# Medicine®

## CLINICAL CASE REPORT

# Rapid and Complete Remission of Metastatic Adrenocortical Carcinoma Persisting 10 Years After Treatment With Mitotane Monotherapy
## Case Report and Review of the Literature

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**Abstract:** Mitotane has been used for more than 5 decades as therapy for adrenocortical carcinoma (ACC). However its mechanism of action and the extent of tumor response remain incompletely understood. To date no cases of rapid and complete remission of metastatic ACC with mitotane monotherapy has been reported.

A 52-year-old French Canadian man presented with metastatic disease 2 years following a right adrenalectomy for stage III nonsecreting ACC.

He was started on mitotane which was well tolerated despite rapid escalation of the dose. The patient course was exceptional as he responded to mitotane monotherapy after only few months of treatment. Initiation of chemotherapy was not needed and he remained disease-free with good quality of life on low maintenance dose of mitotane during the following 10 years. A germline heterozygous *TP53* exon 4 polymorphism c.215C>G (p. Pro72Arg) was found. Immunohistochemical stainings for IGF-2 and cytoplasmic β-catenin were positive.

Advanced ACC is an aggressive disease with poor prognosis and the current therapeutic options remain limited. These findings suggest that mitotane is a good option for the treatment of metastatic ACC and might result in rapid complete remission in selected patients.

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**Abbreviations**: ACC = adrenocortical carcinoma, CBG = cortisol-binding globulin, CT = computed tomography, DFS = disease-free survival, ENSAT = European network for the study of adrenal tumors, FDG-PET/CT = fluorodeoxyglucose-fused positron emission tomography with CT, HE = hematoxylin and eosin, IHC = Immunohistochemical, RRM1 = ribonucleotide reductase large subunit 1, SHBG = sex hormone-binding globulin, SNP = single-nucleotide polymorphisms, SUV = standardized uptake value, TBG = thyroxine-binding globulin.

## INTRODUCTION

The annual incidence of adrenocortical carcinoma (ACC) is 0.7 to 2.0 cases per million population with a higher incidence in southern Brazil due to the high prevalence (0.27%) of a specific *TP53* germline mutation (R337H).[1,2] A large proportion of patients present with an advanced stage and have a poor prognosis; 5-year survival ranges from 81% for ENSAT (European network for the study of adrenal tumors) tumor stage I to 13% for tumor stage IV.[3] The most important predictors of survival are tumor grade, tumor stage, surgical treatment, and more recently the proliferative marker Ki67.[1,4] The rarity and the aggressivity of ACC have limited the identification of effective therapeutic options. Mitotane is currently a cornerstone in the management of ACC for which most experts recommend its use in a postoperative adjuvant setting for localized disease with high recurrence risk (ENSAT Stage III, Ki-67 > 10%) as well as in advanced disease either as monotherapy or combined with cytotoxic chemotherapy.[5,6] However, the degree of response to mitotane is variable among patients due to the complexity of its mechanisms of action and pharmacokinetics, which are still not fully elucidated despite 50 years of clinical practice.[6] Only, few cases of complete remission of metastatic ACC treated with mitotane alone following surgical resection have been reported in the literature; in addition, none of them showed drastic response with full recovery after only few months of therapy. We report the case of a 52-year-old man with metastatic ACC who showed complete remission of his tumors to mitotane monotherapy following few months of treatment. He was kept on low maintenance dose and followed regularly with clinical assessment, laboratory testing, computed tomography (CT) scans, and fluorodeoxyglucose-fused positron emission

tomography with CT (FDG-PET/CT) remaining disease-free during the following 10 years.

