New Diagnostic and Therapeutic Approaches in Adrenocortical Cancer

BY

TANWEERA S KHAN

ACTA UNIVERSITATIS UPSALIENSIS
UPPSALA 2004
Dissertation presented at Uppsala University to be publicly examined in Enghoffsalen, Entrance 50, ground floor, Uppsala, Wednesday, May 26, 2004 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

Abstract

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, o,p'-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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ISSN 0282-7476
ISBN 91-554-5954-4
urn:nbn:se:uu:diva-4243 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-4243)
To my beloved family
Munna, Israa and Shahir
List of Papers


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<td>ACC</td>
<td>Adrenocortical cancer</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AML</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>Bq</td>
<td>Becquerel</td>
</tr>
<tr>
<td>¹¹C</td>
<td>Carbon-11</td>
</tr>
<tr>
<td>CAP</td>
<td>Cyclophosphamide, Adriamycin, cisplatin</td>
</tr>
<tr>
<td>CML</td>
<td>Chronic myeloid leukemia</td>
</tr>
<tr>
<td>CR</td>
<td>Complete response</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenyltrichloroethane</td>
</tr>
<tr>
<td>DFI</td>
<td>Disease-free interval</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>Dehydroepiandrosterone sulfate</td>
</tr>
<tr>
<td>EDP</td>
<td>Etoposide, doxorubicin, cisplatin</td>
</tr>
<tr>
<td>EP</td>
<td>Etoposide, cisplatin</td>
</tr>
<tr>
<td>¹⁸F</td>
<td>Fluorine-18</td>
</tr>
<tr>
<td>FDG</td>
<td>Fluoro-deoxy-glucose</td>
</tr>
<tr>
<td>5-FU</td>
<td>5-fluorouracil</td>
</tr>
<tr>
<td>FNA</td>
<td>Fine-needle aspiration</td>
</tr>
<tr>
<td>GCT</td>
<td>Germ cell tumor</td>
</tr>
<tr>
<td>GIST</td>
<td>Gastrointestinal stromal tumor</td>
</tr>
<tr>
<td>Grb2</td>
<td>Growth factor receptor bound protein 2</td>
</tr>
<tr>
<td>Hs</td>
<td>Hot spot</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>JM</td>
<td>Juxtamembrane</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug resistance</td>
</tr>
<tr>
<td>MPD</td>
<td>Myeloproliferative disease</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NP-59</td>
<td>¹³¹I-6β-iodomethyl-19-norcholesterol</td>
</tr>
<tr>
<td>o,p'-DDD</td>
<td>1,1-dichloro-diphenyl-dichloroethylene</td>
</tr>
<tr>
<td>OPEC</td>
<td>Vincristine, cyclophosphamide, teniposide, cisplatin</td>
</tr>
<tr>
<td>PDGFR</td>
<td>Platelet-derived growth factor receptor</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PFI</td>
<td>Progression-free interval</td>
</tr>
<tr>
<td>PR</td>
<td>Partial response</td>
</tr>
<tr>
<td>PD</td>
<td>Progressive disease</td>
</tr>
<tr>
<td>RTK</td>
<td>Receptor tyrosine kinase</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SCF</td>
<td>Stem cell factor</td>
</tr>
<tr>
<td>SD</td>
<td>Stable disease</td>
</tr>
<tr>
<td>SNL</td>
<td>Sinonasal lymphoma</td>
</tr>
<tr>
<td>SO</td>
<td>Streptozocin, o,p’-DDD</td>
</tr>
<tr>
<td>SUV</td>
<td>Standardized uptake value</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>VOI</td>
<td>Volume of interest</td>
</tr>
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</table>
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 and aldosterone 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.\(^2\) 11β-hydroxylase and 21β-hydroxylase are the essential enzymes in cortisol- and aldosterone synthesis.\(^2\) The basic structure of a steroid is a cyclopentenoperhydrophenanthrene 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.\(^1,3\)
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 the United States, ACC affects two inhabitants per 1 million per year, accounting for an approximately 0.05-0.2% of all malignancies, whereas in Sweden, 16 new cases are diagnosed each year. The prevalence of non-functioning tumors has been estimated to be 0.35-5% among incidentally discovered adrenal masses, that are found on abdominal imaging, performed for reasons other than suspected adrenal disease. Autopsy studies show that approximately 5-15% of the general adult population may have adrenal incidentalomas. Approximately one per 1500 adrenal tumors is malignant.
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.\(^\text{15}\)

### 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.\(^\text{19}\)

Scintigraphy using NP-59 (\(^{131}\text{I}-6\beta\)-iodomethyl-19-norcholesterol) and \(^{75}\text{Se}-\text{selenomethyl-norcholesterol}\) 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 \textit{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).

