

Management of Adrenocortical Carcinoma

**Bruno Allolio*, Stefanie Hahner, Dirk Weismann,
Martin Fassnacht***

Endocrinology and Diabetes Unit, Dept of Medicine, University of Wuerzburg,
Germany

* supported by Deutsche Forschungsgemeinschaft (Al 203/7-3,4)

Correspondence:

Prof. Bruno Allolio, M.D.

Endocrine and Diabetes Unit; Dept. of Medicine

University of Wuerzburg

Josef-Schneider-Str. 2

97080 Wuerzburg

Germany

Tel.: +49-931-201-36788

Fax: +49-931-20136283

allolio_b@medizin.uni-wuerzburg.de

Abstract

Adrenocortical carcinoma (ACC) is a rare neoplasm with poor prognosis. Patients present with signs of steroid hormone excess (e.g. Cushing's syndrome, virilisation) or an abdominal mass. Tumour size at presentation (mean diameter at diagnosis >10 cm) is the most important indicator of malignancy. In addition, computed tomography typically demonstrates an inhomogeneous adrenal lesion with irregular margins and variable enhancement of solid components after iv contrast media. Magnetic resonance imaging is equally effective as CT and is particularly helpful to visualise invasion into large vessels. Complete tumour removal (R₀ resection) offers by far the best chance for long-term survival and, therefore, surgery is the treatment of choice in stage I-III ACC. Despite tumour resection for cure most patients will eventually develop local recurrence or distant metastases. Thus adjuvant treatment options need to be evaluated in high risk patients (e.g. radiation therapy of the tumour bed and / or chemotherapy). In tumour recurrence re-operation should always be considered. In metastatic disease (stage IV ACC) not amenable to surgery mitotane (o,p'DDD) remains the first line therapy. Drug monitoring is needed for effective treatment aiming at concentrations between 14-20 mg/L. Patients not responding to mitotane may benefit from cytotoxic chemotherapy (23% partial remissions, 4% complete remissions). Only large prospective multi-centre trials comparing different treatment options will allow to make systematic progress in the management of ACC.

Adrenocortical carcinoma (ACC) is a rare and highly malignant neoplasm with poor prognosis. The incidence is approximately 1-2 per million population per year (National Cancer Institute 1975, Dackiw, *et al* 2001) leading to 0.2 % of cancer deaths according to data from the United States (Wajchenberg, *et al* 2000). The age distribution is bimodal with a first peak in childhood and a higher second peak in the 4th to 5th decade (Luton, *et al* 1990, Wooten and King 1993, Wajchenberg, *et al* 2000). In a recent review of 920 adult patients with ACC the mean age at diagnoses was around 45 years (Wajchenberg, *et al* 2000). In a meta-analysis by Wooten and King (Wooten and King 1993) including more than 1800 cases ACC was somewhat more frequent in women (59 %) than in men. Epidemiologic studies have suggested an increased risk of ACC with the use of oral contraceptives and smoking (Hsing, *et al* 1996). In addition, adrenal tumours including ACC have been associated with poorly treated congenital adrenal hyperplasia (Allolio 2001).

Pathogenesis

The molecular pathogenesis of adrenal tumours has been the topic of recent reviews (Reincke 1998, Kirschner 2002, Kjellman, *et al* 2001, Koch, *et al* 2002). Despite significant advances in the understanding of adrenal tumour development the underlying sequence of events remains to be elucidated. Some insight comes from hereditary tumour syndromes associated with the development of adrenocortical cancer. In the Li-Fraumeni syndrome the frequency of ACC is about 1 % (Sameshima, *et al* 1992). Affected patients have germline mutations of the p53 tumour suppressor gene located at the 17p13 locus (Malkin, *et al* 1990, Wagner, *et al* 1994) and may develop a variety of malignancies (e.g. breast cancer, sarcomas). In the tumour the second p53 allele is inactivated by a somatic mutation leading to complete loss of wild-type p53 activity (McNicol, *et al* 1997a, McNicol, *et al* 1997b). An exciting recent

observation is the demonstration of a specific germline point mutation of p53 encoding an R337H amino acid substitution in children with ACC from Brazil (Ribeiro, *et al* 2001). In contrast to patients with the typical Li-Fraumeni syndrome only ACC has been associated with this mutation indicating a tissue-specific effect. This is the first demonstration of a germline p53 mutation, which contributes to cancer in a tissue-specific manner (DiGiammarino, *et al* 2002). Intriguingly, the mutated R337H p53 protein functioned normally in some in vitro studies. However, it was found that the function changed in a pH-sensitive and temperature-dependent-manner (Lee, *et al* 2003). How these physico-chemical abnormalities predispose to ACC remains to be elucidated (Hainaut 2002). Mutations in the p53 gene have also been demonstrated in a large percentage of patients with sporadic ACC (Reincke, *et al* 1994, Wachenfeld, *et al* 2001, Barzon, *et al* 2001, Gicquel, *et al* 2001) and accumulation of abnormal p53 protein correlates with a more aggressive clinical behaviour in ACC (Sredni, *et al* 2003).

Another hereditary syndrome associated with ACC is the Beckwith-Wiedeman syndrome (BWS). BWS has been mapped to the 11p15.5 region and is associated also with other malignancies (e.g. Wilm's tumour, hepatoblastoma). The 11p15.5. locus includes the IGF-II, H19, and p57/Kip2 genes which show functional imprinting. Whereas normally the paternal IGF-II allele is transcribed, H19 and the p57 tumour suppressor gene are expressed by the maternal allele (Koch, *et al* 2002). Uniparental paternal isodisomy for this locus associated with IGF-II overexpression has been found in BWS. Similarly, in sporadic ACC rearrangement at the 11p15 locus with overexpression of IGF-II is frequently observed caused either by duplications of the paternal 11p15 allele or by loss of the maternal allele containing the H19 gene, which is involved in IGF-II suppression (Gicquel, *et al* 1994, Gicquel, *et al* 1997, Hao, *et al* 1993, Ilvesmaki, *et al* 1993, Leighton, *et al* 1995, Weber, *et al* 2000). Increased expression of IGF-II was recently also demonstrated by Giordano *et al.* in 90 % of sporadic ACCs using DNA microarray analysis (Giordano, *et al* 2003). The magnitude of

increased IGF-II expression and the lack of other signal transduction related changes observed in this transcriptional survey suggest that IGF-II overexpression is of particular importance for ACC progression and, therefore, may be a promising therapeutic target. However, this new technical approach not only confirmed the role of IGF-II but also identified some other genes that might be relevant to ACC pathogenesis (e.g. cyclins).

Of interest is also the role of pro-opiomelanocortin (POMC) and its receptors in adrenal tumourigenesis, as the trophic function of POMC for the adrenals has been well documented. Sequencing of the ACTH receptor (ACTH-R) gene in adrenal tumours did not reveal constitutive activating mutations (Latronico, *et al* 1995). Tumours rather demonstrated loss of heterozygosity of the ACTH-R with reduced expression of ACTH-R mRNA, in particular in some malignant adrenal tumours (Beuschlein, *et al* 2001, Reincke, *et al* 1997a, Reincke, *et al* 1997b). These findings support the concept derived from in vitro studies that ACTH acts as a differentiation factor at the adrenal level. Accordingly, it was recently demonstrated that ACTH inhibits growth of Y1 ACC in mice in vivo (Zwermann, *et al* 2003). On the other hand, it has been reported that peptides derived from the N-terminus of POMC play a role for adrenal growth (Estivariz, *et al* 1982, Lowry, *et al* 1983). These peptides may be activated at the adrenal level by the “adrenal secretory protease” (AsP) (Bicknell, *et al* 2001). This view is supported by in vitro data demonstrating a growth stimulating effect of N-POMC on adrenocortical cancer cells in vitro via an unknown receptor (Fassnacht, *et al* 2003). Obviously these findings raise the question whether suppression of POMC (by exogenous glucocorticoids) may play a role in the management of some patients with ACC.

Chromosomal instability has been observed in both benign and malignant adrenal tumours indicating defects in the mitogenic machinery (Dohna, *et al* 2000). Accordingly, the number of centrosomes is increased (Kjellman, *et al* 2001). A transition to an aneuploid state has been described in tumours larger than 4 cm, and the genetic alterations detected by comparative

genomic hybridisation correlate with tumour size and malignancy with frequent gene amplifications (Kjellman, *et al* 1996, Dohna, *et al* 2000, Kjellman, *et al* 2001).

Clinical presentation

Functioning ACC (approximately 60 % of cases) often presents with signs and symptoms of adrenal steroid hormone excess, although hypersecretion of androgens in males or estrogens in females may go unnoticed. The same holds true for hypersecretion of steroid precursors (e.g. 17- α -hydroxy-progesterone; deoxycorticosterone) which is frequently detectable in seemingly non-functioning tumours. Cushing's syndrome (CS) with or without virilization is the most frequent presentation in functioning ACC (Didolkar, *et al* 1981, Favia, *et al* 2001, Icard, *et al* 2001, Pommier and Brennan 1992, Wajchenberg, *et al* 2000). Rapid development of CS with skin atrophy, muscle weakness, hyperglycaemia, hypertension, and psychiatric symptoms is common. Androgen excess in women leads to hirsutism, male pattern baldness, deepening voice, breast atrophy and menstrual abnormalities. The anabolic action of concomitantly secreted androgens may counteract the glucocorticoid-induced muscle atrophy. Estrogen secreting ACCs in males usually present with gynaecomastia and testicular atrophy, in women breast tenderness and irregular menstrual bleeding may occur (Scheingart and Homan 2001). Aldosterone hypersecretion in ACC is rare and may lead to hypokalaemia and hypertension which may also occur in severe adrenal Cushing's syndrome with massive hypercortisolaemia leading to incomplete renal inactivation by 11 β -dehydrogenase type II and hence mineralocorticoid excess (Stewart, *et al* 1995). In children adrenal sex steroid excess is common and may lead to virilisation and precocious pseudo-puberty (Ribeiro, *et al* 2000, Wajchenberg, *et al* 2000). Due to low efficiency in steroid production clinical abnormalities may be subtle in a significant percentage of patients.

Patients with a non-functioning ACC usually present with symptoms related to the local mass effect like abdominal fullness, pain, indigestion, nausea and vomiting (Dackiw, *et al* 2001, Pommier and Brennan 1992, Samaan and Hickey 1987). In a minority of patients also weight loss, low grade fever, and weakness may occur (Bodie, *et al* 1989, Kasperlik-Zaluska, *et al* 1998). Due to the large tumour size at diagnosis an abdominal mass may be palpable in a significant percentage of patients. The initial manifestation may also be related to metastatic disease (e.g. pathologic fracture, bone pain). A substantial and apparently increasing fraction of patients is diagnosed incidentally by abdominal imaging (Icard, *et al* 1992, Kasperlik-Zaluska, *et al* 1998).

A peculiar finding in ACC is in our experience a low prevalence of non-specific tumour symptoms (e.g. anorexia, weight loss) even in the presence of a large tumour burden. This absence of a systemic inflammatory response may contribute to the late diagnosis in many patients with ACC.

