Cabozantinib monotherapy for advanced adrenocortical carcinoma: a single-arm, phase 2 trial

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Summary

Background Adrenocortical carcinoma is a rare malignancy with poor response to systemic chemotherapy. Mitotane is the only approved therapy for adrenocortical carcinoma. Cabozantinib is a multikinase inhibitor approved in multiple malignancies. This is the first prospective trial to explore the anti-tumour activity, safety, and pharmacokinetic profile of cabozantinib in patients with advanced adrenocortical carcinoma.

Methods This investigator-initiated, single-arm, phase 2 trial in adult patients (aged ≥18 years) with advanced adrenocortical carcinoma was done at the University of Texas MD Anderson Cancer Center (Houston, TX, USA). Eligible patients had histologically confirmed adrenocortical carcinoma, were not candidates for surgery with curative intent, had measurable disease, had an estimated life expectancy of at least 3 months, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 with adequate organ function. Patients who had used mitotane within 6 months of study participation were required to have a serum mitotane level of less than 2 mg/L. Patients were given oral cabozantinib 60 mg daily with the option of dose reduction to manage adverse events. The primary endpoint was progression-free survival at 4 months, assessed in all patients who received at least one dose of study drug per protocol. This study is registered with ClinicalTrials.gov, NCT03370718, and is now complete.

Findings Between March 1, 2018, and May 31, 2021, we enrolled 18 patients (ten males and eight females), all of whom received at least one dose of study treatment. Of the 18 patients, eight (44%) had an ECOG performance status of 0, nine (50%) patients had a performance status of 1, and one (6%) patient had a performance status of 2. Median follow-up was 36·8 months (IQR 30·2–50·3). At 4 months, 13 (72·2%; 95% CI 46·5–90·3) of 18 patients had progression-free survival and median progression-free survival was 6 months (95% CI 4·3 to not reached). One patient remains on treatment. Treatment-related adverse events of grade 3 or worse occurred in 11 (61%) of 18 patients. The most common grade 3 adverse events were lipase elevation (three [17%] of 18 patients), elevated γ-glutamyl transferase concentrations (two [11%] patients), elevated alanine aminotransferase concentrations (two [11%] patients), hypophosphatemia (two [11%] patients), and hypertension (two [11%] patients). One (6%) of 18 patients had grade 4 hypertension. No treatment related deaths occurred on study.

Interpretation Cabozantinib in advanced adrenocortical carcinoma showed promising efficacy with a manageable and anticipated safety profile. Further prospective studies with cabozantinib alone and in combination with immune checkpoint therapy are ongoing.

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Introduction

Adrenocortical carcinoma is a rare endocrine malignancy with an annual incidence of about one case per million population globally.[1,2] Generally, adrenocortical carcinoma is associated with a poor prognosis and limited response to currently used treatments.[3–5] Mitotane is the only approved systemic therapy for adrenocortical carcinoma and is often combined with other cytotoxic chemotherapy drugs due to its limited efficacy. The standard of care for metastatic adrenocortical carcinoma is the combination of cisplatin, etoposide, doxorubicin, and mitotane. This regimen is associated with substantial toxicity and a suboptimal objective response rate of 23% with a median time to progression of about 5 months.[6] Tyrosine kinase inhibitors have shown clinical efficacy in improving survival across many solid malignancies but have not shown meaningful efficacy in adrenocortical carcinoma.[7] The poor efficacy of tyrosine kinase inhibitors in adrenocortical carcinoma could be partly because of previous use of mitotane, which increases their hepatic clearance through cytochrome P450 3A4 (CYP3A4) induction.[8,9] Cabozantinib, an oral tyrosine kinase inhibitor with targets including VEGF, c-Met, AXL, and RET receptors, has been found to suppress adrenocortical carcinoma cell line proliferation and tumour growth in vitro and in adrenocortical carcinoma mice xenografts.[10] Cabozantinib primarily disrupts tumour vasculature and increases tumour hypoxia in preclinical models.[10] In a

Research in context

Evidence before this study

We searched PubMed from database inception to Nov 13, 2023, for reports published in English using the search terms "adrenocortical carcinoma AND trial AND phase NOT review". The search yielded 52 articles, of which 25 were prospective clinical studies of patients with adrenocortical carcinoma. Of the 24 prospective studies, one study had investigated mitotane, 11 studies focused on chemotherapy regimens alone or in combination with mitotane, four studies were of immunotherapy drugs, and eight studies were of targeted therapy. Chemotherapy with etoposide, doxorubicin, and cisplatin with mitotane is the standard of therapy based on a phase 3 study, which showed that patients given this combination had an improved objective response rate and progression free survival compared with those given the combination with mitotane plus streptozocin, but the study was underpowered to show improvement in overall survival. Two phase 2 studies with pembrolizumab met the primary endpoint of the studies, which supported use in adrenocortical carcinoma. The targeted therapy studies including those targeting the insulin like growth factor receptor, tyrosine kinase inhibitors targeting vascular endothelial growth factor receptors, and others including epidermal growth factor receptor and cyclin dependent kinases. To date, studies of targeted therapy in adrenocortical carcinoma have shown limited treatment responses and have not yielded substantial evidence of improvements in progression-free survival or overall survival.

