Sunitinib in Refractory Adrenocortical Carcinoma: A Phase II, Single-Arm, Open-Label Trial

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Context: Treatment of refractory adrenocortical carcinoma (ACC) is not established. Animal experiments pointed toward adrenal toxicity of sunitinib.

Objective: The objective of the study was to determine the antitumor effects of sunitinib in refractory ACC.

Design: This was a phase II, open-label trial using a two-stage accrual design.

Setting: The study was conducted at two tertiary referral centers.

Patients: Thirty-eight patients with refractory ACC progressing after mitotane and one to three cytotoxic chemotherapies participated in the study.

Intervention: The intervention included sunitinib at a standard dose (50 mg/d, 4 wk on, 2 wk off).

Main Outcome Measure: Response was defined as progression-free survival (PFS) of 12 wk or longer (first tumor evaluation).

Results: Thirty-five patients could be evaluated for response. Five patients experienced stable disease, 24 had progressive disease, and six patients died from ACC before the first evaluation (naïve estimate five of 35 = 14.3%, median unbiased response rate 15.4%, 95% confidence interval 5.0–33.4%). The median PFS was 2.8 months. In responders, PFS ranged between 5.6 and 11.2 months and overall survival between 14.0 and 35.5 months. Of 36 serious adverse events, only nine were possibly related to sunitinib. Concomitant mitotane appeared to negatively impact on outcome. Furthermore, a negative correlation between the serum concentrations of sunitinib plus its active metabolite *N*-desethylsunitinib (SU12662) and mitotane ($r = -0.650$; $P = 0.114$) was observed in seven evaluable patients suggestive of a relevant drug interaction.

Conclusion: Sunitinib has modest activity in advanced refractory ACC, which compares favorably with other targeted treatments in these patients. Sunitinib serum levels might have been profoundly reduced by mitotane induced cytochrome P450-3A4 activity attenuating its antitumor activity and adverse effects. Together these findings suggest that sunitinib deserves further investigation in mitotane-naïve ACC patients. **(*J Clin Endocrinol Metab* 97: 3495–3503, 2012)**

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Abbreviations: ACC, Adrenocortical carcinoma; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; PFS, progression-free survival; RECIST, Response Evaluation Criteria of Solid Tumors; VEGFR, vascular endothelial growth factor receptor.

Adrenocortical carcinoma (ACC) is a rare malignancy with an annual incidence of about one per million (1, 2). Patients frequently suffer from severe Cushing's syndrome and hirsutism due to excess glucocorticoid and/or androgen secretion (3–6). In patients with metastatic disease, prognosis is poor, with a 5-yr survival less than 15% (3, 7–10). Mitotane (*o*,*p*′-dichlorodiphenyldichloroethane, *o*,*p*′-DDD) has been in use for the treatment of ACC since 1959 (11) and is the only approved drug for this disease. It is a derivative of the insecticide dichlorodiphenyl-trichloroethane, disrupts steroid synthesis, and induces cell death specifically in the adrenal cortex (12). There is evidence that a mitotane serum level of 14–20 mg/l is predictive for a higher response rate with acceptable toxicity, and therefore, drug monitoring is recommended (13, 14). Very recently the results of the First International Randomized Trial in Locally Advanced and Metastatic Adrenocortical Carcinoma Treatment trial became available, indicating that the combination of mitotane with etoposide, doxorubicine, and cisplatin is superior to treatment with streptozotozin and mitotane (15). However, the objective response rate, even in the group treated with combination of mitotane with etoposide, doxorubicine, and cisplatin, was only 23%, and long-term disease control was achieved in less than 15% of patients (15). Therefore, several studies investigated targeted therapies in ACC patients. Nineteen patients were treated with the epidermal growth factor receptor (EGFR) inhibitor gefitinib (16) and four patients with imatinib (17) without response. Studies from our groups investigated the combination of cytotoxic drugs and drugs targeting vascular endothelial growth factor receptor (VEGFR) or EGFR, respectively, but both of these regimens failed to show any relevant response (18, 19). Recently a phase II trial of oral daily sorafenib in combination with weekly paclitaxel was even stopped after the enrollment of 10 patients because in all of the patients, progressive disease was detected at the time of the first tumor evaluation (20).

