Ther Adv Med Oncol

2023, Vol. 15: 1–14

DOI: 10.1177/
17588359231197957

Mitochondrially targeted tamoxifen as anticancer therapy: case series of patients with renal cell carcinoma treated in a phase I/Ib clinical trial

Zuzana Bielcikova, Lukas Werner, Jan Stursa, Vladimir Cerny, Ludmila Krizova, Jan Spacek, Stanislav Hlousek, Michal Vocka, Olga Bartosova, Michal Pesta, Katarina Kolostova, Petr Klezl, Vladimir Bobek, Jaroslav Truksa, Sona Stemberkova-Hubackova, Lubos Petruzelka, Pavel Michalek and Jiri Neuzil

***Abstract*:** Mitochondrially targeted anticancer drugs (mitocans) that disrupt the energy-producing systems of cancer are emerging as new potential therapeutics. Mitochondrially targeted tamoxifen (MitoTam), an inhibitor of mitochondrial respiration respiratory complex I, is a first-in-class mitocan that was tested in the phase I/Ib MitoTam-01 trial of patients with metastatic cancer. MitoTam exhibited a manageable safety profile and efficacy; among 37% (14/38) of responders, the efficacy was greatest in patients with metastatic renal cell carcinoma (RCC) with a clinical benefit rate of 83% (5/6) of patients. This can be explained by the preferential accumulation of MitoTam in the kidney tissue in preclinical studies. Here we report the mechanism of action and safety profile of MitoTam in a case series of RCC patients. All six patients were males with a median age of 69 years, who had previously received at least three lines of palliative systemic therapy and suffered progressive disease before starting MitoTam. We recorded stable disease in four, partial response in one, and progressive disease (PD) in one patient. The histological subtype matched clear cell RCC (ccRCC) in the five responders and claro-cellular carcinoma with sarcomatoid features in the non-responder. The number of circulating tumor cells (CTCs) was evaluated longitudinally to monitor disease dynamics. Beside the decreased number of CTCs after MitoTam administration, we observed a significant decrease of the mitochondrial network mass in enriched CTCs. Two patients had long-term clinical responses to MitoTam, of 50 and 36 weeks. Both patients discontinued treatment due to adverse events, not PD. Two patients who completed the trial in November 2019 and May 2020 are still alive without subsequent anticancer therapy. The toxicity of MitoTam increased with the dosage but was manageable. The efficacy of MitoTam in pretreated ccRCC patients is linked to the novel mechanism of action of this first-in-class mitochondrially targeted drug.

Keywords: case series, efficacy, mechanism of action, mitocans, mitochondria, mitochondrially targeted tamoxifen, MitoTam, phase I/Ib trial, safety, renal cell carcinoma

Received: 28 March 2023; revised manuscript accepted: 3 August 2023.

Correspondence to:
Zuzana Bielcikova
Department of Oncology, General Faculty Hospital, U Nemocnice 499/2, Prague 2, 128 08, Czech Republic
zuzana.bielcikova@vfn.cz

Jiri Neuzil
School of Pharmacy and Medical Science, Griffith University, Southport, Qld 4222, Australia

Department of Pediatrics and Inherited Metabolic Diseases, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic

Department of Physiology, Faculty of Science, Charles University, and General University Hospital, Prague, Czech Republic

Institute of Biotechnology, Czech Academy of Sciences, Prumyslova 595, Prague-West 252 50, Czech Republic
j.neuzil@griffith.edu.au; jiri.neuzil@ibt.cas.cz

Lukas Werner
Institute of Biotechnology, Czech Academy of Sciences, Prumyslova 595, Prague-West 252 50, Czech Republic

Diabetes Centre, Institute for Clinical and Experimental Medicine, Prague 4, Czech Republic
lukaswerner@ibt.cas.cz

Jan Stursa
Sona Stemberkova-Hubackova
Institute of Biotechnology, Czech Academy of Sciences, Prague-West, Czech Republic

Diabetes Centre, Institute for Clinical and Experimental Medicine, Prague 4, Czech Republic

Introduction

Tumor metabolism is generally characterized by the phenotypic heterogeneity of the tumor cells that make up the tumor.[1] Good examples of this are the metabolic cooperativity that exists between oxidizing and glycolytic cancer cells in many tumor types, and the observation that the metastatic progenitor cells and circulating stem cells in a tumor

Vladimir Cerny
Department of Radiodiagnostics, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic

Ludmila Krizova
Jan Spacek
Stanislav Hlousek
Michal Vocka
Lubos Petruzelka
Department of Oncology, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic

Olga Bartosova
Institute of Pharmacology, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic

Michal Pesta
Department of Probability and Mathematical Statistics, Faculty of Mathematics and Physics, Charles University, Prague, Czech Republic

Katarina Kolostova
Vladimir Bobek
Laboratory of Personalized Medicine, Oncology Clinic, Faculty Hospital Kralovske Vinohrady, Prague, Czech Republic

Petr Klezl
Laboratory of Personalized Medicine, Oncology Clinic, Faculty Hospital Kralovske Vinohrady, Prague, Czech Republic

Urology Clinic, Third Faculty of Medicine, Charles University and Faculty Hospital Kralovske Vinohrady, Prague, Czech Republic

Jaroslav Truksa
Institute of Biotechnology, Czech Academy of Sciences, Prague-West, Czech Republic

Pavel Michalek
Department of Anesthesiology and Intensive Care, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic

exhibit different metabolic behaviors to that of the multiple tumor cell population within the tumor.[2] In the overall metabolic map of cancer, mitochondria function as powerhouses, and as dynamic signaling organelles controlling cell survival and death, motility, and resistance to treatment. The molecular definition of 'oncogenic mitochondria', that mitochondria carry or could carry malignant information, should be a priority for basic research.

Mitochondria are emerging as plausible therapeutic targets because they differ between normal and malignant cells, and are essential for the initiation/progression of primary and metastatic tumors.[3–5] To date, only one mitochondria-targeting agent, the BH3 mimetic venetoclax, which disrupts anti-apoptotic signaling of Bcl-2 (B-cell lymphoma 2) family proteins, has been approved for the treatment of chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML).[6] AG221, an inhibitor of mutant isocitrate dehydrogenase-2 (IDH2), showed promising results in patients with aberrant IDH2 AML and is currently being evaluated in a phase Ib/II trial.[7] However, the development of some promising drug candidates has been discontinued. For example, IACS-010759, which targets oxidative phosphorylation (OXPHOS), was tested in a phase I clinical trial in individuals with AML and solid tumors, and showed a narrow therapeutic index with dose-limiting toxicities (DLT) that included neurotoxicity and increased blood lactate.[8] Furthermore, BAY240223423,[9] an inhibitor of mitochondria-associated *de novo* pyrimidine synthesis, and CPI-613 (devimistat),[10] a tricarboxylic acid cycle inhibitor, were discontinued due to a lack of benefit against AML and pancreatic cancer, respectively. Drugs that target mitochondrial pathways were associated with adverse events (AEs) including hematotoxicity (neutropenia, anemia, thrombocytopenia), neurotoxicity, upper respiratory tract infection, diarrhea, nausea, fatigue, fever, and serious AEs (pneumonia, febrile neutropenia, autoimmune hemolytic anemia, and tumor lysis syndrome) in prior trials.