Added value of this study

This is the first, prospective, phase 2 study in patients with adrenocortical carcinoma that supports the use of a targeted therapy. In this study, which included patients who had previous treatment and hormonally productive tumours (including cortisol-producing tumours), cabozantinib resulted in most patients having disease control at 4 months with 13 (72%) of 18 patients having progression-free survival for longer than 4 months. Cabozantinib was safe and the toxicity profile was consistent with that observed in previous studies in other cancer types, which was important considering that 11 of 18 study participants had cortisol-producing tumors contributing to baseline hypertension. Any previous mitotane exposure lowered pharmacokinetic concentrations of cabozantinib, supporting the need to control for mitotane exposure in patients who will receive drugs that are metabolised by the cytochrome P450 3A4 pathway.

Implications of all the available evidence

This study supports the continuation of an ongoing cabozantinib study in Germany (NCT03612232) to generate more data and subsequent studies exploring cabozantinib in combination with immune checkpoint therapy.

retrospective, off-label analysis, in patients with metastatic adrenocortical carcinoma, cabozantinib use was associated with disease stabilisation or responses in eight (50%) of 16 patients, with an acceptable adverse effect profile.[11]

This phase 2 trial was designed to investigate the activity and safety of cabozantinib in adult patients with advanced adrenocortical carcinoma.

Methods

Study design and participants

We did an investigator-initiated, single-arm, phase 2 clinical trial at the University of Texas MD Anderson Cancer Center (Houston, TX, USA). Eligible patients were adults (≥18 years) with histological confirmation of adrenocortical carcinoma, who were not candidates for surgery with curative intent, had measurable disease, had an estimated life expectancy of at least 3 months, and Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 with adequate organ function. Sex, race, and ethnicity data were self-reported. Concomitant mitotane use was not permitted, and patients who had received mitotane within 6 months of study participation were required to have a mitotane serum level of less than 2 mg/L as an inclusion criterion. Complete inclusion and exclusion criteria are provided in the appendix (pp 1–3). All patients provided written informed consent before study enrolment. The study protocol (appendix pp 25–87) was approved by institutional review board of the University of Texas MD Anderson Cancer Center (2016-0741), in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. The institutional review board of the MD Anderson Cancer Center approved a waiver of consent to publish deidentified clinical data.

See Online for appendix

Procedures

Patients were given oral cabozantinib (Exelixis, Alameda, CA, USA), starting at a dose of 60 mg daily, with the option of dose reduction to 40 mg and 20 mg, or dose interruptions in case of adverse events. Treatment was provided until disease progression or unacceptable toxicity. If patients discontinued treatment due to toxicity, they remained eligible for follow-up for survival analysis. The dose was selected on the basis of the single agent dosing of cabozantinib approved by the US Federal Drug Administration for use in metastatic renal cell carcinoma. Considering the possibility of mitotane induction of CYP3A4, which can affect cabozantinib concentrations, we did a a pharmacokinetic study to measure cabozantinib levels on day 1 and day 29 of cabozantinib treatment. Cabozantinib levels were measured by liquid chromatography–mass spectrometry assay at a validated laboratory (Worldwide Clinical Trials, Austin,

TX, USA). Samples were collected also for future pharmacogenomic studies.

Imaging with CT or MRI was done at baseline and every 8 weeks. Response to therapy was assessed by the Quantitative Imaging Analysis Core at MD Anderson Cancer Center using the Response Evaluation Criteria In Solid Tumors (RECIST; version 1.1) at baseline and every 8 weeks.[12]

Participants were monitored for adverse events from the first study intervention until their final follow-up visit (30 days after the last dose of cabozantinib treatment), and all adverse events, regardless of causality, were reported. Adverse events were graded as per Common Terminology Criteria for Adverse Events (CTCAE; version 4.0). Laboratory assessment and examination occurred within 28 days of study treatment, day 1 of weeks 3 and 5, and then every 4 weeks thereafter (plus or minus 5 days).

For cytokine detection, we used plasma from the enrolled patients who received cabozantinib (appendix p 4). To examine the variations in cytokine, chemokine, and soluble protein expression over time, we did a multiplex assay with the Immune Monitoring 65-Plex Human ProcartaPlex Panel (Thermo Fisher Scientific, Waltham, MA, USA) using Luminex 200 (Luminex, Austin, TX, USA) at baseline screening, at week 9 after cabozantinib treatment, and at disease progression (appendix p 5).

For flow cytometry analysis, peripheral blood mononuclear cells were used. List of antibodies in the flow cytometry panels and the gating strategy of each panel used is provided in the appendix (pp 6, 15). Samples were acquired using the BD LSRFortessa X-20 (BD Biosciences, Franklin Lakes, NJ, USA) and analysed using FlowJo software (version 10.8.1).