\textbf{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. The presence of increased FDG uptake in cancer cells may be related to proliferative tissue activity and the number of viable cells. 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. Although FDG-PET appears effective in distinguishing adrenal adenoma from adrenal carcinoma, 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.\textsuperscript{25,26}

\textbf{\textsuperscript{11}C-Metomidate PET}

Recently, \textsuperscript{11}C-etomidate and \textsuperscript{11}C-metomidate, the inhibitors of 11\textbeta-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. \textsuperscript{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. \textit{In vitro} frozen section autoradiography with \textsuperscript{11}C-etomidate and \textsuperscript{11}C-metomidate showed a very high uptake in normal adrenal cortex from rat, pig and man.\textsuperscript{2} \textit{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.\textsuperscript{2} Moreover, \textsuperscript{11}C-metomidate-PET could differentiate adrenocortical from adrenomedullary tumors \textit{in vivo}.\textsuperscript{26,28}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{metomidate.png}
\caption{The chemical structure of metomidate.}
\end{figure}

\textbf{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.

\textbf{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.\textsuperscript{29,30}
Table 1: Diagnosis of ACC

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


Staging

The MacFarlane system later modified by Sullivan is the basis for the staging of ACC.\(^5,31\) 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).\(^3^4\)

Table 2: The surgical staging of ACC

<table>
<thead>
<tr>
<th>Stages</th>
<th>Extent of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tumor less than 5 cm without local invasion, nodal, or distant metastases</td>
</tr>
<tr>
<td>II</td>
<td>Same as stage I except tumor more than 5 cm</td>
</tr>
<tr>
<td>III</td>
<td>Tumor with local invasion or positive lymph nodes</td>
</tr>
<tr>
<td>IV</td>
<td>Tumor with local invasion and positive lymph nodes or distant metastases</td>
</tr>
</tbody>
</table>

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.\(^3^2,3^4\) 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.\textsuperscript{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.\textsuperscript{34} Metastases occur most often in liver, lung, lymph nodes and bone. Therefore, cytotoxic treatment is necessary for these patients.

Medical Treatment

\textbf{o,p'-DDD}

\textit{o,p'-DDD} (1,1-dichloro-diphenyl-dichloroethane, Figure 4), also called mitotane, is an isomer of the insecticide \textit{DDT},\textsuperscript{35} the only drug so far known to have adrenolytic action. It alters mitochondrial function, inhibits cholesterol side-chain cleavage, blocks 11\textbeta-hydroxylation leading to suppression of steroid production, and thus decreases plasma as well as urinary steroid levels.\textsuperscript{7} Mitotane increases extra-adrenal metabolism of cortisol leading to a reduction in urinary 17-OH-corticosteroids and increased formation of 6\textbeta-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.\textsuperscript{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.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure4.png}
\caption{The chemical structure of \textit{o,p'-DDD}}
\end{figure}

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.\textsuperscript{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 \textit{o,p'-DDD} treatment due to its toxicity in dose ranges considered therapeutic.\textsuperscript{38-40} Monitoring of serum mitotane levels may be helpful during therapy.\textsuperscript{7,37,41} However, lack of an asso-
Association has been observed between mitotane concentrations and the response that occurred before mitotane levels reached the therapeutic range. Some studies suggest that mitotane should be kept at a critical threshold plasma level to prolong survival. 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. In a series of studies, 13.5-35% of patients have shown regression of both primary tumor and metastases (Table 3), although there is no evidence that this improves survival. The role of mitotane as an adjuvant agent after surgical resection is still not known even though some experts recommend its use. Moreover, multidrug resistance (MDR) mediated by \textit{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, opening for the exploration of mitotane use in combination with chemotherapy 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.