Here, we report the initial clinical data for mitochondrially targeted tamoxifen (MitoTam), our novel proprietary compound that inhibits mitochondrial complex I (CI) and disrupts the respiratory chain and energy generation, which are essential for tumor cells.[11,12] MitoTam, a member of the group of *mitocans* (standing for 'mitochondria and cancer'),[11–15] is tagged with the mitochondria-targeting vector triphenylphosphonium (TPP^+), originally used for mitochondrial delivery of coenzyme Q.[16] Due to the lipophilic delocalized cationic TPP^+, MitoTam behaves as an uncharged compound in many biological environments. Because the potential across the inner mitochondrial membrane is considerably higher in cancer cells than in non-malignant counterparts,[17] MitoTam preferentially accumulates in the mitochondria of malignant cells. It prevents the transfer of electrons from the catalytic centre of CI to the ubiquinone molecule, which interacts with molecular oxygen to promote the formation of superoxide, triggering apoptosis.[11]

MitoTam showed high anticancer efficacy in several mouse models of cancer.[11,12] Preclinical studies documented preferential accumulation of MitoTam in the kidney, adrenal gland, lungs, spleen, and liver, at levels 2- to 10-fold greater than the administered dose. High accumulation of MitoTam in kidney tissue, with a half-life estimated to be longer than several weeks, may substantially contribute to its efficacy against renal cell carcinoma (RCC). Based on our research and preclinical data,[11,12] we performed a phase I/Ib MitoTam trial (registered 1 November 2017 with EudraCT number 2017-004441-25), the results of which were recently published.[18] The key findings of the trial are as follows. The safety profile of MitoTam included hematological toxicities (50% of patients in phase Ib), hyperthermia/fever (58% of patients in phase Ib), and thromboembolic (TE) complications (13% of patients in phase Ib). The only significant dose-dependent adverse event (AE) was anemia in phase Ib. Of 14 patients who experienced a clinical benefit of repeated administration of MitoTam, 5 had RCC. The recommended dosage for phase II studies was determined to be 3.0 mg/kg.

Here, we describe a series of six patients with RCC enrolled in phase Ib to demonstrate the safety profile of MitoTam and the rationale for the high efficacy of MitoTam against RCC, which may be considered surprising because kidney tumors rarely respond to treatment with cytostatic drugs. The standard of care is currently represented by immune checkpoint inhibitors (ICIs) or tyrosine kinase inhibitors (TKIs) with antiangiogenic activity.[19] We provide additional data that are not described in our earlier report, including the experimental detection of circulating tumor cells (CTCs) and detailed descriptions of patients with clear cell RCC (ccRCC) who benefited from MitoTam treatment. Owing to the importance of discussing limitations and possible

toxicities when introducing new therapeutic agents, we believe our data will support future trials of MitoTam. To the best of our knowledge, MitoTam is the first cytostatic drug showing effectiveness against renal tumors. Our findings that preferential accumulation of MitoTam in the kidney and a novel mode of action that targets metabolically active OXPHOS are highly supportive of a prospective phase II trial.

Methods

Study design and participants

MitoTam-01 was an open-label, single-arm, non-randomized, single-center, phase I/Ib trial carried out at the Department of Oncology, General Faculty Hospital, Charles University in Prague (Czech Republic) between 23 May 2018 and 22 July 2020.

The trial protocol and the study endpoints are reported in detail in our prior report.[18] The major inclusion criteria were locally advanced inoperable or metastatic tumors after standard therapy, life expectancy >3 months, age 18–75 years, and Eastern Cooperative Oncology Group performance status (PS) of 0–2.

The hypotheses that MitoTam might influence the number of CTCs, mitochondrial network morphology, functionality, and mitochondrial metabolism associated genes expression, were tested as research intention. Complete CTCs data will be published separately.

The CARE case reporting guideline was used when preparing this article.[20]

Procedures

The phase Ib part tested three different repeatedly administered dosages (1.0, 3.0, and 4.0 mg/kg in regimens 1–3) of MitoTam in two schemes (weekly and biweekly). The individual dosages were derived from the results of the phase I part (not discussed here). MitoTam was administered intravenously *via* a tunneled centrally inserted central venous catheter or peripherally inserted central catheter (PICC) to prevent peripheral vein irritation and inflammation.

For experimental determination of the blood CTCs count, blood samples [two ×8 mL, Ethylenediaminetetraacetic acid disodium salt (solution)] were obtained in the screening period, and then in day (D) 5 of cycles 1 and 4 in regimen 1 and in D1 of cycle 6 in regimens 2 and 3. In subsequent cycles, additional blood samples were collected on analogous days (D5 of cycles 5 and 8, 9 and 12, 13 and 16 in regimen 1; and D1 of cycle 12 in regimen 2). Size-based separation (using a MetaCell®) was used to enrich CTCs.[21] After separation, the CTCs were cultured *in vitro* (3–5 days) for subsequent cellular and molecular analysis. Cytomorphological analysis of CTCs involved vital fluorescent staining of the nucleus (NucBlue™), cytoplasm (CellTracker™), and the mitochondria (MitoTracker™). 'Significant elevation' in the number of CTCs was defined as a two-fold increase in the number of CTCs between consecutive assessments or between baseline and the final dose of MitoTam. If the number of CTCs remained stable or decreased, it was considered as a 'reduction of the CTC-positive rate'. The number of CTCs at the end of the study was compared to the number of CTCs determined at study entry (screening), and compared to the clinical benefit rate (CBR). A reduction of the CTC-positive rate was also considered if the mitochondrial activity of CTCs was changed, according to the results obtained using MitoTracker™. Thus, a reduction of the CTC-positive rate applied to cases with non-vital mitochondria or a significant reduction in the mitochondria network, even if the number of CTCs increased.

To explore the treatment efficacy, patients underwent computed tomography (CT) scans during the screening period and after every four cycles in regimen 1 or every six cycles in regimens 2 and 3. The CBR was defined as the percentage of patients with a complete response (CR), partial response (PR), or stable disease (SD). Patients with SD or PR continued treatment, with additional four cycles, to a maximum of 16 cycles in regimen 1 or to 12 cycles in regimen 2. It was not possible for responders in regimen 3 to continue therapy due to the time restriction linked to the COVID-19 pandemic. Patient enrollment into regimen 3 was postponed at the beginning of 2020. Due to planned end of the study responders had no possibility to repeat the MitoTam therapy.

Results

Patient characteristics and disease course

A total of 38 patients were enrolled into the phase Ib part of the MitoTam-01 trial between 11

Table 1. Summary of the main clinical and pathological characteristics, treatment course, and clinical response to MitoTam of the six patients with RCC.