Diagnosis

Hormonal Evaluation

Hormonal evaluation is mandatory in all patients with suspected ACC (table 1) and may be associated with improved survival (Icard, *et al* 1992). Unfortunately hormone concentrations are usually of limited help in predicting malignancy. However, in the presence of an adrenal lesion elevated serum dehydroepiandrosterone sulphate (DHEAS) levels suggest an ACC, as benign adrenocortical tumours often exhibit low DHEAS concentrations (Flecchia, *et al* 1995, Osella, *et al* 1994, Terzolo, *et al* 2000a). In addition, elevated serum 17 β -oestradiol is a rare but rather typical marker of oestrogen secreting ACC in men. Accordingly, in male patients with an adrenal tumour and elevated serum 17 β -oestradiol an ACC should be assumed until

proven otherwise (Gabrilove, *et al* 1965). As cortisol hypersecretion is the most common hormone excess in ACC, evaluation for adrenal CS is essential including an overnight dexamethasone suppression test, assessment of urinary free cortisol excretion and determination of plasma ACTH. In case of subclinical CS a corticotropin releasing hormone test will predict the risk of adrenal insufficiency after complete tumour removal (Reincke, *et al* 1992).

Aldosterone-secreting ACCs are rare and usually present with hypokalaemia and very high serum aldosterone concentration. Aldosterone-secreting tumours smaller than 4 cm with only moderately elevated aldosterone levels are suggestive of a benign adenoma.

At presentation additional steroids should be measured, as they may also serve as tumour markers during follow-up: urinary excretion of 17-ketosteroids, 17- α -hydroxyprogesterone, 11-deoxycortisol, deoxycorticosterone, and in women with virilisation also androstenedione and testosterone. In advanced ACC serum LDH may serve as a marker of disease progression. Measurement of urinary catecholamine excretion or plasma metanephrines is mandatory to exclude pheochromocytoma prior surgery.

Imaging

The size of the adrenal mass, as measured by computed tomography (CT) or magnetic resonance imaging (MRI) remains the single best indicator of malignancy. In a recent series from France (Icard, *et al* 2001) mean tumour size at diagnosis was 12.0 ± 6.0 cm (n=223) and mean tumour weight was 689 ± 822 g (n=202). Similar results have been found in earlier series (Didolkar, *et al* 1981) and were confirmed recently (Stojadinovic, *et al* 2002, Vassilopoulou-Sellin and Schultz 2001). The likelihood of ACC increases to 35 – 98 % in patients with an adrenal mass > 6 cm (Ross and Aron 1990). However, in recent years additional imaging

features (e.g. attenuation coefficients) have been used to discern malignancy in adrenal tumours.

A thin-collimation CT is the imaging method of choice for adrenal masses and for differentiation of benign from malignant lesions. Nonadenomatous lesions typically have higher CT density values due to their lower lipid content (Korobkin, *et al* 1996). ACCs are typically inhomogeneous with irregular margins and irregular enhancement of solid components after iv contrast media. Sometimes calcifications are visible. Dependent on the threshold value of the Hounsfield units sensitivity and specificity for characterisation of an adrenal lesion as a benign adenoma in unenhanced CT ranged from 47 % and 100 %, at a threshold of 2 HU, to 88 % and 84 %, respectively, at a threshold of 20 HU (Boland, *et al* 1998). Recent studies suggest that delayed contrast-enhanced CT scans can be used to further characterise lesions with higher HU in unenhanced scans. As early as 3 min and up to 60 min after contrast enhancement, the mean CT attenuation value of adenomas is substantially lower than that of non-adenomas. Therefore adrenal lesions with an attenuation value of more than 10 HU in unenhanced CT or an enhancement washout of less than 50 % and a delayed attenuation of more than 35 HU (on 10-15 min delayed enhanced CT) are suspicious for malignancy (Caoili, *et al* 2000, Korobkin, *et al* 1998, Lee, *et al* 1991, Pena, *et al* 2000, Szolar and Kammerhuber 1998). Local invasion or tumour extension into inferior vena cava as well as lymph node or other metastasis (lung and liver) is often found in advanced ACC.

MRI is equally effective as CT in distinguishing malignant from benign lesions (Outwater, *et al* 1996, NIH state of science statement 2002). With the advent of dynamic gadolinium enhanced- and chemical shift-technique in the last decade, MR characterisation of adrenal masses has improved significantly. ACCs are typically isointense to liver on T1 and show intermediate to increased intensity on T2 (Fig. 1). The enhancement after gadolinium is distinct and the washout is usually slow. However, most MRI studies for adrenal lesions focused on differentiating adenoma from metastases, rather than from ACC. In these studies

the sensitivity of MR imaging for differentiation of benign and malignant adrenal masses ranged between 81 % and 89 % with specificity between 92 and 99 % (Bilbey, *et al* 1995, Heinz-Peer, *et al* 1999, Honigschnabl, *et al* 2002, Korobkin, *et al* 1995). Whether chemical-shift MRI can reliably differentiate adenoma from carcinoma has not yet been established (Dunnick and Korobkin 2002). MRI is superior to CT in detecting tumour extension into the inferior vena cava (Goldfarb, *et al* 1990).

Adrenal scintigraphy (NP-59) is not widely available, time consuming (3-5 days), and the diagnostic value beyond CT and MRI is controversial. Therefore, we do not recommend scintigraphy in patients with presumed ACC. In contrast, ¹⁸F-FDG-PET has demonstrated good performance in differentiating malignant from benign adrenal lesion in retrospective studies (Becherer, *et al* 2001, Boland, *et al* 1995, Maurea, *et al* 1999, Yun, *et al* 2001). Moreover, FDG-PET can be used to detect metastatic disease. Prospective studies are needed to further validate the role of FDG-PET. ¹¹C-metomidate PET has been successfully used for imaging of non-necrotic ACC (Khan, *et al* 2003). Major disadvantages are the limited availability and high costs of PET methods.

Fine-needle aspiration (FNA)/ cut biopsy is not recommended to establish the diagnosis of ACC due to the risk of complications (up to 12 %) (Kloos, *et al* 1995), in particular needle tract metastases, (Mody, *et al* 1995) and its controversial diagnostic value. However, in a recent prospective study adrenal cut biopsy was investigated in an “ex-vivo” approach in 220 consecutive adrenal lesions after surgical removal (Saeger, *et al* 2003). The overall sensitivity and specificity were 94.6 and 95.3 %, respectively, suggesting significant diagnostic potential. However, despite ideal conditions for biopsy in 10 cases the material was insufficient or not representative. Moreover, these data arose from an ex-vivo approach with no risk of

complications (e.g. tumour spillage). Thus an in-vivo study would be needed to evaluate whether similar results can be obtained in a clinical setting.

For **staging** of established ACC we recommend a high resolution CT of thorax and abdomen. FDG-PET may occasionally be helpful to differentiate metastasis from benign lesions. At the time of diagnosis and in case of bone pain, a bone scintigraphy with consecutive conventional X-ray studies of regions with an increased uptake is performed. Hormone measurements are also occasionally important: after presumed complete tumor removal in patients with ACC and Cushing's syndrome postoperative endogenous cortisol should be subnormal, otherwise stage IV should be assumed even if no metastases in imaging are detected in MRI and/or CT.

Pathohistology

Even after surgical removal of the adrenal tumour the diagnosis may remain difficult. As with tumour size in adrenal imaging tumour weight is important, since most adenomas weigh between 20 and 50 g, while most malignant cortical tumours weigh more than 100 g (Saeger 2000). For diagnosis of ACC different diagnostic scores (Hough, *et al* 1979, van Slooten, *et al* 1985, Weiss 1984, Weiss, *et al* 1989) (table 2) have been developed. Typical histopathological markers of malignancy are a high number of mitoses, atypical mitoses, vessel or capsule invasion and necroses. Molecular markers have been widely studied in recent years (Wachenfeld, *et al* 2001). However, no single marker is diagnostic of ACC. A Ki-67 staining index of more than 5 % in adrenocortical tumours is suggestive for an ACC. To differentiate metastases from ACC or atypical pheochromocytomas immunostaining and the comparison with the primary extraadrenal tumour is often necessary (Saeger 2000). The marker D11 is useful, since it is positive in almost all cortical but negative in medullary

adrenal tumors. To identify a pheochromocytoma or a neuroendocrine carcinoma chromogranin A is the best marker. Ceratin filaments are usually demonstrable in metastases from carcinomas.

Staging

For staging of ACC the system of McFarlane (MacFarlane 1958) modified by Sullivan (Sullivan, *et al* 1978) is most frequently used and predicts the prognosis (Barzon, *et al* 1997, Icard, *et al* 2001, Kendrick, *et al* 2001, Luton, *et al* 2000, Soreide, *et al* 1992, Wajchenberg, *et al* 2000). However, modifications proposed by Lee *et al.* (Lee, *et al* 1995) and Icard *et al.* (Icard, *et al* 1992) are plausible, as they may better reflect the natural history of the disease and correlate more closely with other staging systems used for solid tumours (Dackiw, *et al* 2001) (see table 3). In the majority of patients with stage I to III complete tumour removal may be achievable, whereas this is highly unlikely in the presence of distant metastases (stage IV). In this revised staging system stage IV is defined by the presence of distant metastasis.

While in older series (Wooten and King 1993) most patients were diagnosed in advanced disease (stage IV), some more recent studies have reported the highest percentage of patients in stage II (Icard, *et al* 2001, Kendrick, *et al* 2001) probably reflecting improved and more widely available imaging technology.

Distant metastases affect most often liver and lung (see table 4)

Therapy

Surgery

Complete surgical resection continues to be the treatment of choice for ACC (Dackiw, *et al* 2001). A margin free resection (R₀ resection) is a strong predictor of long-term survival (Icard, *et al* 2001, Kendrick, *et al* 2001, Khorram-Manesh, *et al* 1998). It is best performed by an experienced surgeon using a transabdominal or even a thoraco-abdominal approach (Dackiw, *et al* 2001, Icard, *et al* 2001). To avoid tumour spillage the tumour capsule must remain intact. Invasion by or adherence of the carcinoma into adjacent organs often requires en-bloc excision of the kidney, the spleen, partial hepatectomy or pancreatectomy (Icard, *et al* 2001). In addition, lymphadenectomy has often to be included. The presence of a tumour thrombus in the renal vein or the inferior vena cava does not preclude a complete resection, although cardiac bypass technique may be necessary for successful removal of tumour tissue extending into the inferior vena cava or even the right atrium (Cheung and Thompson 1989, Hedican and Marshall 1997, Moul, *et al* 1991). The role of tumour debulking in the presence of metastatic disease is a matter of debate. Incomplete resection of the primary tumour or metastatic disease not amenable to surgery is associated with a particular poor prognosis. In most studies the median survival is below 12 months (Crucitti, *et al* 1996, Icard, *et al* 1992) (Lee, *et al* 1995, Zografos, *et al* 1994). However, tumour debulking may help to control hormone excess and may in individual cases facilitate other therapeutic options. Even if complete resection has been achieved, local recurrence and metastatic disease during follow-up is common. Risk factors include stage III tumours, a tumour diameter above 12 cm, a high mitotic index and intra-tumoural haemorrhage (Harrison, *et al* 1999, Stojadinovic, *et al* 2002). Laparoscopic resection of benign adrenal tumours has been a major improvement in adrenal surgery. However, in our view a laparoscopic approach should not be used for a presumable ACC, because of the risk of tumour capsule violation, tumour fragmentation (Dackiw, *et al*

2001, Iino, *et al* 2000) and the potential difficulty to perform a definite margin free R₀ resection.