Sunitinib targets several tyrosine kinase receptors on tumor cells and tumor vessels, in particular VEGFR1 and VEGFR2, c-KIT, Fms-like tyrosine kinase 3, and platelet-derived growth factor receptor (21, 22). Thus, the drug combines the direct antitumor effects with antiangiogenic activity and is now approved for several tumors (23–25).

Intriguingly, sunitinib induced adrenal hemorrhage in animal experiments, leading to adrenal insufficiency (26). Some patients treated with this drug within phase I–III clinical trials developed a reduced response to ACTH stimulation indicative of impaired adrenocortical function (27). This finding resulted in a safety note in the summary of product characteristics of sunitinib. Moreover, we recently demonstrated expression of the key target molecules vascular endothelial growth factor and VEGFR2 in ACC tumor samples (28). *In vitro*, sunitinib inhibits proliferation of adrenocortical cancer cells and impairs steroidogenesis by the down-regulation of 3β-hydroxysteroid dehydrogenase II (28).

Here we describe the largest phase II trial investigating prospectively a tyrosine kinase inhibitor in ACC. In this single-arm study in two German tertiary referral centers, we evaluated the clinical activity and safety of sunitinib in advanced ACC progressing after mitotane and one to three cytotoxic chemotherapies. Furthermore, in a *post hoc* analysis, we investigated the interaction of sunitinib and mitotane and its impact on toxicity and clinical efficacy.

Patients and Methods

The study drug, sunitinib L-malate, was provided by Pfizer Pharma (Karlsruhe, Germany) as capsules containing 12.5-, 25-, and 50-mg equivalents of the sunitinib-free base.

Patients

Patients were eligible for the study if they had histologically confirmed ACC not amenable to radical surgery with disease progressing after mitotane treatment and one to three cytotoxic chemotherapy regimens including a platin-based protocol. Further inclusion criteria were age 18 yr or older, Eastern Cooperative Oncology Group (ECOG) performance status 0 to 2, life expectancy 3 months or longer, radiologically monitorable disease according to Response Evaluation Criteria of Solid Tumors (RECIST) (29) and adequate bone marrow reserve as well as a negative pregnancy test and effective contraception. Key exclusion criteria included uncontrolled prior malignancies, severe renal or hepatic insufficiency, a macrovascular ischemic or thromboembolic event, relevant cardiac disease, hemorrhage of Common Terminology Criteria for Adverse Events grade 3, hypertension refractory to medical treatment, and concomitant treatment with known cytochrome P450-3A4 inducers. Furthermore, any other anticancer treatment was excluded with the exception of mitotane, which was permitted at the discretion of the treating physician. All patients provided written informed consent. Approval was obtained from the ethics committees of the participating universities and the German Federal Institute for Drugs and Medical Devices.

Study design and assessments

This study was a two-center, single-arm, open-label, phase II clinical trial. The imaging was performed at 12-wk intervals until the discontinuation of the study drug by a contrast-enhanced spiral computed tomography or magnetic resonance imaging scan of the abdomen, chest, and pelvis. Radiological assessment was done according to RECIST criteria version 1.0 (29) at each center and reviewed centrally by an independent radiologist.

Treatment and dose modification

Sunitinib was self-administered with a starting dose of 50 mg once daily in a continuous regimen for 4 wk followed by a 2-wk

off-period (corresponding to one treatment cycle). Patient adherence to sunitinib was assessed by a health professional-recorded dispensing log throughout the treatment duration. Treatment continued until disease progression, intolerable toxicity, or withdrawal of consent. Patients were advised to take capsules once daily without regard to meals. In case the patient experienced signs of drug toxicity, dose levels were adjusted in a stepwise manner to 37.5 and 25 mg daily. Patients experiencing dose-limiting toxicity were temporarily withdrawn from treatment for 1 wk and study drug administration continued at reduced level if required.

Pretreatment evaluation and safety assessment

Pretreatment evaluation and assessment at d 1, 14, and 28 of each cycle comprised assessment of concomitant treatment, physical examination including blood pressure, and extensive laboratory tests. Complete thyroid function tests and ECOG performance test were done at screening and at every evaluation after 12 wk of treatment; 12-lead electrocardiogram and echocardiography were performed at screening and every second evaluation or when heart failure was clinically suspected. Adverse events were rated using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (see http://ctep.cancer.gov/reporting/ctc.html).

