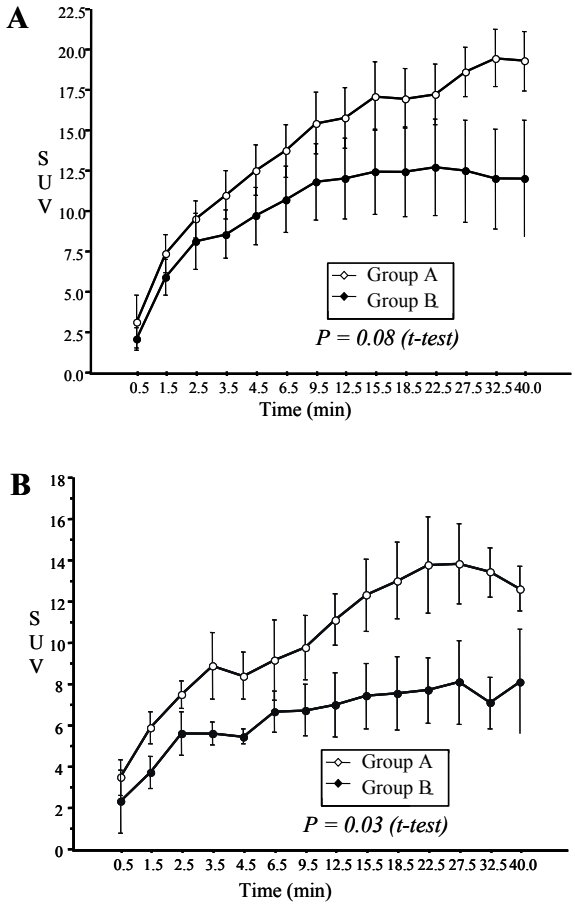

Figure 11. Uptake kinetics of ^{11}C-metomidate in the two groups: all tumor tissues (A) and normal adrenal (B). Tracer accumulation is expressed as SUV over time; the standard error of mean is indicated by the error bars

Table 15: *Treatment effects on ^{11}C-metomidate PET in group B*

Drugs	No. of Study	No. of Tumor lesions	Mean ROI_{hs} SUV
Streptozocin	3	2	13.4 ± 3.4
SO therapy	2	2	22.8 ± 5.8
5-FU	1	2	8.7 ± 0.8
5-FU, ketoconazole, metyrapone	1	2	7.7 ± 2.1

DFI

The median DFI for all radically operated patients (n = 28) was 31 months (mean, 49 ± 10 months). Patients in the adjuvant group had longer DFI (median, 49 months) than the non-adjuvant group (median, 12 months) (P = 0.02, Fig 12A). Five patients in the adjuvant group were free of disease (median, 14 years) at last follow-up without any recurrence or metastasis and one patient died of other cause after having a DFI of 11.8 years while 11 patients developed recurrence or metastases with a median DFI of 28 months. Two of latter mentioned patients who underwent a second operation due to recurrence, continued the SO treatment, and had a DFI of 36 and 50 months, respectively. The remaining patients received other regimens due to disease progression. One patient in the non-adjuvant group who was treated with SO therapy was free of disease for 36 months after a second operation, underwent a third surgical procedure for re-recurrence, and had a DFI of 12 years at last follow-up.

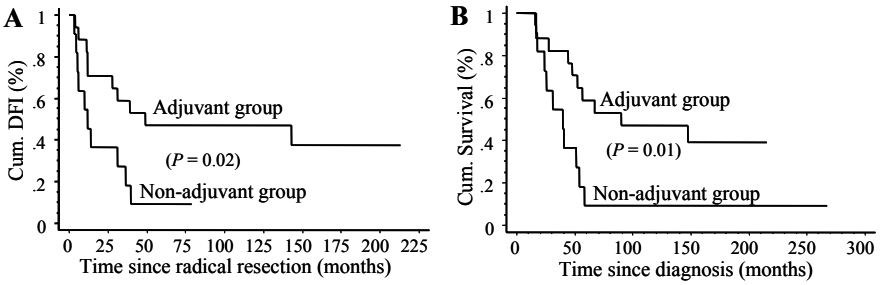

Figure 12. Adjuvant group, n = 17 vs. non-adjuvant group, n = 11. (A) Cumulative DFI rates from the date of radical resection of the primary tumor until tumor recurrence, (B) Cumulative survival rates from the date of diagnosis of ACC.

Therapeutic Responses

One patient with recurrent ACC who underwent radical surgery and did not have any detectable disease either biochemically or radiologically prior to SO therapy was excluded from the evaluation of therapeutic responses. Therapeutic responses were measured in 22 patients with advanced ACC (recurrent, n = 10, metastatic, n = 12) with measurable disease. CR or PR was observed in 36.4% of patients (median, 8 months) while 18.2% had SD (median, 2.75 months). Four patients with PR who underwent radical resection had a median DFI of 81 months. One of them had a DFI for more than 22.5 years at last follow-up and one died of breast cancer after having a DFI of 10 years. The third patient had SD with a PFI of 3 years, underwent re-operation and the fourth patient had 5 months of DFI before re-recurrence.

Side Effects

Side effects of SO therapy are shown in Table 16. In two patients, o,p'-DDD was withdrawn due to side effects such as allergic skin rash, however, continuation with streptozocin alone for 9 months could maintain the effect.

Table 16: *Side effects of SO therapy*

Toxicity	No. of Patients	
	Streptozocin	*o,p'-DDD*
Gastrointestinal	24	20
Disturbances in liver enzymes	-	25
Neurological	-	15
Renal	28	-
Others[a]	-	16

[a]autoimmune hepatitis, gynecomastia, hemorrhagic cystitis, microscopic hematuria, or adrenolytic symptoms

Survival

The overall median survival of 40 patients was 49.5 months (Figure 13A). Twenty-eight patients died because of complications of progressive disease while two died due to unrelated causes. Eleven adjuvantly-treated patients (64%) ultimately developed metastases in distant organs. Tumors of stage III-IV at initial diagnosis was found to have poor survival (P = 0.02). The overall 2-year and 5-year survival rates were 70% and 35%, respectively.

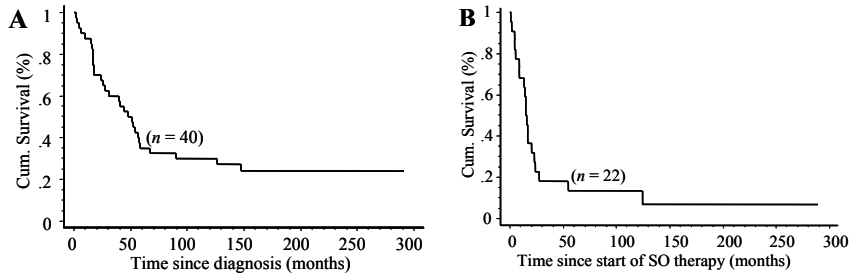

Figure 13. Cumulative overall survival curves, (A) from the date of diagnosis in all patients (n = 40) and (B) from the start of SO therapy in patients with advanced disease (n = 22)

The median survival for all radically operated patients was 53 months. The difference of survival between adjuvant and non-adjuvant group was significant (adjuvant, median, 90 months; non-adjuvant, median, 40 months; P = 0.01, Figure 12B). The median survival of 10 survivors was 15 years (free of disease, n = 8; SD, n = 1; recurrence, n = 1) at last follow-up. The patients

with measurable disease had a median survival of 18 months since diagnosis while it was 15.4 months since the start of SO therapy (Figure 13B); however, among these, the responders (CR/PR, $n = 8$) had longer survival than non-responders (SD/PD, $n = 14$) ($P = 0.05$).

OPEC Combination (III)

Therapeutic Responses

The therapeutic responses are shown in Table 17. All patients had measurable disease both radiologically and biochemically except two patients (nos. 1, 9) who underwent radical resection, however, still had pathological steroid profiles or abnormal estrogen level before OPEC was started. One patient (no. 11) who died before the defined evaluation time was excluded from the assessment. Responses were observed in ten patients. Overall PR was observed in two patients (median, 8.1 months; range, 6.75 – 9.5 months) and SD in seven patients with a median duration of 6 months (range, 3 – 11 months).

Table 17: *Therapeutic responses with OPEC regimen in eleven patients with advanced ACC*

Patient No.	**Total no. of cycles**	**Duration of treatment**	**Responses**		
			Biochemical	*Radiological*	*Overall*
		(months)			
1	6	5.5	SD (4.5)	PFI (3)	SD (3)
2	8	8.5	SD (8.5)	SD (8.25)	SD (8.25)
3	6	6	PR (6)	SD (6)	SD (6)
4	3	3.5	PR (3.75)	SD (3.5)	SD (3.5)
5	7	7.5	SD (7.5)	SD (7.5)	SD(7.5)
6	8	11	PR (11)	SD (11)	SD (11)
7	4	3	SD (5.25)	SD (3)	SD (3)
8	8	9	PR (4), CR (7.5)	PR (9.5)	PR (9.5)
9	6	8.5	PR (21)	PFI (6.75)	PR (6.75)
10	3	3	SD (3)	PD	PD
11	1	0.5	NE	NE	NE

PFI-progression-free interval, CR-complete response, PR-partial response, SD-stable disease, PD-Progressive disease, NE-not evaluable.