Surgical resection of recurrent disease is an important therapeutic option associated with prolonged survival (Bellantone, *et al* 1997, Jensen, *et al* 1991, Schulick and Brennan 1999b), although cure is seldom achieved. Surgery for recurrent disease includes locoregional recurrence as well as isolated hepatic and pulmonary metastases. The most frequent indication for re-operation is locoregional disease (> 65 %) (Jensen, *et al* 1991) (Favia, *et al* 2001). Recently also successful thermoablation for recurrent or metastatic ACC has been reported (Wood, *et al* 2003).

Surgery related mortality has improved but remains substantial (5 %) (Icard, *et al* 2001). It is particularly high for stage III disease with invasion of adjacent organs.

Radiation therapy

The role of radiotherapy in ACC has not been well defined and is usually regarded as of limited benefit (Schulick and Brennan 1999a). However, palliative radiotherapy for metastatic disease was effective in a significant percentage of patients (Didolkar, *et al* 1981, Percarpio and Knowlton 1976) and is the treatment choice for bone metastases (30-40 Gy). More importantly, radiotherapy may have a role as adjuvant postoperative radiation therapy in patients at high risk for local recurrence. Based on a small series of patients with stage III the survival was higher than expected from historic series (Markoe, *et al* 1991). Local tumour recurrence is common in ACC and the most frequent cause for re-operation (Jensen, *et al* 1991) (Favia, *et al* 2001). Of note, in a series of children with ACC metastatic disease was invariably preceded by local recurrence of the disease (Ribeiro, *et al* 2000). Postoperative radiation of the tumour bed (50-60 Gy) may, therefore, improve the long-term outcome in stage III ACC or high risk stage II patients (tumour diameter > 12 cm, high mitotic index,

violation of the tumour capsule or frank evidence of tumour spillage during surgery). Modern treatment concepts with CT-planning, high voltage radiation (4-6 MeV) and multiple fields are required for optimum results.

Medical therapy

Medical therapy aims at the control of hormone hypersecretion and – more importantly – partial or complete remission of tumour spread.

Mitotane

More than 40 years ago Bergenstal et al. (Bergenstal, *et al* 1959) reported the first successful use of o,p'-DDD (mitotane) in patients with metastatic ACC. Mitotane (1,1 dichloro-2(o-chlorophenyl)-2-(p-chloro-phenyl)ethane) is an isomer of the insecticide p,p'-DDD and a chemical congener of the insecticide DDT. It is an adrenolytic compound with specific activity on the adrenal cortex (Schteingart 2000). Its therapeutic effects depend on intradrenal metabolic transformation. Mitotane is hydroxylated in the mitochondria at the β -carbon and further transformed into an acyl-chloride. It has been reported that the active metabolites cause toxicity by oxygen activation with superoxide formation or by covalent binding to specific proteins (Schteingart 2000).

The clinical efficacy of mitotane remains disputed. Hutter and Kayhoe (Hutter and Kayhoe 1966) collected a series of 138 patients and reported that 34 % of 59 patients with evaluable measurable disease had objective tumour regression. Wooten and King (Wooten and King 1993) have reviewed 551 cases in the English literature and reported a response rate of 35 % with mostly partial and transient responses and only an occasional complete remission. More recent series have reported lower response rates (Baudin, *et al* 2001, Khorram-Manesh, *et al* 1998). In our own experience also only a minority of patients exhibits objective tumour regression. However, in two patients with documented metastatic disease we observed a

lasting complete remission, which in both cases persists for now more than 7 years after mitotane withdrawal. Similar cases have been described in the literature (Remond, *et al* 1992).

The role of mitotane as adjuvant therapy after complete surgical removal of ACC remains a matter of debate (Barzon, *et al* 1999, Icard, *et al* 1992, Kasperlik-Zaluska 2000, Khorram-Manesh, *et al* 1998, Venkatesh, *et al* 1989). Due to the high rate of locoregional or metastatic recurrence after seemingly curative resection adjuvant treatment options are clearly needed. However, in some series adjuvant mitotane was associated with a poorer outcome (Vassilopoulou-Sellin, *et al* 1993). It is important to note that differences or similarities in survival of patients with ACC cannot be assessed without prospective randomised trials with a sufficient number of patients and, therefore, the value of adjuvant mitotane remains uncertain (Ahlman, *et al* 2001).

Mitotane is either given as tablets (Lysodren®, Bristol Meyer Squibb) in doses > 3 g/day or as capsules of micronized mitotane mixed with cellulose acetylphthalate, with a lower absorption rate, but, possibly, a better gastrointestinal tolerance (usually higher doses up to 12 g/day) (Luton 1990, Baudin, *et al* 2001). Drug monitoring is important. It has been found that drug levels > 14 mg/l are required to induce tumour regression. An objective response in metastatic disease was found in 31 % (Baudin, *et al* 2001), 55 % (Haak, *et al* 1994) or > 80 % (van Slooten, *et al* 1984) of patients achieving this level, whereas no response was seen in patients with a lower serum concentration. As side effects are more frequent with drug levels > 20 mg/l (van Slooten, *et al* 1984) and drug concentrations in serum are not closely related to drug dose (Terzolo, *et al* 2000b), drug monitoring may also improve quality of life during mitotane treatment by avoiding over-treatment. Due to the long half-life of o,p'DDD the highest trough levels are achieved only after several months of therapy (Baudin, *et al* 2001). Accordingly, in our experience mitotane side effects may become more pronounced with ongoing treatment despite a constant mitotane dose as drug levels gradually increase. Side

effects of mitotane occur frequently and are often dose limiting (table 5). These effects are mainly gastrointestinal (diarrhoea, nausea, anorexia) or concern the central nervous system (lethargy, somnolence, ataxia, dizziness, confusion) (Hutter and Kayhoe 1966, Schteingart, *et al* 1982). Patients rarely tolerate doses > 6 g/day for long-term therapy.

Due to its adrenolytic activity long-term mitotane treatment induces adrenal insufficiency. As its action is more pronounced in fasciculata cells, glucocorticoid deficiency precedes mineralocorticoid deficiency. Inadequately treated adrenal insufficiency enhances mitotane-induced side-effects and reduces mitotane tolerance (Kasperlik-Zaluska 2000). Since an increased metabolic clearance of glucocorticoids (e.g. dexamethasone) has been reported (Robinson, *et al* 1987) high dose glucocorticoid replacement is needed. Hydrocortisone is the treatment of choice (Kasperlik-Zaluska 2000, Robinson, *et al* 1987) and the glucocorticoid replacement is monitored best with careful clinical assessment and measurements of plasma ACTH levels, which should not be elevated. A daily dose of 50 mg hydrocortisone (20-20-10 mg) and more may be needed. Fludrocortisone may be added depending on blood pressure, serum potassium levels and plasma renin activity.

Increases in hepatic gamma glutamyl transaminase levels are frequent (Luton, *et al* 1990, Neuman, *et al* 2001) and in most cases do not require withdrawal of the drug. However, serious hepatotoxicity has also been described. Mitotane increases serum cholesterol mainly by increasing LDL-cholesterol. This increase may be amenable to statin therapy (Maher, *et al* 1992). In addition, mitotane can prolong the bleeding time by changing platelet aggregation response (Haak, *et al* 1991).

Of particular importance are mitotane-induced endocrine abnormalities. Mitotane strongly increases hormone binding globulins (e.g. cortisol-binding globulin, sex hormone binding globulin). Thus, measurement of total hormone concentrations may give normal results in the presence of clearly impaired bioavailability of free hormones (van Seters and Moolenaar

1991). Additionally, total thyroxine levels may be reduced as mitotane competes with endogenous thyroxine for thyroxine-binding globulin binding sites (Marshall and Tompkins 1968). In some patients also free thyroid hormone concentrations decrease and thyroxine replacement may become necessary.

For management of nausea 5-HT blockers may be useful. In case of significant neuropsychiatric side effects drug treatment is interrupted for a minimum of one week and restarted with a lower dose. Due to the long half-life significant serum concentrations may persist for weeks to months after cessation of therapy.

Cytotoxic chemotherapy

In patients with advanced local or metastatic disease not amenable to surgical resection cytotoxic chemotherapy has been investigated (see table 6).

In ACC strong expression of the multi-drug-resistance gene *mdr-1* has been observed (Abraham, *et al* 2002) resulting in high levels of P-glycoprotein which acts as a drug efflux pump and may cause chemotherapy failure (Ahlman, *et al* 2001). In vitro studies have shown that mitotane may partially reverse multi-drug resistance by inhibition drug efflux (Bates, *et al* 1991). This observation has led to protocols combining mitotane with cytotoxic chemotherapy, although a recent study casts doubts on the efficacy of mitotane to act as an effective p-glycoprotein antagonist in vivo (Abraham, *et al* 2002). Several cytotoxic agents have been used as single drugs or in combination to treat patients with advanced ACC (see table) including cisplatin, doxorubicin, etoposide, vincristine, 5-fluorouracil, and streptozocin (Ahlman, *et al* 2001). Although the results are variable, there is evidence that cisplatin alone or in combination with etoposide has some activity in advanced ACC (Berruti, *et al* 1998, Bonacci, *et al* 1998, Bukowski, *et al* 1993, Burgess, *et al* 1993, Williamson, *et al* 2000). Bonacci *et al.* treated 18 patients with etoposide (100mg/m²/d on days 1-3) and cisplatin

(100mg/m²/d on day 1) every 4 weeks maintaining mitotane therapy with an overall response of 33 %. Similarly Burgess et al. using the same drugs without mitotane reported a response rate of 46 % (Burgess, *et al* 1993). However, results of a more recent study in 45 patients with non-resectable or metastatic carcinoma using etoposide (100 mg/m²/d on day 1-3) and cisplatin (50 mg/m²/d on day 1 and 2) were clearly inferior with objective response in only 11 % of patients (Williamson, *et al* 2000). In this study mitotane was withheld or interrupted during cytotoxic chemotherapy. The highest response rate so far has been observed in a phase II multi-centre trial from Italy using the combination of etoposide (100 mg/m²/d on day 5-7), doxorubicin (20 mg/m²/d on days 1 and 8) and cisplatin (40 mg/m²/d on days 1 and 9) every 4 weeks (3-8 cycles) given together with continuous mitotane (planned dose 4 g/d). According to WHO criteria an overall response rate of 53.5 % was achieved (2 complete and 13 partial responses in 28 patients). Due to mitotane side-effects a reduced mitotane dose (2-3 g/d) was given in the majority of these patients. Recently, Khan et al. (Khan, *et al* 2000) have evaluated the efficacy of streptozocin plus mitotane in ACC. Oral mitotane (1-4 g/d) was given together with intravenous streptozocin (1g/d for 5 days, thereafter 2g once every 3 weeks). Complete or partial responses were obtained in 36.4 % (8 out of 22) of patients with measurable disease. Importantly, in this paper Khan et al. (Khan, *et al* 2000) provide evidence of a possible efficacy of this regime in an adjuvant setting after surgery. In a non-randomised study streptozocin plus mitotane significantly increased survival compared to patients who did not receive treatment after complete tumour resection. Again, such finding needs confirmation in a prospective randomised trial, as selection bias is likely.