Assessment of mitotane and sunitinib serum levels

Routine blood sampling was done at study inclusion and at the end of each treatment cycle after 2 wk of treatment interruption. For further analysis (*e.g.* drug monitoring of sunitinib and mitotane), samples were stored at −80 C until assessment. Mitotane levels at baseline were measured as described by using a gas chromatographic-electron capture detection assay (30). Determinations of serum levels of sunitinib and its active metabolite *N*-desethylsunitinib (SU12662) were performed by liquid chromatography-tandem mass spectrometry as described (31) in patients, in whom blood samples collected 12–24 h after administration of 50 mg sunitinib were available (n = 7).

Endpoints, statistics, power, and sample size considerations

Because progression-free survival (PFS) of more than 12 wk is a very rare event in patients with refractory progressive ACC, we considered stabilization of disease as a result of sunitinib treatment. The primary end point was response defined as PFS at the time of first tumor evaluation at 12 wk. In the protocol, the null-hypothesis of a response rate of 5% (H) was prespecified to be tested confirmatory at a one-sided type I error level of 5%, choosing an optimized two-stage Simon design. The alternative hypothesis of a response rate of 20% (A) should be detected with a power of 80%. This design requires 29 patients assessed for response. Considering a dropout rate of 20%, a sample size of 36 patients was planned. At least one response at the interim analysis based on the first 10 assessed patients and at least four of 29 responses at final analysis will claim rejection of H in favor of A. Coping with the group sequential design, the median unbiased estimate and exact 90 and 95% two-sided confidence intervals for the response rate are calculated by stage-wise ordering according to Clopper and Pearson (40). The naïve-biased estimate is presented in addition. Secondary end points were PFS, overall survival, objective response rate, and toxicity.

Results

Patient characteristics

Between July 2007 and September 2009, a total of 39 patients were enrolled in the study. After the documentation of stable disease at 12 wk in the third assessed patient, the study entered the second phase (also the seventh patient experienced stable disease). Patient characteristics are summarized in Table 1. All patients had progressed despite prior cytotoxic chemotherapy and suffered from significant tumor burden. In 19 patients autonomous hormone excess was documented. Eleven patients were participants of the First International Randomized Trial in Locally Advanced and Metastatic Adrenocortical Carcinoma Treatment trial (15); all other patients were referred for radiologically diagnosed disease progression. Twenty-four patients received mitotane treatment at study inclusion.

Four patients were excluded from the per-protocol analysis (Fig. 1): in one patient (PID 16), the review of tumor specimens by the reference pathologist revealed misdiagnosis of a malignant pheochromocytoma as ACC. This patient had stable disease at 12 wk, progressed after 164 d, and died 63 wk after starting sunitinib treatment. One patient (PID 29) experienced a serious adverse event (dyspnea due to heart failure) unrelated to the study treatment and withdrew further study treatment 42 d after enrollment. One patient (PID 21) had a myocardial infarction considered to be possibly treatment related, which led to the discontinuation of the study drug after 9.5 wk of treatment. Imaging outside the study suggested progressive disease, and the patient died 2 months later. One patient (PID 5) was excluded from the study due to incompliance with the study procedures after 8 wk. However, the appearance of a new metastatic skin lesion suggested progressive disease, and the patient died after 43 wk. Thus, 35 patients were analyzed for response on a per-protocol basis.

Tumor response and survival analysis

The primary end point of the study was 12-wk PFS in patients treated per protocol. Of these 35 patients, six patients died of progressive disease before the first radiological evaluation at 12 wk. Of the remaining 29 patients, five patients experienced stable disease, and 23 patients had progressive disease (Fig. 2) at first evaluation. No partial or complete tumor response according to RECIST criteria was observed. Of the five patients with stable disease at first evaluation, three patients showed disease progression at the second evaluation. One patient (PID 7) had progressive disease after 11.2 months of treatment and in PID 12 sunitinib was withdrawn after the diagnosis of progressive disease was made at the second evaluation.