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>No. of Patients</th>
<th>Responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Slooten et al</td>
<td>1984</td>
<td>34</td>
<td>PR, 8 (23.5%)</td>
</tr>
<tr>
<td>Luton et al</td>
<td>1990</td>
<td>59</td>
<td>PR, 8 (13.5%)</td>
</tr>
<tr>
<td>Decker et al</td>
<td>1991</td>
<td>36</td>
<td>CR, 2, PR, 6 (22%)</td>
</tr>
<tr>
<td>Pommier et al</td>
<td>1992</td>
<td>29</td>
<td>PR, 7 (24%)</td>
</tr>
<tr>
<td>Wooten and King et al</td>
<td>1992</td>
<td>551</td>
<td>CR, PR (35%)</td>
</tr>
<tr>
<td>Haak et al</td>
<td>1994</td>
<td>55</td>
<td>CR, 8, PR 7 (27%)</td>
</tr>
<tr>
<td>Barzon et al</td>
<td>1997</td>
<td>11</td>
<td>PR, 2 (18%)</td>
</tr>
<tr>
<td>Baudin et al</td>
<td>2001</td>
<td>13</td>
<td>CR, 1, PR, 3 (31%)</td>
</tr>
</tbody>
</table>

CR, complete response; PR, partial response

**Streptozocin**

Streptozocin is a member of a group of alkylating antineoplastic agents known as alkyl nitrosoureas (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. 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.\(^{35}\)

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 \(\beta\) cells. Moreover, it has been shown to concentrate in the adrenal cortex in mice.\(^{36}\) 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.\(^{37}\) By using this combination therapy, the dosages of both drugs can be decreased to more tolerable levels.\(^{27}\)

\[
\text{CH}_2\text{OH}
\]

\[
\begin{array}{c}
\text{H} \\
\text{O} \\
\text{H} \\
\text{O} \\
\text{H} \\
\text{O} \\
\text{H} \\
\text{O} \\
\text{O}
\end{array}
\]

\[
\begin{array}{c}
\text{NO} \\
\text{C} \\
\text{N} \\
\text{CH}_2
\end{array}
\]

Figure 5. The chemical structure of streptozocin

\textbf{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.\(^{31,58}\)

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.  

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Drugs</th>
<th>No. of Patients</th>
<th>Responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Slooten et al</td>
<td>1983</td>
<td>CAP</td>
<td>11</td>
<td>PR, 2 (18%)</td>
</tr>
<tr>
<td>Schlumberger et al</td>
<td>1991</td>
<td>FDP</td>
<td>13</td>
<td>CR, 1, PR, 2 (23%)</td>
</tr>
<tr>
<td>Bukowski et al</td>
<td>1993</td>
<td>P</td>
<td>37</td>
<td>CR, 1, PR, 10 (30%)</td>
</tr>
<tr>
<td>Berruti et al</td>
<td>1998</td>
<td>EDP</td>
<td>28</td>
<td>CR, 2, PR, 13 (53.5%)</td>
</tr>
<tr>
<td>Bonacci et al</td>
<td>1998</td>
<td>EP</td>
<td>18</td>
<td>CR, 3, PR, 3 (33%)</td>
</tr>
<tr>
<td>Williamson et al</td>
<td>2000</td>
<td>EP</td>
<td>45</td>
<td>PR, 5 (11%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cytotoxic drugs</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>Renal toxicity, impaired hearing, tingling and numbness (neuropathy)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>Etoposide</td>
<td>Liver toxicity, skin rash, hypersensitivity</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Skin rash, hypersensitivity</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Constipation, abdominal pain, bone pain, peripheral neuropathy</td>
</tr>
<tr>
<td>Fluorouracil</td>
<td>Mucositis, arrhythmia, cerebral ataxia</td>
</tr>
</tbody>
</table>

Symptomatic Treatment

Adrenal steroid inhibitors may be indicated for symptomatic relief of functioning and metastatic or inoperable disease, although they have no antitumor effects. 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. 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. Spiro-
nolactone, amiloride, and various antihypertensive drugs are used in hyper-
aldosteronism. Spironolactone usually corrects hypokalemia but is fre-
quently inadequate in controlling hypertension. Ketoconazole and spiro-
nolactone also have specific antiandrogenic effects. Adrenal insufficiency is
a risk when using all these agents, and replacement steroids such as hydro-
cortisone and fludrocortisone may therefore be required.68

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 exten-
sive 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 devel-
opment 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 Mo-
lecular 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 altera-
tions in oncogenes and tumor-suppressor genes in ACC in different studies.

Deregulation of signal transduction pathways are frequent during malig-
nant cellular transformation.71 Critical molecules involved in signal trans-
duction constitute proto-oncogenes; some tyrosine kinases are very potent
oncogenes that are susceptible to mutations and can activate multiple down-
stream signaling pathways thereby altering cell phenotypes. Although sev-
eral 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

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

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). 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). 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. 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. 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. 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.

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. Phosphorylated tyrosine residues act as specific binding sites for downstream signaling proteins containing Src homology 2 (SH2) domains. The Src tyrosine kinases containing SH2 domain may be involved in the adrenal cell steroidogenesis.