Side Effects

Sixty cycles of OPEC were administered to eleven patients. In 24 cycles (40%), the dosages were according to the protocol. Four patients (nos. 3, 6–

8; Table 17) were able to complete four or more cycles (median, 4 cycles, range, 4-6 cycles) without any reduction of the doses or withdrawal of drugs. Dose reduction (cisplatin and/or vincristine) was made in 25 cycles (n = 7) and withdrawal of the drug (cisplatin, vincristine, or teniposide) was required in 23 cycles (n = 8) because of adverse reactions. Table 18 shows the side effects of OPEC therapy with its grading according to NCI's common toxicity criteria.

Table 18: *OPEC toxicity*

Side effects	Grading[a]	No. of Patients	No. of Cycles (%)
Renal toxicity	Grade1	11	33 (55%)
Alopecia	Grade 2	10	39 (65%)
Peripheral neuropathy	Grade 1-2	8	24 (40%)
Leukopenia	Grade 1	4	9 (15%)
	Grade 2	1	1 (1.5%)
	Grade 3	1	1 (1.5%)
Anemia	Grade 2	3	8 (13%)
Ototoxicity	Grade 2	2	8 (13%)
Allergic reactions	Grade 2	2	2 (3%)
	Grade 3	1	1 (1.5%)
Gastrointestinal toxicity			
a. Nausea	Grade 1-2	2	5 (8%)
b. Vomiting	Grade 1	1	2 (3%)
Tiredness	Grade 1	2	3 (5%)
Dry skin	Grade 2	1	2 (3%)
Weight loss	Grade 1	1	4 (6.5%)
Fever	Grade 1	1	1 (1.5%)

[a]according to Common Toxicity Criteria of the National Cancer Institute (NCI)

Grade 1–2 toxicity occurred in all patients in 57 cycles while grade 3 toxicities were observed in two patients in only two cycles. Cisplatin had to be reduced (20–75%) in 25 cycles in seven patients whereas it was withdrawn in the last two of eight cycles in one patient. The dose of vincristine was reduced 50% in one cycle in one patient, while the drug had to be withdrawn completely in 18 cycles in six patients. Three patients exhibited allergic reactions after administration of teniposide, which was withdrawn from the remaining one to four cycles in these patients. The regimen had to be discontinued due to peripheral neuropathy and/or renal toxicity in four patients after six to eight cycles (median, 7.5 cycles) and in the remaining patients after three to eight cycles (median, 5 cycles) because of disease progression.

Survival

The overall median survival of all patients from diagnosis was 44 months (range, 4.5 – 66 months). All patients died because of metastatic diseases. Figure 14A shows the overall cumulative survival data for all patients. The estimated overall 2-year and 5-year survival rates were 82% and 9%, respectively. The median overall survival of these patients was 26 months since SO therapy (range, 3.75 – 55 months) and it was 21 months following the start of second-line OPEC treatment (range, 2 weeks – 48 months, Figure 14B). Two patients with PR had a median survival of 33.8 months. Moreover, the median survival in nine patients (nos. 1 – 9, Table 17) with PR or SD from diagnosis was 52 months (range, 26 – 66 months).

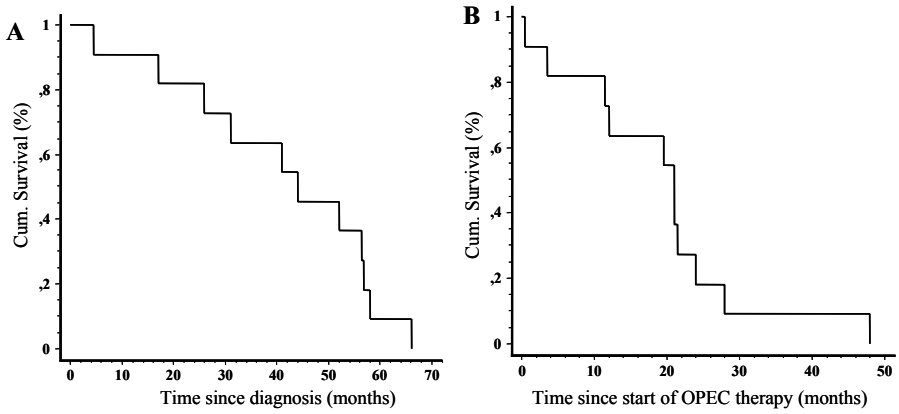

Figure 14. The overall survival curves of eleven patients with recurrent and/or metastatic adrenocortical cancer **(A)** from diagnosis (median, 44 months) and **(B)** from the start of OPEC regimen (median, 21 months).

Receptor Tyrosine Kinases in ACC (IV)

Table 19 illustrates the intensities and patterns of immunoreactivities of all 21 ACCs as evaluated. Among the three antibodies tested, PDGFRβ immunoreactivity tended to be most sensitive and more intense. All ACCs were positive for at least one antibody tested. Of them, six ACCs expressed only c-Kit and two ACCs shown PDGFRβ expression alone. Concurrently, the remaining 13 ACC were positive for two antibodies (Table 19).

Expression of c-Kit

Normal Adrenal Cortex

Diffuse and moderate immunoreactivity with c-Kit antibody was detected in normal adrenal cortex (Figure 15A). The staining pattern was both cyto-

plasmic and membranous, demonstrating unique patterns of expression in different zones of adrenal cortex, more in the outer zona glomerulosa.

ACC

Nineteen of 21 ACCs (90.5%) expressed c-Kit staining. The pattern of immunoreactivity was mainly cytoplasmic whereas in three ACCs, it was both cytoplasmic and membranous, as shown in Figure 15B. The staining intensity was weak to moderate in all c-Kit positive ACC (Table 19).

Expression of phospho-c-Kit

Normal Adrenal Cortex

Diffuse and weak cytoplasmic staining was found in both samples of normal adrenal cortex, as shown in Figure 16A.

Table 19. *The intensity and pattern of staining in 21 patients with ACC*

No. of Patients	c-Kit	Phospho-c-Kit	PDGFRβ
1	++	-	+[a]
2	+	-	+
3	++	-	++
4	++	-	+
5	+	-	+
6	+	-	-
7	+	-	-
8	-	-	+[a]
9	++	+	-
10	+	-	-
11	+	-	+[a]
12	-	-	+
13	+	-	-
14	+	-	+
15	+	-	-
16	+	-	-
17	+	-	+++[a]
18	+[a]	-	+[a]
19	+	+	+
20	+[a]	+	+[a]
21	+[a]	-	+[a]

[a]both cytoplasm and cell membrane staining; +, weak staining; ++, moderate staining; +++, strong staining

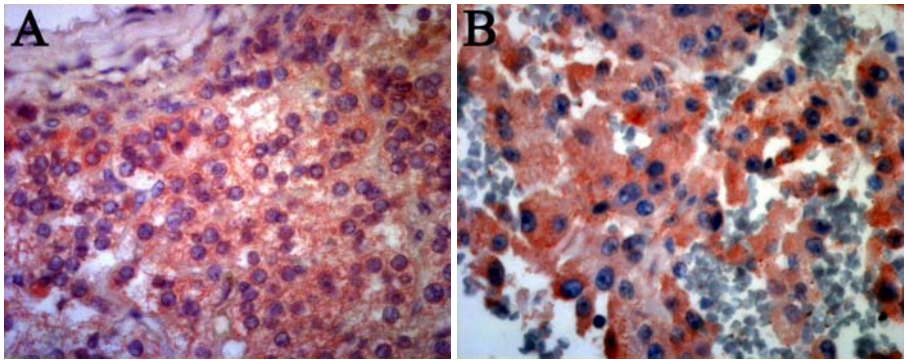

Figure 15. Immunohistochemical staining of normal adrenal cortex (**A**) and ACC (**B**) showing positive immunoreactivity in both cytoplasm and cell membrane with an antibody against c-Kit. X400.

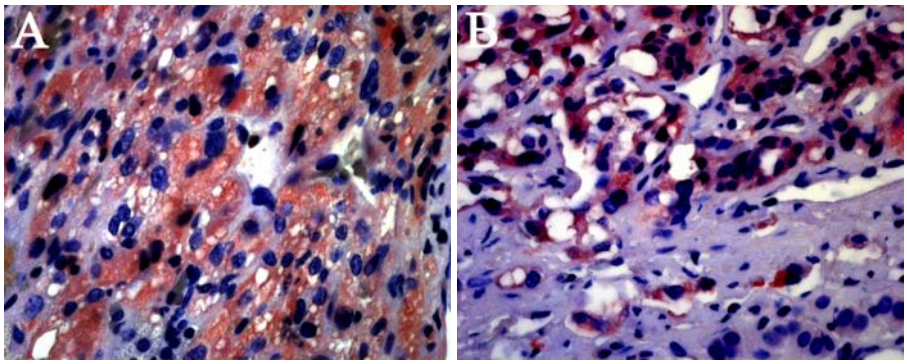

Figure 16. Immunohistochemical staining of normal adrenal cortex (**A**) and ACC (**B**) showing positive immunoreactivity in cytoplasm with an antibody against phospho-c-Kit. X400.