## CASE PRESENTATION

A 52-year-old French Canadian man was referred to our adrenal tumor care center 2 years following a right adrenalectomy for stage III ACC. The tumor largest diameter was 21 cm (T3N0M0). Biochemical investigations preoperatively were suggestive of a nonsecreting tumor. Histology confirmed ACC with the presence of capsular invasion but without clear vascular invasion (Ki 67 of 12% and modified Weiss score 7/7). Postoperative recovery was uneventful and he did not receive any type of adjuvant therapy. Follow-up thoracic and abdominal CT scans alternating with FDG-PET-CT scans were unremarkable until 2 years later when the patient noticed a subcutaneous nodule of the right abdominal wall that was confirmed to measure 13 mm in diameter on CT scan. Fine needle aspiration of the nodule was positive for malignant cells compatible with metastatic ACC. A 5-mm hepatic lesion as well as an 18-mm mediastinal and a 12-mm retroperitoneal lymph nodes metastases were also identified on CT scan (Figure 1A) and FDG-PET-CT scan (Figure 2A). The patient was referred to our center and was started initially on 1 g of mitotane as monotherapy with dose increments up to 4 g daily over 8 weeks. Treatment was well tolerated. Therapeutic levels between 14 and 20 μg/mL were achieved within 2 months of initiation of therapy. No trials of chemotherapy or radiotherapy were attempted because the patient reported clinical disappearance of the palpable subcutaneous nodule after 4 weeks of initiation of therapy. Complete regression of all ACC metastatic lesions was observed on FDG-PET scan after 4 months of initiation of therapy (Figure 2B) and on CT scan after 7 months (Figure 1B and C). Thus, the metabolic response of the FDG-PET scan was more rapid than the radiologic response. The patient was maintained on 3.5 g of mitotane per day and it was well tolerated; his last mitotane level being 14.4 μg/mL. He developed hypothyroidism, adrenal insufficiency, and hypogonadism, which were supplemented by levothyroxine, hydrocortisone (50 mg), and testosterone respectively as well as hypercholesterolemia managed by statin. No other adverse events related to mitotane were reported. Surveillance with thoracic and abdominal CT scans and FDG-PET-CT scans being performed every 6 months confirmed that the patient remained disease-free 10 years after his relapse. The patient had

no familial history of cancers except for his sister who was diagnosed with a colorectal cancer at the age of 50 years old.

The patient gave written informed consent for further genetic investigations. Genomic DNA was extracted from peripheral-blood lymphocytes using the standard protocol. He was tested by MLPA and by direct sequencing of polymerase chain reaction products of the *Tp53* gene (exons 2 to 11 and intron-exon boundaries) (NCBI reference sequence NC_000017.9). Genetic analysis of the *TP53* gene in his lymphocyte DNA did not show any germline mutation. Only a heterozygous *TP53* exon 4 polymorphism c.215C>G (p. Pro72Arg) was found. Immunohistochemical analysis (IHC) was also conducted. Original and new hematoxylin and eosin (HE) slides of the ACC tumor were reviewed by an experienced pathologist (M.L.) to confirm the presence of ACC and to determine the Weiss score. β-Catenin, IGF-2, and TP53 stainings were performed on 3-mm thick sections of deparaffinized tissue and antigens were retrieved. The slides were incubated with mouse monoclonal antibodies (Ventana BenchMark system) against β-catenin (clone b-catenin 1, 1:200 dilution) (Dako), IGF2 protein (clone SIF2, 1:100 dilution) (Millipore), and TP53 (clone Pab1801, 1:50 dilution) (Leica). External positive controls were performed. ACC tumor was scored semi-quantitatively as 0 (negative), 1+ (focally or weakly positive), 2+ (moderate staining), 3+ (diffuse strong staining), or 4+ (intense diffuse staining). In our patient, IHC analysis showed diffuse, mildly granular cytoplasmic staining for IGF-2 (2+) and diffuse membranous cytoplasmic staining with membranous enhancement for β-catenin (2+) but no nuclear staining. TP53 staining was negative (Figure 3 A–D).

## DISCUSSION

Despite recent advances in early detection of adrenal tumors and surgical techniques, the relapse rate in ACCs is high and overall survival of advanced ACC remains generally poor.[7] A MEDLINE search of all peer-reviewed previously published cases of advanced stage ACC with long-term remission after only mitotane treatment in the English literature (case reports, original articles, and reviews) allowed us to identify only 8 cases described previously between 1974 and 2014[8–14] and we report here 1 new case. Three cases of metastatic ACC responding to mitotane monotherapy were also described in the pediatric population;[9,15,16] however, we focused our review here on adult ACC cases because pediatric ACC have different behavior than adult ACC.



