Chapter 13
Adrenal Cortical Carcinoma: Mitotane and Beyond

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Introduction

Adrenocortical carcinoma (ACC) is a rare endocrine tumor characterized by a poor prognosis as the 5-year survival rate after diagnosis is less than 40% [1–3]. A limited range of therapeutic options is available for ACC: its rarity and aggressiveness have concurred to hamper progress in the development of treatment beyond surgery. In this grim scenario, mitotane remains a cornerstone in the management of patients with ACC. More than 50 years have passed since the introduction of mitotane in clinical practice; however, we still have many uncertainties on how to use this old drug and what we may expect in terms of activity [4]. Mitotane is currently used both in a postoperative adjuvant setting and in advanced disease. However, no data from randomized prospective trials are available to guide management.

Mechanism of Action of Mitotane

Mitotane, [1,1-dichlorodiphenildichloroethane (o,p'-DDD)], a parent compound of the insecticide dichlorodiphenyltrichloroethane (DDT), has been widely employed to treat ACC [1–3]. Mitotane has a profound effect on steroidogenesis [5, 6], but the specific mechanisms are not fully understood. The effect on adrenal steroidogenesis has been associated with the inhibition of a number of

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mitochondrial cytochrome P450-dependent enzymes: cholesterol side chain cleavage (CYP11A1), 11β-hydroxylase (CYP11B1), and 18β-hydroxylase (CYP11B2) [7, 8], as well as P450-independent enzymes, such as 3 β-hydroxysteroid-dehydrogenase [9]. Lin et al. [10] explored the effect of non-cytotoxic concentrations of mitotane on cortisol production by an immortalized clone of human ACC cell line (National Cancer Institute-Human 295 [NCI-H295] cells) and found that mitotane interferes with gene transcription of a number of steroidogenic enzymes. Steroidogenic acute regulatory (protein) (StAR) and CYP11A1, which are involved in the rate-limiting step of steroidogenesis, are most sensitive to mitotane, although at drug concentrations close to the therapeutic range (20–40 μM, i.e., 6.4–12.8 mg/L) (Fig. 13.1). Mitotane effect on 11β-hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) was biphasic, more stimulatory than inhibitory, contradicting early reports of a strong suppression of CYP11B1 activity [11]. These data are conflicting but may concur to explain why aldosterone synthesis is less affected than other steroid pathways. The anti-steroidogenetic effect of mitotane was also recently evaluated by van Koetsveld et al. [12], who investigated the effect of mitotane and interferon β in primary cultures of ACC and found that both drugs strongly inhibited mRNA expression of StAR, CYP11A1, 17α-hydroxylase (CYP17A1), and CYP11B1. Combination of mitotane and interferon β induced an additive inhibitory effect on cellular DNA number and cortisol secretion, suggesting that treatment with interferon β may increase sensitivity of ACC cells to mitotane. Lehmann et al. [13] studied the effect of a 24-h mitotane treatment on NCI-H295R cell viability and expression of genes involved in adrenal steroidosynthesis. It was found that mitotane markedly inhibited expression of genes coding for enzymes involved in generation of cortisol and dehydroepiandrosterone sulfate (CYP11A1 and CYP17A1). Moreover, mitotane reduced viability of NCI-H295R cells inducing cell apoptosis triggered by increased caspase 3 and caspase 7 activities. The mitotane-induced repression of genes of the steroidogenetic pathway has been confirmed by another study in the same cell line [14]. Chortis et al. [15] studied the steroid inhibitory effect of mitotane in vivo, using a novel steroidobolomic approach, to analyze 24-h urine samples from ACC patients receiving mitotane for adjuvant treatment or metastatic disease. It was found that mitotane downregulated the initial steps of steroidogenesis but did not influence CYP11B1 activity. As previously discussed, in vitro data are controversial about the mitotane effect on this enzymatic step. Moreover, mitotane was found to be a strong inducer of CYP3A4 activity leading to glucocorticoid inactivation and a consequent sharp rise in 6β-hydroxycortisol urinary excretion. It was calculated that mitotane is able to inactivate 50% of administered hydrocortisone, and this explains why patients on mitotane have an increased dose requirement of steroid replacement. Finally, mitotane proved to be a strong inhibitor of 5α-reductase activity, and this effect prompts to use 5α-dihydrotestosterone as androgen substitution in mitotane-treated men. An important mitotane-induced derangement of cortisol and testosterone metabolism has been also shown in a similar study [16]. To evaluate which are the intracellular targets of mitotane, Poli et al. [17] performed electron microscopy on human ACC H295R and SW13 cell lines. Increasing concentrations of mitotane

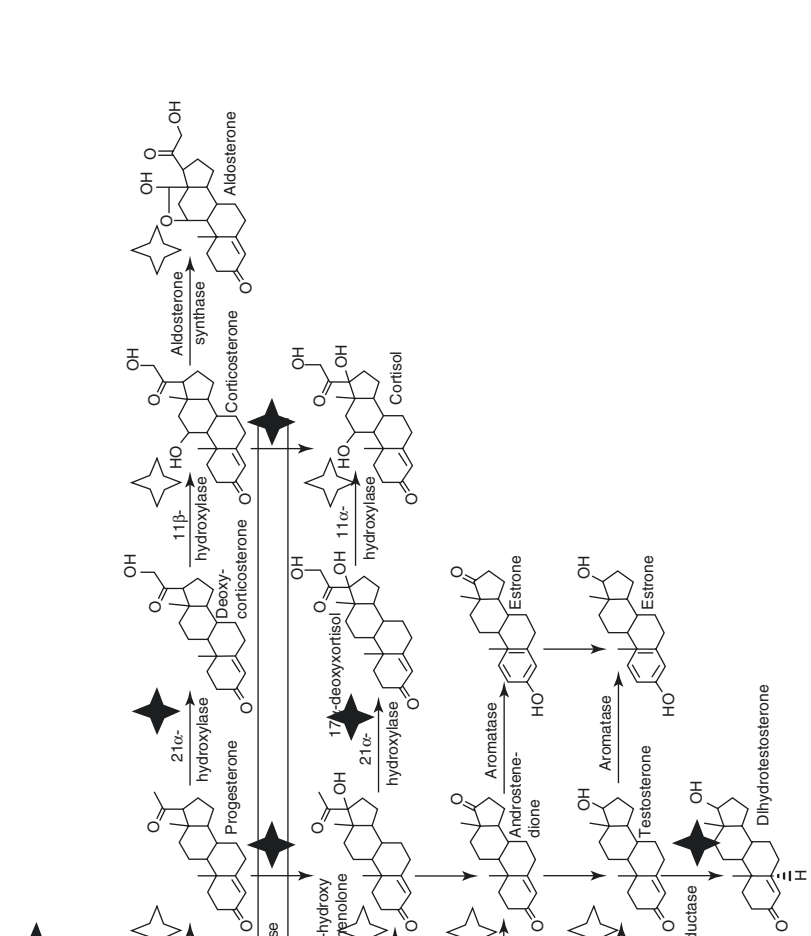

Fig. 13.1 Effects of mitotane on mRNA expression of several steroidogenic enzymes. Black diamonds identify a definitive inhibitory effect, whereas diamonds identify mixed effects

caused marked alterations in the morphology of mitochondria in a dose- and time-dependent manner. Mitochondria were finally disrupted leading to a drastic reduction of cell oxygen consumption. Mitotane was converted by the mitotane-sensitive H295R cells in its active metabolites and exerted cytostatic and cytotoxic effects at doses corresponding to the therapeutic window (30–50 μM, i.e., 9.6–16 mg/L). This study showed that mitotane effects seem to be mainly mediated by the mitochondria damage that activates an apoptotic process involving caspase 3 and caspase 7. Further data showing that mitotane affects mitochondrial function have been reported by Hescot et al. [18]. In H295R and SW13 cell lines, mitotane inhibited cell proliferation in a dose- and a time-dependent manner and suppressed cortisol and 17-hydroxyprogesterone through inhibition of a number of genes involved in steroidogenesis (StAR, CYP11A1, HSD3B2, CYP11B1, and CYP11B2). Mitotane hampered the mitochondrial respiratory chain function complex IV (cytochrome c oxidase), and this was accompanied by enhanced mitochondrial mass, as a compensatory mechanism in response to the respiratory chain defect. Furthermore, mitotane induced morphologic fragmentation of the mitochondrial membranes that are required for respiratory chain activity and presumably steroidogenesis.