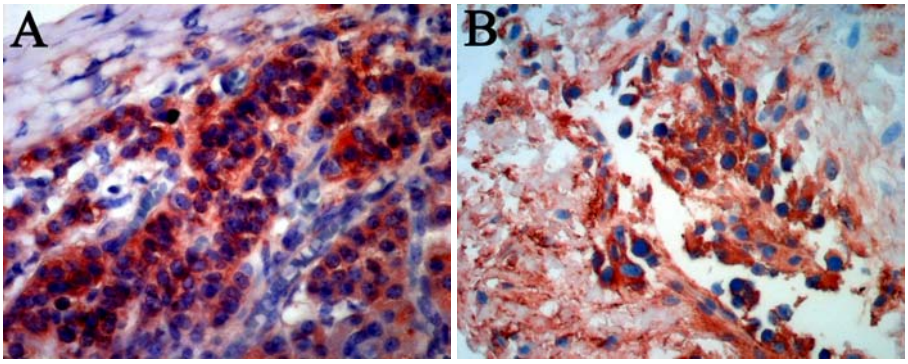

Figure 17. Immunohistochemical staining of normal adrenal cortex (A) and ACC (B) showing positive immunoreactivity in both cytoplasm and cell membrane with an antibody against PDGFRβ. X400.

ACC

Only three c-Kit positive ACCs expressed weak immunoreactivity for phospho-c-Kit antibody. The expression pattern was cytoplasmic (Figure 16B, Table 19).

Expression of PDGFRβ

Normal Adrenal Cortex

Positive immunoreactivity was observed in normal adrenal cortex. The staining pattern was diffuse, and strong. The staining was present in both cytoplasm and cell membrane demonstrating unique patterns of expression in different zones of adrenal cortex (Figure 17A).

ACC

Fourteen ACCs (66.7%) exhibited expression of PDGFRβ (Figure 17B). All of them had cytoplasmic staining while seven of them had both cytoplasmic and cell membrane staining. The intensity of staining was mostly weak except two. One of them had moderate and the other had strong immunoreactivity (Table 19).

Mutation Analysis

Since 90.5% of ACC were found to express c-Kit, we examined the tumors further to find out whether they carried any mutations. Only the most common site of mutation (exon 11) was analyzed in 13 c-Kit-positive ACC and two c-Kit-negative ACC. None of the 15 tumors exhibited a mutation within the coding region of exon 11 of the c-*Kit* gene.

Discussion

Diagnostic Approaches

An accurate diagnosis of ACC is difficult to achieve, because surgical exploration and detailed hormonal evaluation are not possible or feasible in all patients. Due to the nonspecific cancer symptoms, 70% of patients with ACC have advanced disease at the time of diagnosis and thus prognosis and survival are poor.[13,14,45] Depending on the nature of the adrenal mass, different treatment regimens are required. It is important from a clinical point of view to differentiate the benign from the malignant lesions and the non-functioning from the functioning adrenal masses. Due to the difficulties in the imaging and characterization of adrenal masses, some patients are currently undergoing possibly unnecessary surgical intervention. However, according to a standardized diagnostic program, all functioning tumors and all non-metastatic adrenal masses larger than 3 cm should be removed regardless of the patient's hormonal profile.[19]

The increase of abdominal imaging procedures, mainly by CT and US, has lead to a more frequent incidental detection of adrenal lesions, so called "incidentalomas", which need to be characterized. CT may establish the diagnosis of a benign cortical adenoma based on the combination of a low attenuation of the lesion at native scanning, indicating fat content, and contrast material accumulation following intravenous contrast enhancement. Since native scanning is not always performed routinely, a repeated CT is often required in order to carry out these attenuation measurements. MRI using dedicated signal sequences to show fat content is more sensitive than CT in this respect.

NP-59 scintigraphy may differentiate an adenoma from a possible carcinoma, however, it requires an interval of at least 3-7 days from tracer injection to imaging.[140] Kloos et al suggests FNA cytology if tumors showed discordant patterns in NP-59 scintigraphy.[16] However, the cytological appearance following FNA may not differentiate between benign and malignant adrenal cortical tumors and there is always a substantial fear of dissemination of malignant cells when the ACC capsule is broken. FNA should not be performed on any adrenal mass before excluding a possible pheochromocytoma, as the method may otherwise induce a potentially fatal crisis.

During the last few years PET has evolved as a very powerful functional imaging modality which also has been applied for imaging and characteriza-

tion of adrenal tumors,[25] but little has so far been done in ACC. ^{18}F-FDG-PET may differentiate a benign from a malignant lesion,[24] however, it does not specify whether the tumor is of adrenocortical or non-adrenocortical origin. Recently, ^{11}C-metomidate has been developed as a PET tracer.[2] Excellent clinical imaging of adrenocortical tumors with PET using ^{11}C-metomidate has been reported.[26] In a clinical trial in patients with incidentalomas using ^{11}C-metomidate PET, very high uptake has been observed in lesions of adrenocortical origin including two ACC, but not in non-adrenocortical lesions.[26]

In paper I, the imaging potential of ^{11}C-metomidate PET was evaluated in eleven patients with localized or advanced ACC. We found that all viable tumors, primary tumors as well as metastases, could be clearly visualized due to high uptake of the tracer. However, necrotic or fibrotic tumors, in which very few viable tumor cells were found at histopathology, were devoid of tracer accumulation and thus represented false negative PET findings. Medical treatment (cytostatic, steroid synthesis inhibitor) was found to decrease the tumor tracer uptake and could therefore potentially hamper lesion detection. Our study suggests that caution should be practiced when analyzing ^{11}C-metomidate PET results in patients on treatment, although the data indicate that uptake is merely diminished and not eliminated.

Almost all PET findings (18 true positive, 3 false negative and 1 true negative) could be compared to CT, and the diagnosis was established by histopathology. The metomidate uptake in the viable tumors was higher than in the normal adrenal, liver and other normal tissues. Although the metomidate uptake was high in the normal liver, the tracer accumulation was even higher in the liver metastases allowing detection of these lesions as well. ^{11}C-metomidate PET may thus be used for staging purposes and for follow-up after surgical resection to allow early diagnosis of recurrent and metastatic disease.

Nowadays the ^{11}C-metomidate PET technique enables whole-body PET examinations for staging of ACC patients (Figure 18), especially with the development of PET-CT where the PET- and CT- images may be viewed separately or as a fused image set where both the morphological information from CT and the functioning information from PET are provided. Whole-body ^{11}C-metomidate PET can reveal extraadrenal tumor sites for accurate disease staging and characterization that helps both surgeon and oncologist to select the appropriate treatment for the patient.

We do not yet have experience from ^{18}F-FDG and ^{11}C-metomidate PET in a sufficient number of patients with ACC in order to draw firm conclusions regarding their relative impact for tumor visualization in ACC. Most likely, both tracers have a role in the clinical work-up of these patients. One possible approach would be to start with ^{11}C-metomidate PET as a primary examination since it allows differentiation between adrenocortical and non-adrenocortical lesions, whereas FDG as a second examination may be per-

formed in patients with non-adrenocortical tumors to detect the primary tumor.

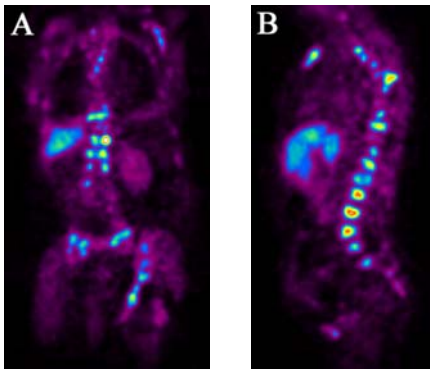

Figure 18. Whole-body ^{11}C-metomidate PET images in a patient with recurrent ACC having disseminated bone metastases: coronal view (**A**) and sagittal view (**B**).