	Patient no. and MitoTam dosage level (mg/kg)					
	P1-1.0	**P6-1.0**	**P20-1.0**	**P3-3.0**	**P6-3.0**	**P9-3.0**
Regimen	1	1	1	2	2	2
MitoTam weight-based doses (mg)	89–97	84–87	58–65	207–222	309–315	216–225
Sex	Male	Male	Male	Male	Male	Male
Age (years)	63	71	62	72	73	67
ECOG PS at screening	0	1	0	0	0	1
Histological type of RCC	ccRCC	ccRCC	CCSF	ccRCC	ccRCC	ccRCC
Lines of palliative treatment	3× TKI	3× TKI, 1× ICI	2× TKI, 1× ICI, 1× CTx	3× TKI	2× TKI, 1× IFN	4× TKI
Sites of metastatic disease at screening	Right kidney, T12, muscles	Local recurrence, left adrenal gland, tumor near spleen, skeleton (C7, Th12, ilium, hip bones)	Local recurrence, others in RP, lungs, LN in chest and RP	Pancreas, right adrenal gland, lungs, mediastinum, tumor near bladder	Pancreas, right renal fascia, right kidney, right adrenal gland, lungs, LN in chest	Left pleural effusion, left pleura, lungs, mediastinum, right adrenal gland, LN in RP and chest
Number of cycles of MitoTam	16	12	4	12	6	11
Best clinical response according to RECIST 1.1.	SD	PR	PD	SD	SD	SD

This table includes some data reported in our prior publication and reprinted under a CC-BY license.[18] In regimen 1, each cycle consisted of three administrations of MitoTam on D1, D3, and D5 in 1 week. A total of four biweekly cycles were performed over 8 weeks followed by the control CT scan. Responders (patients with CR, PR, or SD) could continue with another four cycles to a maximum of 16 cycles. In regimen 2, MitoTam was administered once per week, six times, followed by the control CT scan. Responders (patients with CR, PR, or SD) could continue treatment for another six cycles to a maximum of 12 cycles.
RCC, renal cell carcinoma; C, cervical vertebra; ccRCC, clear cell renal cell carcinoma; CCSF, claro-cellular carcinoma with sarcomatoid features; CR, complete response; CT, computed tomography; CTx, chemotherapy; D, day; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitor; IFN, interferon alfa; L, lumbar vertebra; LN, lymph node; PD, progressive disease; PR, partial response; PS, performance status; RECIST, Response Evaluation Criteria in Solid Tumors; RP, retroperitoneum; SD, stable disease; Th, thoracic vertebra; TKI, tyrosine kinase inhibitor.

February 2019 and 22 July 2020, of which 6 were diagnosed with RCC. Three patients received regimen 1 and three received regimen 2.

The main patient and disease characteristics are summarized in Table 1. The medical, family, and psychosocial history (Supplemental Table S1), main demographic data, PS, disease symptoms (Supplemental Table S2), disease history, and staging of RCC (Supplemental Table S3) are presented in the Supplemental Materials.

Overall, six males with RCC and a median age of 69 years were treated with MitoTam in phase Ib. All patients had a PS of 0 or 1 and had no or minimal symptoms of the main disease (Supplemental Table S2). Five patients were originally diagnosed with non-metastatic (M0)

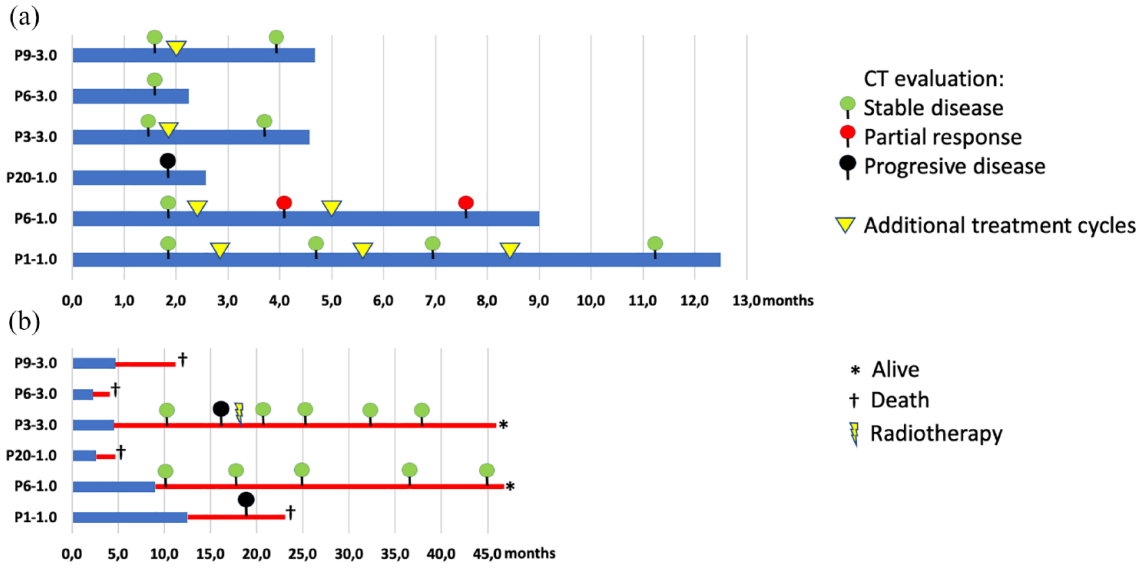

Figure 1. Timeline of MitoTam therapy and clinical responses in the phase Ib trial (a) and in the follow-up period after the trial (b). (a) Blue lines indicate MitoTam therapy during the phase Ib trial. In regimen 1 (dose of 1.0 mg/kg), CT scans were performed after four biweekly cycles. In regimen 2 (dose of 3.0 mg/kg), CT scans were performed after six weekly cycles. Of the six patients with RCC, five experienced a clinical benefit from MitoTam therapy (CBR 83%), comprising PR in one patient (P6-1.0) and SD in four patients. Patient P6-3.0 did not continue treatment due to worsening PS during the follow-up period after the first six cycles. (b) Red lines indicate the follow-up period after completing the trial. Four patients died, two (P6-1.0 and P3-3.0) were still alive at the time of writing. The patient (P3-3.0) with PD subsequently underwent RT of the liver and paracardial metastasis was found after the MitoTam-01 trial.
C, cycle; CBR, clinical benefit rate; CT, computed tomography; D, day; PD, progressive disease; PR, partial response; RCC, renal cell carcinoma; RT, radiotherapy; SD, stable disease.

disease, but all underwent radical nephrectomy (RN) of a single kidney. The median disease-free survival (DFS) of the M0 patients was 7 years; the longest DFS was 11 years (P1-1.0) and the shortest DFS was 5 years (P3-3.0). Two patients (P1-1.0 and P6-1.0) had favorable prognosis according to the Memorial Sloan-Kettering Cancer Center (MSKCC) score for metastatic RCC (Supplemental Table S3). All patients had visceral metastases at the time of screening and their disease was progressing after the previous therapy. Patients were previously treated with at least three lines of palliative systemic therapy, which included a TKI in all patients and an ICI in two patients (Table 1). Only two patients were pretreated with ICI due to the availability of ICI in the Czech Republic from October 2017 and the approved indication at the time of treatment.