**FIGURE 1.** (A) Baseline (2004) abdominal scan showed multiple metastatic lesions: right abdominal wall: (arrow), hepatic, mediastinal, and retroperitoneal lymph nodes. (B) At 4 months following initial administration of mitotane, a decrease in size of metastatic lesions was observed, and at 7 months, (C) all lesions had disappeared.
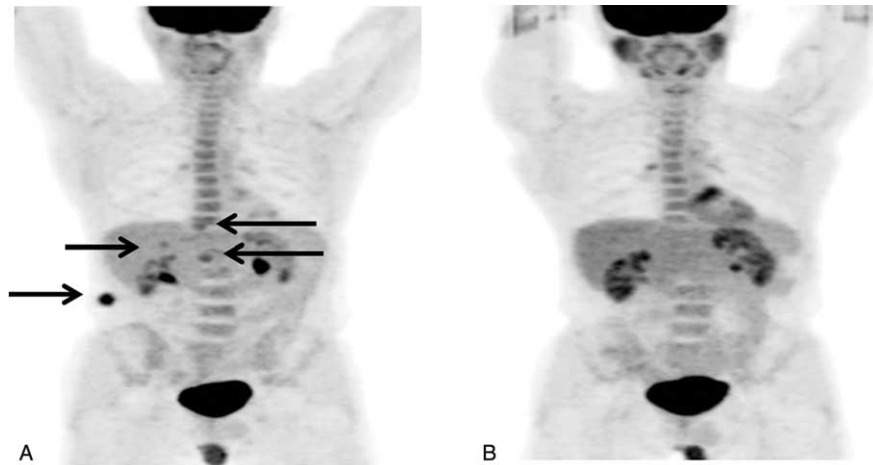
**FIGURE 2.** (A) Baseline (2004) FDG-PET showing a subcutaneous metastasis of the right abdominal wall with intense metabolic activity. Hepatic, mediastinal, and retroperitoneal lymph node metastasis were also present. (B) Complete regression of all neoplastic lesions following 4 months of mitotane treatment. FDG-PET/CT = fluorodeoxyglucose-fused positron emission tomography with CT.

In the series of adult patients with ACC including our patient and the 8 selected cases from literature search, they were 2 men and 7 women. They had a mean age of $52 \pm 12$ years. The mean size of the tumors was of $15 \pm 5$ cm (Table 1). All cases were evaluated for hormonal secretion; 3 were nonsecreting, 1 was cortisol-secreting, 3 had virilizing tumors, and 2 were co-secreting cortisol and androgens. No clinical specific characteristic seems to predict a complete response to mitotane. In all cases, metastases were localized either in the liver, lungs, lymph nodes, or in the abdomen. Only our patient had a subcutaneous metastasis. The longest time of disease free survival was 25 years.[13] In cases described previously, mitotane treatment was stopped after 5 and 6 months and 1, 3, 6.5, and 8 years of therapy; however, in the remaining 2 cases, patients had lifelong mitotane therapy (4 and 16 years). As for our patient he is still on mitotane enjoying a good quality of life since 10 years (Table 1).
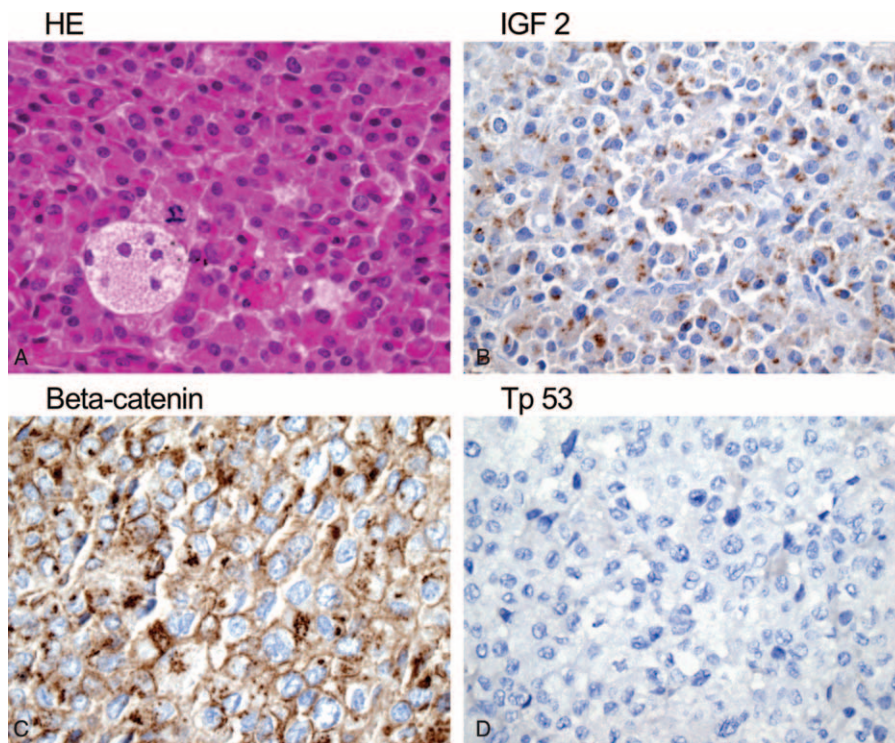