Outcomes

The primary endpoint was 4-month progression-free survival (defined as the proportion of patients alive and progression free at 4 months). Progression-free survival was calculated until a patient changed therapy, had disease progression, death, or withdrew consent. Secondary endpoints were overall response rate (proportion of patients with complete response or partial response, per RECIST version 1.1), overall survival (calculated as time from trial consent to date of death or withdrawal of consent), and safety as per CTCAE (version 4.0). Median follow-up time was calculated using the reverse Kaplan Meier technique. Exploratory outcomes were pharmacokinetics of cabozantinib plasma concentrations in response to therapy, the effect of cabozantinib on angiogenic proteins, cytokines, and chemokines in circulation, and steroid hormone biomarkers as markers of disease response. Samples were collected for pharmacogenomic analyses, however, data had not analysed at the time of writing, and results will be reported in a future study.

Statistical analysis

A priori, we expected that cabozantinib would achieve a minimum 4-month progression-free survival of 20% while the maximum toxicity (defined as having to discontinue therapy due to a serious protocol-defined dose limited toxicity) would be below 30%. The sample size was selected on the basis of the width of the 90% credible interval for 4-month progression-free survival from previous tyrosine kinase studies,[4,13] which indicated lack of benefit. With a sample size of 18 participants, a 90% credible interval for the primary endpoint of 4-month progression-free survival had a width of 0·271, assuming that three patients achieved 4-month progression-free survival and a Beta (0·2,0·8) prior distribution on 4-month progression-free survival and adverse event rates. 4-month progression-free survival and adverse events were monitored simultaneously using the Bayesian approach of Thall, Simon, and Estey,[14] as extended by Thall and Sung.[15] Beta prior distributions were placed on the probability of 4-month progression-free survival and toxicity. Patients were monitored in cohorts of three, starting at six patients. Interim early stopping boundaries due to futility and toxicity were calculated using Multc Lean design software (version 2.1.0). Neither early stopping for futility nor early stopping for toxicity occurred, resulting in full accrual of 18 patients.

Primary, secondary, and safety outcomes were assessed in all participants who received at least one dose of study treatment. The primary endpoint of progression-free survival and the secondary endpoint of overall survival were estimated using Kaplan-Meier curves. Overall response rate was estimated using a proportion and a 95% Clopper-Pearson exact CI. Continuous variables were summarised using descriptive statistics. Frequency tables were used for categorical variables. Time-to-event endpoints were compared between subgroups using the log-rank test. Patients were censored due to consent withdrawal and discontinuing the study. Censoring was non-informative. p values of less than 0·05 were deemed to indicate a statistically significant difference. All statistical tests were two-sided.

Post-hoc analyses were done to assess disease control rate (proportion of patients with a complete response, partial response, and stable disease), response assessment in cortisol producing tumours, response assessment in patients with a history of mitotane exposure, and impact of mitotane on pharmacokinetics, angiogenic proteins, cytokines, and chemokines.

For pre-specified exploratory analysis of pharmacokinetics of cabozantinib plasma levels in response to therapy, the area under the curve was calculated using the linear trapezoid method for each patient on day 1 and 29, and the medians and IQR were reported. Statistical analyses of flow cytometry and cytokine assays were performed using non-parametric Wilcoxon matched-pairs signed rank test and two-way ANOVA using GraphPad Prism (version 9.0.0). The Investigational New

Drug Office at MD Anderson Cancer Center monitored this study. The study is registered with ClinicalTrials.gov, NCT03370718.

Role of the funding source

The study funder was involved in study design, but had no role in data collection, data analysis, data interpretation, or writing of the report.

Results

Between March 1, 2018, and May 31, 2021, 28 patients were assessed for eligibility, of whom 19 patients met the eligibility criteria. One patient withdrew consent before starting treatment to join another experimental treatment (figure 1). 18 participants received at least one dose of cabozantinib and were included in this analysis. Ten (56%) of the 18 study participants were men and eight (44%) were women, and the median age at the consent date was 55·5 years (IQR 46·3–64·8; table 1). The median time between the initial adrenocortical carcinoma diagnosis and the start of cabozantinib therapy was 23·8 months (IQR 12·4–46·2), 11 (61%) of 18 patients had adrenal surgery before joining the study, and 16 (89%) were treated with previous systemic therapy. 15 (83%) of 18 patients had hormonally active adrenocortical carcinoma, and 11 (61%) had cortisol overproduction.

Patients started cabozantinib treatment at a median of 17·5 days (IQR 14·3 to 22·0) after the consent date. The median duration of exposure to cabozantinib was 5·7 months (2·5 to 7·2). Patients who had received previous treatment with mitotane within 6 months of cabozantinib dosing had a shorter treatment duration than patients who were treated with mitotane more than 6 months before study start. The median follow-up for participants was 36·8 months (95% CI 33·2 to not reached). Two (11%) of 18 patients discontinued study treatment due to surgical procedures unrelated to cabozantinib treatment, one (6%) patient withdrew consent due to an adverse event (grade 2 osteonecrosis of the jaw), and one (6%) patient was taken off the study due to grade 4 hypertension both adverse events were treatment-related. One (6%) of 18 patients remains on study treatment at the time of writing with stable disease 26·9 months after the start of treatment (figure 1).