More recently, Sbiera et al. [19] demonstrated that mitotane is an inhibitor of sterol-O-acyl-transferase 1 (SOAT1) leading to accumulation of free cholesterol at toxic levels for the cell. The fact that SOAT1 is predominantly expressed by the adrenals confers the specificity of action to mitotane. By inhibiting SOAT1, mitotane downregulates steroidogenesis and exerts its cytotoxic effect due to lipid-induced endoplasmic reticulum stress. In a small number of ACC tissues, SOAT1 expression correlated with the response to mitotane treatment, i.e., low SOAT1 expression was associated with poor response. Targeting cancer-specific lipid metabolism can then open new avenues for treatment of ACC. We should pay attention to potential drug binding, since mitotane is a lipophilic drug that accumulates in lipoproteins and induces dyslipidemia (hypercholesterolemia and/or hypertriglyceridemia). Previous studies suggested that the lipoprotein profile may influence mitotane drug distribution [20]. Moreover, high plasma mitotane levels have been described in dyslipidemic patients who did not exhibit any side effect, suggesting either methodological issues, or that plasma mitotane distribution in lipoprotein subtypes is a major determinant of its distribution in tissues [21]. Indeed, Hescot et al. recently reported that plasma mitotane levels were correlated with o,p′-DDD measured in HDL and LDL fractions [22], and in a subsequent case report, they showed the case of an ACC patient with severe dyslipidemia and very high levels of plasma mitotane but without any neurological side effects [23]. They demonstrated that dyslipidemia causes an overestimation of plasma mitotane levels explained by a so-called matrix effect. On this basis, only lipoprotein-free mitotane should be considered the therapeutically active fraction. This concept has been confirmed in vitro by Kroiss et al. [24] by means of demonstration of activity of mitotane inhibited by lipoprotein binding. However, measurement of lipoprotein-free mitotane levels has still to enter clinical practice even if the methodology is not technically demanding.

Mitotane in the Adjuvant Setting

The main predictor of outcome for ACC patients is the possibility of a radical surgery; still, fully half of the tumors that have been completely extirpated are doomed to relapse [25–30]. Since even stages I–II tumors recur frequently, surgical failure cannot be the only reason. Several potential predictive factors of recurrence in radically resected ACC have been identified [31, 32], but the issue of defining prognostic factors is complicated by the great variability of clinical presentation and biological heterogeneity of ACC. A so high recurrence rate has prompted to consider the use of systemic adjuvant therapy following ACC removal. However, the literature is conflicting for a variety of reasons (Table 13.1). First, most studies [26, 33, 38, 45] had limited statistical power. Second, many studies [26, 29, 37–39, 45] did not include a concomitant matched control group of untreated patients, whereas in some series a number of patients underwent multiple adjuvant treatments [28]. In addition, the definition of recurrence-free survival (RFS) has not been uniform, and the duration of response has been sometimes unclear. Finally, all studies but one [39] were retrospective and employed different formulations of mitotane at doses ranging from 3 to 20 g daily, which were given for different times.

Table 13.1 Outcome of adjuvant mitotane treatment

References	Patients treated with mitotane	Outcome
Schteingart [29]	4	Mean survival of 74 ± 33 months in patients who received adjuvant MIT. No control group
Venkatesh et al. [30]	7	After 1–4 years from surgery, 6/7 patients treated with adjuvant MIT are still alive. No control group
Bodie et al. [33]	21	No difference in survival between patients with or without ($n = 25$) adjuvant MIT. No information on DFS is given
Pommier and Brennan [28]	7	Mean DFS was 2.4 years for 10 patients treated adjuvantly (MIT in 7 and radiotherapy in 3 patients) and 2.5 years for 43 untreated patients (NS)
Vassilopoulou-Sellin et al. [34]	8	Median DFS was 10 months for the patients treated with adjuvant MIT vs 23 months for 6 untreated patients ($P < 0.01$). MIT was discontinued early in 5 patients for toxicity
Haak et al. [35]	11	Median survival of the patients treated with adjuvant MIT was 51 vs 61 months for untreated patients ($n = 15$) (NS). Six patients had MIT levels >14 mg/L
Barzon et al. [36]	7	Median DFS of 8 months in the patients treated with adjuvant MIT vs 13 months for untreated patients ($n = 11$) (NS). Nevertheless, 5/7 patients in MIT group are disease-free at the last follow-up (range 5–54 months), in contrast to 3/11 in the control group

(continued)

Table 13.1 (continued)

References	Patients treated with mitotane	Outcome
Dickstein et al. [37]	4	DFS ranged 18–68 months. No control group
Kasperlik-Zaluska et al. [38][a]	55	At the last follow-up, 18/32 (56%) patients treated immediately after surgery are alive vs 6/27 (22%) patients treated with delay. Only 1/8 (12%) untreated patient is surviving. Adjuvant MIT was given irrespective of staging and completeness of surgery
Icard et al. [26][b]	83	Adjuvant MIT did not have an independent effect on survival. It is not reported whether the patients in MIT group had comparable prognostic factors with the untreated patients. No information on DFS is given
Baudin et al. [39]	11	Recurrence developed in 8 patients within 1 year; 6 of them had MIT levels >14 mg/L. No control group
Terzolo et al. [40]	47	Increased risk of recurrence in two concomitant control groups of untreated patients (group 2, $n = 55$ and group 3, $n = 75$) compared to the MIT group (group 1): group 2 vs group 1, HR 3.79 (2.77–6.32); group 3 vs group 1, HR 2.93 (1.74–4.94); $P < 0.001$ at multivariable analysis Increased risk of death in group 2 vs group 1 (HR 2.47, 1.26–4.85) and group 3 vs group 1 (HR 1.96, 1.00–3.87); $P = 0.03$ at multivariable analysis
Grubbs et al. [41]	22	Increased risk of recurrence in the control group of untreated patients ($n = 196$) than in the MIT group: HR 1.95 (1.06–3.59); $P = 0.03$ at multivariable analysis
Fassnacht et al. [42]	35	Reduced risk of death in the MIT group than in the control group of untreated patients ($n = 114$): HR 0.38 (0.12–1.28); $P = 0.11$ at multivariable analysis
Wangberg et al. [43]	37	Reduced risk of death in the high-level MIT group ($n = 24$) than in the low-level MIT group ($n = 13$): HR 0.25 (0.06–1.00); $P = 0.049$ at Poisson regression
Else et al. [44]	105	Reduced risk of recurrence in the MIT group than in the control group ($n = 159$): HR 0.72 (0.53–0.98); $P = 0.037$ at multivariable analysis

DFS disease-free survival, *MIT* mitotane, *NS* not significant, *HR* hazard ratio
[a]The study includes the patients reported previously by Kasperlik-Zaluska et al. [45]
[b]The study includes the patients reported previously by Icard et al. [46]