It is important to do a full evaluation in all patients with an adrenal tumor larger than 1 cm to determine whether the tumor is functioning or nonfunctioning. Libe *et al* have recommended CT of the adrenals every 6 months for at least 2 years, thereafter yearly for all non-functioning adrenal masses to detect any increase in tumor size.[141] However, a Swedish prospective study suggests CT after 3-6 months following the diagnosis of a non-functioning adrenal incidentaloma, then yearly to detect any increase in tumor size.[19] In addition, periodic analyses of hormonal profiles should be performed after 1 year and thereafter every 1-2 years.[15]

The Role of Adjuvant Therapy

Surgery is the first-line treatment for ACC. Curative resection of ACC may succeed in stages I, II, or III and removal of all gross tumors should be attempted to reduce the tumor burden. As ACC recurs frequently and metastasize rapidly even after intented curative surgery, many reports have suggested the use of o,p'-DDD as adjuvant therapy.[36,44,46,48,49] Icard P et al have reported the use of o,p'-DDD alone as adjuvant chemotherapy after complete resection. However, they did not report any difference in outcome compared to patients who did not receive this drug,[49] indicating the lack of its efficacy as a single agent. Combinations of cytotoxic agents may therefore be considered. Cisplatin-containing combination chemotherapy has not proven to be effective in some studies.[42,67]

In a previous report from our group, o,p'-DDD combined with streptozocin has shown beneficial effects in two out of three patients with advanced ACC.[57] Based on this study,[57] we continued to treat more patients over the

past 25 years. In paper II, we observed that the patients in the adjuvant group had longer DFI ($P = 0.02$) as well as survival ($P = 0.01$) than the nonadjuvant group. These results suggested that the new combination for adjuvant therapy increased the DFI; in addition, there was a significant positive correlation between the DFI and the observed survival of patients receiving the SO regimen following surgery with curative intention.

Luton et al reported a median DFI of 12 months in radically resected patients who received mitotane therapy,[36] and it was 49 months in our study in the adjuvant group. Khorram-Manesh et al demonstrated a longer DFI of 59 months in five of 18 patients who underwent a repeat surgery after treating with adjuvant mitotane.[6] On the other hand, in our study, five patients in the adjuvant group were free of disease at last follow-up with a median DFI of 14 years.

The median survival was reported to be less than 2 years in other studies,[33,42,43,51,67,142] while it was more than 4 years in our study. Moreover, the mean overall survival in our study exceeded 6 years, which is longer than the other studies reported.[13,68] Furthermore, seven patients in the adjuvant group were still alive for 10.8 to 18 years after diagnosis (median, 14 years). The five-year survival rate was 35%; it has not exceeded 38% in other studies,[11,36,44,45,49,143] with the exception of a study reporting 58%, demonstrating the value of repeated surgery in recurrent disease.[6]

To avoid undesirable toxic side effects we used a median o,p'-DDD dose of 3 g/d that was tolerated by most patients. It has been suggested that the dose be increased to achieve a better therapeutic effect, but very often with intolerable side effects.[13,37,41] Addition of streptozocin to low dose o,p'-DDD in this study was able to demonstrate that our combination might have a better antitumor effect, reflecting a synergistic action of these drugs. We also found that in two patients in whom o,p'-DDD had to be withdrawn because of side effects, streptozocin alone for 9 months maintained the therapeutic effect, indicating that streptozocin might have effects of its own. Corticosteroid replacement in addition to the use of antiemetics was helpful to overcome the adverse events caused by the combination. Furthermore, the prolonged survival in the adjuvant group indicated that it was advantageous to continue the therapy for one year or until the disease progression.

Thus, the use of SO therapy following complete resection of the primary tumor as an adjuvant therapy appears to have a beneficial effect on DFI as well as on survival. Our findings on DFI showed a significant P value of 0.02 where the power of the test was 66%. Therefore, a prospective adjuvant study with larger patient materials in a randomized trial, probably a multicenter study, is needed to evaluate further this treatment. To achieve a significance level of 0.05 and 90% power of the test for DFI, at least 20 patients should be included in each group to do a randomized study.

Treatment Strategies in Advanced ACC

Since ACC is a highly malignant and rapidly progressing tumor, the therapeutic decision is complex and controversial especially in advanced tumor stages.[46] Despite surgery with curative intent, most of the patients die from recurrent disease. However, it has been shown that repeated surgical resection followed by chemotherapy using o,p'-DDD can prolong survival in patients with recurrent or metastatic disease.[6,142]

o,p'-DDD has been used in the treatment of recurrent or metastatic ACC as first-line medical therapy due to its adrenolytic functions. However, o,p'-DDD alone or in combination with other cytotoxic drugs has not been shown to be effective.[14] In paper II, we studied 23 patients with recurrent and/or metastatic ACC where SO therapy was given as first-line medical therapy. The patients with measurable disease had a median survival of 15 months since the start of therapy; however, the responders (CR/PR, n = 8, 36.4%) had a longer survival than nonresponders did (P = 0.05). We also found that the patients diagnosed at an advanced stage had a poor survival (P = 0.02). These observations indicate that other combinations of drugs may be required for the patients who do not respond to SO therapy as a first-line treatment.

Since cisplatin-containing chemotherapy generally is preferred as second-line medical treatment,[7,59,67] in paper III, we treated eleven recurrent and/or metastatic ACC patients with OPEC combination after the failure of SO therapy. Of them, ten patients were evaluable and PR was observed in two patients (median duration of response, 8.1 months). The median overall survival was 44 months while it was 26 months following SO therapy and 21 months after the start of OPEC, indicating the usefulness of second-line treatment in advanced ACC. The response rate did not exceed 50% in most studies when the patients with recurrent or advanced ACC were treated with o,p′-DDD and/or cisplatin-containing chemotherapy.[7,36,42,51,67] In a study using cisplatin and mitotane in combination, the median duration of response was 7.9 months and the overall response was 30%, however, the median survival was only 11.8 months.[51] Abraham et al. described a median overall survival of 13.5 months after the start of o,p′-DDD, doxorubicin, etoposide, and vincristine combination therapy as first-line medical treatment, producing objective responses in only 22%.[42] An Italian multicenter phase II trail using EDP combination therapy plus o,p'-DDD as a first-line medical treatment observed a response rate of 53.5% with a median time to progression of 24.4 months.[58] Their combination was associated with undesirable grade 3-4 toxicities,[58] although they have tried to lower the adverse events by dividing the doses in a nine-days schedule per cycle. Conversely, our regimen was restricted within four days and toxicities were mainly restricted to Grade 1-2 while grade 3 toxicities were observed only in 2 (3%) of 60 cycles. There was no grade 4 toxicity found in our study. This study also supports

the results of a recent multicenter study regarding the adverse effects of OPEC where this combination has been suggested as a well-tolerated therapy for stage 4 neuroblastoma.[144] The total dose of teniposide was less than the other study used,[58] and the drug did not need to be reduced in any patient but was withdrawn in three patients because of allergic reactions. On the other hand, cisplatin dose was higher, needed to be reduced in 41.6% of cycles, and was withdrawn in 3% of cycles due to toxicity. Since vincristine was withdrawn completely from OPEC regimen in 30% of cycles, its addition in a combination to treat an advanced ACC is disappointing. It might be possible that our patients already received streptozocin that had nephrotoxic effects and four patients had nephrectomy before starting OPEC, making it difficult to complete cycles, and thus decreased the response rate.

In this OPEC study, o,p'-DDD was continued in one of eleven patients together with OPEC therapy (no. 6, Table 17), and moreover, the median interval between discontinuation of SO therapy and the start of OPEC treatment in our study was 1.8 months. Therefore, o,p'-DDD might have a confounding effect on the therapeutic response as mitotane is characterized by a prolonged half-life. However, two patients who received SO therapy as an adjuvant treatment (nos. 1 – 2, Table 17) and OPEC therapy at relapse after 30.75 months and 16 months, respectively, had SD suggesting that this combination maintained the effects of its own.

Our study indicates that the OPEC combination may be used as second-line medical therapy after the discontinuation of SO therapy since grade 1–2 toxicities were considerable. The individual dose adjustment of cisplatin/vincristine is necessary to optimize the therapy. However, further evaluation of this regimen is also needed in larger groups of patients preferably in a randomized multicenter trial.

Only a few specialized national centers have been treating the ACC patients and so far the best results have been achieved by the combination of EDP with mitotane with a response rate of 53.5%, including individual complete responses.[58] To be able to make progress in treating advanced ACC disease, a phase III clinical trial has recently been planned on a randomized multinational basis to compare our SO combination with the combination of EDP plus mitotane regimen.