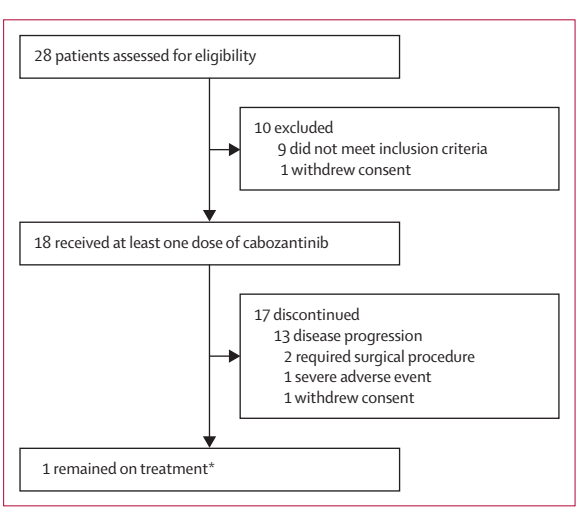

Figure 1: **Trial profile**
*One patient (with stable disease according to Response Evaluation Criteria in Solid Tumours version 1.1) remained on the study treatment at the time of writing.

	Participants (n=18)
Sex	
Female	8 (44%)
Male	10 (56%)
Age, years	55·5 (46·3–64·8)
Race	
Asian	2 (11%)
Black	1 (6%)
White	14 (78%)
Other	1 (6%)
Time from initial adrenocortical carcinoma diagnosis, months	23·8 (12·4–46·2)
Hormonal activity of tumour	
Androgen-producing	4 (22%)
Cortisol-producing (including mixed hormonal production)	11 (61%)
Non-functioning	3 (17%)
Number of metastatic sites at start of cabozantinib	
1	8 (44%)
2	5 (28%)
3	5 (28%)
Sites of metastases	
Lungs	10 (56%)
Liver	9 (50%)
Bone	6 (33%)
Other	8 (44%)
ECOG performance status	
0	8 (44%)
1	9 (50%)
2	1 (6%)
Number of lines of previous systemic therapy	
0	2 (11%)
1–2	7 (39%)
3–4	5 (28%)
≥5	4 (22%)
Systemic therapies before cabozantinib	
Platinum-based chemotherapy	12 (67%)
Mitotane	12 (67%)
Immunotherapy	10 (56%)
Other	5 (28%)
Previous adrenalectomy	11 (61%)

Data are n (%) or median (IQR). ECOG=Eastern Cooperative Oncology Group.

Table 1: **Baseline demographic and clinical characteristics of the enrolled patients**

At 4 months, 13 (72·2%; 95% CI 46·5 to 90·3) of 18 study participants had progression-free survival and the median progression-free survival was 6 months (95% CI 4·3 months to not reached; figure 2A). Post-hoc analysis of progression-free survival based on previous mitotane exposure was performed (appendix pp 19–20). The median overall survival was 24 months (95% CI 15·6 months to not reached; figure 2B).

Two (11%) of 18 patients discontinued study treatment due to severe adverse events and early clinical progression and did not have on-study radiological evaluation. Hence, tumour growth pattern was evaluated in 16 patients (89%) of 18 patients (figure 3A). Two (11% [95% CI 1·4–35·0]) of 18 patients had an overall response (complete response or partial response), and 14 (78% [52–94]) of 18 study participants had a complete response, partial response, or stable disease. Regarding the best radiological response achieved during the treatment, 12 (75%) of 16 patients had stable disease for a median 3·7 months (IQR 3·1–7·3), and two (13%) of 16 patients had a confirmed partial response for a median of 8·9 months (5·8–11·9). Among 10 patients with cortisol-producing tumours who had restaging on study, two (20%) had disease progression, seven (70%) had stable disease, and one patient had a confirmed partial response (figure 3B).

11 (61%) of 18 patients had a dose reduction from 60 mg daily to 40 mg per day due to adverse events, and three (17%) required an additional reduction to 20 mg per day. The most frequent treatment-related adverse events occurring in at least 33% of study participants are shown in table 2. Full list of all adverse events, treatment-related adverse events, and severe treatment-related adverse events are provided in the appendix (pp 7–13). 11 (61%) of 18 patients had grade 3 or 4 treatment-related