Several other agents have been used in the treatment of advanced ACC. Suramin, an anti-trypansomal agent, may induce transient remission in occasional patients (Allolio, *et al* 1989) but its use is limited by significant toxicity (Arlt, *et al* 1994). Gossypol, a plant toxin from cotton seed oil, induced a partial remission in 3 out of 18 patients (17 %) with metastatic

ACC. However, 3 patients died of their disease without achieving the intended drug levels and had been eliminated from the analysis (Flack, *et al* 1993).

Inhibition of steroidogenesis

Since hormone excess (in particular hypercortisolism) is associated with a decreased of quality of life and an increased risk of complications, it is essential that patients do not suffer from Cushing's syndrome. Adrenostatic drugs other than mitotane may be needed to control endocrine activity (Luton, *et al* 1990). Meyrapone, ketoconazole, etomidate and aminoglutethimide inhibit P450 steroidogenic enzymes like 11 β -hydroxylase and side-chain cleavage enzyme (Feldman 1986). Aminoglutethimide is also an inhibitor of aromatase activity. Ketoconazole (400-1200mg/d) is most frequently used and may even possess anti-proliferative activity in some patients with ACC (Contreras, *et al* 1985). Adrenal insufficiency requiring hormone replacement and hepatotoxicity are the most frequent side effects. It is important to note, that ketoconazole may impair the adrenolytic action of mitotane (Schteingart 2000)

Intravenous etomidate is the most potent adrenostatic drug available (Allolio, *et al* 1988) and is probably the treatment of choice to rapidly control severe life-threatening hypercortisolism also in ACC. Again, there is evidence that etomidate and also aminoglutethimide possess some anti-proliferative activity in adrenocortical tumour cells (Fassnacht, *et al* 2000).

The use of adrenostatic drugs – including mitotane – always requires supervision by an experienced endocrinologist.

Follow-up

Close follow-up is of vital importance in ACC to detect recurrence at a time when surgical intervention is still possible. In our experience this aspect is often neglected after complete tumour removal in stage I and II patients as cure is prematurely assumed. Unfortunately, recurrence is common and most patients eventually succumb to their disease (Icard, *et al* 2001, Vassilopoulou-Sellin and Schultz 2001, Wajchenberg, *et al* 2000). Staging using CT should be performed every 3-4 months during the first two years after complete tumour removal. Intervals may then increase with disease free time from surgery. In functioning tumours hormonal marker (e.g. DHEAS) may rise again after surgery long before tumour tissue becomes detectable by imaging techniques. The duration of follow-up has not been standardised but should probably be indefinite.

Prognosis

There is some evidence that in the last two decades earlier diagnosis and improved surgical management have both led to a significantly better outcome (Icard, *et al* 2001, Vassilopoulou-Sellin and Schultz 2001). It is also important to bear in mind that ACC is a heterogenous disease with some patients surviving for more than 10 years despite metastatic disease whereas others die within a few months from a rapidly progressive disease not responding to any available therapy.

In general the prognosis for ACC is still grim. McFarlane has reported that patients with untreated ACC have a median survival of 3 months only (MacFarlane 1958). In treated ACC overall 5 year survival ranged between 23 % and 60 % in different series (Venkatesh, *et al* 1989, Haak, *et al* 1993, Haak, *et al* 1990, Icard, *et al* 1992, Luton, *et al* 1990, Nader, *et al*

1983, Vassilopoulou-Sellin and Schultz 2001, Icard, *et al* 2001). Patients with stage I and II have a similar prognosis which is significantly better than that for stage III and IV patients (Wajchenberg, *et al* 2000). In a recent series including 253 patients from France the 5 year actuarial survival rates were 60 % for stage I, 58 % for stage II, 24 % for stage III and 0 % for stage IV (Icard, *et al* 1992). The overall rate was 38 % with a rate of 50 % in patients who underwent resection for cure. At present the most important prognostic factors remain, therefore, early stage and complete tumour removal aiming at cure (Schulick and Brennan 1999a, Schulick and Brennan 1999b, Soreide, *et al* 1992, Zografos, *et al* 1994, Pommier and Brennan 1992). Accordingly, in a recent series reported by Vassilopoulou-Sellin and Schultz long-term survivors (> 5 years) had significantly less extensive disease at diagnosis ($p < 0.001$) in comparison to patients with the shortest survival (<11 months) (Vassilopoulou-Sellin and Schultz 2001) . In contrast, they found no differences in age, gender and functionality. Tumour size may be important, as patients with completely resected large (> 12 cm) tumours had significantly reduced survival (Harrison, *et al* 1999). Recently, Stojadinovic *et al* (Stojadinovic, *et al* 2002) have analysed additional parameters in a series of 124 patients using molecular expression profiles and morphologic patterns in tissue specimens. In their analysis tumour necrosis ($p < 0.01$), a mitotic rate of more than 5 of 50 high power fields ($p = 0.004$) and atypic mitotic figures ($p = 0.008$) were associated with reduced disease free survival. In addition, high proliferative activity as assessed by Ki-67 staining and evidence for mutated p53 are associated with advanced stage ACC and poor prognosis (Stojadinovic, *et al* 2002). Endocrine activity of ACC has no general influence on prognosis as compared to non-functioning ACC (Favia, *et al* 2001). However, there is some evidence, that tumours secreting androgens or steroid precursors only have a better prognosis than cortisol secreting ACC or tumours secreting both cortisol and androgens (Icard, *et al* 1992, Ribeiro, *et al* 2000).

Future directions

Progress in the management of ACC is hampered by its low incidence and heterogeneity. Not a single prospective randomised study (phase III trial) has been performed to directly compare different treatment modalities. Accordingly, claims of improved survival for certain treatment options (e.g. mitotane for stage IV disease (Icard, *et al* 1992) are not well founded, as selection bias, are likely. Only multi-centre and probably multinational (e.g. European) efforts will change this picture. To this end a number of consecutive steps need to be taken: patients with ACC should be treated in a few specialised national centres to provide the necessary optimum interdisciplinary care. These centres should then build a national ACC registry to further enhance patient recruitment and to standardise patient care. Some registries have already been established in France and Italy, and a German registry is under way. These national registries should be harmonised and could serve as a multinational nucleus for prospective trials of sufficient size. In our view, two important areas should be the focus of such trials. The first concerns adjuvant therapy after surgical resection for cure, as the majority of these patients will develop local recurrence or metastatic disease. Possible options are mitotane with or without streptozocin and/or adjuvant radiotherapy of the tumour bed. An untreated control group is needed, because for none of these treatment options a beneficial effect has been established. The other area is stage IV ACC. Here it is important to compare mitotane alone with a combination of mitotane plus cytotoxic drugs.

Undoubtedly, innovative treatment options need to be developed based on a better understanding of the molecular pathogenesis of ACC. At present inhibition of IGF-II signalling (e.g. by small molecule IGF-I receptor antagonists) seems to be a promising approach. In addition, antiangiogenic drugs and immunotherapy should be investigated in patients with progressive disease.

In conclusion, at this time it is our responsibility not only to provide the best possible care for individual patients with ACC but also to set up structures, which will allow us to make systematic progress in the management of this dreadful disease.

References

- Abraham, J., Bakke, S., Rutt, A., Meadows, B., Merino, M., Alexander, R., Schrupp, D., Bartlett, D., Choyke, P., Robey, R., Hung, E., Steinberg, S.M., Bates, S. & Fojo, T. (2002) A phase II trial of combination chemotherapy and surgical resection for the treatment of metastatic adrenocortical carcinoma: continuous infusion doxorubicin, vincristine, and etoposide with daily mitotane as a P-glycoprotein antagonist. *Cancer*, **94**, 2333-2343.
- Ahlman, H., Khorram-Manesh, A., Jansson, S., Wangberg, B., Nilsson, O., Jacobsson, C.E. & Lindstedt, S. (2001) Cytotoxic treatment of adrenocortical carcinoma. *World J Surg*, **25**, 927-933.
- Allolio, B. (2001) Adrenal Incidentalomas. In: *Adrenal Disorders* (ed. by A.N. Margioris & G.P. Chrousos), pp. 249-261. Humana Press, Totawa, NJ.
- Allolio, B., Reincke, M., Arlt, W., Deuss, U., Winkelmann, W. & Siekmann, L. (1989) Suramin for treatment of adrenocortical carcinoma. *Lancet*, **2**, 277.
- Allolio, B., Schulte, H.M., Kaulen, D., Reincke, M., Jaursch-Hancke, C. & Winkelmann, W. (1988) Nonhypnotic low-dose etomidate for rapid correction of hypercortisolaemia in Cushing's syndrome. *Klin Wochenschr*, **66**, 361-364.
- Arlt, W., Reincke, M., Siekmann, L., Winkelmann, W. & Allolio, B. (1994) Suramin in adrenocortical cancer: limited efficacy and serious toxicity. *Clin Endocrinol (Oxf)*, **41**, 299-307.
- Barzon, L., Chilosi, M., Fallo, F., Martignoni, G., Montagna, L., Palu, G. & Boscaro, M. (2001) Molecular analysis of CDKN1C and TP53 in sporadic adrenal tumors. *Eur J Endocrinol*, **145**, 207-212.
- Barzon, L., Fallo, F., Sonino, N., Daniele, O. & Boscaro, M. (1997) Adrenocortical carcinoma: experience in 45 patients. *Oncology*, **54**, 490-496.
- Barzon, L., Scaroni, C., Sonino, N., Fallo, F., Paoletta, A. & Boscaro, M. (1999) Risk factors and long-term follow-up of adrenal incidentalomas. *J Clin Endocrinol Metab*, **84**, 520-526.
- Bates, S.E., Shieh, C.Y., Mickley, L.A., Dichek, H.L., Gazdar, A., Loriaux, D.L. & Fojo, A.T. (1991) Mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (mdr-1/P-glycoprotein) which is also expressed by adrenocortical carcinomas. *J Clin Endocrinol Metab*, **73**, 18-29.