Mitotane has a narrow therapeutic index [3, 35, 39] and can cause significant toxicity; thus, it is not an ideal drug to treat patients free of disease. This concept coupled with a limited evidence of efficacy in the literature [26, 28, 33–36, 39] made adjunctive treatment with mitotane less appealing until the last 10 years. As a matter of fact, no recommendation in favor or against adjuvant treatment was formulated at a consensus conference on ACC held at Ann Arbor, Michigan, USA, in

2003 [47]. In 2007, however, we published a retrospective analysis involving a large cohort of ACC patients, followed for up to 10 years at different institutions in Italy and Germany which challenged this view [40]. In that study, adjuvant mitotane was given to 47 Italian patients after radical surgery, and RFS in these patients was compared with that of two concomitant, independent groups of 55 Italian and 75 German patients who were left without any postsurgical treatment. RFS (the primary outcome of the study) was significantly prolonged in the mitotane group (42 months), as compared with the two groups of untreated patients (10 and 25 months, respectively) who had a significantly higher recurrence rate than those receiving mitotane. The mitotane group and the Italian control group were highly comparable for the clinical characteristics known to affect outcome, whereas the control group from Germany had better prognostic factors making mitotane effects even more impressive. Indeed, multivariate analysis confirmed that mitotane treatment gave a significant advantage for RFS. The benefit on OS was less evident, although being significant after adjusting for the difference in prognostic factors [40]. An important finding of the study is that a favorable effect was achieved with low doses of mitotane (1–5 g per day), which were associated with an acceptable toxicity [40]. Conversely, severe and disabling toxicity was observed in the previous series employing high doses of mitotane [28, 34].

Following publication of our study, Bertherat et al. [48] reported that in a cohort of 166 patients, mitotane use following complete tumor removal was not associated with any improvement in DFS. Since mitotane was given to only half of the patients referred to the authors' institution, a selection bias may be anticipated, implying that patients with unfavorable prognostic factors were selected for adjuvant mitotane treatment. This is a major difference with our study [40], in which the choice to recommend mitotane was made according to a predefined center policy irrespective of patient or tumor characteristics. The predefined treatment assignment and the inclusion of well-matched control groups were considered to be the major advantages of our study as compared with other studies that had less clear treatment assignments and often used historical controls or no controls at all [4]. Bertherat et al. [48] raised also the question whether the efficacy of mitotane may change as a function of the secretory activity of ACC since in a previous report by the same group a beneficial effect of mitotane in patients with Cushing's syndrome was reported [32]. It is biologically plausible that hypercortisolism may contribute to an unfavorable outcome in patients with advanced ACC and complicates management. By instance, susceptibility to infections poses a great challenge to application of chemotherapeutic protocols in patients with severe Cushing. However, a recent multicentric retrospective study showed that cortisol excess portends a worse prognosis also when tumors can be completely removed and Cushing be cured [49]. This implies that the negative prognostic effect of cortisol excess persists after its resolution; it is likely that secreting tumors have some still unknown biological characteristics that confer higher aggressiveness. At present, there is no firm evidence that controlling cortisol excess by employing steroid-inhibiting drugs (i.e., ketoconazole, metyrapone) improves prognosis of affected patients, although this is pursued in clinics.

The retrospective nature of our study, however, does not allow concluding definitively that adjuvant mitotane treatment is beneficial [50]. Arguments against are based on the methodological flaws of the available evidence, toxicity and complexity of mitotane treatment, and lack of factors predicting response to treatment [51]. Following our study, new evidence on the value of adjuvant mitotane has been published [41–43]. A study from the M.D. Anderson Cancer Center claimed that a state-of-the-art surgical approach may provide a similar survival to surgery plus adjuvant mitotane, but the lack of adjuvant mitotane treatment was a factor predicting a higher risk of recurrence [42]. Moreover, patients treated with adjuvant mitotane showed significantly better RFS even if they were mostly treated by less experienced surgeons in the community [41]. Fassnacht and colleagues [42] found that survival was improved in patients with stage II ACC who were managed by a specialized center early after surgery compared to patients who were referred at a larger stage, usually after tumor recurrence. Adjuvant mitotane was more frequently used in the first group and was associated with a survival advantage [42]. Wangberg and colleagues [43], reviewing their experience with ACC, showed that an aggressive surgical approach was associated with a satisfactory disease-specific survival. The benefit of mitotane was evident for patients with high-stage ACC and circulating drug levels >14 mg/L [43].

The availability of mitotane measurement across Europe, as a free service offered by the company distributing mitotane (info@lysodren-europe.com), gives the possibility to guide dose adjustments and to prevent severe toxicity. Mitotane monitoring is key for an appropriate management of adjuvant treatment giving the possibility to guide dose adjustments and target mitotane concentrations that have been associated with therapeutic effect. Results of our group demonstrated that plasma mitotane concentrations matter also for patient outcome in adjuvant setting [52]. We did a retrospective analysis of 122 ACC patients who were radically operated on and then treated adjuvantly with a monitored mitotane treatment, targeting concentrations of 14–20 mg/L. The concentrations were attained and maintained during a median follow-up of 36 months in only 63 patients (52%). These patients showed a prolonged RFS compared with the remainders [hazard ratio of recurrence 0.497, $P < 0.01$], while a nonsignificant increase in OS was observed (hazard ratio of death, 0.511, $P = 0.06$). The rather limited duration of follow-up and the low number of events may explain why OS was not significantly changed. Mitotane concentration of 14 mg/L, or higher, was a predictor of RFS in multivariable analysis, and this finding supports the strategy of targeting a cutoff value of 14 mg/L when giving mitotane for adjunctive purposes, which was previously recommended on an expert opinion basis [5, 53–55]. However, the study also demonstrated that maintaining mitotane concentrations at target for a long time is a difficult task requiring firm commitment by both patients and physicians. The patients included in this study were treated with different dosing regimens of mitotane, according to the policies at each center. However, there was no difference between low-dose and high-dose regimens in the probability of reaching the target concentrations after 3 months of treatment, suggesting that individual factors may be as important as pharmacologic ones [52]. Treatment-related toxicity was overall acceptable and manageable with

temporary treatment discontinuation or dose reduction. Although a retrospective analysis may underestimate adverse events, it is likely that the monitoring of mitotane concentrations contributed to limit severe unwanted effects, which may be linked to circulating mitotane levels exceeding 20 mg/L [5, 53–55].

Very recently, a retrospective analysis at the University of Michigan reported on 389 patients followed from 1979 to 2013, of whom 105 patients treated postoperatively with mitotane [44]. Despite the fact that the adjuvant group had a worse risk profile than the control group, mitotane treatment was associated with a significantly improved RFS (hazard ratio 0.7, $P < 0.05$). However, treatment failed to prolong significantly OS. The lack of effect on OS may be due to the relatively short follow-up duration (25.6 months in the overall series). Despite the usual limits of being a retrospective analysis, this study has the merit of including a large cohort of well-characterized patients from a single center. Lacking data from controlled prospective trials, the results of this study add further evidence in favor of the use of mitotane in an adjuvant setting.

Controversy on adjuvant mitotane is deemed to continue unless results of prospective controlled studies become available. Therefore, we have launched the first randomized trial in an adjuvant setting for ACC, the ADIUVO study (http://www.adiuvo-trial.org) under the endorsement of the European Network for the Study of Adrenal Tumors (ENS@T). The study's aim is to assess the efficacy of adjuvant mitotane treatment in prolonging RFS in ACC patients at low-intermediate risk of recurrence. Results of ADIUVO may not be expected before 2019.