Moreover, the autophosphorylation 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.

c-Kit is expressed in a variety of normal human tissue such as hematopoietic stem cells, mast cells, melanocytes, primordial germ cells, Leydig cell of Sertoli, interstitial cells of Cajal, breast and ovarian epithelial cells.

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). In addition, it has been seen in some endocrine tumors, such as thyroid cancers, malignant endocrine pancreatic tumors, testicular and ovarian cancers. However, their role in the pathogenesis of these malignancies has not been defined.
**c-Kit Mutation**

Mutation in the c-Kit or SCF loci in the mouse have deleterious effects, 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. A linkage has been found between c-Kit mutations and the pathogenesis of some non-haemopoietic tumors such as GISTs and seminoma. Most of the GISTs expressing the c-Kit protein have mutations leading to constitutive or higher activation of this kinase. 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%. Mutations have also been described in exons 13 and 17, which encodes the intracellular part of the receptor.
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. PDGFRβ plays an essential role in haematopoiesis, probably in megakaryocytogenesis, in the poiesis of B and T lymphocytes, natural killer (NK) cells and other haematopoietic cells. The biological role of PDGF signaling can vary from autocrine stimulation of cell growth to more subtle paracrine interactions involving adjacent stroma and angiogenesis. PDGFRβ is normally expressed in fibroblasts, smooth muscle cells, glial cells, chondrocytes, multipotent stem cells, mast cells, myeloid progenitor cell lines, T lymphocytes and NK cells.

Receptor Tyrosine Kinase Inhibitors

Emerging new treatment modalities are targeted to specific tyrosine kinases of cancer cells in the signaling pathway of tumor cells. 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). 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. 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.

Complete or partial responses have been reported in 53-54% of GISTs with imatinib treatment, which has been linked to the presence of c-Kit mutation in exon 11. PDGFRβ expression is associated with tumor neoangiogenesis that can be inhibited by imatinib. Clinical responses in CML with PDGFRβ fusion oncogene have been observed with imatinib treatment. An effect of imatinib in MPDs with a translocation involving PDGFRβ has been described, and it is suggested that any neoplasm arising from an abnormality of PDGFRβ should respond to this drug. However, the Asp816 mutant isoform has been identified as resistant to imatinib. Imatinib has shown a synergistic cell killing effect with cisplatin on non-small cell lung cancer cells. This indicates that the utility of imatinib and/or
related compounds might extend well beyond CML, so that once again fur-
ther 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%. The stage and ag-
gressiveness 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.12 Re-
gardless 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>
<thead>
<tr>
<th>Extent of disease</th>
<th>No. of Patients</th>
<th>No. of Tumor lesions at CT</th>
<th>Patients on treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I-II</td>
<td>2(^a)</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>2(^a)</td>
<td>4(^b)</td>
<td>1(^c)</td>
</tr>
<tr>
<td>Recurrent</td>
<td>7</td>
<td>15(^b)</td>
<td>6(^c)</td>
</tr>
</tbody>
</table>

\(^{a}\)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 (\( n = 40 \))

<table>
<thead>
<tr>
<th></th>
<th>Uppsala</th>
<th>Stockholm</th>
<th>Lund</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I-II</td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>13</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Recurrent</td>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total no. of Patients</td>
<td>34</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

**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.
**11C-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 11C-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-11C] metomidate was performed as previously described (Figure 7). 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. 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 11C-metomidate, a 45-min dynamic examination sequence was started.

\[
\text{[11C]CH}_3\text{I} + \text{TBA}^+ \xrightarrow{\text{anhydrous DFM}} \text{[11C]CH}_3\text{I} + \text{TBA}^+ \xrightarrow{130^\circ C, 4 \text{ min}} \text{[11C]CH}_3\text{I} + \text{TBA}^+
\]

*Figure 7. Synthesis of 11C-metomidate from 11C-methyl iodide (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\textsubscript{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\textsubscript{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**

\(^{11}\text{C-}\)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. \(^{11}\text{C-}\)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 \(^{11}\text{C-}\)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**

<table>
<thead>
<tr>
<th>Extent of disease at study entry</th>
<th>Total no. of patients</th>
<th>Radical resection</th>
<th>Measurable disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Partial resection</td>
</tr>
<tr>
<td>Primary tumor and/or metastasis</td>
<td>29</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Recurrence and/or metastasis*</td>
<td>11</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*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>
<thead>
<tr>
<th>Extent of disease</th>
<th>No. of patients</th>
<th>Median duration of treatment (months)</th>
<th>Therapeutic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I-II</td>
<td>3</td>
<td>11</td>
<td>28 DFI</td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>3</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Recurrent</td>
<td>5(^a)</td>
<td>2.25</td>
<td>-</td>
</tr>
</tbody>
</table>