CBR and correlation with number of CTCs

Of the six patients with RCC, a clinical benefit was observed in five, with a CBR of 83%. The five responders had ccRCC and the non-responding patient had CCSF. Figure 1(a) shows the timelines of the patients' disease course during MitoTam therapy. The duration of response, measured from the first cycle of MitoTam treatment to D28 of follow-up after the last cycle, was 19 weeks, with individual values of 50, 36, 18, 9, and 19 weeks in P1-1.0, P6-1.0, P3-3.0, P6-3.0, and P9-3.0, respectively. Four patients died after completing the trial [Figure 1(b)], of which two were due to PD (P1-1.0 and P20-1.0), one due to COVID-19-related pneumonia whose disease status was unknown at the time of the death (P9-3.0), and one was due to epileptic seizure and right-sided hemiparesis of unknown etiology (P6-3.0). The latter patient refused to undergo CT of the central nervous system. Since the CT scans of the chest, abdomen, and pelvis after six cycles of MitoTam indicated SD, this patient was considered to be a responder.

We also investigated the correlation between the CTCs results and the clinical responses (Supplemental Table S4). In general, the reduction of CTC-positive rate reflected the clinically assessed SD/PR after cycle 12 for regimens 1 and 2, and after cycle 16 for regimen 1. In comparison,

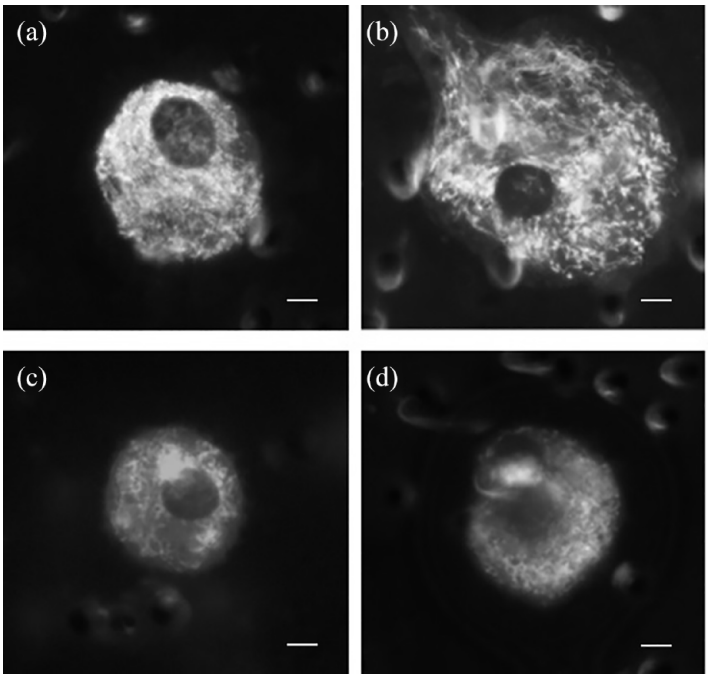

Figure 2. Mitochondria of CTCs from patients with ccRCC grew on the separation membrane. The composite figure depicts the mitochondria that were visualized using MitoTracker™ in CTCs enriched from the peripheral blood from patients with ccRCC and subjected to short-term cultured on a separation membrane (MetaCell®). (a, b) CTCs isolated from patients before starting MitoTam therapy. The mitochondria are fused and have formed a significant mitochondrial network. (c, d) CTCs obtained after MitoTam therapy. There is a loss of active mitochondria and disintegration of the mitochondrial network is evident relative to (a, b). Bar size = 8 μm.
ccRCC, clear cell renal cell carcinoma; CTCs, circulating tumor cells.

earlier during regimen 1, a significant elevation of CTC-positive rate was observed compared to the clinical results seen after cycle 4 (8 weeks of therapy), indicating that the CTC results did not correspond to the CBR. However, in patients with higher numbers of CTCs, we detected CTCs that were missing active mitochondria or had a huge nucleus, especially in RCC patients treated for a long time (Figure 2). Regarding the number of CTCs, the non-responder (P20-1.0) had a much higher CTC count at the start of the study than the other responders (Supplemental Figure S1).

Clinical course of two patients with long-term MitoTam therapy

Patient P1-1.0 was a 63-year-old male with ccRCC of the left kidney. He underwent RN in 1999 but relapsed in 2011. He was indicated for total thyroidectomy because of metastatic disease located in the thyroid gland, and subsequently received three lines of palliative systemic therapy and palliative radiotherapy (RT) through to November 2018 (Supplemental Table S3). He was enrolled in MitoTam-01 in December 2018 (Figure 3). MitoTam was administered *via* a PICC line inserted during the screening period. Control CT scans were performed after cycles 4, 8, 12, and 16, the last cycle occurring in January 2020. Despite experiencing PD during TKI treatment and RT, we observed SD throughout MitoTam treatment [Figure 4(a)]. The time from D1 of the first cycle to D28 of cycle 16 was 50 weeks. MitoTam therapy was discontinued due to AEs (see below) rather than due to lack of effect in January 2020. Therefore, MitoTam was effective for approximately 1 year as the fourth line of palliative therapy. He was diagnosed with disease progression in October 2020, after the end of the trial, and died in January 2021 [Figure 1(b)].

Patient P6-1.0 was a 73-year-old male with ccRCC of the left kidney diagnosed in 2007. He underwent RN and remained disease-free until 2014, when he was diagnosed with local recurrence and bone metastasis. The patient underwent deliberation of the dural sac, followed by RT, and subsequently received four lines of palliative systemic therapy, the most recent pretrial treatment being an ICI (Supplemental Table S3).

2011 – 09/2018	05.12.18 – 06.01.19	07.01.19 – 11.02.19	18.02.19 – 06.05.19	06.05.19 – 20.01.20
Prior to the MitoTam-01 trial	**Sign of IC Screening period**	**Phase I : 1 cycle, control D14+D28**	**Phase Ib: cycles 1-4 control D14+D28**	**Phase Ib: cycles 5-16 control D14+D28**

CT SCAN (date):	**9/18**		**02.01.19**		**10.04.19**	**02.07.19**	**16.10.19**	**15.01.20**
TARGET LESIONS:	**[mm]**		**[mm]**		**[mm]**	**[mm]**	**[mm]**	**[mm]**
Right kidney 1	35x23		35x31		35x31	36x30	36x30	37x30
Right kidney 2	6		9		10	10	10	11
Th12*	23x21		29x25		29x25	28x26	28x26	28x26
RESULT:	**PD**		**PD**		**SD**	**SD**	**SD**	**SD**

Figure 3. Timeline of MitoTam therapy in patient P1-1.0 in the MitoTam-01 trial. The patient was diagnosed with ccRCC of the left kidney in 1999 and underwent RN. He experienced disease relapse in 2011 and received three lines of palliative TKI therapy. Metastases found in the screening period of the MitoTam-01 trial comprised two lesions in the right kidney and one lesion in Th12. The patient underwent 16 cycles of MitoTam therapy in phase Ib, with two follow-up visits after every 4 cycles of therapy. The disease remained stable throughout MitoTam therapy. Treatment was discontinued due to PE, which was classified as a serious AE, and possibly associated with the treatment. Responses were evaluated according to RECIST 1.1.
*Lytic bone lesion with soft tissue component measurable according to RECIST 1.1.
ccRCC, clear cell renal cell carcinoma; RN, radical nephrectomy; TKI, tyrosine kinase inhibitor; Th12, thoracic vertebra Th12; RECIST, Response Evaluation Criteria in Solid Tumors; IC, informed consent; D14 and D28, follow-up visits at days 14 and 28 after day 5 in cycles 4, 8, 12 and 16; CT, computed tomography; SD, stable disease; PR, partial response; PD, progressive disease; PE, pulmonary embolism.

