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Antineoplastic activity of Artemisin in Adrenocortical Carcinoma

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Response to Reviewers:	Professor Sebastiano Filetti Editor in Chief Endocrine Dear professor Filetti Here attached please find the revised version of the paper entitled “Antineoplastic activity of Artemisin in Adrenocortical Carcinoma.” By Luigi Lorini et al that we wish to resubmit as Research Letter to Endocrine. As itemized below, in the new version all the minor comments/chriticisms raised by the refree 2 were addressed. Looking forward to the decision of the Editorial Office we remain Yours sincerely Luigi Lorini on behalf of all co-authors Replies to the Reviewer #2 comments I read with attention the manuscript entitled "antineoplastic activity of artemisin in adrenocortical carcinoma". It addresses an interesting case of a possible

	antineoplastic activity of artemisin in a patient with advanced adrenocortical carcinoma. Despite, as mentioned by the authors, is difficult to estabilish if artemisin has had a role in slowering the disease progression, this is a promising observation that could warrant the implementation of preclinical and clinical studies in the following years, considering the limited availability of anticancer drugs in the treatment of adrenal carcinoma, expecially in advanced patients. We thank the reviewer for the appreciation of our paper I suggest to report how was PET (or CT or RM) after 33 months of EDP-M scheme To address this issue the following sentence was added: “In November 2011 an abdomen MRI showed a hypervascularized nodule attached to the left diaphragmatic side, confirmed by a subsequent PET with fdg .” (page 3 lines 11-12) What do you mean by indolent progression? Please specify the radiologic findings during the subsequent chemotherapy lines and if chemotherapy was going on when there was disease progression We have better clarified the point and the following paragraph was added in the text: “Second line chemotherapy with carboplatin + paclitaxel was introduced leding to a radiological response lasting 10 months of both diaphragmatic (15mm vs 23mm) and mesenteric (5 mm vs 10mm) lesions. At disease progression, Gemcitabine plus capecitabine was administered but this regimen was interrupted after 2 months due to unefficacy. Mitotane therapy was never interrupted. In December 2013, a further disease progression was observed: at TC scan the 2 abdominal lesions converged in a large left adrenal lesion (51x12mm). During the subsequent months, the disease showed only limited progression. Therefore, due to the rather indolent behavior of the disease, in March 2015 the patient underwent surgery with radical intent. The histological examination confirmed the ACC recurrence” (page 3, lines 13-20).

Antineoplastic activity of Artemisin in Adrenocortical Carcinoma.

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INTRODUCTION

Artemisia annua is a medicinal plant that is used in China to treat various diseases for over two millenniums. Preclinical studies revealed that the compound has a cytotoxic activity against various types of cancer cells(1)

Data on anticancer activity of artemisin in humans are limited, but case reports and small case series have shown encouraging results in patients affected by metastatic breast cancer, colorectal cancer and uveal melanoma (2).

The main mechanism of artemisin antineoplastic activity is not fully elucidated. This drug and relevant derivatives display a multi-target activity, as frequently observed in most natural products (3). Indeed, artemisin compounds could block the activation of intracellular pathways such as Wnt/β-catenin, BCR/ABL, or by inducing cell cycle arrest, alteration of DNA repair mechanisms and inducing death via apoptosis and non-apoptotic pathways.

Concerning the artemisin safety profile, reported adverse effects, such as renal, hepatic and neurological toxicity were unfrequent and mild. Only one case of toxic brainstem encephalopaty was observed in a patient after only 2 week treatment of artemisin (400mg)(4).

The good safety profile and the multi-target mechanism of action makes this drug potentially useful in association with current available therapies, in order to potentiate their efficacy. In particular, the observed activity of artemisin in reversing resistance to chemotherapy is suggestive for the potential synergism of action between artemisin and cytotoxic drugs (5). Indeed, the drug administered in association with gemcitabine or cyclophosphamide was found to improve the response rate in patients with pancreatic cancer and lung cancer, respectively (6) (7). Further synergistic effects were observed with others antineoplastic compounds, such as cisplatin, carboplatin, doxorubicin and temozolomide (8).