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\(^\text{®}\), Novartis Pharma AG, Basel, Switzerland), betametasone (Betapred\(^\text{™}\), 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\(^\text{®}\), Amgen Inc, CA, USA) was used if the neutrophil count was below \( 2.0 \times 10^9/L \) (normal value, \( 2.5 - 6.0 \times 10^9/L \)). Metyrapone or ketoconazole was allowed for symptomatic relief of symptoms.
Table 11: **OPEC regimen**

<table>
<thead>
<tr>
<th>Day</th>
<th>Drugs</th>
<th>Doses</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Cyclophosphamide</td>
<td>600 mg/m² mixed with distilled water (20 mg/mL), 3–5 min brief infusion</td>
<td>Sendoxan, Baxter Oncology GmbH, Halle, Germany</td>
</tr>
<tr>
<td></td>
<td>Vincristine</td>
<td>1.5 mg/m², maximum dose 2.0 mg, 3–5 min brief infusion</td>
<td>Oncovin, Eli Lilly and Company Ltd., Indianapo-</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>100 mg/m² mixed with 1000 mL NaCl over 24 hours as a continuous infusion</td>
<td>Platinol®, Bristol-Myers Squibb, Princeton, NJ, USA</td>
</tr>
<tr>
<td>Day 4</td>
<td>Teniposide (VM-26)</td>
<td>150 mg/m² mixed with NaCl, 30–60 min infusion</td>
<td>Vumon, Bristol-Myers Squibb, Princeton, NJ, USA</td>
</tr>
</tbody>
</table>

**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 $\geq 3.5 \times 10^9$/L and platelet count $\geq 150 \times 10^9$/L), normal serum creatinine level, and adequate renal function (normal urinary albumin, calculated creatinine clearance $\geq 75$ 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.\(^3\) 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 \(\geq 50\%\) 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 \(\geq 25\%\), 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.

<table>
<thead>
<tr>
<th>Grading</th>
<th>Severity of Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Mild adverse events</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Moderate adverse events</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Severe adverse events</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Life-threatening or disabling adverse events</td>
</tr>
<tr>
<td>Grade 5</td>
<td>Death related to adverse events</td>
</tr>
</tbody>
</table>

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% H₂O₂. 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 H₂O₂ 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**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Type</th>
<th>Working dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Kit</td>
<td>rabbit polyclonal</td>
<td>1:150</td>
<td>sc-168, Santa Cruz Biotechnology, CA, U.S.A</td>
</tr>
<tr>
<td>Phospho-c-Kit</td>
<td>rabbit polyclonal</td>
<td>1:200</td>
<td>Zymed Laboratories, CA, USA</td>
</tr>
<tr>
<td>(Tyr-703)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGFRβ</td>
<td>rabbit polyclonal</td>
<td>1:200</td>
<td>sc-19, Santa Cruz Biotechnology, CA, U.S.A</td>
</tr>
</tbody>
</table>

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 independent-
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’ AGCCCTGTTCATACTGAC-3’. The PCR amplification reaction was carried out in a volume of 25 μl containing 0.125 μl Taq DNA polymerase and 3 mM MgCl2 (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)**

**11C-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 11C-metomidate PET with corresponding CT and/or histopathology

<table>
<thead>
<tr>
<th>Tumor lesions</th>
<th>PET</th>
<th>CT</th>
<th>Histopathology</th>
<th>PET diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 tumor lesions a</td>
<td>Positive</td>
<td>Positive</td>
<td>Viable tumors</td>
<td>True +ve</td>
</tr>
<tr>
<td>1 suspected lymph node metastasis</td>
<td>Positive</td>
<td>Negative</td>
<td>Viable lymph node metastasis</td>
<td>True +ve</td>
</tr>
<tr>
<td>1 local recurrence</td>
<td>Positive</td>
<td>Negative</td>
<td>Not done</td>
<td>True +ve</td>
</tr>
<tr>
<td>1 suspected liver metastasis</td>
<td>Negative</td>
<td>Positive</td>
<td>Fat vacuolation</td>
<td>True -ve</td>
</tr>
<tr>
<td>3 tumor lesions c</td>
<td>Negative</td>
<td>Positive</td>
<td>Necrotic/fibrotic tumors d</td>
<td>False -ve</td>
</tr>
<tr>
<td>1 lymph node metastasis (1cm)</td>
<td>Negative</td>
<td>Positive</td>
<td>Not done</td>
<td>Uncertain</td>
</tr>
</tbody>
</table>

a(3 primary tumors, 6 liver metastases, 2 lung metastases, 2 lymph node metastases and 3 local recurrences); bfound 1 month later at follow-up CT; c(1 suspected local recurrence, a 5-cm primary tumor and a 4-cm mesenteric metastasis); dfound 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) 11C-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 contrast-enhancing right adrenal tumor is seen to have a necrotic centre.