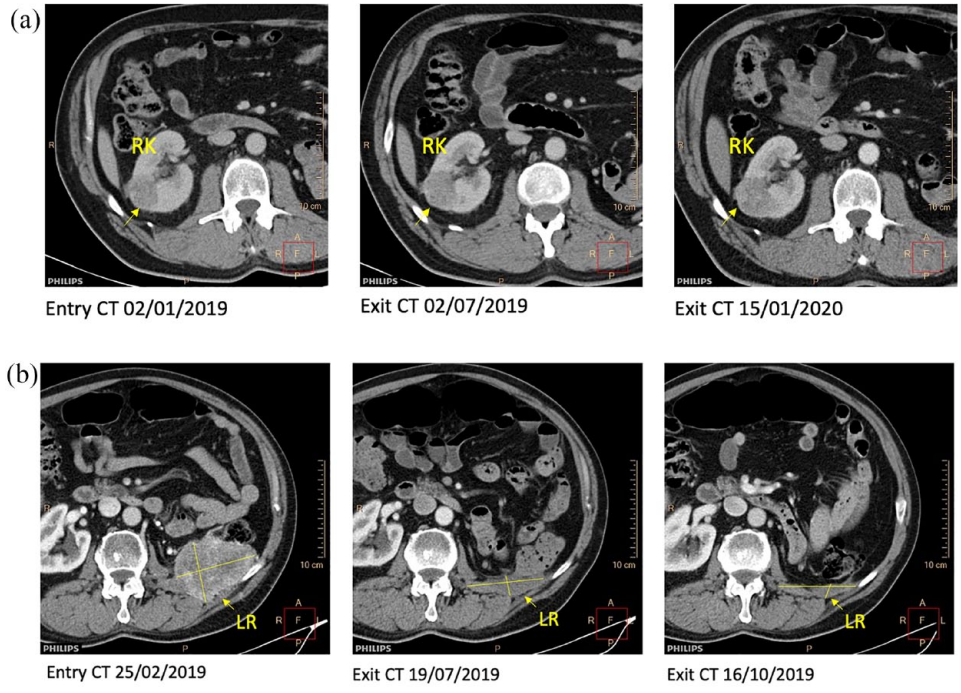

Figure 4. CT findings of patients with long-term MitoTam therapy. (a) Stable disease of the target lesion in the right kidney (RK) in patient P1-1.0. This lesion is marked as 'right kidney 1' in Figure 3. (b) Partial response of the local recurrence after left nephrectomy in patient P6-1.0. This lesion is marked as 'local recurrence' in Figure 5.
CT, computed tomography.

2014-11/2018		14.01.19– 10.03.19	Not enrolled	11.03.19 – 27.05.19		27.05.19 – 18.10.19	
Prior to the MitoTam-01 trial		**Sign of IC Screening period**	**Phase I**	**Phase Ib: cycles 1-4 control D14+D28**		**Phase Ib: cycles 5-12 control D14+D28**	
CT SCAN (date):	**11/18**	**25.02.19**		**06.05.19**		**19.07.19**	**24.10.19**
TARGET LESIONS:	**[mm]**	**[mm]**		**[mm]**		**[mm]**	**[mm]**
Local recurrence	73x56	82x68		82x68		57x15	45x14
Tumor near spleen	not present	12x12		17x12		0	0
Left adrenal gland	19x13	22x19		14x8		12x5	10x5
Th12*	27x12	27x12		27x12		27x12	0
Right hip bone*	38x19	38x20		38x20		38x20	0
RESULT:	**PD**	**PD**		**SD**		**PR**	**PR**

Figure 5. Timeline of MitoTam therapy in patient P6-1.0 in the Mitotam-01 trial. The patient was diagnosed with left kidney ccRCC in 2007 and underwent RN. Disease relapse was found in 2014, and he was treated with three lines of TKI and one line of ICI in a palliative setting. Metastases found in the screening period were located in the skeleton, left adrenal gland, near the spleen, and a massive local recurrence. The patient underwent 12 cycles of MitoTam therapy in phase Ib, and two follow-up visits after every four cycles of treatment. The disease was stabilized after the first four cycles and then regressed significantly. MitoTam was discontinued due to PE, which was classified as a serious AE, and possibly associated with the treatment. Responses were evaluated according to RECIST 1.1.
*Lytic bone lesion with soft tissue component measurable according to RECIST 1.1.
ccRCC, clear cell renal cell carcinoma; CT, computed tomography; D14 and D28, follow-up visits at days 14 and 28 after day 5 in cycles 4, 8, and 12; IC, informed consent; ICI, immune checkpoint inhibitor; RECIST, Response Evaluation Criteria in Solid Tumors; RN, radical nephrectomy; TKI, tyrosine kinase inhibitor; Th12, thoracic vertebra Th12; PD, progressive disease; PR, partial response; SD, stable disease.

He started MitoTam in March 2019 and underwent a total of 12 cycles until October 2019 (Figure 5). MitoTam was administered *via* a PICC. The first whole-body control CT scan in May 2019 indicated SD, and the next two CT scans revealed PR. His local recurrence was stable after cycle 4 and substantially decreased after cycles 8 and 12. This lesion responded with a 40-fold reduction in volumetric terms [Figure 4(b)]. The time from D1 in cycle 1 to D28 in cycle 12 was 36 weeks, and the duration of response was thus 9 months. At the time of drafting the manuscript in January 2023 the patient was alive, having been on no therapy for cancer for more than 36 months since the last dose of MitoTam [Figure 1(b)].

Toxicities of MitoTam in RCC patients

Hematological toxicities, fever, and TE complications were the most common AEs documented in MitoTam-01.[18] The AEs that occurred in these six patients with RCC are listed in Table 2. Two patients developed deep vein thrombosis and pulmonary embolism (PE), which were classified as serious AEs and led to treatment discontinuation. Both patients were asymptomatic and the AEs were incidentally diagnosed at the restaging CT after cycle 16 in P1-1.0 and cycle 12 in P6-1.0. In both cases, the PICCs were removed, 11 months after their insertion for P1-1.0 and 9 months for P6-1.0. Therapy with low-molecular-weight heparin was indicated and initiated. Prior to these events, only one case of grade 1 (G1) leukopenia was recorded in P1-1.0 and one case of G1 hypertension was recorded in P6-1.0 (Table 2). Because hypertension occurred only once throughout the study, we believe it was related to the main diagnosis. Similarly, the elevation of creatinine in P9-3.0 may be related to chronic kidney disease. Conjunctivitis occurred in two patients (P3-3.0 and P9-3.0) on the same day. Hyperthermia and/or fever occurred in three patients. Loss of appetite, weight loss, and/or fatigue were mainly observed in regimen 3 and appeared to be dose-dependent.