Practical Guidelines to Adjunctive Mitotane Therapy

At San Luigi Hospital, we advise to start adjunctive mitotane treatment as soon as possible after surgery, at the very last within 3 months, in patients at high risk of recurrence, while the remainders are encouraged to enter the ADIUVO trial. Although our capability of predicting future risk of ACC recurrence after radical surgery and death is limited, it is generally agreed that stages III–IV ACC, margin-positive resection, and an elevated mitotic index are all factors portending an unfavorable prognosis [56]. Stage IV ACC may be susceptible to complete removal of the primary and metastatic tumor sites, but this condition may be assimilated to a margin-positive resection. Even if solid evidence is lacking, it is usually thought that these patients with stage IV tumors require postoperative medical treatment [57]. An elevated mitotic index is increasingly recognized as a negative prognostic factor, and studies showed that cutoff values of 10% for Ki-67 or nine mitoses per high-power microscopic field were able to categorize patients at high risk of recurrence [57, 58].

We do not institute mitotane therapy before surgery, as advocated by Dickstein et al. [59]. In our practice, we use a low-dose regimen (starting dose of 1 g daily with daily increments of 0.5 g every 4 days until the maximal tolerated dose, usually less ≤6 g/daily), because it is better tolerated with less impact on the quality of life

of the patients. In some centers, however, mitotane is currently administered at high, rapidly escalating doses (up to 6–9 g daily) [60]. Although a high-dose regimen is able to provide therapeutic plasma concentrations of mitotane within 1 month in about one-third of the patients [21], we are more cautious with dose escalation. A high-dose regimen requires an intensive follow-up, combining clinical and mitotane level monitoring, and may be more frequently associated with side effects, while our schedule is better tolerated.

The most common unwanted effects are gastrointestinal manifestations that appear early, independently on mitotane levels [60]. They can be managed with temporary dose reduction, or delay of dose increments, and supportive therapy. Elevated c-glutamyltransferase levels are also frequently observed but are not actually troublesome unless values are exceedingly elevated. Clinically significant liver toxicity is characterized by a marked increase in transaminases and bilirubin but is infrequently observed in the absence of predisposing conditions [3, 7]. Central neurologic toxicity (cerebellar symptoms, disturbed cognitive performance) is more closely associated with elevated mitotane concentrations (20 mg/L), but subtler symptoms, such as memory impairment or attention deficit, may be observed in some patients even when they are exposed to lower drug concentrations [7, 38, 39]. A great individual variability in the susceptibility to mitotane-related unwanted effects is apparent for causes that are still unknown. A general measure to deal with mitotane toxicity is a step-down to the previously tolerated dose or temporary drug withdrawal in the event of severe manifestations. However, well-informed and motivated patients are able to cope with side effects and maintain compliance to treatment [3, 61]. To accomplish this task, it is important to establish a close patient-physician relationship to induce and maintain adherence to treatment. Patients seek advice frequently, also because their local physicians are unfamiliar with mitotane use and its attendant complications, and it is necessary to give a timely counseling to keep patients on treatment.

Because of the adrenolytic effect of mitotane, all patients should receive glucocorticoid replacement to prevent adrenal insufficiency. Steroid doses are typically higher than in Addison's disease, due to an enhanced metabolic clearance rate of glucocorticoids induced by mitotane [3, 61, 62]. An inadequate treatment of adrenal insufficiency increases mitotane-related toxicity, particularly gastrointestinal side effects, and reduces tolerance [3, 38, 54]. Mineralocorticoid supplementation is not mandatory in all patients because the zona glomerulosa is partly spared by the toxic effect of mitotane [3, 54]. This may be also the result of the biphasic action of mitotane on aldosterone synthase, as previously mentioned. Moreover, mitotane affects thyroid and gonadal function in a complex way by mechanisms that are still to be completely elucidated. Mitotane administration is associated with low FT4 levels without a compensatory rise in TSH, an effect that becomes apparent early in the course of treatment. This prompts thyroxin replacement, even if the benefit of this measure is difficult to appreciate [54, 61]. In women, gonadal function is usually preserved, and most female patients have regular cycles unless PRL levels are significantly increased [54, 57, 61] due to a weak estrogen-like action of mitotane [63]. Conversely, in men mitotane treatment causes sexual dysfunction as a late but com-

mon unwanted effect, due to inhibition of testosterone secretion. Sex steroid replacement may become necessary to treat hypogonadism in some patients but may worsen gynecomastia [54, 57, 61].

The optimal duration of therapy remains undefined. The time to first recurrence after complete tumor resection is highly variable from some months to more than 10 years, but most recurrences occur within 2 years of primary surgery [1–3, 28, 31, 54, 57]. In our own series, about 70% of relapses took place in the first 2 years of follow-up, whereas the frequency of late (>5 year) relapses was less than 1% [40]. It is our current practice to accommodate patient preferences between a range of possibilities (2, 5, or even more years of therapy) in a shared decision-making depending on tumor and patient characteristics. However, we are eager to prolong treatment if well tolerated in patients at elevated risk.

Selection of Patients to Adjuvant Mitotane

Despite the limits of the available evidence, adjuvant mitotane therapy is currently recommended in many expert centers whenever the patients present an elevated risk of recurrence. Differences do exist in the criteria used to define a high-risk condition, as exemplified in a recent position of an international panel of experts who agreed on stages I–II, complete (R0) resection, and ki-67 < 10% as markers of good prognosis, but a consensus was not found on stage III R0 ACC [56]. In patients with good prognostic markers, the decision on adjuvant mitotane therapy may be individualized, whereas adjuvant mitotane is mandatory in the high-risk category [56]. Following the ENS@T ACC staging system, stage III applies to locally invasive tumors characterized by infiltration in surrounding tissue, positive regional lymph nodes, or a neoplastic thrombus in the vena cava or vena renalis [64]. It is biologically plausible that tumor spread in regional lymph nodes or in the vein system may portend to a higher risk of recurrence than local infiltration, and it is our opinion that subgroups at different risk of recurrence do exist among stage III ACC. Infrequently, a stage IV ACC, defined by presence of distant metastases [64], may be completely resected and has to be considered at a high risk of recurrence. The lowest risk applies to stage I and II ACC, being tumors localized in the adrenal gland with a size of ≤5 cm or >5 cm, respectively [64]. Recent data suggest that the proliferation activity of the tumor is the most important factor predicting risk of recurrence following R0 surgery. Assessment of the proliferation index Ki-67 is currently used to assess proliferation, despite some problems to harmonize immunohistochemical readings among different pathologists. In a European multicentric study, a threshold value at 10% was found to separate patients at good or worse prognosis with a hazard ratio of recurrence of 1.042 per each % increase [65]. Although the results of this study have still to be considered as preliminary, the availability of a large patient cohort totaling more than 500 patients represents a solid database to confirm the view that tumor proliferation is a strong determinant of patient survival. The value of ACC proliferation has been already appreciated in smaller series by the use of mitosis

count [31, 58], which is likely the single most predictive factor of Weiss score. Conversely, Weiss score as a whole does not clearly indicate the probability of tumor recurrence [58, 66]. Resection status is another established adverse risk factor, being Rx (unknown), R1 (microscopically positive margins), and R2 (macroscopically positive margins) associated with progressively reduced RFS irrespectively of other risk factors [57, 67–72]. A number of molecular markers, like matrix metallo-proteinase type 2 [73], glucose transporter GLUT1 [74], SF1 [75], and BUB1B and PINK1 [76], might potentially emerge in the future as powerful outcome predictors, but none of them has yet found a place in current management of ACC.