TABLE 1. Patient characteristics at study inclusion of the entire study cohort (n = 39)

Characteristic	No. of patients
Sex	
Male	17
Female	22
Age (yr)	
Median	51.4
Range	22–72
ECOG performance status	
0	16
1	20
2	3
Mitotane therapy	
Patients (n)	24
Mitotane plasma level	
Median	11.6
Range	<1.0–33.7
Steroid hormone secretion	
Glucocorticoid excess	
Clinically apparent	7
Biochemical only	2
Androgen excess	
Clinically apparent	10
Biochemical only	7
Estrogen excess	
Clinically apparent	3
Biochemical only	4
Mineralocorticoid excess	
Biochemical only	1
Weiss score (n = 35)	
Median	6
Range	4–9
Ki67 index (n = 35)	
Median	20%
Range	2–50%
Prior cytotoxic chemotherapies	
EDP	
Patients (n)	38
Median (no. of cycles)	5
Range (no. of cycles)	1–10
Streptozotocin	
Patients (n)	35
Median (no. of cycles)	4
Range (no. of cycles)	1–18
Other	
Patients (n)	6
Median (no. of cycles)	5
Range (no. of cycles)	1–13
Baseline target lesions (RECIST)	
Median	207
Range	60–351
Baseline target lesions (n)	
Median	7
Range	2–10
Sites of target lesions (no. of patients)	
Adrenal	15
Liver	27
Local lymph nodes	5
Distant lymph nodes	12
Lung	26
Peritoneum	10
Kidney	4
Skin and soft tissue	5
Spleen	2
Bone	1[a]
Baseline nontarget lesions (no. of patients)	
Lung	21
Bone	5
Liver	3
Kidney	1
Peritoneum	2
Spleen	2
Other	2

EDP, Etoposide, doxorubicine and cisplatin.

[a] PID 15, the patient with malignant pheochromocytoma.

However, a central review later indicated stable disease leading to censoring of this patient at this time point. The patient finally progressed after 5.7 months and died from ACC after 35.5 months. Thus, the null-hypothesis (5% response rate) could be rejected ($P = 0.0247$, one sided), and the estimated response rate was 14.3% in a naïve estimate. The median unbiased estimate was 15.4% [90% confidence interval (CI) 6.1–30.0%, 95% CI 5.01–33.4%]. In addition, we performed an intention-to-treat analysis with all 39 patients, assessing one patient (PID 29) conservatively as a nonresponder. This sensitivity analysis leads to $P = 0.0107$ (one sided) and an estimated response rate of 15.4% naïve and 16.3% (90% CI 7.2–30.2%, 95% CI 6.1–33.5%) unbiased.

In the cohort of the 35 per-protocol-evaluated patients, the median PFS was 83 d (95% CI 80–85 d, Fig. 3A), exactly the time of the first evaluation. The median overall survival was 5.4 months (95% CI 3.2–7.6 months, Fig. 3B). At the time of the closing of the collection of data

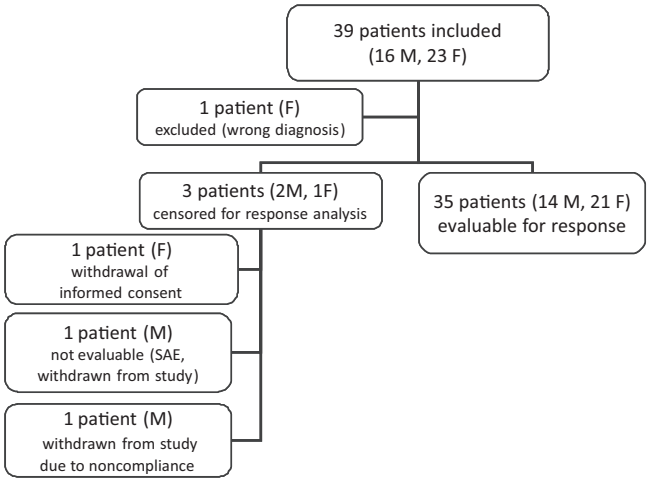

FIG. 1. CONSORT diagram. Of the 39 patients initially included in the study, one did not meet inclusion criteria. Thirty-five patients were analyzed for response on a per-protocol basis. M, Male; F, female.