Table 2. AEs related to MitoTam therapy in patients with RCC.

	Patient no. and MitoTam dosage level (mg/kg)					
	P1-1.0	**P6-1.0**	**P20-1.0**	**P3-3.0**	**P6-3.0**	**P9-3.0**
AE (grade)	Leukopenia (G1)[a] DVT and PE (G3)[b]	Hypertension (G1) DVT and PE (G3)[b]	Hyperthermia[c] Weight loss (G1) Fatigue (G1) Loss of appetite (G1) Anemia (G2)[a]	Conjunctivitis (G1)	Fever (G1) Hyperthermia[c]	Loss of appetite (G1) Conjunctivitis (G1) Fever (G1–G2)[a] Hyperthermia[a,c] Fatigue (G1) Back pain (G1) Headache (G1) Creatinine elevation (G1) Weight loss (G1)

AEs were recorded as those related to the study treatment.
[a]AEs that occurred repeatedly.
[b]Reported as serious AEs.
[c]Hyperthermia was defined as a temperature of 37.1°C–37.9°C according to the study protocol.
AE, adverse event; DVT, deep vein thrombosis; G, grade; PE, pulmonary embolism; RCC, renal cell carcinoma.

Discussion

In this case series report, we have described the therapeutic effects of MitoTam, a CI inhibitor belonging to a novel class of potential anticancer drugs bearing the TPP^+ mitochondria-targeting moiety. Although mitochondrial targeting appears to be clinically challenging, considering the recently failed clinical trials,[8,10,22] we assume that this pessimistic view is unfounded and that there is a 'silver lining' for mitochondrial targeting as an anticancer strategy.

Although it is difficult to compare agents across different stages of disease development, it seems that MitoTam can offer lower toxicity while maintaining effective anti-mitochondrial activity. At the recommended dose (3.0 mg/kg) almost all of the observed AEs were G1. Unlike the OXPHOS inhibitor IACS-010759, which showed a narrow therapeutic index in a phase I clinical trial[8] in individuals with AML and/or solid tumors, the therapeutic window of MitoTam is sufficiently wide and DLT occurred at a dose of 6.0 mg/kg, approximately double the effective dose of 3.0 mg/kg. Asymptomatic PE was infrequently observed, and this event may be related to greater biodistribution in lung tissue, as observed in the preclinical model, and the presence of lipid droplets in the cytoplasm of lung cells is common for chemical entities with amphiphilic properties.[23] Other clinically relevant, yet reversible AEs, were related to hematotoxicities. However, neither of these reversible or asymptomatic AEs that occurred in this phase Ib study seem to be more serious than those reported for venetoclax, a BH3 mimetic that disrupts anti-apoptotic signaling *via* Bcl-2 family members, and is currently the only approved mitochondria-targeting agent.[6] Although side effects associated with venetoclax monotherapy include neutropenia, diarrhea, nausea, anemia, thrombocytopenia, fatigue, fever, upper respiratory tract infection (including pneumonia), febrile neutropenia, autoimmune hemolytic anemia, anemia, and tumor lysis syndrome, it was approved for therapy of CLL and AML. In this respect, MitoTam is well tolerated even over long-term administration.

The toxicity profile of MitoTam also compares well relative to other mitochondrial agents BAY240223423, an inhibitor of mitochondria-associated *de novo* pyrimidine synthesis,[9] and CPI-613 (devimistat), an inhibitor of tricarboxylic acid cycle in mitochondria.[10] AEs reported for agents include hematological toxicities, while the most common G3-4 non-hematological AEs were hyperglycemia, hypokalemia, peripheral sensory neuropathy, diarrhea, and abdominal pain. Clinical testing of BAY240223423 and CPI-613 was discontinued due to lack of efficacy, not toxicity. Another mitochondrial agent is AG221, an inhibitor of mutant IDH2 proteins, that showed promising results in a phase Ib/II trial in patients with mutant-IDH2 AML, but limiting toxicities were not mentioned.[7] For MitoTam, local toxicity is an important consideration, particularly venous phlebitis when administered peripheral. We believe this is due to its lipophilic properties, allowing it to readily penetrate the surrounding tissue and epithelium at the injection site. However, this side effect can be entirely avoided by an alternative administration route (i.e. intravenous port or PICC). Overall, the safety profile of MitoTam indicates a sufficient therapeutic window and its side effects, which are well manageable, are similar to those

reported for other mitochondrial agents. Some of the adverse reactions seem to be non-specific, reversible, and likely related to the lipophilic nature of the molecule.

Regarding the efficacy of MitoTam, we have described the outcomes of six patients with metastatic RCC, of which five were classified as ccRCC and experienced a clinical benefit (i.e. PR or SD) during the phase Ib MitoTam trial. This clinical benefit of MitoTam is underscored by the fact that all patients had previously undergone at least three lines of palliative systemic therapy and their disease was progressing at the time of screening. Only two patients had previously received an ICI, of which one had PR during treatment with MitoTam. Although syngeneic effects of MitoTam with an ICI were observed in our recent study in a mouse model of RCC,[24] we can only speculate on the efficacy of MitoTam in a patient who progressed on ICI treatment. Mitochondrial dynamics influence many functions of the immune system involved in the efficacy and/or toxicity of ICIs.[25] An experimental study showed that venetoclax increases intratumoral effector T-cells.[26] Some mitochondrial metabolites can promote tumor immune escape[27] and mitochondrial genes (MTGs) related to oxidative stress may have a prognostic significance in ccRCC.[28] It was shown that activation of mitochondrial function can inhibit tumor progression[27] and the expression levels of prognostic MTGs were significantly associated with drug sensitivity in ccRCC.[28] Recently, Marquardt *et al.* revealed a unique histologically independent subgroup of RCC that was characterized by enhanced mitochondria and weakened angiogenesis-related gene signatures.[29] Further research into the mitochondrial metabolism of such tumors and their microenvironment is needed.