It would be interesting to identify a molecular signature that may predict mitotane efficacy. In a study by our group, the ribonucleotide reductase large subunit (RRM1) gene expression was able to predict efficacy of adjuvant mitotane [77]. The RRM1 gene encodes for an enzyme essential for the production of deoxyribonucleotides prior to DNA synthesis in S phase of dividing cells. It is located in an important tumor-suppressor gene region. Alterations in this region have been associated with the Beckwith-Wiedemann syndrome, Wilms tumor, rhabdomyosarcoma, adrenocortical carcinoma, and lung, ovarian, and breast cancer. This gene may play a role in the pathogenesis of such malignancies. High RRM1 gene expression was associated to shorter disease-free survival (DFS) and overall survival at both univariate and multivariate analysis. In patients with low RRM1 gene expression, adjuvant mitotane was associated with improved DFS, whereas this effect was lost in cases with high RMM1 expression. In vitro mitotane induced strong upregulation of RRM1 transcription (up to 25-fold increase) in mitotane-insensitive human ACC cell line SW-13 but not in mitotane-sensitive human ACC cell line H295R cells. Furthermore, RRM1 silencing in SW-13 cells induced sensitivity to mitotane. The efficacy of this marker for predicting response to mitotane still deserves validation in prospective studies.

Mitotane for Advanced Adrenocortical Carcinoma

The management of ACC patients with recurrent and metastatic disease is challenging and the prognosis is often poor. However, ACC is a heterogeneous disease and, a subset of patients bear a less aggressive tumor and may have longer survival perspective, although most patients are destined to die of disease progression within 1–2 years. Several prognostic factors such as time since diagnosis, presence of distant metastases, number of metastatic lesions and number of tumoral organs involved, high mitotic rate (20 per 50 high-power field), and atypical mitoses in the primary tumor have been found to predict survival in patients with metastatic ACC [78, 79]. Two previous reports identified cortisol secretion as a negative prognostic factor in metastatic ACC patients. In a large single-institution French series including 202 patients with different disease stages, cortisol excess was found to be an independent prognostic factor for OS and was predictive of subsequent metastatic disease in the subset of patients with stages I–III [32]. However, the study does not

provide demonstration that treating cortisol excess improves prognosis by itself. In clinical practice, it is difficult to discriminate between the effect of tumor shrinkage and cortisol reduction. Similar results were obtained from a series of 72 Italian patients with metastatic ACC submitted to chemotherapy with EDP (etoposide, doxorubicin, and cisplatin) plus mitotane [80].

The treatment of advanced/metastatic patients includes locoregional approaches such as surgery, radiofrequency ablation (RFA), and chemoembolization in addition to systemic therapies in patients with slowly progressive disease and low metastatic burden. RFA and chemoembolization have been found to be of potential utility in advanced ACC [81, 82], and we are currently using these techniques in association with mitotane in the more favorable clinical setting. In the presence of isolated locoregional recurrence or oligo-metastatic disease, surgery can lead to improved survival [25], so an aggressive surgical approach may be advisable whenever complete resection (R0) can be envisaged. Conversely, tumor debulking offers little benefit and may be considered in patients with functional tumors not controlled by medical treatment.

Mitotane alone or mitotane plus chemotherapy are the currently adopted systemic strategies. Chemotherapy plus mitotane is currently recommended for patients with aggressive disease and multiple metastases. However, in the presence of isolated locoregional recurrence, or metastatic disease involving a limited number of organs, mitotane monotherapy can be a reasonable systemic option. Single agent mitotane is active, and response rates between 13% and 31% have been reported (Table 13.2.). Most of the responses are of limited duration, and complete responses rarely occur. The key concept of mitotane treatment in patients with advanced/meta-

Table 13.2 Outcome of mitotane monotherapy in patients with advanced ACC

References	Daily dose (g)	Patients	OR (no, % and CI)	CR (no, % and CI)	Duration (months)
Retrospective studies					
Henley et al. [84]	NR	24	6 OR (25%, 7–43)	None	3–24
Venkatesh et al. [30]	NR	72	21 OR (29%, 18–40)	None	NA
Luton et al. [85]	3–20	37	5 OR (13%,2–24)	None	5–25
Pommier et al. [28]	NA	29	7 OR (24%, 8–40)	None	NA
Haak et al. [35]	4–8	55	15 OR (27%, 15–39)	8 CR (15%, 5–25)	2–190
Barzon et al. [36]	4–8	11	2 OR (18%, 0–41)	None	40–64
Williamson et al. [86]	4–10	16	2 OR (13%, 0–30)	None	NA
Total		244	58 OR (24%, 18–30)	8 CR (3%, 1–5)	
Prospective studies					
Decker et al. [87]	6	36	8 OR (22%, 8–36)	2 CR (6%, 0–14)	3–82
Baudin et al. [39]	6–12	13	4 OR (33%, 7–59)	1 CR (8%, 0–23)	10–48
Total		49	12 OR (24%, 12–36)	3 CR (6%, 0–13)	

OR overall response, *CR* complete response, *NA* not available, *NR* not retrieved

static disease is that plasma mitotane concentration ranging between 14 and 20 mg/L should be targeted in any patient. It was demonstrated that disease responses are mainly confined in patients attaining and maintaining over time serum levels within this therapeutic range [35, 39]. This concept has been validated more recently in a retrospective series of 91 patients receiving mitotane for unresectable or metastatic ACC [53]. In this study, mitotane level above 14 mg/L was associated with tumor response and better survival irrespective of whether mitotane was administered as monotherapy or in combination with chemotherapy. Besides its antitumor effect, mitotane is a strong inhibitor of adrenal steroidogenesis, and it has a compelling indication in patients with endocrine symptoms, although the rate of success in controlling hormone excess is not well known [57, 67]. Owing to the latency of mitotane to attain the therapeutic range, mitotane monotherapy is indicated in the management of patients with a low tumor burden and/or more indolent disease. For patients whose disease shows an aggressive behavior, cytotoxic chemotherapy is required. Chemotherapy in the management of advanced ACC is usually administered in association with mitotane not only in patients with treatment-naïve disease but also in patients with disease progression to mitotane therapy, when mitotane is usually maintained at the same doses if tolerated. Despite that combining mitotane with classic cytotoxic agents is a commonly used strategy, the evidence supporting a synergism between mitotane and chemotherapy is weak. Mitotane may have a synergistic effect on chemotherapy activity thanks to the ability to reverse multidrug resistance mediated by P-glycoprotein expression. ACC produces high levels of the multidrug resistance protein MDR1 (also known as P-glycoprotein) which functions as an ATP-dependent drug efflux pump, transporting out of the cell hydrophobic cytotoxic agents such as doxorubicin, vinblastine, and paclitaxel. Overcoming MDR gene, mitotane may enhance the cytotoxicity of anthracyclines, etoposide, and taxanes [83, 88] whose activity is hampered by MDR gene expression. However, the effect of mitotane on MDR has been questioned [89]. Indirect comparisons of response rates obtained in non-randomized Phase II trials showed greater activity of chemotherapy regimens, including mitotane, as recently reviewed [90]. However, no randomized study has tested prospectively the efficacy of mitotane plus chemotherapy vs chemotherapy alone.