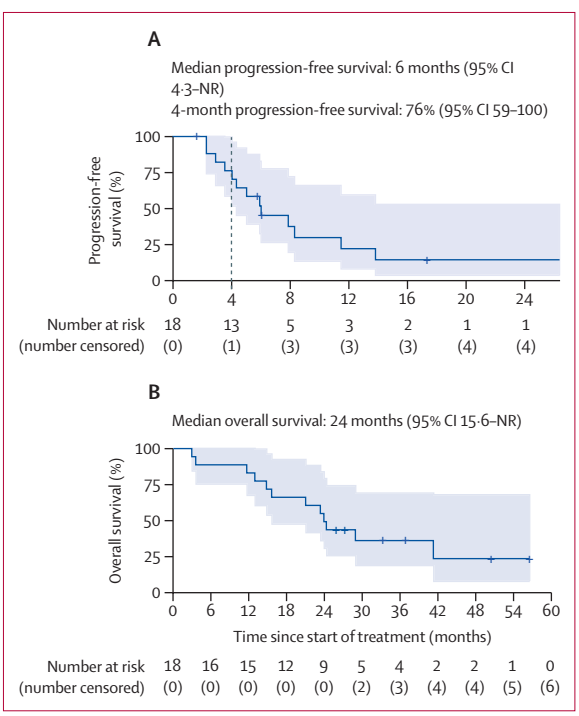

Figure 2: **Progression-free survival (A) and overall survival (B)**
NR=not reached.

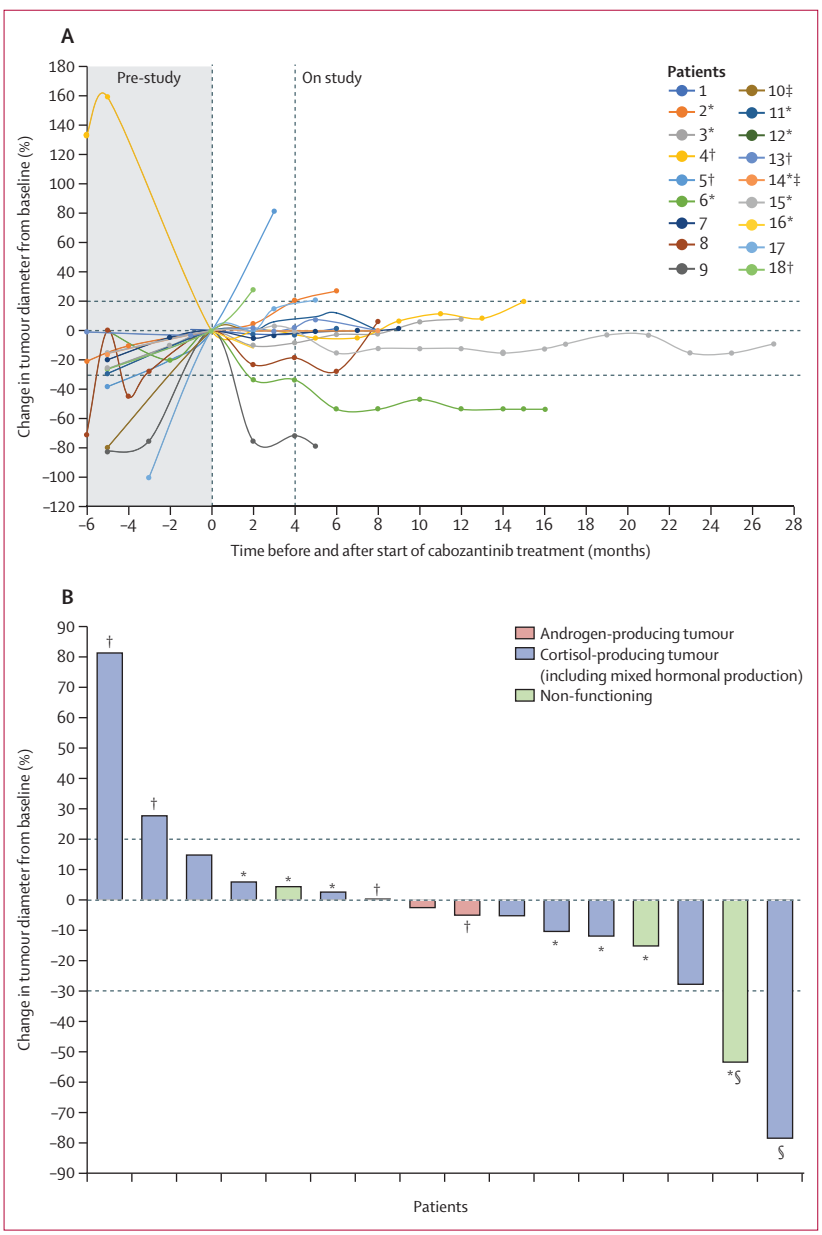

Figure 3: **Tumour response during the study**
(A) Tumour growth evaluated on radiological assessment 6 months before (grey background) and after start of cabozantinib treatment (white background) relative to baseline. Patient 4 had a significant decrease in tumour volume due to systemic therapy before participating in this study. This patient was enrolled to receive cabozantinib since they presented with a residual tumour. (B) Best overall response achieved by 16 patients. Change in tumour diameter of between −30% and 20% from baseline was considered stable disease (within dotted lines), as per RECIST (version 1.1). An increase of more than 20% in diameter from baseline was considered progressive disease, and a decrease of more than 30% in diameter from baseline was considered a partial response. *Patients who received mitotane more than 6 months before cabozantinib treatment. †Patients who received mitotane within 6 months of starting cabozantinib. ‡Patients 10 and 14 did not have radiological evaluation after cabozantinib treatment due to adverse events and early clinical progression. §Confirmed partial response.