- Baudin, E., Pellegriti, G., Bonnay, M., Penfornis, A., Laplanche, A., Vassal, G. & Schlumberger, M. (2001) Impact of monitoring plasma 1,1-dichlorodiphenildichloroethane (o,p'DDD) levels on the treatment of patients with adrenocortical carcinoma. *Cancer*, **92**, 1385-1392.
- Becherer, A., Vierhapper, H., Potzi, C., Karanikas, G., Kurtaran, A., Schmaljohann, J., Staudenherz, A., Dudczak, R. & Kletter, K. (2001) FDG-PET in adrenocortical carcinoma. *Cancer Biother Radiopharm*, **16**, 289-295.
- Bellantone, R., Ferrante, A., Boscherini, M., Lombardi, C.P., Crucitti, P., Crucitti, F., Favia, G., Borrelli, D., Boffi, L., Capussotti, L., Carbone, G., Casaccia, M., Cavallaro, A., Del Gaudio, A., Dettori, G., Di Giovanni, V., Mazziotti, A., Marrano, D., Masenti, E., Miccoli, P., Mosca, F., Mussa, A., Petronio, R., Piat, G., Marazano, L. & et al. (1997) Role of reoperation in recurrence of adrenal cortical carcinoma: results from 188 cases collected in the Italian National Registry for Adrenal Cortical Carcinoma. *Surgery*, **122**, 1212-1218.
- Bergental, D., Lipsett, M., Moy, R. & Hertz, R. (1959) Regression of adrenal cancer and suppression of adrenal function in men by o,p-DDD. *Transactions of American Physicians*, **72**, 341.
- Berruti, A., Terzolo, M., Pia, A., Angeli, A. & Dogliotti, L. (1998) Mitotane associated with etoposide, doxorubicin, and cisplatin in the treatment of advanced adrenocortical carcinoma. Italian Group for the Study of Adrenal Cancer. *Cancer*, **83**, 2194-2200.
- Beuschlein, F., Fassnacht, M., Klink, A., Allolio, B. & Reincke, M. (2001) ACTH-receptor expression, regulation and role in adrenocortical tumor formation. *Eur J Endocrinol*, **144**, 199-206.
- Bicknell, A.B., Lomthaisong, K., Woods, R.J., Hutchinson, E.G., Bennett, H.P., Gladwell, R.T. & Lowry, P.J. (2001) Characterization of a serine protease that cleaves pro-gamma-melanotropin at the adrenal to stimulate growth. *Cell*, **105**, 903-912.
- Bilbey, J.H., McLoughlin, R.F., Kurkjian, P.S., Wilkins, G.E., Chan, N.H., Schmidt, N. & Singer, J. (1995) MR imaging of adrenal masses: value of chemical-shift imaging for distinguishing adenomas from other tumors. *AJR Am J Roentgenol*, **164**, 637-642.
- Bodie, B., Novick, A.C., Pontes, J.E., Straffon, R.A., Montie, J.E., Babiak, T., Sheeler, L. & Schumacher, P. (1989) The Cleveland Clinic experience with adrenal cortical carcinoma. *J Urol*, **141**, 257-260.

- Boland, G.W., Goldberg, M.A., Lee, M.J., Mayo-Smith, W.W., Dixon, J., McNicholas, M.M. & Mueller, P.R. (1995) Indeterminate adrenal mass in patients with cancer: evaluation at PET with 2-[F-18]-fluoro-2-deoxy-D-glucose. *Radiology*, **194**, 131-134.
- Boland, G.W., Lee, M.J., Gazelle, G.S., Halpern, E.F., McNicholas, M.M. & Mueller, P.R. (1998) Characterization of adrenal masses using unenhanced CT: an analysis of the CT literature. *AJR Am J Roentgenol*, **171**, 201-204.
- Bonacci, R., Gigliotti, A., Baudin, E., Wion-Barbot, N., Emy, P., Bonnay, M., Cailleux, A.F., Nakib, I. & Schlumberger, M. (1998) Cytotoxic therapy with etoposide and cisplatin in advanced adrenocortical carcinoma. Réseau Comete INSERM. *Br J Cancer*, **78**, 546-549.
- Bukowski, R.M., Wolfe, M., Levine, H.S., Crawford, D.E., Stephens, R.L., Gaynor, E. & Harker, W.G. (1993) Phase II trial of mitotane and cisplatin in patients with adrenal carcinoma: a Southwest Oncology Group study. *J Clin Oncol*, **11**, 161-165.
- Burgess, M.A., Legha, S.S. & Sellin, R.V. (1993) Chemotherapy with cisplatin and etoposide (UP16) for patients with advanced adrenal cortical carcinoma (ACC). *Proc Ann Soc Clin Oncol*, **12**, 188.
- Caoli, E.M., Korobkin, M., Francis, I.R., Cohan, R.H. & Dunnick, N.R. (2000) Delayed enhanced CT of lipid-poor adrenal adenomas. *AJR Am J Roentgenol*, **175**, 1411-1415.
- Cheung, P.S. & Thompson, N.W. (1989) Right atrial extension of adrenocortical carcinoma. Surgical management using hypothermia and cardiopulmonary bypass. *Cancer*, **64**, 812-815.
- Contreras, P., Rojas, A., Biagini, L., Gonzalez, P. & Massardo, T. (1985) Regression of metastatic adrenal carcinoma during palliative ketoconazole treatment. *Lancet*, **2**, 151-152.
- Crucitti, F., Bellantone, R., Ferrante, A., Boscherini, M. & Crucitti, P. (1996) The Italian Registry for Adrenal Cortical Carcinoma: analysis of a multiinstitutional series of 129 patients. The ACC Italian Registry Study Group. *Surgery*, **119**, 161-170.
- Dackiw, A.P., Lee, J.E., Gagel, R.F. & Evans, D.B. (2001) Adrenal cortical carcinoma. *World J Surg*, **25**, 914-926.
- Decker, R.A., Elson, P., Hogan, T.F., Citrin, D.L., Westring, D.W., Banerjee, T.K., Gilchrist, K.W. & Horton, J. (1991) Eastern Cooperative Oncology Group study 1879: mitotane and adriamycin in patients with advanced adrenocortical carcinoma. *Surgery*, **110**, 1006-1013.

- Didolkar, M.S., Bescher, R.A., Elias, E.G. & Moore, R.H. (1981) Natural history of adrenal cortical carcinoma: a clinicopathologic study of 42 patients. *Cancer*, **47**, 2153-2161.
- DiGiammarino, E.L., Lee, A.S., Cadwell, C., Zhang, W., Bothner, B., Ribeiro, R.C., Zambetti, G. & Kriwacki, R.W. (2002) A novel mechanism of tumorigenesis involving pH-dependent destabilization of a mutant p53 tetramer. *Nat Struct Biol*, **9**, 12-16.
- Dohna, M., Reincke, M., Mincheva, A., Allolio, B., Solinas-Toldo, S. & Lichter, P. (2000) Adrenocortical carcinoma is characterized by a high frequency of chromosomal gains and high-level amplifications. *Genes Chromosomes Cancer*, **28**, 145-152.
- Dunnick, N.R. & Korobkin, M. (2002) Imaging of adrenal incidentalomas: current status. *AJR Am J Roentgenol*, **179**, 559-568.
- Estivariz, F.E., Iturriza, F., McLean, C., Hope, J. & Lowry, P.J. (1982) Stimulation of adrenal mitogenesis by N-terminal proopiomelanocortin peptides. *Nature*, **297**, 419-422.
- Fassnacht, M., Hahner, S., Beuschlein, F., Klink, A., Reincke, M. & Allolio, B. (2000) New mechanisms of adrenostatic compounds in a human adrenocortical cancer cell line. *Eur J Clin Invest*, **30 Suppl 3**, 76-82.
- Fassnacht, M., Hahner, S., Hansen, I.A., Kreutzberger, T., Zink, M., Adermann, K., Jakob, F., Troppmair, J. & Allolio, B. (2003) N-terminal Proopiomelanocortin acts as a mitogen in adrenocortical tumor cells and decreases adrenal steroidogenesis. *J Clin Endocrinol Metab*, **88**, 2171-9.
- Favia, G., Lumachi, F. & D'Amico, D.F. (2001) Adrenocortical carcinoma: is prognosis different in nonfunctioning tumors? results of surgical treatment in 31 patients. *World J Surg*, **25**, 735-738.
- Feldman, D. (1986) Ketoconazole and other imidazole derivatives as inhibitors of steroidogenesis. *Endocr Rev*, **7**, 409-420.
- Flack, M.R., Pyle, R.G., Mullen, N.M., Lorenzo, B., Wu, Y.W., Knazek, R.A., Nisula, B.C. & Reidenberg, M.M. (1993) Oral gossypol in the treatment of metastatic adrenal cancer. *J Clin Endocrinol Metab*, **76**, 1019-1024.
- Flecchia, D., Mazza, E., Carlini, M., Blatto, A., Olivieri, F., Serra, G., Camanni, F. & Messina, M. (1995) Reduced serum levels of dehydroepiandrosterone sulphate in adrenal incidentalomas: a marker of adrenocortical tumour. *Clin Endocrinol (Oxf)*, **42**, 129-134.
- Gabrilove, J., Sharma, D., Watz, H. & Dorfman, R. (1965) Feminizing adrenocortical tumors in the male: a review of 52 cases including a case report. *Medicine*, **44**, 37-39.

- Gicquel, C., Bertagna, X., Gaston, V., Coste, J., Louvel, A., Baudin, E., Bertherat, J., Chapuis, Y., Duclos, J.M., Schlumberger, M., Plouin, P.F., Luton, J.P. & Le Bouc, Y. (2001) Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors. *Cancer Res*, **61**, 6762-6767.
- Gicquel, C., Bertagna, X., Schneid, H., Francillard-Leblond, M., Luton, J.P., Girard, F. & Le Bouc, Y. (1994) Rearrangements at the 11p15 locus and overexpression of insulin-like growth factor-II gene in sporadic adrenocortical tumors. *J Clin Endocrinol Metab*, **78**, 1444-1453.
- Gicquel, C., Raffin-Sanson, M.L., Gaston, V., Bertagna, X., Plouin, P.F., Schlumberger, M., Louvel, A., Luton, J.P. & Le Bouc, Y. (1997) Structural and functional abnormalities at 11p15 are associated with the malignant phenotype in sporadic adrenocortical tumors: study on a series of 82 tumors. *J Clin Endocrinol Metab*, **82**, 2559-2565.
- Giordano, T.J., Thomas, D.G., Kuick, R., Lizyness, M., Misek, D.E., Smith, A.L., Sanders, D., Aljundi, R.T., Gauger, P.G., Thompson, N.W., Taylor, J.M. & Hanash, S.M. (2003) Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis. *Am J Pathol*, **162**, 521-531.
- Goldfarb, D.A., Novick, A.C., Lorig, R., Bretan, P.N., Montie, J.E., Pontes, J.E., Strem, S.B. & Siegel, S.W. (1990) Magnetic resonance imaging for assessment of vena caval tumor thrombi: a comparative study with venacavography and computerized tomography scanning. *J Urol*, **144**, 1100-1103; discussion 1103-1104.
- Haak, H.R., Caekebeke-Peerlinck, K.M., van Seters, A.P. & Briet, E. (1991) Prolonged bleeding time due to mitotane therapy. *Eur J Cancer*, **27**, 638-641.
- Haak, H.R., Cornelisse, C.J., Hermans, J., Cobben, L. & Fleuren, G.J. (1993) Nuclear DNA content and morphological characteristics in the prognosis of adrenocortical carcinoma. *Br J Cancer*, **68**, 151-155.
- Haak, H.R., Hermans, J., van de Velde, C.J., Lentjes, E.G., Goslings, B.M., Fleuren, G.J. & Krans, H.M. (1994) Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients. *Br J Cancer*, **69**, 947-951.
- Haak, H.R., van Seters, A.P. & Moolenaar, A.J. (1990) Mitotane therapy of adrenocortical carcinoma. *N Engl J Med*, **323**, 758.
- Hainaut, P. (2002) Tumor-specific mutations in p53: the acid test. *Nat Med*, **8**, 21-23.
- Hao, Y., Crenshaw, T., Moulton, T., Newcomb, E. & Tycko, B. (1993) Tumour-suppressor activity of H19 RNA. *Nature*, **365**, 764-767.