The first prospective multinational trial on treatment of ACC (FIRM-ACT) ever published has recently set a standard of care for advanced/metastatic ACC [91]. In this trial, the association of etoposide, doxorubicin, and cisplatin plus mitotane (EDP-M) was found to be superior to streptozotocin plus mitotane (SZ-M) in terms of disease response rate and progression-free survival (PFS). On the bases of the results of this study, the EDP-M scheme is actually recommended as the standard approach for ACC patients by international guidelines [70]. The efficacy of EDP-M in this multinational Phase III trial, however, was modest: the response rate was low (23%), and the median PFS and OS were of only 5 and 14.8 months, respectively. The FIRM-ACT trial also provided some evidence that mitotane levels at target could improve patient outcome [91]. Mitotane efficacy is not immediate, and the so-called therapeutic range is usually attained within 2–3 months, so disease progression may precede the time when mitotane levels are at target. Chemotherapy

may be effective in the first weeks of therapy, and this is a pragmatic point favoring a functional synergism between mitotane and chemotherapy in patients with aggressive disease. On the other hand, mitotane may be also important in the long-term disease control. In the randomized trial FIRM-ACT, a few patients were free of progression after 4 years in both EDP-M and SZ-M arms. In these patients, mitotane could have contributed to the long-term delay of disease progression.

Conclusion

Whenever ACC is completely removed, we should face the dilemma to use adjuvant therapy or not. In our opinion, adjuvant mitotane is the preferable approach in most cases, because the majority of patients referred to our institution following adrenalectomy have an elevated risk of recurrent disease. A better understanding of factors that influence prognosis and response to treatment [92, 93] will help in stratifying patients according to their probability of benefiting from adjuvant mitotane, with the aim of sparing unnecessary toxicity to patients who are likely unresponsive. However, until significant advancements take place, we have to deal with uncertainty using our best clinical judgment and personal experience in the clinical decision process. Our current policy, then, is to recommend adjuvant mitotane after extirpation of ACC. Patients at low risk of recurrence (R0, stage I–II, Ki-67 < 10%) are offered to participate in the ADIUVO trial and are randomized between mitotane treatment and observation. A monitored mitotane treatment is followed targeting levels between 14 and 20 mg/L. Our scheme of low-dose mitotane treatment is given in Table 13.3. Minimal duration of treatment for high-risk patients is 2 years, but we strive continuing for 4–5 years in most cases. The strategy of treatment of advanced ACC is chosen considering a number of prognostic factors (tumor burden,

Table 13.3 Practical guidelines for giving low-dose adjuvant mitotane treatment

• Start with 1 g daily and increase mitotane dose every ≈ 4 days up to 6–8 g daily or the maximum tolerated dose. Give mitotane in split doses with meals or snacks
• Accommodate mitotane schedule to patient's tolerance aiming at serum mitotane concentrations of 14–20 mg/L (therapeutic levels)
• Check mitotane levels every 4–8 weeks to adjust dosage until reaching target levels
• At target, clinical assessment, biochemical and hormonal evaluation, and monitoring of mitotane levels every 3–4 months or in case of significant side effects. Adjust mitotane dose according to circulating levels and tolerability
• In case of slight unwanted effects, continue mitotane and treat symptoms (e.g., nausea, diarrhea)
• In case of moderate side effects, step down to the previously tolerated dose and use symptomatic therapy
• In case of severe side effects, discontinue mitotane and institute specific treatment. Duration of treatment stop depends on clinics and mitotane levels. After interruption, restart with a lower dose

type of progression, secretion, proliferation index) and the clinical conditions. If a patient is fit and carries bad prognostic factors, we recommend the polychemotherapy regimen EDP plus mitotane. In case of compromised conditions, platinum plus mitotane is an alternative. Patients at perceived good prognosis may be treated with mitotane monotherapy, and EDP is added on in case of disease progression.

References

1. Wajchenberg BL, Albergaria Pereira MA, Medonca BB, et al. Adrenocortical carcinoma: clinical and laboratory observations. Cancer. 2000;88:711–36.
2. Dackiw AP, Lee JE, Gagel RF, et al. Adrenal cortical carcinoma. World J Surg. 2001;25:914–26.
3. Allolio B, Fassnacht M. Adrenocortical carcinoma: clinical update. J Clin Endocrinol Metab. 2006;60:273–87. A comprehensive review providing an excellent update on the clinical management of ACC
4. Schteingart DE. Adjuvant mitotane therapy of adrenal cancer: use and controversy. N Engl J Med. 2007;356:2415–8.
5. Fassnacht M, Kroiss M, Allolio B. Update in adrenocortical carcinoma. J Clin Endocrinol Metab. 2013;98:4551–64.
6. Daffara F, De Francia S, Reimondo G, et al. Prospective evaluation of mitotane toxicity in adrenocortical cancer patients treated adjuvantly. Endocr Relat Cancer. 2008;15:1043–53.
7. Schteingart DE. Conventional and novel strategies in the treatment of adrenocortical cancer. Br J Med Biol Res. 2000;33:1197–200.
8. Asp V, Ulleras E, Lindstrom V, et al. Biphasic hormonal responses to the adrenocorticolytic DDT metabolite 3-methylsulfonyl-DDE in human cells. Toxicol Appl Pharmacol. 2010;242:281–9.
9. Hart MM, Reagan RL, Adamson RH. The effect of isomers of DDD on the ACTH-induced steroid output, histology and ultrastructure of the dog adrenal cortex. Toxicol Appl Pharmacol. 1973;24:127–59.
10. Lin CW, Chang YH, Pu HF. Mitotane exhibits dual effects on steroidogenic enzymes gene transcription under basal and cAMP-stimulating microenvironments in NCI-H295 cells. Toxicology. 2012;298:14–23.
11. Brown RD, Nicholson WE, Chick WT, et al. Effect of o,p'DDD on human adrenal steroid 11b-hydroxylation activity. J Clin Endocrinol Metab. 1973;36:730–3.
12. van Koetsveld PM, Vitale G, Feelders RA, et al. Interferon-b is a potent inhibitor of cell growth and cortisol production in vitro and sensitizes human adrenocortical carcinoma cells to mitotane. Endocr Relat Cancer. 2013;20:443–54.
13. Lehmann TP, Wrzesinski T, Jagodzinski PP. The effect of mitotane on viability, steroidogenesis and gene expression in NCI-H295R adrenocortical cells. Mol Med Rep. 2013;7:893–900.
14. Zsippai A, Szabò DR, Tombol Z, et al. Effects of mitotane on gene expression in the adrenocortical cell line NCI-H295R: a microarray study. Pharmacogenomics. 2012;13:1351–61.
15. Chortis V, Taylor AE, Schneider P, et al. Mitotane therapy in adrenocortical cancer induces CYP3A4 and inhibits 5a-reductase, explaining the need for personalized glucocorticoid and androgen replacement. J Clin Endocrinol Metab. 2013;98:161–71.
16. Ghataore L, Chakraborti I, Aylwin SJ, et al. Effects of mitotane treatment on human steroid metabolism: implications for patient management. Endocr Connect. 2012;1:37–47.
17. Poli G, Guasti D, Rapizzi E, et al. Morphofunctional effects of mitotane on mitochondria in human adrenocortical cancer cells. Endocr Relat Cancer. 2013;20:537–50.
18. Hescot S, Slama A, Lombès A, et al. Mitotane alters mitochondrial respiratory chain activity by inducing cytochrome c oxidase defect in human adrenocortical cells. Endocr Relat Cancer. 2013;20:371–81.