New Treatment Approaches

Molecular biology and genetics have been used to identify and characterize the components of signaling pathways of normal and neoplastic adrenocortical cells to exploit the pathophysiology of ACC.[70,145] Conventional chemotherapies have not achieved significant improvement in the survival of patient with advanced ACC.[14] Tumor cell proliferation is mediated through different signaling pathways where tyrosine kinases are playing very impor-

tant role. Therefore, well-known tyrosine kinase inhibitors are used that are specifically targeted towards small molecules whose functions are essential for maintenance of the cancer phenotype.[111,122,126] RTKs such as c-Kit and PDGFRβ are the therapeutic targets of imatinib, a relatively non-toxic tyrosine kinase inhibitor currently being used with considerable success to treat GIST and CML.[131,146] The profiling of expression of these RTKs in ACC may provide knowledge of potential therapeutic targets of this malignancy.

c-Kit expression and activation are well documented in gonads including GCTs,[103,104,108,110,113] and there is evidence that both adrenal cortex and gonads originate from a novel adreno-genital primordium and both have in common the function of synthesizing steroid hormones.[147] In paper IV, we have studied the expression of c-Kit in ACC as well as in normal adrenal cortex. c-Kit immunoreactivity was found in both the normal adrenal cortex and about 90% of ACC, suggesting that the c-Kit/SCF signaling pathway might required for normal adrenocortical cellular growth and proliferation. Most recently, Zhang PJ et al also reported the presence of c-Kit immunoreactivity, however, only in one of nine ACC they studied.[148]

To determine whether our finding of c-Kit expression in ACC had a role in downstream signal transduction in cell proliferation, we stained all samples with an antibody specific for phosphorylated c-Kit, Tyr-703. We observed positive phospho-c-Kit expression with weak intensity in only three ACCs that expressed c-Kit as well, indicating that phosphorylation of c-Kit at Tyr-703 might be lost during malignant transformation of cells. However, further confirmation of this finding is warranted and phospho-c-Kit activation through other phosphorylated tyrosine kinases must be excluded.

The most common c-Kit mutation in GISTs are located in exon 11 and less frequently in exons 9, 13 and 17.[115-117,146] However, imatinib appears to inhibit various types of activating mutant kit found in GISTs.[98] The present study indicates that although the majority of ACC expressed c-Kit, c-Kit mutations within exon 11 do not occur commonly in ACC, consistent with other findings of a low prevalence of oncogene and tumor-suppressor gene mutations in ACC.[69] However, Kit mutation has been identified in different exons, mainly in AML. Point mutations in c-Kit are most common in the phosphotransferase kinase domain in mastocytosis, myeoproliferative syndromes, AML and GCTs.[105,107,108,149] Other mutations are also identified in mast cell disease or AML in kinase domain.[149] c-Kit mutations in several exons are found in various tumors such as NK or T cell lymphoma, idiopathic myelofibrosis (MPD) or in CML.[93,109,120,149] Thus, we could not excluded the possibility of mutations in nucleotides that are close to exons that might alter c-Kit transcription, or that mutations in the other exons or regulatory regions of the Kit gene and might have a role in tumorigenesis of ACC.

The effect of imatinib in MPDs and a translocation involving PDGFRβ has been described[128] and suggested that a neoplasm that arises from an abnormality of the tyrosine kinase PDGFRβ should respond to imatinib.[136]

Moreover, PDGFRβ expression is associated with tumor neoangiogenesis that can be inhibited by imatinib.[135] In the current study, we demonstrated that 66.7% of ACC expressed PDGFRβ whereas 50% of them stained diffusely in both cytoplasm and cell membrane, opening for the exploration of its inhibitor in the treatment of ACC.

Therefore, positive immunostaining of PDGFRβ and c-Kit in ACC does not exclude the possibility of use of their inhibitors, as these are well-established molecules of cell proliferation signaling pathway. Moreover, absence of mutation in c-Kit gene at its exon 11 does not rule out the possibility of finding mutations at other sites that might be sensitive to imatinib. Therefore, further studies based on our current results, will be needed to evaluate other mutations that can be targeted by imatinib.

Conclusions

ACC is a rare disease and difficult to treat. It is very important to improve both diagnostic and therapeutic approaches for these patients in order to reduce their sufferings and thereby help to prolong their survival. ^{11}C-metomidate PET has shown its specificity to identify viable adrenal masses. In this small number of patients, ^{11}C-metomidate PET added to the radiological staging of ACC. Moreover, ^{11}C-metomidate PET allowed differentiation of a non-adrenocortical lesion from ACC. We were able to show that SO combination chemotherapy given as an adjuvant treatment can delay recurrence and thereby increase survival. Metastatic ACC showed merely a 36.4% response with this combination therapy. Therefore, new treatment alternatives are needed. We have shown that OPEC could be one of the options, as a second line treatment to optimize therapy by individual dose adjustment. Although our study with a small number of patients is not conclusive, we expect it will help to find alternative new combination chemotherapy in the future. Molecule-targeted therapy is an upcoming modern approach of treating various tumors. Although our study did not show any c-Kit mutation in exon 11, the findings regarding the expression of c-Kit, phospho-c-Kit and PDGFRβ indicate that further studies might be necessary to evaluate other changes in their regulatory pathways.

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References

1. **Williams GH, Dluhy RG**. Disorders of Adrenal Cortex. In: Braunwald E, Fauci AS, Isselbacher KJ, et al., eds. Harrison's Principles of Internal Medicine. New York, NY: The McGraw-Hill Companies, 2003: Chapter 331.
2. **Bergstrom M, Bonasera TA, Lu L, *et al.*** In vitro and in vivo primate evaluation of carbon-11-etomidate and carbon-11-metomidate as potential tracers for PET imaging of the adrenal cortex and its tumors. *J Nucl Med* 1998;39(6)**:**982-9.
3. **Macfarlane DA**. Cancer of the adrenal cortex; the natural history, prognosis and treatment in a study of fifty-five cases. *Ann R Coll Surg Engl* 1958;23(3)**:**155-86.
4. **National Cancer Institute**. Third National Cancer Survey: Incidence Data. In: US Department of Health. Education and Welfare: Public Health Service NIoH, ed. NCI Monograph. Bethesda, Maryland: DHEW Publication No. (NIH) 75-787, 1975: 41.
5. **Grondal S, Cedermark B, Eriksson B, *et al.*** Adrenocortical carcinoma. A retrospective study of a rare tumor with a poor prognosis. *Eur J Surg Oncol* 1990;16(6)**:**500-6.
6. **Khorram-Manesh A, Ahlman H, Jansson S, *et al.*** Adrenocortical carcinoma: surgery and mitotane for treatment and steroid profiles for follow-up. *World J Surg* 1998;22(6)**:**605-11; discussion 611-2.
7. **Ahlman H, Khorram-Manesh A, Jansson S, *et al.*** Cytotoxic treatment of adrenocortical carcinoma. *World J Surg* 2001;25(7)**:**927-33.
8. **Kloos RT, Gross MD, Shapiro B, *et al.*** Diagnostic dilemma of small incidentally discovered adrenal masses: role for 131I-6beta-iodomethyl-norcholesterol scintigraphy. *World J Surg* 1997;21(1)**:**36-40.
9. **Copeland PM**. The incidentally discovered adrenal mass. *Ann Intern Med* 1983;98(6)**:**940-5.
10. **Stratakis CA, Chrousos GP**. Adrenal cancer. *Endocrinol Metab Clin North Am* 2000;29(1)**:**15-25, vii-viii.
11. **Nader S, Hickey RC, Sellin RV, Samaan NA**. Adrenal cortical carcinoma. A study of 77 cases. *Cancer* 1983;52(4)**:**707-11.
12. **Ng L, Libertino JM**. Adrenocortical carcinoma: diagnosis, evaluation and treatment. *J Urol* 2003;169(1)**:**5-11.
13. **Wooten MD, King DK**. Adrenal cortical carcinoma. Epidemiology and treatment with mitotane and a review of the literature. *Cancer* 1993;72(11)**:**3145-55.

14. **Norton JA, Le HN**. Adrenal Tumors. In: DeVita VTJ, Hellman S, Rosenberg SA, eds. Cancer: Principles & Practice of Oncology. 6th ed. Philadelphia, Pa: Lippincott Williams & Wilkins, 2000: 1770-1788.
15. **Grondal S, Eriksson B, Hagenas L, *et al.*** Steroid profile in urine: a useful tool in the diagnosis and follow up of adrenocortical carcinoma. *Acta Endocrinol (Copenh)* 1990;122(5)**:**656-63.
16. **Kloos RT, Gross MD, Francis IR, *et al.*** Incidentally discovered adrenal masses. *Endocr Rev* 1995;16(4)**:**460-84.
17. **Erasmus JJ, Patz EF, Jr., McAdams HP, *et al.*** Evaluation of adrenal masses in patients with bronchogenic carcinoma using 18F-fluorodeoxyglucose positron emission tomography. *AJR Am J Roentgenol* 1997;168(5)**:**1357-60.
18. **McNicholas MM, Lee MJ, Mayo-Smith WW, *et al.*** An imaging algorithm for the differential diagnosis of adrenal adenomas and metastases. *AJR Am J Roentgenol* 1995;165(6)**:**1453-9.
19. **Bulow B, Ahren B**. Adrenal incidentaloma--experience of a standardized diagnostic programme in the Swedish prospective study. *J Intern Med* 2002;252(3)**:**239-46.
20. **Rubello D, Rufini V, Casara D, *et al.*** Clinical role of positron emission tomography (PET) in endocrine tumours. *Panminerva Med* 2002;44(3)**:**185-96.
21. **Minn H, Joensuu H, Ahonen A, Klemi P**. Fluorodeoxyglucose imaging: a method to assess the proliferative activity of human cancer in vivo. Comparison with DNA flow cytometry in head and neck tumors. *Cancer* 1988;61(9)**:**1776-81.
22. **Boland GW, Goldberg MA, Lee MJ, *et al.*** Indeterminate adrenal mass in patients with cancer: evaluation at PET with 2-[F-18]-fluoro-2-deoxy-D-glucose. *Radiology* 1995;194(1)**:**131-4.
23. **Maurea S, Mainolfi C, Bazzicalupo L, *et al.*** Imaging of adrenal tumors using FDG PET: comparison of benign and malignant lesions. *AJR Am J Roentgenol* 1999;173(1)**:**25-9.
24. **Becherer A, Vierhapper H, Potzi C, *et al.*** FDG-PET in adrenocortical carcinoma. *Cancer Biother Radiopharm* 2001;16(4)**:**289-95.
25. **Trampal C, Engler H, Juhlin C, *et al.*** Pheochromocytomas: detection with 11C hydroxyephedrine PET. *Radiology* 2004;230(2)**:**423-8.
26. **Bergstrom M, Juhlin C, Bonasera TA, *et al.*** PET imaging of adrenal cortical tumors with the 11beta-hydroxylase tracer 11C-metomidate. *J Nucl Med* 2000;41(2)**:**275-82.
27. **Bergstrom M, Sorensen J, Kahn TS, *et al.*** PET with [11C]-Metomidate for the Visualization of Adrenocortical Tumors and Discrimination from Other Lesions. *Clin Positron Imaging* 1999;2(6)**:**339.
28. **Eriksson B, Bergstrom M, Sundin A, *et al.*** The role of PET in localization of neuroendocrine and adrenocortical tumors. *Ann N Y Acad Sci* 2002;970**:**159-69.