AdrenoCortical Carcinoma (ACC) is a rare and aggressive neoplasm. The prognosis of metastatic patients is poor(9)(10)

Mitotane or mitotane plus cytotoxic chemotherapy (etoposide, doxorubicin and cisplatin—EDP-M scheme) are the standard systemic treatments in the management of metastatic ACC patients (11). No effective second-line therapies are available (12)(13)(14). Modern molecular target therapies and immunotherapies failed to demonstrate a significant activity (15)(16). On these basis, new treatment strategies are needed.

p53 mutation and wnt-β catenin amplification are frequently involved in the ACC carcinogenesis. Since both pathways were reported to be targeted by artemisin and its derivatives, this observation makes artemisin potentially active in the management of ACC(17).

We present in this paper the case of a male patient, bearing ACC with liver and peritoneal metastases, who obtained a durable disease response after oral assumption of artemisin, taken after failure of several antineoplastic therapies.

CASEPRESENTATION

A 51 years old patient underwent left adrenalectomy for ACC in May 2008. Surgery was complicated by capsular rupture. The histological examination showed: adrenal lesion of 7 x 5.5 x 4.5 cm. No adjuvant mitotane therapy was prescribed.

Disease progression occurred 18 months later, when a FDG PET, performed in November 2009, showed local recurrence (45mm) together with multiple metastatic lesions located between spleen and diaphragma. The patient was submitted to EDP-M scheme followed by maintenance of mitotane therapy, leading to a partial response lasting 33 months. In November 2011 a MRI showed hypervascularized nodules on left diaphragma and mesenterial tissue near the spleen, confirmed by a subsequent FDG-PET.

Second line chemotherapy with carboplatin + paclitaxel was introduced leding to a radiological response lasting 10 months of both diaphragmatic (15mm vs 23mm) and mesenteric (5 mm vs 10mm) lesions. At disease progression, Gemcitabine plus capecitabine was administered but this regimen was interrupted after 2 months due to unefficacy. Mitotane therapy was never interrupted.

In December 2013, a further disease progression was observed: at TC scan the 2 abdominal lesions converged in a large left adrenal lesion (51x12mm). During the subsequent months, the disease showed only limited progression. Therefore, due to the rather indolent behavior of the disease, in March 2015 the patient underwent surgery with radical intent. The histological examination confirmed the ACC recurrence.

A newer disease progression was observed in September 2015 when a diaphragmatic mass (42 mmm) was detected. This lesion underwent a local regional therapy with gamma knife attaining a 20% size reduction. The disease remained stable till April 2017 when a MRI showed in T2 weighted images (T2W) two slightly hyperintense metastatic lesions in liver segment 8 (S8) and S7 (8 mm and 7 mm) with restricted diffusion in diffusion weighted images (DWIb800). The lesions were hypointense in ADC (apparent diffusion coefficient) map and hepatobiliary phase post Gadoxetic acid injection (figure 1a)

In May 2017, after a consultation to an herbalist, the patient started taking Artemisia Annua tabs

(Artemisin 99%) (600mg die for five days followed by 5 days off). The treatment was well tolerated, the patient did not suffer from any symptoms and no clinical sign of toxicity was observed at clinical examination.

In July 2017, a MRI showed a marked size reduction of the 2 metastases in S8 and S7 as confirmed at DWIb800 and ADC map (5 mm vs 8 mm and 5 mm vs 7 mm) (figure 1b).

The response obtained was maintained at the MRI performed in January 2018 showing stable disease of the lesion in S7 and S8 at T2W and DWI/ ADC (figure 1c).

Artemisin assumption was prudentially interrupted in February 2018, as recommended by the herbalist, due to the occurrence of an Herpes Zoster episode on the left side of abdomen.