19. Sbiera S, Leich E, Liebisch G, et al. Mitotane inhibits sterol-o-acyl transferase 1 triggering lipid-mediated endoplasmic reticulum stress and apoptosis in adrenocortical carcinoma cells. Endocrinology. 2015;156:3895–908.
20. Pohland RC, Counsell RE. The role of high density lipoproteins in the biodistribution of two radioiodinated probes in the rat. Toxicol Appl Pharmacol. 1985;77:47–57.
21. Mauclère-Denost S, Leboulleux S, Borget I, et al. High-dose mitotane strategy in adrenocortical carcinoma: prospective analysis of plasma mitotane measurement during the first 3 months of follow-up. Eur J Endocrinol. 2012;166:261–8.
22. Hescot S, Seck A, Guerin M, et al. Lipoprotein-free mitotane exerts high cytotoxic activity in adrenocortical carcinoma. J Clin Endocrinol Metab. 2015;100:2890–8.
23. Paci A, Hescot S, Seck A, et al. Dyslipidemia causes overestimation of plasma mitotane measurements. Endocrinol Diab and Metab. 2016;2016:150135.
24. Kroiss M, Plonné D, Kendl S, et al. Association of mitotane with chylomicrons and serum lipoproteins: practical implications for treatment of adrenocortical carcinoma. Eur J Endocrinol. 2016;174:343–53.
25. Bellantone R, Ferrante A, Boscherini M, et al. Role of reoperation in recurrence of adrenal cortical carcinoma: results from 188 cases collected in the Italian National Registry for Adrenal Cortical Carcinoma. Surgery. 1997;122:1212–8.
26. Icard P, Goudet P, Charpenay C, et al. Adrenocortical carcinomas: surgical trends and results of a 253-patient series from the French Association of Endocrine Surgeons study group. World J Surg. 2001;25:891–7.
27. Schulick RD, Brennan MF. Long-term survival after complete resection and repeat resection in patients with adrenocortical carcinoma. Ann Surg Oncol. 1999;6:719–26.
28. Pommier RF, Brennan MF. An 11-year experience with adrenocortical carcinoma. Surgery. 1992;112:963–70.
29. Schteingart DE, Motazedi A, Noonan RA, Thompson NW. Treatment of adrenal carcinomas. Arch Surg. 1982;117:1142–6.
30. Venkatesh S, Hickey RC, Sellin RV, et al. Adrenal cortical carcinoma. Cancer. 1989;64:765–9.
31. Stojadinovic A, Ghossein RA, Hoos A, et al. Adrenocortical carcinoma: clinical, morphologic, and molecular characterization. J Clin Oncol. 2002;20:941–50.
32. Abiven G, Coste J, Groussin L, et al. Clinical and biological features in the prognosis of adrenocortical cancer: poor outcome of cortisol-secreting tumors in a series of 202 consecutive patients. J Clin Endocrinol Metab. 2006;91:2650–5.
33. Bodie B, Novick AC, Pontes JE, et al. The Cleveland Clinic experience with adrenal cortical carcinoma. J Urol. 1989;141:257–60.
34. Vassilopoulou-Sellin R, Guinee VF, Klein MJ, et al. Impact of adjuvant mitotane on the clinical course of patients with adrenocortical cancer. Cancer. 1993;71:3119–23.
35. Haak HR, Hermans J, van de Velde CJ, et al. Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients. Br J Cancer. 1994;69:947–51.
36. Barzon L, Fallo F, Sonino N, et al. Adrenocortical carcinoma: experience in 45 patients. Oncology. 1997;54:490–6.
37. Dickstein G, Shechner C, Arad E, et al. Is there a role for low doses of mitotane (o,p 0 -DDD) as adjuvant therapy in adrenocortical carcinoma? J Clin Endocrinol Metab. 1998;83:3100–3.
38. Kasperlik-Zaluska AA. Clinical results of the use of mitotane for adrenocortical carcinoma. Braz J Med Biol Res. 2000;33:1191–6.
39. Baudin E, Pellegriti G, Bonnay M, et al. Impact of monitoring plasma 1, 1-dichlorodiphenildi chloroethane (o,p 0 -DDD) levels on the treatment of patients with adrenocortical carcinoma. Cancer. 2001;92:1385–92.
40. Terzolo M, Angeli A, Fassnacht M, et al. Adjuvant mitotane treatment for adrenocortical carcinoma. N Engl J Med. 2007;356:2372–80.
41. Grubbs EG, Callender GG, Yan Xing Y, et al. Recurrence of adrenal cortical carcinoma following resection: surgery alone can achieve results equal to surgery plus mitotane. Ann Surg Oncol. 2010;17:263–70.

42. Fassnacht M, Johanssen S, Fenske W, et al. Improved survival in patients with stage II adrenocortical carcinoma followed up prospectively by specialized centers. J Clin Endocrinol Metab. 2010;95:4925–32.
43. Wängberg B, Khorram-Manesh A, Jansson S, et al. The long-term survival in adrenocortical carcinoma with active surgical management and use of monitored mitotane. Endocr Relat Cancer. 2010;17:265–72.
44. Else T, Williams AR, Sabolch A, et al. Adjuvant therapies, patient and tumor characteristics associated with survival of adult patients with adrenocortical carcinoma. J Clin Endocrinol Metab. 2014;99:455–61.
45. Kasperlik-Zaluska AA, Migdalska BM, Zgliczynski S, et al. Adrenocortical carcinoma. A clinical study and treatment results of 52 patients. Cancer. 1995;75:2587–91.
46. Icard P, Chapuis Y, Andreassian B, et al. Adrenocortical carcinoma in surgically treated patients: a retrospective study on 156 cases by the French Association of Endocrine Surgery. Surgery. 1992;112:972–9.
47. Schteingart DE, Doherty GM, Gauger PG, et al. Management of patients with adrenal cancer: recommendations of an international consensus conference. Endocr Relat Cancer. 2005;12:667–80.
48. Bertherat J, Coste J, Bertagna X. Adjuvant mitotane in adrenocortical carcinoma [letter to the editor]. N Engl J Med. 2007;357:1256–9.
49. Berruti A, Fassnacht M, Haak H, et al. Prognostic role of overt hypercortisolism in completely operated patients with adrenocortical cancer. Eur Urol. 2014;65:832–8.
50. Huang H, Fojo T. Adjuvant mitotane for adrenocortical cancer a recurring controversy. J Clin Endocrinol Metab. 2008;93:3730–2.
51. Veytsman I, Nieman L, Fojo T. Management of endocrine manifestations and the use of mitotane as a chemotherapeutic agent for adrenocortical carcinoma. J Clin Oncol. 2009;27:4619–29.
52. Terzolo M, Baudin AE, Ardito A, et al. Mitotane levels predict the outcome of patients with adrenocortical carcinoma treated adjuvantly following radical resection. Eur J Endocrinol. 2013;169:263–70.
53. Hermsen IG, Fassnacht M, Terzolo M, et al. Plasma concentrations of o,p'DDD, o,p'DDA, and o,p'DDE as predictors of tumor response to mitotane in adrenocortical carcinoma: results of a retrospective ENS@T multicenter study. J Clin Endocrinol Metab. 2011;96:1844–51.
54. Terzolo M, Berruti A. Adjunctive treatment of adrenocortical carcinoma. Curr Opin Endocrinol Diabetes Obes. 2008;15:221–6.
55. Terzolo M, Ardito A, Zaggia B, et al. Management of adjuvant mitotane therapy following resection of adrenal cancer. Endocrine. 2012;42:521–5.
56. Berruti A, Fassnacht M, Baudin E, et al. Adjuvant therapy in patients with adrenocortical carcinoma: a position of an international panel. J Clin Oncol. 2010;28:e401–2.
57. Fassnacht M, Libé R, Kroiss M, et al. Adrenocortical carcinoma: a clinician's update. Nat Rev Endocrinol. 2011;7:323–35.
58. Volante M, Bollito E, Sperone P, et al. Clinicopathological study of a series of 92 adrenocortical carcinomas: from a proposal of simplified diagnostic algorithm to prognostic stratification. Histopathology. 2009;55:535–43.
59. Dickstein G, Shechner C, Natif O. Adjuvant mitotane in adrenocortical carcinoma. letter to the editor. N Engl J Med. 2007;357:1256–7.
60. Faggiano A, Leboulleux S, Young J, et al. Rapidly progressing high o,p'-DDD doses shorten the time required to reach the therapeutic threshold with an acceptable tolerance: preliminary results. Clin Endocrinol. 2006;64:110–3. A study reporting feasibility of a high-dose regimen of mitotane
61. Daffara F, De Francia S, Reimondo G, et al. Prospective evaluation of mitotane toxicity in adrenocortical cancer patients treated adjuvantly. Endocr Relat Cancer. 2008;4:1043–53.
62. Hague RV, May W, Cullen DR. Hepatic microsomal enzyme induction and adrenal crisis due to o,p'-DDD therapy for metastatic adrenocortical carcinoma. Clin Endocrinol. 1989;31:51–7.