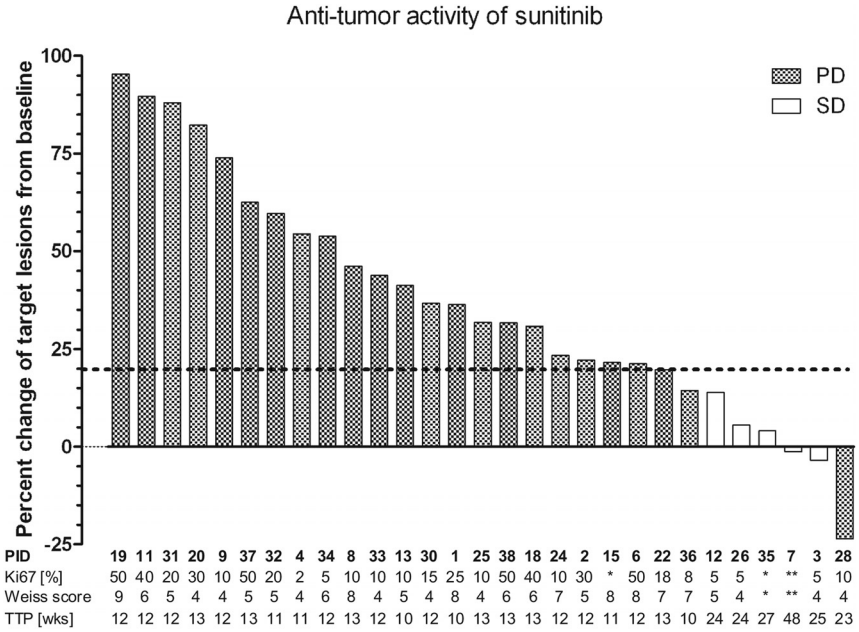

FIG. 2. Waterfall plot (n = 29) of tumor response according to RECIST. The plot shows the best percentage change from baseline in the size of the target lesions in each patient. Stable disease was observed in five patients (*white bars*). PID 22, PID 36, and PID 28 experienced the appearance of new lesions. *Dashed line* indicates 20% increase of target lesions and definition of progressive response according to RECIST. *, Not available; **, biopsy only. SD, Stable disease; PD, progressive disease; TTP, time to progression.

(August 1, 2011), one patient was still alive with a maximum follow-up of 2.5 yr.

It is noteworthy that among the patients who responded to the treatment at first evaluation, PFS ranged between 5.6 and 11.2 months and overall survival between 14.0 and 35.5 months (Fig. 3B).

Safety and tolerability

A total of 158 adverse events were recorded, with a median number of adverse events per patient of 4.0 (range 0–10). The majority of adverse events were Common Terminology Criteria grade 1 or 2 (66%), with the most common nonhematological adverse events being polyneuropathy (n = 11 in 10 patients), pain (n = 19 in 12 patients), infections (n = 15 in 10 patients), and diarrhea (n = 9 in nine patients). Surprisingly, treatment-related adverse events typically observed with multityrosine kinase inhibitors, such as fatigue (n = 3), hand-foot reactions, rash or discolored nails (n = 9), and mucositis (n = 4) were generally mild or absent (hypertension). Hematological laboratory abnormalities were also only mild or moderate. There was one grade 4 hypoglycemia, which was considered to be possibly related to sunitinib but was probably related to high glucose use by a large tumor mass. Forty-four serious adverse events were recorded, but only 10 were judged to be possibly related to the study drug (Table 2). In total, only 42 adverse events were considered to be related to sunitinib treatment, and only 13 of these adverse events were grade 3 and three grade 4 events (Table 3).

Interaction of sunitinib with mitotane

Because more than half of the patients were treated concomitantly with mitotane, we analyzed whether mitotane cotreatment improved the outcome of patients. Surprisingly, of the five patients with stable disease, only one patient had ongoing mitotane treatment. In contrast, among the 30 patients with progressive disease, 21 had ongoing mitotane treatment leading to an odds ratio for progressive disease of 9.33 (95% CI 0.91–95.63, $P = 0.052$). Because mitotane had been stopped just shortly before inclusion in some patients and the plasma elimination half-life is up to 5 months (12), mitotane levels were reassessed in 34 of 35 patients. In fact, mitotane serum concentrations greater than 7 mg/liter were present in five of 15 patients in whom mitotane treatment had been stopped prior to enrollment. Overall, the median mitotane level at the baseline examination was 11.6 mg/liter (range < 1–33.7 mg/liter).

We next examined blood levels of sunitinib and its active metabolite *N*-desethylsunitinib (SU12662) during sunitinib treatment (n = 7; see Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). The median concentration was 29.3 ng/ml (range < 5–56.6 ng/ml). Only in the single patient not treated with mitotane and with mitotane serum level less than 1 mg/liter, the combined serum concentration of sunitinib and SU12662 was greater than 50 ng/ml, which is considered to be required for therapeutic activity (32). Of note, SU12662 levels were generally higher than sunitinib levels, which are in marked contrast to published data (32) in which the median steady-state concentration of SU12662 was 17.4 ng/ml and sunitinib 40.6 ng/ml. Furthermore, there was evidence for a negative correlation between mitotane and sunitinib serum concentrations, although this correlation was statistically not significant, most likely due to the small number of samples (Fig. 4).