- Harrison, L.E., Gaudin, P.B. & Brennan, M.F. (1999) Pathologic features of prognostic significance for adrenocortical carcinoma after curative resection. *Arch Surg*, **134**, 181-185.
- Hedican, S.P. & Marshall, F.F. (1997) Adrenocortical carcinoma with intracaval extension. *J Urol*, **158**, 2056-2061.
- Heinz-Peer, G., Honigschnabl, S., Schneider, B., Niederle, B., Kaserer, K. & Lechner, G. (1999) Characterization of adrenal masses using MR imaging with histopathologic correlation. *AJR Am J Roentgenol*, **173**, 15-22.
- Honigschnabl, S., Gallo, S., Niederle, B., Prager, G., Kaserer, K., Lechner, G. & Heinz-Peer, G. (2002) How accurate is MR imaging in characterisation of adrenal masses: update of a long-term study. *Eur J Radiol*, **41**, 113-122.
- Hough, A.J., Hollifield, J.W., Page, D.L. & Hartmann, W.H. (1979) Prognostic factors in adrenal cortical tumors. A mathematical analysis of clinical and morphologic data. *Am J Clin Pathol*, **72**, 390-399.
- Hsing, A.W., Nam, J.M., Co Chien, H.T., McLaughlin, J.K. & Fraumeni, J.F., Jr. (1996) Risk factors for adrenal cancer: an exploratory study. *Int J Cancer*, **65**, 432-436.
- Hutter, A.M., Jr. & Kayhoe, D.E. (1966) Adrenal cortical carcinoma. Results of treatment with o,p'DDD in 138 patients. *Am J Med*, **41**, 581-592.
- Icard, P., Chapuis, Y., Andreassian, B., Bernard, A. & Proye, C. (1992) Adrenocortical carcinoma in surgically treated patients: a retrospective study on 156 cases by the French Association of Endocrine Surgery. *Surgery*, **112**, 972-979; discussion 979-980.
- Icard, P., Goudet, P., Charpenay, C., Andreassian, B., Carnaille, B., Chapuis, Y., Cougard, P., Henry, J.F. & Proye, C. (2001) Adrenocortical carcinomas: surgical trends and results of a 253-patient series from the French Association of Endocrine Surgeons study group. *World J Surg*, **25**, 891-897.
- Iino, K., Oki, Y. & Sasano, H. (2000) A case of adrenocortical carcinoma associated with recurrence after laparoscopic surgery. *Clin Endocrinol (Oxf)*, **53**, 243-248.
- Ilvesmaki, V., Kahri, A.I., Miettinen, P.J. & Voutilainen, R. (1993) Insulin-like growth factors (IGFs) and their receptors in adrenal tumors: high IGF-II expression in functional adrenocortical carcinomas. *J Clin Endocrinol Metab*, **77**, 852-858.
- Jensen, J.C., Pass, H.I., Sindelar, W.F. & Norton, J.A. (1991) Recurrent or metastatic disease in select patients with adrenocortical carcinoma. Aggressive resection vs chemotherapy. *Arch Surg*, **126**, 457-461.

- Kasperlik-Zaluska, A.A. (2000) Clinical results of the use of mitotane for adrenocortical carcinoma. *Braz J Med Biol Res*, **33**, 1191-1196.
- Kasperlik-Zaluska, A.A., Migdalska, B.M. & Makowska, A.M. (1998) Incidentally found adrenocortical carcinoma. A study of 21 patients. *Eur J Cancer*, **34**, 1721-1724.
- Kendrick, M.L., Lloyd, R., Erickson, L., Farley, D.R., Grant, C.S., Thompson, G.B., Rowland, C., Young, W.F., Jr. & van Heerden, J.A. (2001) Adrenocortical carcinoma: surgical progress or status quo? *Arch Surg*, **136**, 543-549.
- Khan, T.S., Imam, H., Juhlin, C., Skogseid, B., Grondal, S., Tibblin, S., Wilander, E., Oberg, K. & Eriksson, B. (2000) Streptozocin and o,p'DDD in the treatment of adrenocortical cancer patients: long-term survival in its adjuvant use. *Ann Oncol*, **11**, 1281-1287.
- Khan, T.S., Sundin, A., Juhlin, C., Langstrom, B., Bergstrom, M. & Eriksson, B. (2003) 11C-metomidate PET imaging of adrenocortical cancer. *Eur J Nucl Med Mol Imaging*, **30**, 403-410.
- Khorram-Manesh, A., Ahlman, H., Jansson, S., Wangberg, B., Nilsson, O., Jakobsson, C.E., Eliasson, B., Lindstedt, S. & Tisell, L.E. (1998) Adrenocortical carcinoma: surgery and mitotane for treatment and steroid profiles for follow-up. *World J Surg*, **22**, 605-611; discussion 611-602.
- King, D.R. & Lack, E.E. (1979) Adrenal cortical carcinoma: a clinical and pathologic study of 49 cases. *Cancer*, **44**, 239-244.
- Kirschner, L.S. (2002) Signaling pathways in adrenocortical cancer. *Ann N Y Acad Sci*, **968**, 222-239.
- Kjellman, M., Kallioniemi, O.P., Karhu, R., Hoog, A., Farnebo, L.O., Auer, G., Larsson, C. & Backdahl, M. (1996) Genetic aberrations in adrenocortical tumors detected using comparative genomic hybridization correlate with tumor size and malignancy. *Cancer Res*, **56**, 4219-4223.
- Kjellman, M., Larsson, C. & Backdahl, M. (2001) Genetic background of adrenocortical tumor development. *World J Surg*, **25**, 948-956.
- Kloos, R.T., Gross, M.D., Francis, I.R., Korobkin, M. & Shapiro, B. (1995) Incidentally discovered adrenal masses. *Endocr Rev*, **16**, 460-484.
- Koch, C.A., Pacak, K. & Chrousos, G.P. (2002) The molecular pathogenesis of hereditary and sporadic adrenocortical and adrenomedullary tumors. *J Clin Endocrinol Metab*, **87**, 5367-5384.

- Korobkin, M., Brodeur, F.J., Francis, I.R., Quint, L.E., Dunnick, N.R. & Londy, F. (1998) CT time-attenuation washout curves of adrenal adenomas and nonadenomas. *AJR Am J Roentgenol*, **170**, 747-752.
- Korobkin, M., Giordano, T.J., Brodeur, F.J., Francis, I.R., Siegelman, E.S., Quint, L.E., Dunnick, N.R., Heiken, J.P. & Wang, H.H. (1996) Adrenal adenomas: relationship between histologic lipid and CT and MR findings. *Radiology*, **200**, 743-747.
- Korobkin, M., Lombardi, T.J., Aisen, A.M., Francis, I.R., Quint, L.E., Dunnick, N.R., Londy, F., Shapiro, B., Gross, M.D. & Thompson, N.W. (1995) Characterization of adrenal masses with chemical shift and gadolinium- enhanced MR imaging. *Radiology*, **197**, 411-418.
- Latronico, A.C., Reincke, M., Mendonca, B.B., Arai, K., Mora, P., Allolio, B., Wajchenberg, B.L., Chrousos, G.P. & Tsigos, C. (1995) No evidence for oncogenic mutations in the adrenocorticotropin receptor gene in human adrenocortical neoplasms. *J Clin Endocrinol Metab*, **80**, 875-877.
- Lee, A.S., Galea, C., DiGiammarino, E.L., Jun, B., Murti, G., Ribeiro, R.C., Zambetti, G., Schultz, C.P. & Kriwacki, R.W. (2003) Reversible Amyloid Formation by the p53 Tetramerization Domain and a Cancer-associated Mutant. *J Mol Biol*, **327**, 699-709.
- Lee, J.E., Berger, D.H., el-Naggar, A.K., Hickey, R.C., Vassilopoulou-Sellin, R., Gagel, R.F., Burgess, M.A. & Evans, D.B. (1995) Surgical management, DNA content, and patient survival in adrenal cortical carcinoma. *Surgery*, **118**, 1090-1098.
- Lee, M., Hahn, P., Papanicolaou, N., Egglin, T., Saini, S., Mueller, P. & Simeone, J. (1991) Benign and malignant adrenal masses: CT distinction with attenuation coefficients, size, and observer analysis. *Radiology*, **179**, 415-418.
- Leighton, P.A., Ingram, R.S., Eggenschwiler, J., Efstratiadis, A. & Tilghman, S.M. (1995) Disruption of imprinting caused by deletion of the H19 gene region in mice. *Nature*, **375**, 34-39.
- Lowry, P.J., Silas, L., McLean, C., Linton, E.A. & Estivariz, F.E. (1983) Pro-gamma-melanocyte-stimulating hormone cleavage in adrenal gland undergoing compensatory growth. *Nature*, **306**, 70-73.
- Luton, J.P., Cerdas, S., Billaud, L., Thomas, G., Guilhaume, B., Bertagna, X., Laudat, M.H., Louvel, A., Chapuis, Y., Blondeau, P. & et al. (1990) Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. *N Engl J Med*, **322**, 1195-1201.

- Luton, J.P., Martinez, M., Coste, J. & Bertherat, J. (2000) Outcome in patients with adrenal incidentaloma selected for surgery: an analysis of 88 cases investigated in a single clinical center. *Eur J Endocrinol*, **143**, 111-117.
- MacFarlane, D.A. (1958) Cancer of the adrenal cortex: the natural history, prognosis and treatment in the study of fifty cases. *Ann R Coll Surg Engl*, **109**, 613-618.
- Maher, V.M., Trainer, P.J., Scoppola, A., Anderson, J.V., Thompson, G.R. & Besser, G.M. (1992) Possible mechanism and treatment of o,p'DDD-induced hypercholesterolaemia. *Q J Med*, **84**, 671-679.
- Malkin, D., Li, F.P., Strong, L.C., Fraumeni, J.F., Jr., Nelson, C.E., Kim, D.H., Kassel, J., Gryka, M.A., Bischoff, F.Z., Tainsky, M.A. & et al. (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*, **250**, 1233-1238.
- Markoe, A.M., Serber, W., Micaily, B. & Brady, L.W. (1991) Radiation therapy for adjunctive treatment of adrenal cortical carcinoma. *Am J Clin Oncol*, **14**, 170-174.
- Marshall, J.S. & Tompkins, L.S. (1968) Effect of o,p'-DDD and similar compounds on thyroxine binding globulin. *J Clin Endocrinol Metab*, **28**, 386-392.
- Maurea, S., Mainolfi, C., Bazzicalupo, L., Panico, M.R., Imperato, C., Alfano, B., Ziviello, M. & Salvatore, M. (1999) Imaging of adrenal tumors using FDG PET: comparison of benign and malignant lesions. *AJR Am J Roentgenol*, **173**, 25-29.
- McNicol, A.M., Nolan, C.E., Struthers, A.J., Farquharson, M.A., Hermans, J. & Haak, H.R. (1997a) Expression of p53 in adrenocortical tumours: clinicopathological correlations. *J Pathol*, **181**, 146-152.
- McNicol, A.M., Struthers, A.L., Nolan, C.E., Hermans, J. & Haak, H.R. (1997b) Proliferation in Adrenocortical Tumors: Correlation with Clinical Outcome and p53 Status. *Endocr Pathol*, **8**, 29-36.
- Mody, M.K., Kazerooni, E.A. & Korobkin, M. (1995) Percutaneous CT-guided biopsy of adrenal masses: immediate and delayed complications. *J Comput Assist Tomogr*, **19**, 434-439.
- Moul, J.W., Hardy, M.R. & McLeod, D.G. (1991) Adrenal cortical carcinoma with vena cava tumor thrombus requiring cardiopulmonary bypass for resection. *Urology*, **38**, 179-183.
- Nader, S., Hickey, R., Sellin, R. & Samaan, N. (1983) Adrenal cortical carcinoma. A study of 77 cases. *Cancer*, **52**, 707-711.