Figure 9. (A) 11C-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\textsubscript{mean} and ROI\textsubscript{hs} SUVs were measured in all visible tumor lesions and normal adrenals, and ROI\textsubscript{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\textsubscript{hs} in tumor tissues, adrenals and liver. Dotted lines represent the mean SUV](image)

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\textsubscript{mean} and ROI\textsubscript{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.

![Graph A](image)

**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>
<thead>
<tr>
<th>Drugs</th>
<th>No. of Study</th>
<th>No. of Tumor lesions</th>
<th>Mean ROI_{hs} SUV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptozocin</td>
<td>3</td>
<td>2</td>
<td>$13.4 \pm 3.4$</td>
</tr>
<tr>
<td>SO therapy</td>
<td>2</td>
<td>2</td>
<td>$22.8 \pm 5.8$</td>
</tr>
<tr>
<td>5-FU</td>
<td>1</td>
<td>2</td>
<td>$8.7 \pm 0.8$</td>
</tr>
<tr>
<td>5-FU, ketoconazole, metyrapone</td>
<td>1</td>
<td>2</td>
<td>$7.7 \pm 2.1$</td>
</tr>
</tbody>
</table>
Streptozocin – o,p’-DDD Combination Therapy (II)

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.

![Graph A](image1.png)

**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

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Streptozocin</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>24</td>
</tr>
<tr>
<td>Disturbances in liver enzymes</td>
<td>-</td>
</tr>
<tr>
<td>Neurological</td>
<td>-</td>
</tr>
<tr>
<td>Renal</td>
<td>28</td>
</tr>
<tr>
<td>Others&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

*<sup>a</sup>*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)](image)

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

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


**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

<table>
<thead>
<tr>
<th>Side effects</th>
<th>Grading</th>
<th>No. of Patients</th>
<th>No. of Cycles (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal toxicity</td>
<td>Grade 1</td>
<td>11</td>
<td>33 (55%)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>Grade 2</td>
<td>10</td>
<td>39 (65%)</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>Grade 1-2</td>
<td>8</td>
<td>24 (40%)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>Grade 1</td>
<td>4</td>
<td>9 (15%)</td>
</tr>
<tr>
<td></td>
<td>Grade 2</td>
<td>1</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>1</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>Grade 2</td>
<td>3</td>
<td>8 (13%)</td>
</tr>
<tr>
<td>Ototoxicity</td>
<td>Grade 2</td>
<td>2</td>
<td>8 (13%)</td>
</tr>
<tr>
<td>Allergic reactions</td>
<td>Grade 2</td>
<td>2</td>
<td>2 (3%)</td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>1</td>
<td>1 (1.5%)</td>
</tr>
</tbody>
</table>

Gastrointestinal toxicity

<table>
<thead>
<tr>
<th></th>
<th>Grading</th>
<th>No. of Patients</th>
<th>No. of Cycles (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Nausea</td>
<td>Grade 1-2</td>
<td>2</td>
<td>5 (8%)</td>
</tr>
<tr>
<td>b. Vomiting</td>
<td>Grade 1</td>
<td>1</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Tiredness</td>
<td>Grade 1</td>
<td>2</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Dry skin</td>
<td>Grade 2</td>
<td>1</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>Grade 1</td>
<td>1</td>
<td>4 (6.5%)</td>
</tr>
<tr>
<td>Fever</td>
<td>Grade 1</td>
<td>1</td>
<td>1 (1.5%)</td>
</tr>
</tbody>
</table>

*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 memranous, 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>
<thead>
<tr>
<th>No. of Patients</th>
<th>c-Kit</th>
<th>Phospho-c-Kit</th>
<th>PDGFRβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>++</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
<td>−</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>++</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
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<td>−</td>
<td>+</td>
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<td>6</td>
<td>+</td>
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<td>8</td>
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<td>+a</td>
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<td>+</td>
<td>−</td>
</tr>
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<td>15</td>
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<td>−</td>
<td>−</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>−</td>
<td>+++a</td>
</tr>
<tr>
<td>18</td>
<td>+a</td>
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<td>+</td>
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<td>+a</td>
</tr>
<tr>
<td>21</td>
<td>+a</td>
<td>−</td>
<td>+a</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Diagnostic Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>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. 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. 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. Kloos et al suggests FNA cytology if tumors showed discordant patterns in NP-59 scintigraphy. 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-</td>
</tr>
</tbody>
</table>