adverse events. The most common grade 3 treatment-related adverse events were lipase elevation (n=3), elevated gamma-glutamyl transferase (n=2), elevated alanine aminotransferase (n=2), hypophosphataemia (n=2), hypertension (n=2), weight loss (n=1), and decreased white blood cells (n=1). One (6%) of 18 patients developed a sudden-onset grade 4 treatment-related adverse event (hypertensive emergency) and was taken off the study 8 days after starting treatment. No treatment-related deaths occurred. The most frequent treatment-related adverse events of any grade were diarrhoea (12 [67%] of 18 patients), weight loss (11 [61%] patients), fatigue (nine [50%]), and elevation of liver transaminases (13 [72%]). Subsequent anti-cancer treatments received by the patients are mentioned in the appendix (p 14).

We did a post-hoc analysis to assess previous mitotane exposure and adverse events during the study. A median of 18·5 (IQR 15·5–20·0) treatment-related adverse events were reported among the six patients who had never received mitotane, a median of 18 (12·5–24·3) treatment-related adverse events were reported among the eight patients who stopped mitotane more than 6 months before the study, and, a median of 8·5 (5·8–13·3) treatment-related adverse events were reported among the four patients who had received mitotane within 6 months of starting the study.

	Grade 1–2	Grade 3	Grade 4
Diarrhoea	12 (66·7%)	0	0
Weight loss	10 (55·6%)	1 (5·6%)	0
Fatigue	9 (50%)	0	0
Dysgeusia	8 (44·4%)	0	0
Palmar-plantar erythrodysesthesia syndrome	8 (44·4%)	0	0
Hypertension	4 (22·2%)	2 (11·1%)	1 (5·6%)
Nausea	7 (38·9%)	0	0
Anorexia	6 (33·3%)	0	0
Dry skin	6 (33·3%)	0	0
Mucositis oral	6 (33·3%)	0	0
Increased alanine aminotransferase concentrations	11 (61·1%)	2 (11·1%)	0
Increased aspartate aminotransferase concentrations	12 (66·7%)	0	0
Increased alkaline phosphatase concentrations	11 (61·1%)	0	0
Decreased white blood cell count	8 (44·4%)	1 (5·6%)	0
Hypophosphataemia	6 (33·3%)	2 (11·1%)	0
Increased thyroid-stimulating hormone concentrations	8 (44·4%)	0	0
Increased γ-glutamyl transferase concentrations	5 (27·8%)	2 (11·1%)	0
Increased lipase concentrations	4 (22·2%)	3 (16·7%)	0
Decreased platelet count	7 (38·9%)	0	0
Proteinuria	7 (38·9%)	0	0
Anaemia	6 (33·3%)	0	0
Increased amylase concentrations	6 (33·3%)	0	0

Treatment-related adverse events were considered adverse events classified as related, possibly related, and probably related to treatment. Most frequent refers to events that occurred in at least 33% of study participants. There were no grade 5 treatment-related adverse events. The full list of treatment-related adverse events is in the appendix (pp 12–14).

Table 2: **Most frequent treatment-related adverse events during the study (N=18)**

All patients underwent pharmacokinetic assessment on day 1, and 17 patients on day 29 of cabozantinib treatment, and the findings for this exploratory analysis are shown in figure 4.

We assessed whether cabozantinib treatment influenced the presence of angiogenic proteins and selected immune markers in circulation. Although the majority of cytokines and chemokines assessed did not change over time, by week 9 there was a significant increase in the concentrations of VEGF-A (p=0·0081), MMP-1 (p=0·0398), TIM3 (p=0·0068) and CD69 (p=0·0342) and a significant decrease in the concentrations of ENA-78 (p=0·0012),) HGF (p=0·0332), total CD4⁺ T cells (p=0·0049) and CD25⁺Foxp3⁺ regulatory T cells (p=0·0020) relative to baseline; appendix pp 21–22). Other inhibitory receptors, activation markers, myeloid cell lineages and proliferation markers remained unchanged. No change in phenotype was observed in the CD4⁺ non-Treg subsets or the CD8⁺ T cells in circulation (appendix pp 21–22). Post-hoc analysis to assess the influence of previous mitotane treatment on the concentration of five cytokines identified (HGF, VEFG-A, MMP-1, ENA-78, and MDC/CCL22) and the concentration of these cytokines and chemokines showed no differences in cytokine and chemokine concentrations on the basis of previous mitotane treatment (appendix pp 23–24). These findings