After this observation, we compared treatment-related adverse events in patients with and without concomitant mitotane treatment but did not find a statistically meaningful difference (data not shown).

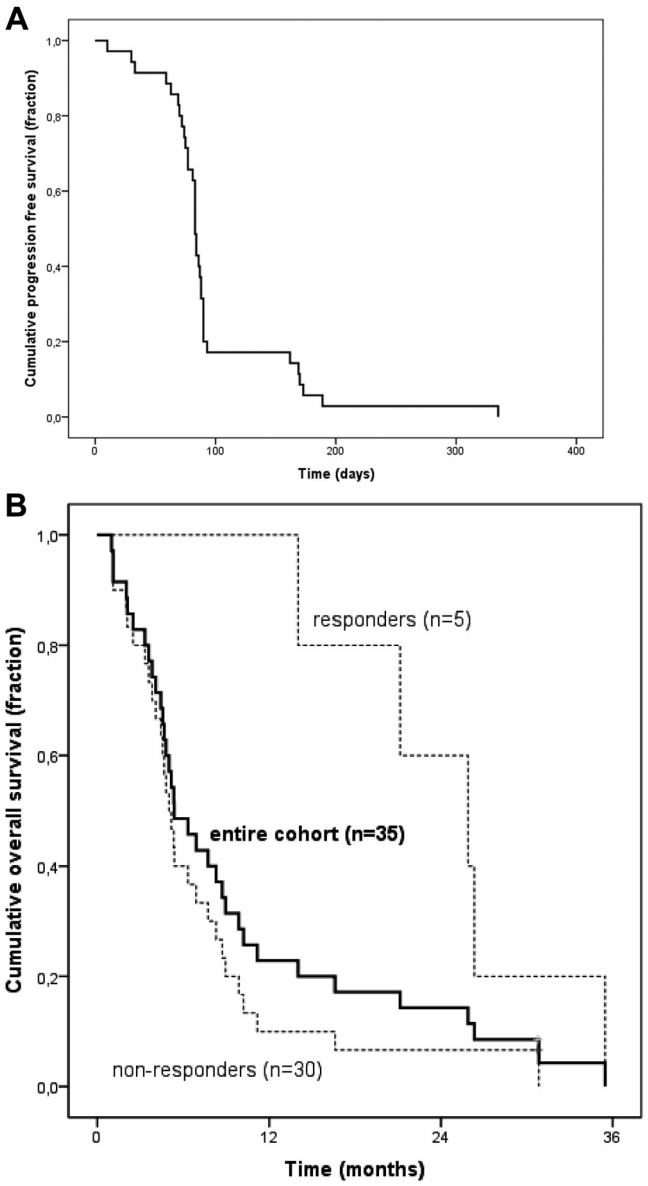

FIG. 3. PFS (A) and overall survival (B) (n = 35) are shown.

Discussion

In this third largest phase II trial in ACC published to date, we observed moderate single-agent activity of the multityrosine kinase inhibitor sunitinib in patients with refractory disease. Five of 35 evaluable patients had disease control for at least 12 wk leading to estimates of response rate of 14.3% (naïve) and 15.4% (median unbiased), respectively. Thus, the null hypothesis (5% response rate) was rejected ($P = 0.0247$). These results appear to compare favorably with other treatment regimens using targeted therapies tested in refractory advanced disease because they all failed to affect disease progression (21). However, no direct comparison has been performed, and all studies included small numbers of patients preventing final conclusions.

TABLE 2. Serious adverse events in 39 patients treated with

Category	Serious adverse event	CTC grade	n
Cardiovascular	Heart failure	3	1
	Hypotension	4	1
	Myocardial infarction	4	1
	Syncope	4	1
Disease progression		5	5
Endocrinology	Hypoglycemia	4	1
	Adrenal insufficiency	3	1
Gastrointestinal	Diarrhea	2, 3	2
	Constipation	2	1
	Oesophagitis	4	1
Hematology	Thrombopenia	2	1
	Anemia	2, 2, 3	3
	Thrombosis	3	1
	Hemorrhage	2	1
Infection	Abscess	4	2
	Sepsis	3, 4	2
	other	2	3
Kidney	Kidney failure	2, 4, 4	3
Urinary tract	Urinary tract obstruction	3	1
Liver	Liver failure	3, 5	2
Neurology	Drowsiness	3, 4	2
	Cauda syndrome	3	1
Pain		3, 4	4
Surgery		3, 4	2
Fatigue		3	1

CTC, Common Terminology Criteria.