53
tion of adrenal tumors,\textsuperscript{25} but little has so far been done in ACC. \textsuperscript{18}F-FDG-PET may differentiate a benign from a malignant lesion,\textsuperscript{24} however, it does not specify whether the tumor is of adrenocortical or non-adrenocortical origin. Recently, \textsuperscript{11}C-metomidate has been developed as a PET tracer.\textsuperscript{2} Excellent clinical imaging of adrenocortical tumors with PET using \textsuperscript{11}C-metomidate has been reported.\textsuperscript{26} In a clinical trial in patients with incidentalomas using \textsuperscript{11}C-metomidate PET, very high uptake has been observed in lesions of adrenocortical origin including two ACC, but not in non-adrenocortical lesions.\textsuperscript{26}

In paper I, the imaging potential of \textsuperscript{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 \textsuperscript{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. \textsuperscript{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 \textsuperscript{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 \textsuperscript{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 \textsuperscript{18}F-FDG and \textsuperscript{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 \textsuperscript{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. 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. In addition, periodic analyses of hormonal profiles should be performed after 1 year and thereafter every 1-2 years.

### 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 intended curative surgery, many reports have suggested the use of o,p'-DDD as adjuvant therapy. Icard *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, 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.

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. Based on this study, 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, 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. 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, 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. 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, with the exception of a study reporting 58%, demonstrating the value of repeated surgery in recurrent disease.

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. 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. 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.

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. 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, 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. 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. 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%. An Italian multicenter phase II trial 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. Their combination was associated with undesirable grade 3-4 toxicities, 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. The total dose of teniposide was less than the other study used, 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. 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. Conventional chemotherapies have not achieved significant improvement in the survival of patients with advanced ACC. 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.\textsuperscript{111,122,126} RTKs such as c-Kit and PDGFR\textbeta are the therapeutic targets of imatinib, a relatively non-toxic tyrosine kinase inhibitor currently being used with considerable success to treat GIST and CML.\textsuperscript{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,\textsuperscript{103,106,108,110,113} 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{115-117,146} However, imatinib appears to inhibit various types of activating mutant kit found in GISTs.\textsuperscript{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.\textsuperscript{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, myeloproliferative syndromes, AML and GCTs.\textsuperscript{105,107,108,149} Other mutations are also identified in mast cell disease or AML in kinase domain.\textsuperscript{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.\textsuperscript{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 PDGFR\textbeta has been described\textsuperscript{128} and suggested that a neoplasm that arises from an abnormality of the tyrosine kinase PDGFR\textbeta should respond to imatinib.\textsuperscript{126}
Moreover, PDGFRβ expression is associated with tumor neoangiogenesis that can be inhibited by imatinib. 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.
Acknowledgments

This study was carried out in the Department of Medical Sciences, Uppsala University, Department of Endocrine Oncology and Uppsala University PET center, University Hospital.

I am very grateful to everyone who has contributed directly or indirectly to the accomplishment of this thesis. I would like to express my sincere gratitude to

Prof. Barbro Eriksson, Head of the Department of Endocrine Oncology, my supervisor, who originally suggested this subject; she has played an important role during the course of this study. Her ideas, experience and interests, as well as perspective of the field of endocrine oncology have always impressed me. I really appreciate your support, guidance and willingness to discuss at any time, as well as giving me the privilege to conduct my research in the direction I preferred.

Prof. Mats Bergström, my co-supervisor, the founder of carbon-11 Metomidate PET imaging, who introduced me to the field of PET; his experience and wide knowledge regarding this area have been very valuable. Thanks for your fruitful discussions and suggestions during the period I worked at preclinical PET lab.

Prof. Kjell Öberg, Dean of the Faculty of Medicine, our group leader, who first got the idea to use the streptozocin and o,p′-DDD combination therapy, whom I know since 1995 when I first came to Uppsala as Hassan’s wife. Thanks a lot for giving me the opportunity to join your group, for your consultation and valuable suggestions, and for your constant support and warm sympathy to our family.