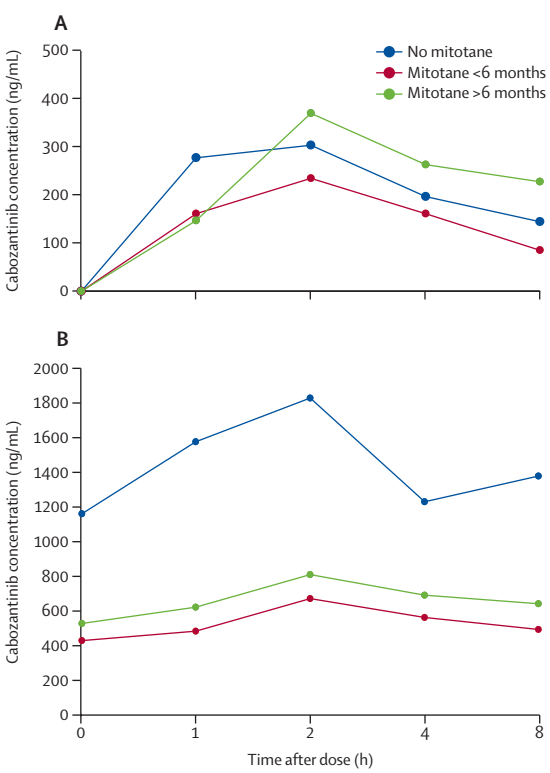

Figure 4: **Pharmacokinetic profile of cabozantinib on day 1 (A) and on day 29 (B) of treatment**
0 h corresponds to the pre-dose plasma concentration of cabozantinib.

must be interpreted with caution considering the small sample size.

Discussion

In this investigator-initiated phase 2 clinical trial, cabozantinib met the prespecified boundary for anti-tumour activity and toxicity establishing for the first time that a tyrosine kinase inhibitor has activity in adrenocortical carcinoma. The majority of participants achieved disease control with cabozantinib, and the adverse events were within the known toxicity profile of cabozantinib. Treatment-related adverse events were manageable with dose reductions and intensive blood pressure management. The side-effect profile is of clinical significance considering the number of patients with cortisol producing tumours who often have baseline secondary hypertension requiring intensive anti-hypertensive management.

The understanding of adrenocortical carcinoma tumorigenesis has evolved; however, little has changed in the availability of treatments for adrenocortical carcinoma in clinical practice since the adoption of etoposide, doxorubicin, and cisplatin with mitotane. Kroiss and colleagues studied the effect of sunitinib in patients with refractory adrenocortical carcinoma in a phase 2 trial.[13] Sunitinib use was associated with a median progression-free survival of 83 days (95% CI 80–85) and a median overall survival of 5·4 months (3·2–7·6), and five (15%) of 35 patients had stable disease as best response.[13] These low response rates were mostly attributed to previous mitotane use.

van Erp et al showed that CYP3A4 induction by mitotane altered the effect of sunitinib, even after 2 months of cessation of mitotane therapy.[9] Our pharmacokinetic data on day 1, which reflect the absorption phase of cabozantinib, did not identify a clear effect of previous mitotane use on cabozantinib concentrations. The pharmacokinetic data on day 29 suggest a lasting effect of previous mitotane use, reducing cabozantinib concentrations. This finding was notable considering our study requirement of a long duration from last mitotane use or low mitotane levels before study entry. The duration of mitotane's effect on CYP3A4 remains unknown. However, considering that mitotane has a half-life ranging from 18 to 159 days, it is not surprising that prolonged CYP3A4 induction can be observed many months after mitotane cessation.[13,16–18]

Considering these findings, we recommend future studies with tyrosine kinase inhibitors alone or in combination with immune checkpoint blockade should consider previous mitotane exposure carefully in the study design.

In terms of efficacy, Kroiss et al published a retrospective cohort of 16 patients with adrenocortical carcinoma treated with cabozantinib and reported a median progression-free survival of 16 weeks (range 3–61) and overall survival of 58 weeks (6–83). Three (19%) of 16 patients had a partial response, five (31%) had stable disease, and eight (50%) had progressive disease.[11] Miller et al evaluated multikinase inhibitors (cabozantinib or lenvatinib) in eight patients with recurrent metastatic adrenocortical carcinoma and reported a partial response rate of 25% (two of eight patients), stable disease rate of 38% (three of eight patients), a progressive disease rate of 38% (three of eight patients), and a clinical benefit rate of 63% (five of eight patients).[19] Our findings, in a heavily pretreated patient population with predominantly cortisol-producing tumours, found that the majority of patients had disease control with cabozantinib and that the treatment was safe. Of the 11 patients with cortisol-producing adrenocortical carcinoma, a well-known poor prognostic factor, eight (73%) had best overall response of stable disease or partial response.[3–5,20]

The most frequent treatment-related adverse events in our study were consistent with the known safety profile of cabozantinib.[21–23] Dose reduction and brief interruptions were helpful to ameliorate most treatment-related adverse events, and only one patient discontinued cabozantinib during the study due to a grade 4 treatment-related adverse event. This grade 4 treatment-related adverse event was a hypertensive emergency and occurred in a 72-year-old woman with a previous history of hypertension. No grade 5 treatment-related adverse events were reported.