29. **Schroder S, Niendorf A, Achilles E, *et al.*** Immunocytochemical differential diagnosis of adrenocortical neoplasms using the monoclonal antibody D11. *Virchows Arch A Pathol Anat Histopathol* 1990;417(2)**:**89-96.
30. **Vanttinen T, Liu J, Kuulasmaa T, *et al.*** Expression of activin/inhibin signaling components in the human adrenal gland and the effects of activins and inhibins on adrenocortical steroidogenesis and apoptosis. *J Endocrinol* 2003;178(3)**:**479-89.
31. **Sullivan M, Boileau M, Hodges CV**. Adrenal cortical carcinoma. *J Urol* 1978;120(6)**:**660-5.
32. **Schulick RD, Brennan MF**. Long-term survival after complete resection and repeat resection in patients with adrenocortical carcinoma. *Ann Surg Oncol* 1999;6(8)**:**719-26.
33. **Tritos NA, Cushing GW, Heatley G, Libertino JA**. Clinical features and prognostic factors associated with adrenocortical carcinoma: Lahey Clinic Medical Center experience. *Am Surg* 2000;66(1)**:**73-9.
34. **Allolio B, Hahner S, Weismann D, Fassnacht M**. Management of adrenocortical carcinoma. *Clin Endocrinol* 2004;60(3)**:**273-287.
35. **Bargenstal DM, Hertz R, Lipsett MB, Moy RH**. Chemotherapy of adrenocortical cancer withp,p´DDD. *Ann Intern Med.* 1960;53**:**672-82.
36. **Luton JP, Cerdas S, Billaud L, *et al.*** Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. *N Engl J Med* 1990;322(17)**:**1195-201.
37. **Baudin E, Pellegriti G, Bonnay M, *et al.*** Impact of monitoring plasma 1,1-dichlorodiphenildichloroethane (o,p'DDD) levels on the treatment of patients with adrenocortical carcinoma. *Cancer* 2001;92(6)**:**1385-92.
38. **Vassilopoulou-Sellin R, Guinee VF, Klein MJ, *et al.*** Impact of adjuvant mitotane on the clinical course of patients with adrenocortical cancer. *Cancer* 1993;71(10)**:**3119-23.
39. **Haak HR, Hermans J, van de Velde CJ, *et al.*** Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients. *Br J Cancer* 1994;69(5)**:**947-51.
40. **Barzon L, Fallo F, Sonino N, *et al.*** Comment--Is there a role for low doses of mitotane (o,p'-DDD) as adjuvant therapy in adrenocortical carcinoma? *J Clin Endocrinol Metab* 1999;84(4)**:**1488-9.
41. **van Slooten H, Moolenaar AJ, van Seters AP, Smeenk D**. The treatment of adrenocortical carcinoma with o,p'-DDD: prognostic implications of serum level monitoring. *Eur J Cancer Clin Oncol* 1984;20(1)**:**47-53.
42. **Abraham J, Bakke S, Rutt A, *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* 2002;94(9)**:**2333-43.

43. **Decker RA, Elson P, Hogan TF, *et al.*** Eastern Cooperative Oncology Group study 1879: mitotane and adriamycin in patients with advanced adrenocortical carcinoma. *Surgery* 1991;110(6)**:**1006-13.
44. **Pommier RF, Brennan MF**. An eleven-year experience with adrenocortical carcinoma. *Surgery* 1992;112(6)**:**963-70; discussion 970-1.
45. **Barzon L, Fallo F, Sonino N, *et al.*** Adrenocortical carcinoma: experience in 45 patients. *Oncology* 1997;54(6)**:**490-6.
46. **Schulick RD, Brennan MF**. Adrenocortical carcinoma. *World J Urol* 1999;17(1)**:**26-34.
47. **Kasperlik-Zaluska AA, Migdalska BM, Zgliczynski S, Makowska AM**. Adrenocortical carcinoma. A clinical study and treatment results of 52 patients. *Cancer* 1995;75(10)**:**2587-91.
48. **Kasperlik-Zaluska AA**. Clinical results of the use of mitotane for adrenocortical carcinoma. *Braz J Med Biol Res* 2000;33(10)**:**1191-6.
49. **Icard P, Goudet P, Charpenay C, *et al.*** Adrenocortical carcinomas: surgical trends and results of a 253-patient series from the French Association of Endocrine Surgeons study group. *World J Surg* 2001;25(7)**:**891-7.
50. **Bates SE, Shieh CY, Mickley LA, *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. *J Clin Endocrinol Metab* 1991;73(1)**:**18-29.
51. **Bukowski RM, Wolfe M, Levine HS, *et al.*** Phase II trial of mitotane and cisplatin in patients with adrenal carcinoma: a Southwest Oncology Group study. *J Clin Oncol* 1993;11(1)**:**161-5.
52. **Dogliotti L, Berruti A, Pia A, *et al.*** Cytotoxic chemotherapy for adrenocortical carcinoma. *Minerva Endocrinol* 1995;20(1)**:**105-9.
53. **Wajchenberg BL, Albergaria Pereira MA, Medonca BB, *et al.*** Adrenocortical carcinoma: clinical and laboratory observations. *Cancer* 2000;88(4)**:**711-36.
54. **Bolzan AD, Bianchi MS**. Genotoxicity of streptozotocin. *Mutat Res* 2002;512(2-3)**:**121-34.
55. **Weiss RB**. Streptozocin: a review of its pharmacology, efficacy, and toxicity. *Cancer Treat Rep* 1982;66(3)**:**427-38.
56. **Tjalve H, Wilander E, Johansson EB**. Distribution of labelled streptozotocin in mice: uptake and retention in pancreatic islets. *J Endocrinol* 1976;69(3)**:**455-6.
57. **Eriksson B, Oberg K, Curstedt T, *et al.*** Treatment of hormone-producing adrenocortical cancer with o,p'DDD and streptozocin. *Cancer* 1987;59(8)**:**1398-403.
58. **Berruti A, Terzolo M, Pia A, *et al.*** Mitotane associated with etoposide, doxorubicin, and cisplatin in the treatment of advanced adrenocortical carcinoma. Italian Group for the Study of Adrenal Cancer. *Cancer* 1998;83(10)**:**2194-200.