63. Nancy Nader N, Raverot G, Emptoz-Bonneton A, et al. Mitotane has an estrogenic effect on sex hormone-binding globulin and corticosteroid-binding globulin in humans. J Clin Endocrinol Metab. 2006;91:2165–70. A study demonstrating an oestrogen-like effect of mitotane
64. Fassnacht M, Johanssen S, Quinkler M, et al. Limited prognostic value of the 2004 international union against cancer staging classification for adrenocortical carcinoma: proposal for a revised TNM classification. Cancer. 2009;115:243–50.
65. Beuschlein F, Obracay J, Saeger W, et al. Prognostic value of histological markers in localized adrenocortical carcinoma after complete resection. Endocr Rev. 2013;34:23–9.
66. Papotti M, Libe R, Duregon E, et al. The Weiss score and beyond-histopathology for adrenocortical carcinoma. Horm Cancer. 2011;2:333–40.
67. Bourdeau I, Mackenzie-Feder J, Lacroix A. Recent advances in adrenocortical carcinoma in adults. Curr Opin Endocrinol Diabetes Obes. 2013;20:192–7.
68. Kebebew E, Reiff E, Duh QY, et al. Extent of disease at presentation and outcome for adrenocortical carcinoma: have we made progress? World J Surg. 2006;30:872–8.
69. Kerkhofs TM, Verhoeven RH, Van der Zwan JM, et al. Adrenocortical carcinoma: a population based study on incidence and survival in the Netherlands since 1993. Eur J Cancer. 2013;49:2579–86.
70. Berruti A, Baudin E, Gelderblom H, et al. Adrenal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. ESMO Guidelines Working Group. Ann Oncol. 2012;23(Suppl 7):VII131–8.
71. Lombardi CP, Raffaelli M, Boniardi M, et al. Adrenocortical carcinoma: effect of hospital volume on patient outcome. Langenbeck's Arch Surg. 2012;397:201–7.
72. Bilimoria KY, Shen WT, Elaraj D, et al. Adrenocortical carcinoma in the United States: treatment utilization and prognostic factors. Cancer. 2008;113:3130–6.
73. Volante M, Sperone P, Bollito E, et al. Matrix metalloproteinase type 2 expression in malignant adrenocortical tumors: diagnostic and prognostic significance in a series of 50 adrenocortical carcinomas. Mod Pathol. 2006;19:1563–9.
74. Fenske W, Volker HU, Adam P, et al. Glucose transporter GLUT1 expression is an stage-independent predictor of clinical outcome in adrenocortical carcinoma. Endocr Relat Cancer. 2009;16:919–28.
75. Duregon E, Volante M, Giorcelli J, et al. Diagnostic and prognostic role of steroidogenic factor 1 in adrenocortical carcinoma: a validation study focusing on clinical and pathologic correlates. Hum Pathol. 2013;44:822–8.
76. De Reynies A, Assie G, Rickman DS, et al. Gene expression profiling reveals a new classification of adrenocortical tumors and identifies molecular predictors of malignancy and survival. J Clin Oncol. 2009;27:1108–15.
77. Volante M, Terzolo M, Fassnacht M, et al. Ribonucleotide reductase large subunit (RRM1) gene expression may predict efficacy of adjuvant mitotane in adrenocortical cancer. Clin Cancer Res. 2012;18:3452–61.
78. Malandrino P, Al Ghuzlan A, Castaing M, et al. Prognostic markers of survival after combined mitotane- and platinum-based chemotherapy in metastatic adrenocortical carcinoma. Endocr Relat Cancer. 2010;17:797–807.
79. Assié G, Antoni G, Tissier F, et al. Prognostic parameters of metastatic adrenocortical carcinoma. J Clin Endocrinol Metab. 2007;92:148–54.
80. Berruti A, Terzolo M, Sperone P, et al. Etoposide, doxorubicin and cisplatin plus mitotane in the treatment of advanced adrenocortical carcinoma: a large prospective phase II trial. Endocr Relat Cancer. 2005;12:657–66.
81. Cazejust J, De Baère T, Auperin A, et al. Transcatheter arterial chemoembolization for liver metastases in patients with adrenocortical carcinoma. J Vasc Interv Radiol. 2010;21:1527–32.
82. Bates SE, Shieh CY, Mickley LA, et al. Mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (mdr-1/P-glycoprotein) which is also expressed by adrenocortical carcinomas. J Clin Endocrinol Metab. 1991;73:18–29.

83. Henley DJ, van Heerden JA, Grant CS, et al. Adrenal cortical carcinoma—a continuing challenge. Surgery. 1983;94:926–31.
84. Luton JP, Cerdas S, Billaud L, et al. Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. N Engl J Med. 1990;322:1195–201.
85. Williamson SK, Lew D, Miller GJ, et al. Phase II evaluation of cisplatin and etopo-side followed by mitotane at disease progression in patients with locally advanced or metastatic adrenocortical carcinoma: a Southwest Oncology Group Study. Cancer. 2000;88:1159–65.
86. Decker RA, Elson P, Hogan TF, et al. Eastern Cooperative Oncology Group study 1879: mitotane and adriamycin in patients with advanced adrenocortical carcinoma. Surgery. 1991;110:1006–13.
87. Villa R, Orlandi L, Berruti A, et al. Modulation of cytotoxic drug activity by mitotane and lonidamine in human adrenocortical carcinoma cells. Int J Oncol. 1999;14:133–8.
88. Berruti A, Terzolo M, Pia A, et al. Mitotane associated with etoposide, doxorubicin, and cisplatin in the treatment of advanced adrenocortical carcinoma. Italian Group for the Study of Adrenal Cancer. Cancer. 1998;83:2194–200.
89. Terzolo M, Daffara F, Ardito A, et al. Management of adrenal cancer: a 2013 update. J Endocrinol Investig. 2014;37:207–17.
90. Fassnacht M, Terzolo M, Allolio B, et al. FIRM-ACT Study Group. Combination chemotherapy in advanced adrenocortical carcinoma. N Engl J Med. 2012;366:2189–97.
91. Xu YZ, Zhu Y, Shen ZJ, et al. Significance of heparanase-1 and vascular endothelial growth factor in adrenocortical carcinoma angiogenesis: potential for therapy. Endocrine. 2011;40:445–51.
92. Ye J, Qi Y, Wang W, et al. Lower expression of ATM and gene deletion is more frequent in adrenocortical carcinomas than adrenocortical adenomas. Endocrine. 2012;41:479–86.
93. Wood BJ, Abraham J, Hvizda JL, et al. Radiofrequency ablation of adrenal tumors and adrenocortical carcinomametastases. Cancer. 2003;97:554–60.