Our translational findings demonstrated an effect of cabozantinib on angiogenic factors, chemotactic proteins, and Treg phenotype in circulation. Collectively, our findings highlight the involvement of cabozantinib in the immune modulation of pro-angiogenetic molecules via Treg-independent mechanisms. The decrease in circulating HGF concentrations was an expected mechanism and correlated with the reduced tumour growth rate observed in this study.[24,25] VEGF-A concentrations showed an inverse response and increased significantly. This effect of cabozantinib on VEGF-A was also observed in the plasma of patients with metastatic castration-resistant prostate cancer.[26] We also identified a unique effect of cabozantinib on the systemic immune system. Treg cells were found in circulation at a relatively high frequency within the total immune population but were found to be significantly reduced following treatment. This is similar to the effect of cabozantinib in the preclinical model by Kwilas and colleagues, where splenic Treg-cell concentrations were significantly reduced following treatment.[27] This study also found an effect of cabozantinib in reducing the suppressive capability of Treg cells. Although we were unable to directly assess the suppressive nature of Treg cells in this setting, we did observe changes in their phenotype that could be associated with their function. Changes in Treg phenotypes were also observed by Apolo and colleagues following treatment with cabozantinib in patients with advanced urothelial cancer.[28] Apolo and colleagues identified a reduced suppressive Treg phenotype, as indicated by increased expression of

PD-1 and decreased expression of TIM3, however, this was not found in our study.

We did not observe any effect on Treg expression of PD-1, but did observe a significant increase in CD69 and TIM3 surface expression on Treg cells after cabozantinib treatment, which suggests the induction of an activated, highly suppressive Treg phenotype.[29] Additionally, although preclinical and clinical studies reported an effect of cabozantinib on $CD8^+$ T-cell frequencies, this was not found in this study. We did not observe any change in $CD4^+$ or $CD8^+$ T-cell phenotypes in circulation. Previous reports have also observed a decrease in suppressive monocyte-derived suppressor cells following cabozantinib; however, we observed these cells to be at low frequency (if detected at all) in circulation, and their frequencies did not change following treatment. This effect on monocyte-derived suppressor cells might be limited to tumour types associated with their presence. Although limited by sample size and use of blood versus on-treatment tumoural tissue, our exploratory translational findings indicate that cabozantinib might have a unique effect on the suppressive function of Treg cells in adrenocortical carcinoma that requires further exploration and validation. Our study attempted to collect on-treatment tumoural biopsies, but these tumour samples were necrotic and not amenable to testing. Future studies incorporating tissue collection is important to verify findings detected in circulation.

Our study, despite the prospective design, was limited by the small sample size that was in part related to the rarity of adrenocortical carcinoma. Because the study was not randomised, it might have been predisposed to biases such as selection bias. Thus, future ongoing studies will be crucial to validate our findings and to further establish the role of cabozantinib in adrenocortical carcinoma. An ongoing study of cabozantinib in adrenocortical carcinoma (NCT03612232) is open in Germany and is continuing to accrue patients. Our study also did not incorporate germline and somatic testing and the impact of cabozantinib across different genetic backgrounds remains unknown.

In summary, cabozantinib in advanced adrenocortical carcinoma showed promising activity and a manageable safety profile. Previous mitotane use led to lower cabozantinib concentrations and might have impaired its efficacy. The possible immunomodulatory effect of cabozantinib, if proven, could have potential for combination therapy with immunotherapy in patients with adrenocortical carcinoma.

Contributors

MTC, CJ, and MAH designed the study. VBB, GT, and MAH collected data. VBB, GT, JPL, and MAH were responsible for clinical data management and quality assurance. MTC, VBB, GT, LPM, and MAH oversaw the protocol. MTC, VBB, CJ, GT, LPM, JV, AYS, JPL, MZ, JO, CH, and MAH analysed and interpreted the data. All authors participated in drafting and reviewing iterations of the manuscript and approved the final draft for submission. MTC, VBB, CJ, GT, LPM, JV, AYS, JPL, MZ, JO, CH, and MAH accessed and verified the underlying data reported in the manuscript. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Declaration of interests

MTC reports institutional research funding from ApricityHealth, AstraZeneca, Exelixis, Janssen, Pfizer, SeaGen, and the United States Department of Defense; speaker's Bureau participation with Curio Science, Dava Oncology, and MJH Life Sciences; and advisory board fees or honorarium from Exelixis, Eisai, and SeaGen. CJ reports research grants paid to their institution from Exelixis, Merck Sharp & Dohme, Progenics pharmaceuticals, and Lantheus pharmaceuticals; and participation of scientific advisory boards for Merck Sharp & Dohme and Lantheus pharmaceuticals. CH reports research grants to their institution from Avenge Bio, Dragonfly Therapeutics, BTG, Sanofi, and Iovance; stock options from BriaCell as a scientific advisory board member; and travel expenses or honoraria from the Hope Foundation and the Society for Immunotherapy of Cancer, outside the scope of the submitted work. MAH reports research grants to their institution from Exelixis, Merck, and Corcept; and honoraria from HRA pharma. All other authors declare no competing interests.

Data sharing

Data collected for the study, including individual participant data, de-identified participant data, and a data dictionary, can be requested by qualified researchers from the corresponding author, and requests will be assessed by a scientific review board.

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