Here, we described the patient and tumor characteristic of the responders. RCC is an aggressive disease that recurs in approximately 20–50% following initial surgical resection of a localized tumor.[30] Factors associated with favorable prognosis include the absence of risk factors according to the MSKCC scoring system,[31,32] clear cell histology compared with the sarcomatoid subtype of RCC, absence of visceral metastases, limited metastatic disease, and/or the length of the disease-free period. A positive effect of cytoreductive nephrectomy in M1 disease has been discussed.[33,34] In our dataset, the histological type was ccRCC in all five responders, of which two had favorable MSKCC scores and four had primary M0 disease and disease-free periods exceeding 1 year (Supplemental Table S3). For two patients, we reported a long-term therapeutic effect, lasting 50 and 36 weeks (P1-1.0 and P6-1.0, respectively). One of these patients (P6-1.0) was alive at the time of writing, after completing the clinical trial in November 2019. Another patient (P3-3.0) completed the MitoTam trial in May 2020 and was also alive. The long-term responders relapsed 12 and 7 years after their initial diagnosis, and were treated for advanced RCC for 8 and 5 years before entering the MitoTam-01 trial. They had no negative prognostic factors according to the MSKCC score at the time of entering the MitoTam-01 trial. A potentially favorable condition that may have contributed to the response of P1-1.0 to MitoTam is the 'small' extent of the disease. This patient had one bone lesion that was irradiated shortly before the trial and two tumors in the right kidney. However, metastatic disease was more extensive in the other four responders. It is therefore unlikely that the small disease extent is a precondition for responsiveness to MitoTam. Visceral metastases were also present in all five responders. Moreover, all of the patients were pretreated with at least three lines of palliative systemic treatment, and had progressed by the time of screening for the MitoTam-01 trial. Therefore, the probability of a positive response to an experimental drug was low.

The high efficacy of MitoTam is likely related to its mechanism of action, that is, generation of reactive oxygen species[11,12] and suppression of respiration that is essential for tumor formation and progression.[4] The prognostic significance of some MTGs[28,29] and their association with drug sensitivity is described above.[28] It is possible that, in clinical settings, ccRCC is sensitive to the disruption of respiration, which appears to be enhanced in advanced ccRCC.[35] Kidney tissue is one of the most sensitive tissues to glucose loss and RCC is strongly dependent on glycolytic metabolism.[36] In addition, the high accumulation of MitoTam in kidney tissue may also contribute to its efficacy.[24] In fact, we previously reported that the accumulation of MitoTam was 2- to 10-fold greater in kidney tissue than in other tissues, and that it persisted for almost 1 week longer in the kidney than in most of the other tissues assessed.[24]

We hypothesized the effect of MitoTam could be monitored by using CTCs isolated from

peripheral blood of patients, and evaluating their number and the morphology of mitochondrial networks (fusion *versus* fission mitochondria). The prognostic significance of CTCs has been confirmed in a number of studies.[37–41] Prior reports have discussed the pharmacodynamic significance of using CTCs for monitoring the response to treatment.[42] Several studies have shown that changes in the number of CTCs in response to treatment provide better prognostic information than the baseline CTC number, and post-treatment persistence of CTCs implies worse prognosis.[43–45] In the future, treatment applications based on CTCs targeting may also be considered, including the targeting of CTCs through metabolic interventions.[46] From this perspective, analyzing the mitochondria of viable CTCs is a completely unique tool for real-time monitoring of drug responses during treatment.

The most widely used methods for CTC enrichment are antigen-dependent. A direct comparison with our study is not possible due to unique method of size-based CTC separation and observation of cells under viable conditions. Cell viability enables us to characterize mitochondria *in situ*, providing a new approach for monitoring the anti-mitochondrial effect of a drug. Considering that we report six patients with different diagnoses who were treated with three different MitoTam regimens and received different numbers of cycles, it is impossible to make general conclusions based on CTCs. Also, this study was not designed to confirm the prognostic or predictive significance of CTCs, but to observe the mechanism of action of MitoTam.

Among these patients with RCC, a significant response to MitoTam therapy was confirmed at the level of CTCs. We showed that the patients had stable numbers of CTCs in peripheral blood, even after 12 cycles of treatment. Interestingly, patients classified as clinically stable (i.e. SD) after MitoTam treatment had significantly fewer CTCs in peripheral blood than the patients with PD. Similarly, patients with high numbers of CTCs at baseline responded less well to treatment than patients with fewer CTCs. In some patients with a significant clinical response, the number of CTCs increased transiently after repeated doses of MitoTam. The elevation in number of CTCs might be explained by the effect of MitoTam on tumor mass, causing CTCs to be shed from the tumor into the circulation in higher numbers. For us, the most significant CTCs evaluation was mitochondria fitness (mitochondria activity depicted using MitoTracker) and the overall shape of the mitochondrial network. MitoTam causes a change in the membrane potential of the internal mitochondrial membrane that may trigger morphological changes of the mitochondria.[47] The observation of missing active mitochondria in CTCs may be associated with broken apoptotic pathways. We detected a significant difference in genes regulating antioxidant pathways of mitochondrial metabolism (unpublished data). We saw a reduction in the actively respiring mitochondrial network in patients with RCC. The mitochondrial network has recently been used to define tumor aggressiveness beyond the standard histopathological parameters, supporting its clinical utility in RCC.[48,49]

There are some limitations of the data presented here. First, a small number of patients with RCC were enrolled in the phase Ib cohort, and they received different treatment sequences depending on year of diagnosis and therapeutic guidelines. Second, two different weekly regimens of MitoTam were used and outcomes are difficult to compare directly. Third, there is a time limitation, with a shorter duration of therapy in regimen 3. Finally, as described in the Introduction and in our previous publication,[18] TE complications occurred in 13% of patients in phase Ib.[18] Two of the six patients with RCC included in this article discontinued therapy because of this AE. Except for the direct effects of MitoTam, we should consider the possible impact of long-term treatment on the PICC line, the personal history of patients, and complications of the malignant disease in different diagnoses. We also recommend preventive anticoagulation therapy in future prospective studies.

Despite the possible limitations mentioned above, our data represent very promising outcomes of MitoTam treatment for patients with RCC. Both the primary (i.e. safety and pharmacokinetic activity) and the secondary endpoints strongly support further testing of this agent in a phase II clinical trial. We recommend that the findings described here should be considered and incorporated into the design of future clinical trials of MitoTam.

Conclusion

MitoTam is a promising drug with an innovative mechanism of action, which involves targeting mitochondria, and achieved significant effectiveness against metastatic ccRCC. We believe these

findings provide a basis for a future phase II trial of MitoTam.

Declarations

Ethics approval and consent to participate

The MitoTam-01 study was performed in accordance with the Declaration of Helsinki and was approved by the Ethical Review Committee of the General Faculty Hospital, Charles University, Reference number 1951/17 S.

Consent for publication

Written informed consent for publication of their clinical details and/or clinical images was obtained from the patients/relatives of the patients. The copies of the consent forms are available for review by the Editor of this journal.

Author contributions

Zuzana Bielcikova: Conceptualization; Formal analysis; Investigation; Methodology; Writing – original draft; Writing – review & editing.

Lukas Werner: Investigation; Methodology; Writing – review & editing.

Jan Stursa: Investigation; Methodology; Visualization.

Vladimir Cerny: Investigation; Visualization.

Ludmila Krizova: Data curation; Investigation.

Jan Spacek: Data curation; Investigation.

Stanislav Hlousek: Data curation; Investigation.

Michal Vocka: Data curation; Investigation.

Olga Bartosova: Data curation.

Michal Pesta: Formal analysis; Investigation.

Katarina Kolostova: Data curation; Investigation.

Petr Klezl: Data curation; Investigation.

Vladimir Bobek: Data curation; Investigation.

Jaroslav Truksa: Investigation; Methodology.

Sona Stemberkova-Hubackova: Investigation; Methodology.

Lubos Petruzelka: Supervision.