**FIGURE 3.** Immunohistochemical analysis performed on HE slides showed diffuse cytoplasmic eosinophilia and atypical mitotic figure, consistent with a diagnosis of ACC (A), diffuse, mildly granular cytoplasmic staining for IGF-2 (2+) (B) and diffuse cytoplasmic staining with membranous enhancement for β-catenin (2+) but no nuclear staining (C). TP53 staining was completely negative (D). ACC = adrenocortical carcinoma; HE = hematoxylin and eosin.

**TABLE 1.** Description of 8 Cases of Metastatic ACC in Adults Reported in the Literature and the Case of this Report that Showed Complete Regression and Long-Term Remission With Mitotane Treatment Alone

| Publications | Age/ Gender | Tumor Diameter/ Location/Type of Secretion | Surgical Resection | Site/Timing of Metastasis | Mitotane Daily Dose Range/ Duration | Time From Mitotane Initiation to Favorable Response | Time From Mitotane Initiation to Complete Remission | DFS |
|---|---|---|---|---|---|---|---|---|
| Downing et al, 1974[8] | 48 y/F | 10 cm/R/Virilizing | Exploratory laparatomy (inoperable) | Lungs/at presentation | 2–6 g/4 y | 2 m | 5 m | 4 y |
| Becker and Schumacher, 1975[9] | 69 y/M | N/A/L/NS | Adrenalectomy | Periadrenal fatty tissue/ at presentation | 1–10 g/6 y + 4 m | N/a | N/A | 7 y + 9 m (17m off therapy) |
| Decker and Kuehner, 1991[10] | 49 y/F | 9 cm/L/CS + Virilizing | Adrenalectomy Nephrectomy Splenectomy Distal pancreatectomy | Abdomen/after the 3rd recurrence | 10 g/1y | N/A | 1 y | 7 y (6 y off therapy) |
| Decker and Kuehner, 1991[10] | 57 y/F | 20 cm/R/Virilizing | Adrenalectomy Nephrectomy, Wedge liver resection | Lungs- Liver/N/A | 3–6 g/6 m | N/A | 5 m | 6 y + 6 m (6 y off therapy) |
| Remond et al, 1992[11] | 56 y/F | 10 cm /L/CS | Inoperable | Lungs- Liver/at presentation | 3–15 g/8 y | N/A | 15 m | 15 y (7 y off therapy) |
| Ilias et al, 2001[12] | 24 y/F | 19 cm/L/CS + Virilizing | Nephrectomy Adrenalectomy | Liver/at presentation | 2.5–8 g/16 y | N/A | N/A | 16 y |
| Chalasani et al, 2009[13] | 57 y/F | 12 cm/R/Virilizing | Nephrectomy Adrenalectomy | Lungs- Liver at presentation | 3–6 g/5 m | N/A | N/A | 25 y |
| Pal et al, 2014[14] | 55 y/F | 16 cm/L/NS | Nephrectomy Adrenalectomy | Lungs-Liver at presentation | 4–8 g/3 (stopped 2 y) then 4–8 g/7 y | 3 m | 1 y | 12 y |
| Our Patient, 2016 | 52 y/M | 21 cm/R/NS | Adrenalectomy | Subcutaneous abdominal nodule- Liver- mediastinal and retroperitoneal lymph nodes/at 2 y post-op | 1–4 g/10 y | 1 m | 4 m | 10 y |

*CS = Cushing's syndrome, DFS = disease-free survival, F = female, g = grams, L = left, M = male, m = months, N/A = data not available, NS = nonsecreting, R = right, y = years.