Very recently a clinical phase II trial in a comparable group of patients with refractory ACC, which used the multityrosine kinase inhibitor sorafenib in conjunction with weekly paclitaxel, was published (20). Sorafenib targets a similar but nonidentical spectrum of tyrosine kinases, in particular with higher relative IC_{50} for c-kit and IGF-I receptor (22), the ligand of which, IGF-II, is overexpressed in the majority of ACC (33–35). However, all nine evaluable patients had progressive disease after 8 wk, and the trial was stopped prematurely due to lack of any positive effect. Furthermore, our own groups examined the combination of the VEGFR inhibitors, bevacizumab and capecitabine, as salvage therapy on a compassionate-use basis (19) in a similar clinical setting of advanced and progressive disease after several cytotoxic chemotherapies. However, in that series, progressive disease occurred in all patients and less than half of the patients reached the time of first evaluation at 12 wk without progression (median PFS 59 d). All but one patient died within 9 months after initiation of bevacizumab and capecitabine (19). Similarly, we treated 10 ACC patients with a combination of the EGFR antagonist erlotinib and gemcitabine as salvage therapy in advanced disease. However, only one of 10 patients had stable disease (10%, 95% CI 0.3%–44.5%) with a PFS of 32 wk, whereas no benefit was seen in the other patients (18). Similar disappointing results

TABLE 3. Treatment-related adverse events in 39 patients treated with sunitinib

Category	Adverse event	CTC1 + 2	CTC3	CTC4
Gastrointestinal	Diarrhea	5	2	0
	Hemorrhoids	1	0	0
Liver	Elevated liver enzymes	0	2	0
	Jaundice	1	0	0
	Liver failure	0	1	0
Dermatology	Mucositis/Stomatitis	4	0	0
	Skin rash	2	0	0
	Hand-foot skin reation	1	0	0
	Dry skin	1	0	0
	Discolored nails	1	0	0
Hematology	Anemia, Thrombopenia, Leukopenia	2	2	0
	Thrombosis	0	1	0
Hemorrhage	1 × gastrointestinal, 1 × respiratory tract, 1 × skin	3	0	0
Endocrinology	Hypoglycemia	0	0	1
	Adrenal insufficiency	0	1	0
Cardiac	Myocardial infarction	0	0	1
	Syncope	0	0	1
Constitutional	Fatigue	0	2	0
	Muscle weakness	1	0	0
Neurology	Dizziness/drowsiness	0	2	0
	Polyneuropathy	3	0	0
Pain	Abdominal pain	1	0	0

CTC, Common Terminology Criteria.

were yielded with gefitinib monotherapy published by now only as an abstract (16).

In this prospective phase II trial using sunitinib, five patients showed stable disease at 12 wk. In these responding patients, median PFS reached 6 months, and the median overall survival was 26 months. This indicates that sunitinib is an active treatment in selected patients with advanced ACC. Moreover, the median change of target lesion in our series was 31% (range −24 to 95%) after 12 wk, whereas it was 60% (range 26–152%) after 8 wk in the trial with sorafenib and paclitaxel (20). Although such a direct comparison is biased, it points again to some efficacy of sunitinib. A limitation arises from the fact that imaging prior to study entry was not standardized. Therefore, we cannot provide data regarding the dynamics of tumor growth before the initiation of sunitinib. Likewise, we cannot exclude that tumors responsive to sunitinib treatment might be biologically less aggressive in view of a relatively low Ki67 index and Weiss score in this group.

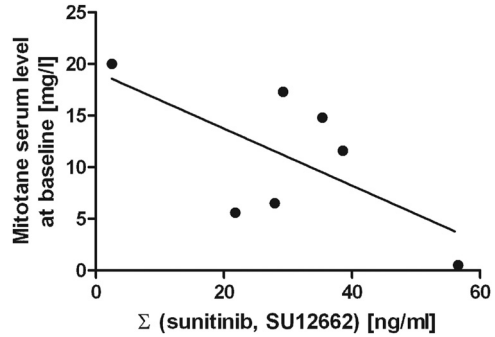

FIG. 4. Inverse correlation of mitotane serum levels at study baseline with sunitinib and its active metabolite SU12662 (Pearson r = −0.650; $P = 0.114$).