59. **Zidan J, Shpendler M, Robinson E**. Treatment of metastatic adrenal cortical carcinoma with etoposide (VP-16) and cisplatin after failure with o,p'DDD. Clinical case reports. *Am J Clin Oncol* 1996;19(3)**:**229-31.
60. **Berruti A, Terzolo M, Paccotti P*, et al.*** Favorable response of metastatic adrenocortical carcinoma to etoposide, adriamycin and cisplatin (EAP) chemotherapy. Report of two cases. *Tumori* 1992;78(5)**:**345-8.
61. **Haq MM, Legha SS, Samaan NA*, et al.*** Cytotoxic chemotherapy in adrenal cortical carcinoma. *Cancer Treat Rep* 1980;64(8-9)**:**909-13.
62. **Arico M, Bossi G, Livieri C*, et al.*** Partial response after intensive chemotherapy for adrenal cortical carcinoma in a child. *Med Pediatr Oncol* 1992;20(3)**:**246-8.
63. **Crock PA, Clark AC**. Combination chemotherapy for adrenal carcinoma: response in a 5 1/2- year-old male. *Med Pediatr Oncol* 1989;17(1)**:**62-5.
64. **van Slooten H, van Oosterom AT**. CAP (cyclophosphamide, doxorubicin, and cisplatin) regimen in adrenal cortical carcinoma. *Cancer Treat Rep* 1983;67(4)**:**377-9.
65. **Schlumberger M, Brugieres L, Gicquel C*, et al.*** 5-Fluorouracil, doxorubicin, and cisplatin as treatment for adrenal cortical carcinoma. *Cancer* 1991;67(12)**:**2997-3000.
66. **Bonacci R, Gigliotti A, Baudin E*, et al.*** Cytotoxic therapy with etoposide and cisplatin in advanced adrenocortical carcinoma. Reseau Comete INSERM. *Br J Cancer* 1998;78(4)**:**546-9.
67. **Williamson SK, Lew D, Miller GJ*, 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* 2000;88(5)**:**1159-65.
68. **Cohn K, Gottesman L, Brennan M**. Adrenocortical carcinoma. *Surgery* 1986;100(6)**:**1170-7.
69. **Reincke M, Beuschlein F, Slawik M, Borm K**. Molecular adrenocortical tumourigenesis. *Eur J Clin Invest* 2000;30 Suppl 3**:**63-8.
70. **Koch CA, Pacak K, Chrousos GP**. The molecular pathogenesis of hereditary and sporadic adrenocortical and adrenomedullary tumors. *J Clin Endocrinol Metab* 2002;87(12)**:**5367-84.
71. **Buchdunger E, O'Reilly T, Wood J**. Pharmacology of imatinib (STI571). *Eur J Cancer* 2002;38 Suppl 5**:**S28-36.
72. **Backlin C, Rastad J, Skogseid B*, et al.*** Immunohistochemical expression of insulin-like growth factor 1 and its receptor in normal and neoplastic human adrenal cortex. *Anticancer Res* 1995;15(6B)**:**2453-9.
73. **Edgren M, Eriksson B, Wilander E*, et al.*** Biological characteristics of adrenocortical carcinoma: a study of p53, IGF, EGF-r, Ki-67

and PCNA in 17 adrenocortical carcinomas. *Anticancer Res* 1997;17(2B)**:**1303-9.

74. **Bernini GP, Moretti A, Bonadio AG*, et al.*** Angiogenesis in human normal and pathologic adrenal cortex. *J Clin Endocrinol Metab* 2002;87(11)**:**4961-5.
75. **Skogseid B, Larsson C, Lindgren PG*, et al.*** Clinical and genetic features of adrenocortical lesions in multiple endocrine neoplasia type 1. *J Clin Endocrinol Metab* 1992;75(1)**:**76-81.
76. **Ilvesmaki V, Kahri AI, Miettinen PJ, Voutilainen R**. Insulin-like growth factors (IGFs) and their receptors in adrenal tumors: high IGF-II expression in functional adrenocortical carcinomas. *J Clin Endocrinol Metab* 1993;77(3)**:**852-8.
77. **Ohgaki H, Kleihues P, Heitz PU**. p53 mutations in sporadic adrenocortical tumors. *Int J Cancer* 1993;54(3)**:**408-10.
78. **Reincke M, Karl M, Travis WH*, et al.*** p53 mutations in human adrenocortical neoplasms: immunohistochemical and molecular studies. *J Clin Endocrinol Metab* 1994;78(3)**:**790-4.
79. **Yashiro T, Hara H, Fulton NC*, et al.*** Point mutations of ras genes in human adrenal cortical tumors: absence in adrenocortical hyperplasia. *World J Surg* 1994;18(4)**:**455-60; discussion 460-1.
80. **Reincke M, Mora P, Beuschlein F*, et al.*** Deletion of the adrenocorticotropin receptor gene in human adrenocortical tumors: implications for tumorigenesis. *J Clin Endocrinol Metab* 1997;82(9)**:**3054-8.
81. **Gicquel C, Raffin-Sanson ML, Gaston V*, 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. *J Clin Endocrinol Metab* 1997;82(8)**:**2559-65.
82. **Liu J, Kahri AI, Heikkila P, Voutilainen R**. Ribonucleic acid expression of the clustered imprinted genes, p57KIP2, insulin-like growth factor II, and H19, in adrenal tumors and cultured adrenal cells. *J Clin Endocrinol Metab* 1997;82(6)**:**1766-71.
83. **Kjellman M, Roshani L, Teh BT*, et al.*** Genotyping of adrenocortical tumors: very frequent deletions of the MEN1 locus in 11q13 and of a 1-centimorgan region in 2p16. *J Clin Endocrinol Metab* 1999;84(2)**:**730-5.
84. **Heppner C, Reincke M, Agarwal SK*, et al.*** MEN1 gene analysis in sporadic adrenocortical neoplasms. *J Clin Endocrinol Metab* 1999;84(1)**:**216-9.
85. **Pilon C, Pistorello M, Moscon A*, et al.*** Inactivation of the p16 tumor suppressor gene in adrenocortical tumors. *J Clin Endocrinol Metab* 1999;84(8)**:**2776-9.
86. **Zhao J, Speel EJ, Muletta-Feurer S*, et al.*** Analysis of genomic alterations in sporadic adrenocortical lesions. Gain of chromosome 17 is an early event in adrenocortical tumorigenesis. *Am J Pathol* 1999;155(4)**:**1039-45.

87. **Zwermann O, Beuschlein F, Mora P, *et al.*** Multiple endocrine neoplasia type 1 gene expression is normal in sporadic adrenocortical tumors. *Eur J Endocrinol* 2000;142(6)**:**689-95.
88. **Dohna M, Reincke M, Mincheva A, *et al.*** Adrenocortical carcinoma is characterized by a high frequency of chromosomal gains and high-level amplifications. *Genes Chromosomes Cancer* 2000;28(2)**:**145-52.
89. **Barzon L, Chilosi M, Fallo F, *et al.*** Molecular analysis of CDKN1C and TP53 in sporadic adrenal tumors. *Eur J Endocrinol* 2001;145(2)**:**207-12.
90. **Wachenfeld C, Beuschlein F, Zwermann O, *et al.*** Discerning malignancy in adrenocortical tumors: are molecular markers useful? *Eur J Endocrinol* 2001;145(3)**:**335-41.
91. **Gicquel C, Bertagna X, Gaston V, *et al.*** Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors. *Cancer Res* 2001;61(18)**:**6762-7.
92. **Stojadinovic A, Ghossein RA, Hoos A, *et al.*** Adrenocortical carcinoma: clinical, morphologic, and molecular characterization. *J Clin Oncol* 2002;20(4)**:**941-50.
93. **Reilly JT**. Class III receptor tyrosine kinases: role in leukaemogenesis. *Br J Haematol* 2002;116(4)**:**744-57.
94. **Ullrich A, Schlessinger J**. Signal transduction by receptors with tyrosine kinase activity. *Cell* 1990;61(2)**:**203-12.
95. **Besmer P, Murphy JE, George PC, *et al.*** A new acute transforming feline retrovirus and relationship of its oncogene v-kit with the protein kinase gene family. *Nature* 1986;320(6061)**:**415-21.
96. **Huang E, Nocka K, Beier DR, *et al.*** The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-kit receptor, the gene product of the W locus. *Cell* 1990;63(1)**:**225-33.
97. **Zhang P, Gao WY, Turner S, Ducatman BS**. Gleevec (STI-571) inhibits lung cancer cell growth (A549) and potentiates the cisplatin effect in vitro. *Mol Cancer* 2003;2(1)**:**1.
98. **Chen H, Isozaki K, Kinoshita K, *et al.*** Imatinib inhibits various types of activating mutant kit found in gastrointestinal stromal tumors. *Int J Cancer* 2003;105(1)**:**130-5.
99. **Porter AC, Vaillancourt RR**. Tyrosine kinase receptor-activated signal transduction pathways which lead to oncogenesis. *Oncogene* 1998;17(11 Reviews)**:**1343-52.
100. **Sirianni R, Carr BR, Pezzi V, Rainey WE**. A role for src tyrosine kinase in regulating adrenal aldosterone production. *J Mol Endocrinol* 2001;26(3)**:**207-15.
101. **Thommes K, Lennartsson J, Carlberg M, Ronnstrand L**. Identification of Tyr-703 and Tyr-936 as the primary association sites for Grb2 and Grb7 in the c-Kit/stem cell factor receptor. *Biochem J* 1999;341 (Pt 1)**:**211-6.
102. **Nocka K, Majumder S, Chabot B, *et al.*** Expression of c-kit gene products in known cellular targets of W mutations in normal and W

mutant mice--evidence for an impaired c-kit kinase in mutant mice. *Genes Dev* 1989;3(6)**:**816-26.