In May 2018 a MRI showed an increase in size and number of the hepatic metastases in S8-S7 as compared with previous MRI (8,2mm vs 5 mm and 6mm vs 5mm) (figure 1d).

In June 2018 the patient resumed the assumption of Artemisia Annua at the same dosage and schedule. However a MRI performed on August 2018 showed moderate progression of disease on the two liver lesions that showed an increased in size at S8 (11vs7mm), S7 (20 vs 8mm). In September 2018, the two lesions at S7 and S8 underwent thermoablation leading to size reduction.

The last MRI performed on March 2019 showed further limited disease progression on the known liver lesions in S7 and S8 (11mm vs 7 mm and 8mm vs 6mm).

At the last follow-up examination on May 2019, the patient was in good condition, he is still continuing taking the assumption of Artemisia Annua at the same dosage without significant side effects without taking any further specific antineoplastic treatment.

DISCUSSION

The present case describes for the first time an antineoplastic activity of Artemisin in an advanced ACC patient. As observed in other rclinical eports, the drug was very well tolerated and the disease response duration (about 12 months) is noteworthy, considering the clinical setting (heavily pre-treated metastatic ACC) in which the drug was taken. It should be underlined that the disease response to artemisin in our patient was longer to that observed with second and third line therapies, previously administered, namely cisplatin + taxol and gemcitabine +capecitabine.

The patient continued taking artemisin after disease progression as the only systemic therapy and the disease displayed a rather indolent behavior, without televant side effects. It is difficult to establish whether this compound has had a role in slowering the disease progression

Due to the very limited availability of anticancer drugs in the treatment of ACC, the activity of artemisin observed in the case presented in this paper warrants the implementation of preclinical and clinical studies, in order to understand the molecular mechanisms of the artemisin cytotoxicity in ACC, as well as its clinical efficacy.

COMPLIANCE WITH ETHICALSTANDARDS:

This study was supported with the contribution of FIRM Foundation (Cremona, Italy).

Conflict of Interest: Dr Luigi Lorini declares that he has no conflict ofinterest

Informed consent was obtained from the patient included in thestudy.

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Legends tofigures

Figure 1: Magnetic Resonance Imaging (T2 weighted sequences) of liver metastases in apatient with adrenocortical carcinoma submitted to artemisin: 1a) baseline condition April 2017, 1b)July 2017 after 3 month treatment, 1c) January 2018, after 9 month treatment, 1d) May 2018 atdisease progression after 12 monthtreatment.

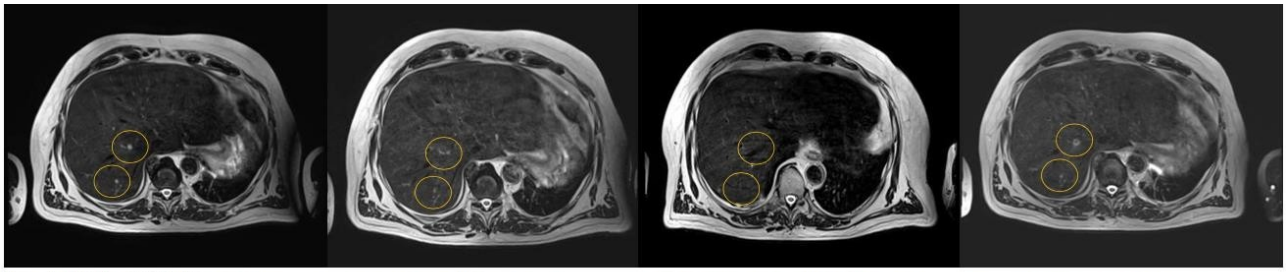

Figure 1a: MRI April 2017 , T2w sequence

Figure 1b: MRI July 2017 T2W seqeunce

Figure 1c: MRI January 2018 T2W sequence

Figure 1d: MRI May 2018 T2W sequence