Importantly, the clinical efficacy of sunitinib in ACC might be underestimated in our trial for several reasons. First, the drug interaction of mitotane with sunitinib may have greatly reduced the exposure to sunitinib, as will be discussed in detail below. Second, extensive pretreatment of ACC with several cytotoxic regimens including cisplatin and mitotane is likely to induce drug resistance and/or selection of multiresistant tumor clones. Our study comprised a selection of highly aggressive tumors because only patients with progressive disease after chemotherapy were eligible. Third, hitherto unknown interindividual variability in drug target expression may account for some proportion of treatment failure.

We found a trend that patients on mitotane were less likely to respond to sunitinib compared with patients without mitotane. Therefore, a *post hoc* analysis on mitotane and sunitinib serum levels was performed in patients from which blood samples were available during sunitinib treatment (excluding the off-phase, n = 7). In line with the observation that mitotane rather negatively affected the clinical outcome, we found higher mitotane levels to be associated with reduced levels of sunitinib and its active metabolite. Despite the clear limitation by the small sample size these results are highly suggestive for a negative correlation between the serum concentration of mitotane and sunitinib. The relatively higher levels of SU12662 compared with sunitinib are in contrast to pub-

lished pharmacokinetic data and suggestive of high CYP3A4 activity (32).

It is well established that sunitinib is metabolized in the liver and intestine by CYP3A4 monooxygenase to the active metabolite SU12662, which is then inactivated in a second step, similar to other tyrosine kinase inhibitors (36). There is growing evidence that the efficacy of sunitinib is reduced by concomitant mitotane treatment. A recent study published after the completion of this trial investigated pharmacokinetic aspects of sunitinib in nine patients (37). By chance, strongly reduced sunitinib exposure was found in two patients who had been treated with mitotane compared with the other seven patients. Additional experiments were performed in a total of four patients on mitotane treatment using oral midazolam as a phenotypic probe of CYP3A4 activity. Based on the significant reduction of midazolam exposure and markedly increased metabolite concentration, it was concluded that mitotane is an extraordinarily strong inducer of CYP3A4 (37). Data published earlier (for review see Ref. 38) are in good agreement with these findings but have been largely neglected in the past.

Strong induction of CYP3A4 by mitotane and therefore reduced exposure to the active drug may also explain the relatively low toxicity in this trial. In comparison with data reported in the literature, some adverse drug effects typical for tyrosine kinase inhibitors were relatively rare in this trial. For instance, in previous trials (24, 39), fatigue was reported in 14–65%, hypertension in 9–33%, and hand-foot syndrome in 5–32% of patients, whereas we have seen these adverse events in only 5, 0, and 3%, respectively. However, the treatment period in our trial was relatively short, biasing a direct comparison.

These findings again suggest that the low toxicity of sunitinib, with few treatment-related serious adverse events and adverse events in our trial, is due to increased metabolic clearance of sunitinib through CYP3A4 induction by mitotane. The fact that we did not find differences in adverse events between mitotane-treated and not mitotane-treated patients is most likely attributable to the overall high rate of adverse effects induced by mitotane compensating the lower sunitinib related toxicity.

All available tyrosine kinase inhibitors are metabolized via CYP3A4 (36), and hence, drug interactions with mitotane may also have influenced the results of previous studies in ACC (16, 18). The trial of sorafenib and paclitaxel (20) indirectly supports the notion of a specific antineoplastic effect of sunitinib in ACC. Mitotane was withdrawn in all patients 1 month before the initiation of sorafenib and mitotane serum levels were below 10 mg/liter in all but one patient at baseline. Therefore, one would expect a less relevant impact of mitotane on sorafenib pharmacokinetics, but sorafenib nevertheless failed to demonstrate any effect.

In conclusion, sunitinib has modest single-agent activity in patients with refractory advanced ACC, although substantial drug interaction appears to have abrogated some antitumor efficacy of sunitinib. This result compares favorably with the results of other targeted therapies and is encouraging for further investigation in mitotane-naïve patients with advanced ACC. Alternatively, further investigations should clarify the daily dosage of sunitinib required to reach therapeutic sunitinib exposure in mitotane treated patients.

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