We report for the first time IHC results in metastatic ACC patients with complete tumoral regression following mitotane therapy. In our patient, IHC staining was positive for IGF-2 and cytoplasmic β-catenin. Unfortunately, no IHC studies were provided in the 8 cases described previously in the literature. Several current histopathological criteria may serve as prognostic markers in ACC;[17] patients with increased IHC staining for TP53, SF-1, β-catenin, or IGF-2 tend to have higher grade tumors, reflected by higher Ki-67 expression, higher tumor stage, and poorer disease-free survival.[17] However no link to response to mitotane has been ever suggested.

The course of this patient was remarkable because he showed full regression of his disease after only a few months of treatment with plasma mitotane concentrations within the recommended therapeutic range. Mitotane targets mitochondria where it downregulates the expression of steroidogenic enzymes; cholesterol side chain cleavage (CYP11A1), 11β-hydroxylase (CYP11B1), 18β-hydroxylase (CYP11B2), and 3β-hydroxysteroid-dehydrogenase.[18–20] Furthermore, it enhances the metabolic clearance of cortisol and a variety of drugs by inducing CYP3A4 gene expression and can inactivate 50% of administered hydrocortisone.[5,21] On the other hand, it increases the levels of sex hormone-binding globulin (SHBG), thyroxine-binding globulin (TBG), and cortisol-binding globulin (CBG) by enhancing their hepatic protein metabolism, which complicates hormonal interpretation.[5,6]

The metabolic response on FDG-PET scan in the reported patient was more rapid than the radiological response similarly to the findings of some studies where changes in metabolic activity on FDG-PET scan can be observed after short courses of chemotherapy before evidence of radiological improvement; these changes can be used to predict the response to specific chemotherapeutic agents[22] thus this may also be the case for mitotane therapy. On the other hand, in all patients in whom disease progression was observed, an increase in the standardized uptake value (SUV) was noted on successive FDG-PET scans.[23,24]

Novel insights on mitotane pharmacokinetics added to the complexity of its mechanism of action. Mitotane concentration of 14 mg/L, or higher, was a predictor of recurrence free survival, but 1 prospective and many retrospective studies showed no difference between low-dose versus high-dose regimens in reaching target therapeutic levels. Therefore, other variables might affect the amount of mitotane plasma concentrations and mitotane-related toxicity.[25–28] Another prospective study showed that patients treated with high-dose regimen reached the therapeutic range in 1 month and thus may be a preferable strategy for patients with advanced stage on mitotane monotherapy.[29] Interestingly, a recent study showed that early-morning trough sampling should be adopted as standard management in monitoring mitotane treatment because random sampling could yield incidentally high levels.[30]

Specific biomarkers predicting the response to mitotane are still lacking. D'Avolio et al[31] showed that the single-nucleotide polymorphism (SNP) CYP2B6 rs3745274GT/TT was a predictor of mitotane concentrations of at least 14 mg/L after 3 months of treatment. Ronchi et al[32] analyzed CYP2W1 expression in a large series of adrenal tissues. They observed that CYP2W1 immunoreactivity was associated with hormonal activity, and that in ACC patients treated with mitotane only, high CYP2W1 immunoreactivity adjusted for the ENSAT stage was associated with longer overall survival and time to progression and with a better response to therapy both as palliative or adjuvant option. Finally, Volante et al[33] evaluated the gene expression of ribonucleotide reductase large subunit 1 (RRM1) in ACC and showed that high RRM1 gene expression was associated to shorter disease-free survival (DFS); in patients with low RRM1 gene expression, adjuvant mitotane was associated with improved DFS, whereas this effect was lost in cases with high RRM1 expression.

## CONCLUSIONS

Patients with advanced ACC have a poor overall prognosis. However, rare cases with complete remission after mitotane monotherapy were reported in the literature. The patient with metastatic ACC described in this article showed exceptional response rapidly after initiation of mitotane lasting for more than years. Prediction of the individual response to mitotane will require a better understanding of its molecular mechanisms of action.

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