- National Cancer Institute (1975) *Third national cancer survey: incidence data*. In: DHEW Publ. No. (NIH) 75-787. NCI monograph., p. 41, Bethesda, National Cancer Institute.
- National Institute of Health: State of the science statement (2002) Management of the Clinically Inapparent Adrenal Mass (Incidentaloma) February 4-6, 2002. http://consensus.nih.gov/ta/021/021_statement.htm.
- Neuman, O., Bruckert, E., Chadarevian, R., Jacob, N. & Turpin, G. (2001) [Hepatotoxicity of a synthetic cortisol antagonist: OP'DDD (mitotane)]. *Therapie*, **56**, 793-797.
- Osella, G., Terzolo, M., Borretta, G., Magro, G., Ali, A., Piovesan, A., Paccotti, P. & Angeli, A. (1994) Endocrine evaluation of incidentally discovered adrenal masses (incidentalomas) [see comments]. *J Clin Endocrinol Metab*, **79**, 1532-1539.
- Outwater, E.K., Siegelman, E.S., Huang, A.B. & Birnbaum, B.A. (1996) Adrenal masses: correlation between CT attenuation value and chemical shift ratio at MR imaging with in-phase and opposed-phase sequences. *Radiology*, **200**, 749-752.
- Pena, C.S., Boland, G.W., Hahn, P.F., Lee, M.J. & Mueller, P.R. (2000) Characterization of indeterminate (lipid-poor) adrenal masses: use of washout characteristics at contrast-enhanced CT. *Radiology*, **217**, 798-802.
- Percarpio, B. & Knowlton, A.H. (1976) Radiation therapy of adrenal cortical carcinoma. *Acta Radiol Ther Phys Biol*, **15**, 288-292.
- Pommier, R.F. & Brennan, M.F. (1992) An eleven-year experience with adrenocortical carcinoma. *Surgery*, **112**, 963-970; discussion 970-961.
- Reincke, M. (1998) Mutations in adrenocortical tumors. *Horm Metab Res*, **30**, 447-455.
- Reincke, M., Beuschlein, F., Latronico, A., Arlt, W., Chrousos, G. & Allolio, B. (1997a) Expression of adrenocorticotrophic hormone receptor mRNA in human adrenocortical neoplasms: correlation with P450scc expression. *Clin Endocrinol (Oxf)*, **46**, 619-626.
- Reincke, M., Karl, M., Travis, W.H., Mastorakos, G., Allolio, B., Linehan, H.M. & Chrousos, G.P. (1994) p53 mutations in human adrenocortical neoplasms: immunohistochemical and molecular studies. *J Clin Endocrinol Metab*, **78**, 790-794.
- Reincke, M., Mora, P., Beuschlein, F., Arlt, W., Chrousos, G. & Allolio, B. (1997b) Deletion of the adrenocorticotropin receptor gene in human adrenocortical tumors: implications for tumorigenesis. *J Clin Endocrinol Metab*, **82**, 3054-3058.
- Reincke, M., Nieke, J., Krestin, G., Saeger, W., Allolio, B. & Winkelmann, W. (1992) Preclinical Cushing's syndrome in adrenal "incidentalomas": comparison with adrenal Cushing's syndrome. *J Clin Endocrinol Metab*, **75**, 826-832.

- Remond, S., Bardet, S. & Charbonnel, B. (1992) [Complete and lasting remission of a metastatic malignant adrenocortical carcinoma under treatment with OP'DDD alone]. *Presse Med*, **21**, 865.
- Ribeiro, R.C., Michalkiewicz, E.L., Figueiredo, B.C., DeLacerda, L., Sandrini, F., Pianovsky, M.D., Sampaio, G. & Sandrini, R. (2000) Adrenocortical tumors in children. *Braz J Med Biol Res*, **33**, 1225-1234.
- Ribeiro, R.C., Sandrini, F., Figueiredo, B., Zambetti, G.P., Michalkiewicz, E., Lafferty, A.R., DeLacerda, L., Rabin, M., Cadwell, C., Sampaio, G., Cat, I., Stratakis, C.A. & Sandrini, R. (2001) An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. *Proc Natl Acad Sci U S A*, **98**, 9330-9335.
- Robinson, B.G., Hales, I.B., Henniker, A.J., Ho, K., Luttrell, B.M., Smee, I.R. & Stiel, J.N. (1987) The effect of o,p'-DDD on adrenal steroid replacement therapy requirements. *Clin Endocrinol (Oxf)*, **27**, 437-444.
- Ross, N.S. & Aron, D.C. (1990) Hormonal evaluation of the patient with an incidentally discovered adrenal mass. *N Engl J Med*, **323**, 1401-1405.
- Saeger, W. (2000) Histopathological classification of adrenal tumours. *Eur J Clin Invest*, **30**, 58-62.
- Saeger, W., Fassnacht, M., Chita, R., Prager, G., Nies, C., Lorenz, K., Barlehner, E., Simon, D., Niederle, B., Beuschlein, F., Allolio, B. & Reincke, M. (2003) High diagnostic accuracy of adrenal core biopsy: results of the German and Austrian adrenal network multicenter trial in 220 consecutive patients. *Hum Pathol*, **34**, 180-186.
- Samaan, N.A. & Hickey, R.C. (1987) Adrenal cortical carcinoma. *Semin Oncol*, **14**, 292-296.
- Sameshima, Y., Tsunematsu, Y., Watanabe, S., Tsukamoto, T., Kawa-ha, K., Hirata, Y., Mizoguchi, H., Sugimura, T., Terada, M. & Yokota, J. (1992) Detection of novel germ-line p53 mutations in diverse-cancer-prone families identified by selecting patients with childhood adrenocortical carcinoma. *J Natl Cancer Inst*, **84**, 703-707.
- Schlumberger, M., Brugieres, L., Gicquel, C., Travagli, J.P., Droz, J.P. & Parmentier, C. (1991) 5-Fluorouracil, doxorubicin, and cisplatin as treatment for adrenal cortical carcinoma. *Cancer*, **67**, 2997-3000.
- Schteingart, D.E. (2000) Conventional and novel strategies in the treatment of adrenocortical cancer. *Braz J Med Biol Res*, **33**, 1197-1200.

- Schteingart, D.E. & Homan, D. (2001) Management of adrenal cancer in *Adrenal disorders* (ed by A.N. Margioris & G.P. Chrousos) pp 231-248. Humana Press, Totowa, New Jersey.
- Schteingart, D.E., Motazed, A., Noonan, R.A. & Thompson, N.W. (1982) Treatment of adrenal carcinomas. *Arch Surg*, **117**, 1142-1146.
- Schulick, R.D. & Brennan, M.F. (1999a) Adrenocortical carcinoma. *World J Urol*, **17**, 26-34.
- Schulick, R.D. & Brennan, M.F. (1999b) Long-term survival after complete resection and repeat resection in patients with adrenocortical carcinoma. *Ann Surg Oncol*, **6**, 719-726.
- Soreide, J.A., Brabrand, K. & Thoresen, S.O. (1992) Adrenal cortical carcinoma in Norway, 1970-1984. *World J Surg*, **16**, 663-667; discussion 668.
- Sredni, S.T., Zerbini, M.C., Latorre, M.R. & Alves, V.A. (2003) p53 as a prognostic factor in adrenocortical tumors of adults and children. *Braz J Med Biol Res*, **36**, 23-27.
- Stewart, P.M., Walker, B.R., Holder, G., O'Halloran, D. & Shackleton, C.H. (1995) 11 beta-Hydroxysteroid dehydrogenase activity in Cushing's syndrome: explaining the mineralocorticoid excess state of the ectopic adrenocorticotropin syndrome. *J Clin Endocrinol Metab*, **80**, 3617-3620.
- Stojadinovic, A., Ghossein, R.A., Hoos, A., Nissan, A., Marshall, D., Dudas, M., Cordon-Cardo, C., Jaques, D.P. & Brennan, M.F. (2002) Adrenocortical carcinoma: clinical, morphologic, and molecular characterization. *J Clin Oncol*, **20**, 941-950.
- Sullivan, M., Boileau, M. & Hodges, C.V. (1978) Adrenal cortical carcinoma. *J Urol*, **120**, 660-665.
- Szolar, D.H. & Kammerhuber, F.H. (1998) Adrenal adenomas and nonadenomas: assessment of washout at delayed contrast-enhanced CT. *Radiology*, **207**, 369-375.
- Terzolo, M., Ali, A., Osella, G., Reimondo, G., Pia, A., Peretti, P., Paccotti, P. & Angeli, A. (2000a) The value of dehydroepiandrosterone sulfate measurement in the differentiation between benign and malignant adrenal masses. *Eur J Endocrinol*, **142**, 611-617.
- Terzolo, M., Pia, A., Berruti, A., Osella, G., Ali, A., Carbone, V., Testa, E., Dogliotti, L. & Angeli, A. (2000b) Low-dose monitored mitotane treatment achieves the therapeutic range with manageable side effects in patients with adrenocortical cancer. *J Clin Endocrinol Metab*, **85**, 2234-2238.
- van Seters, A.P. & Moolenaar, A.J. (1991) Mitotane increases the blood levels of hormone-binding proteins. *Acta Endocrinol (Copenh)*, **124**, 526-533.