Prof. Bengt Långström, for providing me the opportunity to carry out part of this study at PET centre

Associate Prof. Anders Sundin, with whom I spent hours looking through the CT and PET scans, who helped me to improve the manuscripts as well as this thesis remarkably with his constructive criticism, and promptly replied whenever I needed it. This has been very valuable. I am proud of all your cooperation throughout the journey to reach my PhD.

Dr. Hassan Imam, for his tireless guidance, experience and insights in the field of scientific research have been valuable. Thanks for having a great role on this work by sharing your knowledge and experiences whenever I
asked for it, especially for your patience to revising all of my papers as well as this thesis repeatedly.

_Associate Prof. Eva T Janson_, for being a nice examiner in my half-time control, for your delicious dishes and for new ideas in the Monday meetings

_Prof. Britt Skogseid_, for conducting the SO study in a randomized international trial and fruitful discussions regarding the study have been influential. I am proud to see the future of my study!

_Prof. Erik Wilander_, for spending hours by looking through the slides together and for your expert comments that have been helpful, _Associate Prof. Anders Gobl_, who introduced me to the field of Molecular Biology, for teaching me practical things in this area, _Prof. Claes Juhlin_, co-author, for excellent co-operation

_Dr. Steven Lucas_, for linguistic correction of this thesis

_Prof. Otto Peder Rorstad_, Dept. of Medicine, University of Calgary, Calgary, Alberta, Canada, for his valuable discussion and linguistic correction of the summary for my half-time control.

_Assistant Prof. Ranjula Bali Swain_, Dept. of Economics, Uppsala University, for helping me to answer the statistical questions from the reviewers for paper II, and for being a nice friend throughout these years.

_Annette Hägg_ and _Anna Foyer_, for their outstanding help during last years

I would like to show my gratitude to all staff and colleagues at the Department of Endocrine Oncology, especially to

_Dan, Staffan, Marie-Louise, Håkan_, for providing me a nice environment during my work at the Clinic; _Gertrud_, for helping me to find the patients’ files; and special thanks to _Monika_, for letting me do my prayers in her room during my work at the Clinic.

My sincere gratitude to my past and present colleagues at the Lab in Clinical Research Section II, for providing the environment for research, especially to

_Åsa_ and _Rajni_, for helping me with immunohistochemistry, _Yinghua_, for helping me with the task from ‘Molekylärmedicinsk metodik’ course, _Jan_, for expert comments in this thesis and for the discussion regarding receptor tyrosine kinases.

_Associate Prof. Mats Stridsberg_, for having interest in Bangladeshi foods, _Minghui_, for having the patience to hear my daily life, _Apostolos_, for sharing the moments to discuss our ongoing research, _Malin_ for having interest in my research field, _Margareta H., Janet, Juan, Lijun, Wang Shu, Margareta E., Birgitta and Mona_, for always exchanging greetings and asking about my family.
I would like to thank my past and present colleagues at preclinical PET Lab, for keeping the environment lively for research work, especially to 

Elisabeth, for introducing me PET-lab, Ulrika, for teaching me the $^{68}$Ga-Octreotide synthesis and for helping me to avoid the direct contact to radio-tracers throughout my second pregnancy, Gabor, for teaching me in vitro autoradiography, Helena, for teaching me receptor-binding assay, Obaidur, for helping me during ‘Nuclide techniques and the tracer concept’ course, Li, Ray, Sergio, Kayo, Azita, Pasha, Padideh, Irina, for always being friendly during my work at preclinical PET lab and for sharing my joys when I got Shahir, I will never forget your spirit and support.

My special thanks to all of my relatives and friends especially to

Nishi, the only friend from my school and college life, for staying near to me, I am very glad to find you in Sweden. Sangita newly found relative, Juny, Popy, Shetu, Simin, Shewly, Shelly and Rozy, for being friends as well as providing a social and cultural life in Sweden. My friends in Bangladesh especially Lubna, and Mafruha, for being close friends; my parents-in-law, sisters-in-law and brothers-in-law, specially Jemmy and Akmal, Ratna and Bulbul as well as their kids, for staying close to my family, for their support, care and love of my family.

My sincere gratitude to my father Late Suleman Khan, who always wished me to reach this point and whose utmost encouragement inspired me to travel all the way, my mother Arefa Billah, my brothers Sayeed and Saleem, my sisters Shaheeda, Shireena, Tahseena and Tanyeema, for their never ending encouragement and support.

Finally, the special loving thanks go to my husband Munna, my daughter Israa (had a great role for setting up the cover page in this thesis) and my son Shahir, for their love, support, sacrifice, encouragement and understanding during these years. It had not been so worthwhile if I did not have you to share these years.
References


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.


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