103. **Matsui Y, Zsebo KM, Hogan BL**. Embryonic expression of a haematopoietic growth factor encoded by the Sl locus and the ligand for c-kit. *Nature* 1990;347(6294)**:**667-9.
104. **Tsuura Y, Hiraki H, Watanabe K, *et al.*** Preferential localization of c-kit product in tissue mast cells, basal cells of skin, epithelial cells of breast, small cell lung carcinoma and seminoma/dysgerminoma in human: immunohistochemical study on formalin-fixed, paraffin-embedded tissues. *Virchows Arch* 1994;424(2)**:**135-41.
105. **Nagata H, Worobec AS, Oh CK, *et al.*** Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proc Natl Acad Sci U S A* 1995;92(23)**:**10560-4.
106. **Hirota S, Isozaki K, Moriyama Y, *et al.*** Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998;279(5350)**:**577-80.
107. **Gari M, Goodeve A, Wilson G, *et al.*** c-kit proto-oncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia. *Br J Haematol* 1999;105(4)**:**894-900.
108. **Tian Q, Frierson HF, Jr., Krystal GW, Moskaluk CA**. Activating c-kit gene mutations in human germ cell tumors. *Am J Pathol* 1999;154(6)**:**1643-7.
109. **Hongyo T, Li T, Syaifudin M, *et al.*** Specific c-kit mutations in sinonasal natural killer/T-cell lymphoma in China and Japan. *Cancer Res* 2000;60(9)**:**2345-7.
110. **Arber DA, Tamayo R, Weiss LM**. Paraffin section detection of the c-kit gene product (CD117) in human tissues: value in the diagnosis of mast cell disorders. *Hum Pathol* 1998;29(5)**:**498-504.
111. **Heinrich MC, Blanke CD, Druker BJ, Corless CL**. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol* 2002;20(6)**:**1692-703.
112. **Fjallskog ML, Lejonklou MH, Oberg KE, *et al.*** Expression of molecular targets for tyrosine kinase receptor antagonists in malignant endocrine pancreatic tumors. *Clin Cancer Res* 2003;9(4)**:**1469-73.
113. **Schmandt RE, Broaddus R, Lu KH, *et al.*** Expression of c-ABL, c-KIT, and platelet-derived growth factor receptor-beta in ovarian serous carcinoma and normal ovarian surface epithelium. *Cancer* 2003;98(4)**:**758-64.
114. **Fleischman RA**. From white spots to stem cells: the role of the Kit receptor in mammalian development. *Trends Genet* 1993;9(8)**:**285-90.

115. **Rubin BP, Singer S, Tsao C, *et al.*** KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res* 2001;61(22)**:**8118-21.
116. **Taniguchi M, Nishida T, Hirota S, *et al.*** Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. *Cancer Res* 1999;59(17)**:**4297-300.
117. **Hirota S, Nishida T, Isozaki K, *et al.*** Gain-of-function mutation at the extracellular domain of KIT in gastrointestinal stromal tumours. *J Pathol* 2001;193(4)**:**505-10.
118. **Miettinen M, Majidi M, Lasota J**. Pathology and diagnostic criteria of gastrointestinal stromal tumors (GISTs): a review. *Eur J Cancer* 2002;38 Suppl 5**:**S39-51.
119. **Heldin CH, Backstrom G, Ostman A, *et al.*** Binding of different dimeric forms of PDGF to human fibroblasts: evidence for two separate receptor types. *Embo J* 1988;7(5)**:**1387-93.
120. **Scheijen B, Griffin JD**. Tyrosine kinase oncogenes in normal hematopoiesis and hematological disease. *Oncogene* 2002;21(21)**:**3314-33.
121. **Li J, Kleeff J, Guo J, *et al.*** Effects of STI571 (gleevec) on pancreatic cancer cell growth. *Mol Cancer* 2003;2(1)**:**32.
122. **Manley PW, Cowan-Jacob SW, Buchdunger E, *et al.*** Imatinib: a selective tyrosine kinase inhibitor. *Eur J Cancer* 2002;38 Suppl 5**:**S19-27.
123. **Tingstrom A, Reuterdahl C, Lindahl P, *et al.*** Expression of platelet-derived growth factor-beta receptors on human fibroblasts. Regulation by recombinant platelet-derived growth factor-BB, IL-1, and tumor necrosis factor-alpha. *J Immunol* 1992;148(2)**:**546-54.
124. **Funa K, Papanicolaou V, Juhlin C, *et al.*** Expression of platelet-derived growth factor beta-receptors on stromal tissue cells in human carcinoid tumors. *Cancer Res* 1990;50(3)**:**748-53.
125. **Shamah SM, Alberta JA, Giannobile WV, *et al.*** Detection of activated platelet-derived growth factor receptors in human meningioma. *Cancer Res* 1997;57(18)**:**4141-7.
126. **Sawyers CL**. Rational therapeutic intervention in cancer: kinases as drug targets. *Curr Opin Genet Dev* 2002;12(1)**:**111-5.
127. **Druker BJ, Talpaz M, Resta DJ, *et al.*** Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001;344(14)**:**1031-7.
128. **Apperley JF, Gardembas M, Melo JV, *et al.*** Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N Engl J Med* 2002;347(7)**:**481-7.
129. **Tuveson DA, Willis NA, Jacks T, *et al.*** STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications. *Oncogene* 2001;20(36)**:**5054-8.
130. **Magnusson MK, Meade KE, Nakamura R, *et al.*** Activity of STI571 in chronic myelomonocytic leukemia with a platelet-derived

growth factor beta receptor fusion oncogene. *Blood* 2002;100(3)**:**1088-91.

131. **Gambacorti-Passerini CB, Gunby RH, Piazza R*, et al.*** Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. *Lancet Oncol* 2003;4(2)**:**75-85.
132. **Joensuu H, Fletcher C, Dimitrijevic S*, et al.*** Management of malignant gastrointestinal stromal tumours. *Lancet Oncol* 2002;3(11)**:**655-64.
133. **Demetri GD, von Mehren M, Blanke CD*, et al.*** Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002;347(7)**:**472-80.
134. **van Oosterom AT, Judson I, Verweij J*, et al.*** Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* 2001;358(9291)**:**1421-3.
135. **Dudley A, Gilbert RE, Thomas D*, et al.*** STI-571 inhibits in vitro angiogenesis. *Biochem Biophys Res Commun* 2003;310(1)**:**135-42.
136. **Schwartz R**. A molecular star in the wars against cancer. *N Engl J Med* 2002;347(7)**:**462-3.
137. **Frost MJ, Ferrao PT, Hughes TP, Ashman LK**. Juxtamembrane mutant V560GKit is more sensitive to Imatinib (STI571) compared with wild-type c-kit whereas the kinase domain mutant D816VKit is resistant. *Mol Cancer Ther* 2002;1(12)**:**1115-24.
138. **Mitterhauser M, Wadsak W, Langer O*, et al.*** Comparison of three different purification methods for the routine preparation of [11C] Metomidate. *Appl Radiat Isot* 2003;59(2-3)**:**125-8.
139. **Rota Kops E, Herzog H, Schmid A*, et al.*** Performance characteristics of an eight-ring whole body PET scanner. *J Comput Assist Tomogr* 1990;14(3)**:**437-45.
140. **Kurtaran A, Traub T, Shapiro B**. Scintigraphic imaging of the adrenal glands. *Eur J Radiol* 2002;41(2)**:**123-30.
141. **Libe R, Dall'Asta C, Barbetta L*, et al.*** Long-term follow-up study of patients with adrenal incidentalomas. *Eur J Endocrinol* 2002;147(4)**:**489-94.
142. **Jensen JC, Pass HI, Sindelar WF, Norton JA**. Recurrent or metastatic disease in select patients with adrenocortical carcinoma. Aggressive resection vs chemotherapy. *Arch Surg* 1991;126(4)**:**457-61.
143. **Zografos GC, Driscoll DL, Karakousis CP, Huben RP**. Adrenal adenocarcinoma: a review of 53 cases. *J Surg Oncol* 1994;55(3)**:**160-4.
144. **Tweddle DA, Pinkerton CR, Lewis IJ*, et al.*** OPEC/OJEC for stage 4 neuroblastoma in children over 1 year of age. *Med Pediatr Oncol.* 2001;36(1)**:**239-42.
145. **Sidhu S, Gicquel C, Bambach CP*, et al.*** Clinical and molecular aspects of adrenocortical tumourigenesis. *ANZ J Surg* 2003;73(9)**:**727-38.

146. **Heinrich MC, Corless CL, Demetri GD*, et al.*** Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 2003;21(23)**:**4342-9.
147. **Hatano O, Takakusu A, Nomura M, Morohashi K**. Identical origin of adrenal cortex and gonad revealed by expression profiles of Ad4BP/SF-1. *Genes Cells* 1996;1(7)**:**663-71.
148. **Zhang PJ, Genega EM, Tomaszewski JE*, et al.*** The role of calretinin, inhibin, melan-A, BCL-2, and C-kit in differentiating adrenal cortical and medullary tumors: an immunohistochemical study. *Mod Pathol* 2003;16(6)**:**591-7.
149. **Longley BJ, Reguera MJ, Ma Y**. Classes of c-KIT activating mutations: proposed mechanisms of action and implications for disease classification and therapy. *Leuk Res* 2001;25(7)**:**571-6.

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