- van Slooten, H., Moolenaar, A.J., van Seters, A.P. & Smeenk, D. (1984) The treatment of adrenocortical carcinoma with o,p'-DDD: prognostic implications of serum level monitoring. *Eur J Cancer Clin Oncol*, **20**, 47-53.
- van Slooten, H., Schaberg, A., Smeenk, D. & Moolenaar, A.J. (1985) Morphologic characteristics of benign and malignant adrenocortical tumors. *Cancer*, **55**, 766-773.
- van Slooten, H. & van Oosterom, A.T. (1983) CAP (cyclophosphamide, doxorubicin, and cisplatin) regimen in adrenal cortical carcinoma. *Cancer Treat Rep*, **67**, 377-379.
- Vassilopoulou-Sellin, R., Guinee, V.F., Klein, M.J., Taylor, S.H., Hess, K.R., Schultz, P.N. & Samaan, N.A. (1993) Impact of adjuvant mitotane on the clinical course of patients with adrenocortical cancer. *Cancer*, **71**, 3119-3123.
- Vassilopoulou-Sellin, R. & Schultz, P.N. (2001) Adrenocortical carcinoma. Clinical outcome at the end of the 20th century. *Cancer*, **92**, 1113-1121.
- Venkatesh, S., Hickey, R.C., Sellin, R.V., Fernandez, J.F. & Samaan, N.A. (1989) Adrenal cortical carcinoma. *Cancer*, **64**, 765-769.
- Wachenfeld, C., Beuschlein, F., Zwermann, O., Mora, P., Fassnacht, M., Allolio, B. & Reincke, M. (2001) Discerning malignancy in adrenocortical tumors: are molecular markers useful? *Eur J Endocrinol*, **145**, 335-341.
- Wagner, J., Portwine, C., Rabin, K., Leclerc, J.M., Narod, S.A. & Malkin, D. (1994) High frequency of germline p53 mutations in childhood adrenocortical cancer. *J Natl Cancer Inst*, **86**, 1707-1710.
- Wajchenberg, B., Albergaria, P.M., Medonca, B., Latronico, A., Campos, C.P., Ferreira, A.V., Zerbini, M., Liberman, B., Carlos, G.G. & Kirschner, M. (2000) Adrenocortical carcinoma: clinical and laboratory observations. *Cancer*, **88**, 711-736.
- Weber, M.M., Fottner, C. & Wolf, E. (2000) The role of the insulin-like growth factor system in adrenocortical tumorigenesis. *Eur J Clin Invest*, **30 Suppl 3**, 69-75.
- Weiss, L.M. (1984) Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. *Am J Surg Pathol*, **8**, 163-169.
- Weiss, L.M., Medeiros, L.J. & Vickery, A.L., Jr. (1989) Pathologic features of prognostic significance in adrenocortical carcinoma. *Am J Surg Pathol*, **13**, 202-206.
- Williamson, S.K., Lew, D., Miller, G.J., Balcerzak, S.P., Baker, L.H. & Crawford, E.D. (2000) Phase II evaluation of cisplatin and etoposide followed by mitotane at disease progression in patients with locally advanced or metastatic adrenocortical carcinoma: a Southwest Oncology Group Study. *Cancer*, **88**, 1159-1165.

- Wood, B.J., Abraham, J., Hvizda, J.L., Alexander, H.R. & Fojo, T. (2003) Radiofrequency ablation of adrenal tumors and adrenocortical carcinoma metastases. *Cancer*, **97**, 554-560.
- Wooten, M.D. & King, D.K. (1993) Adrenal cortical carcinoma. Epidemiology and treatment with mitotane and a review of the literature. *Cancer*, **72**, 3145-3155.
- Yun, M., Kim, W., Alnafisi, N., Lacorte, L., Jang, S. & Alavi, A. (2001) 18F-FDG PET in characterizing adrenal lesions detected on CT or MRI. *J Nucl Med*, **42**, 1795-1799.
- Zografos, G.C., Driscoll, D.L., Karakousis, C.P. & Huben, R.P. (1994) Adrenal adenocarcinoma: a review of 53 cases. *J Surg Oncol*, **55**, 160-164.
- Zwermann, O., Beuschlein, F., Ortmann, D. & Reincke, M. (2003) ACTH inhibits proliferation of adrenal tumors in vivo. *Exp Clin Endocrinol Diabetes*, **111**, V34.

Table 1:

Hormonal work up in suspected ACC	
•	low dose dexamethasone suppression test; 24h urinary free cortisol excretion; CRH-test (only in subclinical Cushing's syndrome)
•	baseline serum DHEAS, 17 α -OH progesterone
•	baseline serum 17 β -oestradiol (in men only)
•	baseline serum testosterone, androstendione (in virilizing tumours)
•	24h urinary excretion of 17-ketosteroids
•	random serum aldosterone + plasma renin activity (only in patients with hypokalaemia and hypertension)
•	24h urinary catecholamine excretion or plasma metanephrines (for exclusion of pheochromocytoma)

Table 2: Scores used for diagnosis of adrenocortical carcinoma (adapted from Saeger, *et al* 2003)

Criterion	Degree	Hough, <i>et al</i> 1979	Weiss, <i>et al</i> 1989
nuclear atypia	moderate to strong	0.39	1
mitoses	>5/50 HPF		1
	>10/100 HPF	0.69	
atypical mitoses	present		1
clear cells	<25% volume percentage		1
architecture	diffuse growth pattern	0.92	1
veins	tumour invasion		1
sinus	tumour invasion	0.92 ^a	1
tumour capsule	tumour invasion	0.37	1
necroses	present	0.69	1
fibrous bands	present	1.00	
sum		0.17±0.26 benign	1-3 benign
		1±0.58 indeterminate	≥4 malignant
		2.91±0.9 malignant	

^a vessel invasion

Table 3: Staging of ACC

	Sullivan et al. 1978	Lee et al. 1995
I	T ₁ , N ₀ , M ₀	T ₁ , N ₀ , M ₀
II	T ₂ , N ₀ , M ₀	T ₂ , N ₀ , M ₀
III	T ₃ , N ₀ , M ₀ or T ₁₋₂ , N ₁ , M ₀	T _{3/4} , N ₀₋₁ , M ₀ or T ₁₋₂ , N ₁ , M ₀
IV	T ₄ , N ₀ , M ₀ or T ₃ , N ₁ , M ₀ or T ₁₋₄ , N ₀₋₁ , M ₁	T ₁₋₄ , N ₀₋₁ , M ₁

T₁: tumour < 5cm

T₂: tumour > 5cm

T₃: tumour infiltration locally reaching neighbouring organs

T₄: tumour invasion of neighbouring organs

N1: positive lymphnodes

M1: distant metastasis

Table 4: Localisation of distant metastases in ACC

location	Hutter & Kayhoe 1966 (n=127)	King & Lack 1979 (n=29)	Luton et al. 1990 (n=88)
liver	44%	93%	46%
lung	53%	79%	46%
lymph node	18%	73%	40%
peritoneum	16%	79%	40%
pleura	5%	-	3%
bone	7%	24%	17%
CNS	4%	10%	6%
contralateral adrenal	-	7%	3%
kidney	2%	10%	-

Table 5

Side effects of mitotane

- gastrointestinal (diarrhoea; nausea; anorexia)
 - central nervous system (lethargy; somnolence; ataxia; dizziness; confusion)
 - adrenal insufficiency
 - hepatotoxicity
 - hypercholesterolaemia
 - skin rash
 - decreased platelet aggregation
 - leukopenia
 - gynaecomastia
-

Table 6: Cytotoxic chemotherapy studies in ACC

Cytotoxic agent	Mitotane	n	response			Reference
			CR (n)	PR(n)	total	
D, V, E	+	36	1	4	14 %	(Abraham, <i>et al</i> 2002)
S	+	22	1	7	36%	(Khan, <i>et al</i> 2000)
P, E	-	45	-	5	11 %	(Williamson, <i>et al</i> 2000)
E, D, P	+	28	2	13	54 %	(Berruti, <i>et al</i> 1998)
P, E	+	18	3	3	33 %	(Bonacci, <i>et al</i> 1998)
P, E	-	13	-	6	46 %	(Burgess, <i>et al</i> 1993)
P	+	37	1	10	30 %	(Bukowski, <i>et al</i> 1993)
D	-	16	1	2	19 %	(Decker, <i>et al</i> 1991)
D, P, 5-FU	-	13	1	2	23 %	(Schlumberger, <i>et al</i> 1991)
C, D, P	-	11	-	2	18 %	(van Slooten and van Oosterom 1983)
		239	10	54	27 %	

D: doxorubicin, E etoposid, 5-FU 5-fluorouracil, C cyclophosphamide, V vincristin, S streptozocin, P cisplatin

CR complete response

PR partial response

Legends

Figure 1:

A) T2-weighted SE (Spin-Echo)-sequence of a 1 year-old boy with adrenal carcinoma: well defined inhomogenous tumor with cystic areas (arrows) representing necrosis.

B) Corresponding T1-weighted SE image after contrast administration: inhomogenous contrast enhancement with tumor necrosis (arrows).

Figure 2: Proposed flow chart for adrenocortical carcinoma (ACC)*

- a) follow up intervals may increase with duration of remission
- b) drug monitoring is important. aim at mitotane levels > 14 mg/l and < 20 mg/l
- c) see table 6

* It is important to note that for all of the proposed therapeutic interventions results from randomised phase III trials are lacking

Figure 1A



Figure 1B

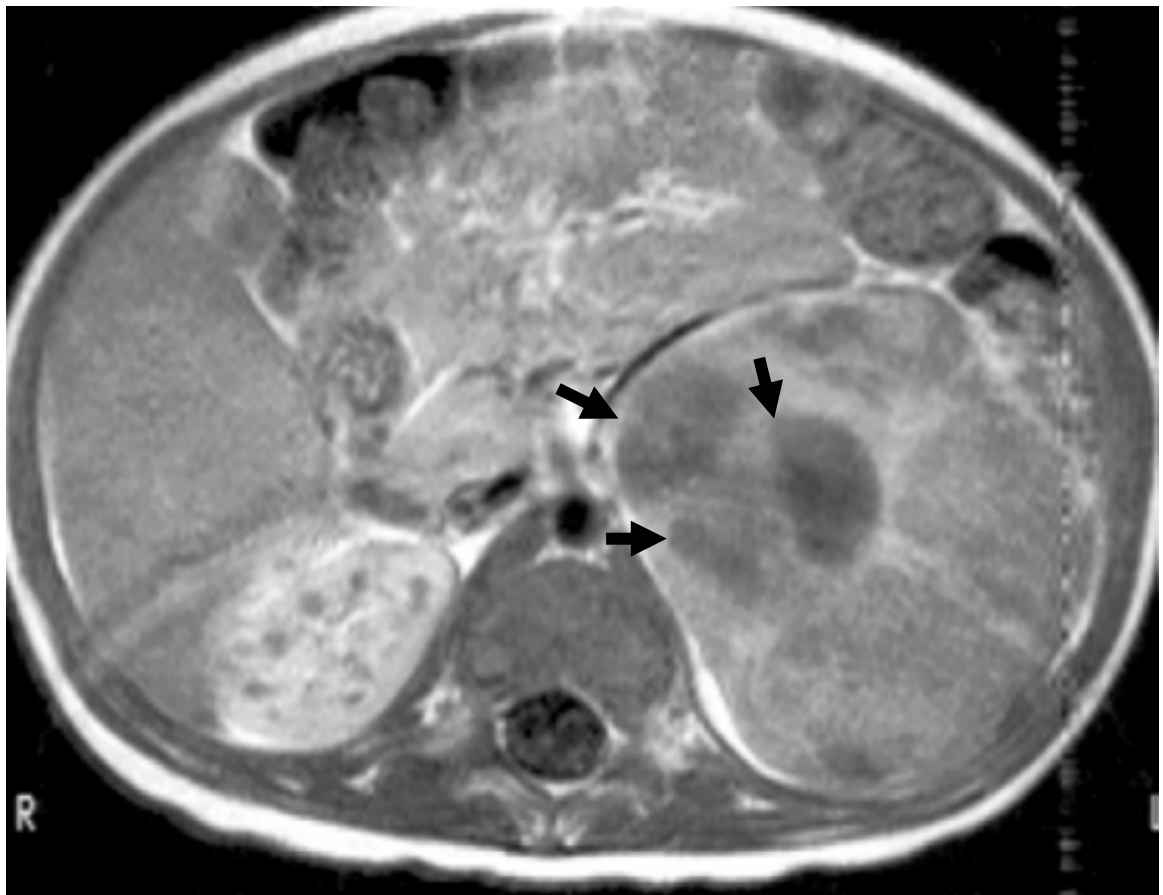


Figure 2