Pavel Michalek: Investigation; Writing – review & editing.

Jiri Neuzil: Supervision; Writing – review & editing.

Acknowledgements

We thank the patients and/or their relatives for their consent to this publication. The authors acknowledge Nicholad D. Smith for English language editing, which was funded by the Czech Health Foundation [NU21-03-00545], Research Programme 18 – Strategy AV21 of the Czech Academy of Sciences, and IKEM.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: MitoTam-01 trial was supported in part by SmartBrain s.r.o. (Czech Republic). The work was supported in part by funding from the Czech Health Foundation (NU21-03-00545), Research Programme 18 – Strategy AV21 of the Czech Academy of Sciences, and IKEM.

Competing interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Jiri Neuzil, Jan Stursa, and Lukas Werner are owners of MitoTax s.r.o. that co-owns the MitoTam intellectual property. The remaining authors declare that they have no conflict of interest.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

ORCID iD

Zuzana Bielcikova https://orcid.org/0000-0001-8434-9848

Supplemental material

Supplemental material for this article is available online.

References

1. Robertson-Tessi M, Gillies RJ, Gatenby RA, *et al*. Impact of metabolic heterogeneity on tumor growth, invasion, and treatment outcomes. *Cancer Res* 2015; 75(8): 1567–1579.
2. Lawson DA, Kessenbrock K, Davis RT, *et al*. Tumour heterogeneity and metastasis at single-cell resolution. *Nat Cell Biol* 2018; 20(12): 1349–1360.

3. Gogvadze V, Orrenius S and Zhivotovsky B. Mitochondria in cancer cells: what is so special about them? *Trends Cell Biol* 2008; 18: 165–173.
4. Bajzikova M, Kovarova J, Coelho A, *et al.* Reactivation of dihydroorotate dehydrogenase by respiration restores tumor growth of mitochondrial DNA-depleted cancer cells. *Cell Metab* 2019; 29: 399–416.
5. Roth KG, Mambetsariev I, Kulkarni P, *et al.* The mitochondrion as an emerging therapeutic target in cancer. *Trends Mol Med* 2020; 26(1): 119–134.
6. Ashkenazi A, Fairbrother WJ, Leverson JD, *et al.* From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. *Nat Rev Drug Discov* 2017; 16: 273–284.
7. DiNardo CD, Schuh AC, Stein EM, *et al.* Enasidenib plus azacitidine versus azacitidine alone in patients with newly diagnosed, mutant-IDH2 acute myeloid leukaemia (AG221-AML-005): a single-arm, phase 1b and randomised, phase 2 trial. *Lancet Oncol* 2021; 22(11): 1597–1608.
8. Yap TA, Daver N, Mahendra M, *et al.* Complex I inhibitor of oxidative phosphorylation in advanced solid tumors and acute myeloid leukemia: phase I trials. *Nat Med* 2023; 29(1): 115–126.
9. Christian S, Merz C, Evans L, *et al.* The novel dihydroorotate dehydrogenase (DHODH) inhibitor BAY 2402234 triggers differentiation and is effective in the treatment of myeloid malignancies. *Leukemia* 2019; 33: 2403–2415.
10. Alistar A, Morris BB, Desnoyer R, *et al.* Safety and tolerability of the first-in-class agent CPI-613 in combination with modified FOLFIRINOX in patients with metastatic pancreatic cancer: a single-centre, open-label, dose-escalation, phase 1 trial. *Lancet Oncol* 2017; 18(6): 770–778.
11. Rohlenova K, Schaphibulkij K, Stursa J, *et al.* Selective disruption of respiratory supercomplexes as a new strategy to suppress Her2^{high} breast cancer. *Antiox Redox Signal* 2017; 26: 84–103.
12. Hubackova S, Rohlenova K, Davidova E, *et al.* Selective elimination of senescent cells by mitochondrial targeting is regulated by ANT2. *Cell Death Differ* 2019; 26: 276–290.
13. Neuzil J, Dong LF, Rohlena J, *et al.* Classification of mitocans, anti-cancer drugs acting on mitochondria. *Mitochondrion* 2013; 13: 199–208.
14. Dong LF, Jameson VJA, Tilly D, *et al.* Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anti-cancer activity via mitochondrial complex II. *J Biol Chem* 2011; 286: 3717–3728.
15. Boukalova S, Stursa J, Werner L, *et al.* Mitochondrial targeting of metformin enhances its activity against pancreatic cancer. *Mol Cancer Ther* 2016; 15: 2875–2886.
16. Smith RA, Hartley RC, Cochemé HM, *et al.* Mitochondrial pharmacology. *Trends Pharmacol Sci* 2012; 33: 341–352.
17. Modica-Napolitano JS and Aprille JR. Delocalized lipophilic cations selectively target the mitochondria of carcinoma cells. *Adv Drug Deliv Rev* 2001; 49: 63–70.
18. Bielcikova Z, Stursa J, Krizova L, *et al.* Mitochondrially targeted tamoxifen in patients with metastatic solid tumours: an open-label, phase I/Ib single-centre trial. *EClinicalMedicine* 2023; 57: 101873.
19. Lalani AA, Heng DYC, Basappa NS, *et al.* Evolving landscape of first-line combination therapy in advanced renal cancer: a systematic review. *Ther Adv Med Oncol* 2022; 14: 17588359221108685.
20. Gagnier JJ, Kienle G, Altman DG, *et al.* The CARE guidelines: consensus-based clinical case reporting guideline development. *Glob Adv Health Med* 2013; 2(5): 38–43.
21. Kolostova K, Broul M, Schraml J, *et al.* Circulating tumor cells in localized prostate cancer: isolation, cultivation in vitro and relationship to T-stage and Gleason score. *Anticancer Res* 2014; 34(7): 3641–3646.
22. Zhang X and Dang CV. Time to hit pause on mitochondria-targeting cancer therapies. *Nat Med* 2023; 29: 29–30.
23. Halliwell WH. Cationic amphiphilic drug-induced phospholipidosis. *Toxicol Pathol* 1997; 25(1): 53–60.
24. Stemberkova-Hubackova S, Zobalova R, Dubisova M, *et al.* Simultaneous targeting of mitochondrial metabolism and immune checkpoints as a new strategy for renal cancer therapy. *Clin Transl Med* 2022; 12(3): e645.
25. Zhang X, Gan Y, Zhu H, *et al.* Role of mitochondrial metabolism in immune checkpoint inhibitors-related myocarditis. *Front Cardiovasc Med* 2023; 10: 1112222.
26. Kohlhapp FJ, Haribhai D, Mathew R, *et al.* Venetoclax increases intratumoral effector T cells and antitumor efficacy in combination with immune checkpoint blockade. *Cancer Discov* 2021; 11(1): 68–79.

27. Du F, Yang LH, Liu J, *et al*. The role of mitochondria in the resistance of melanoma to PD-1 inhibitors. *J Transl Med* 2023; 21(1): 345.

28. Wu Y, Zhang X, Wei X, *et al*. A mitochondrial dysfunction and oxidative stress pathway-based prognostic signature for clear cell renal cell carcinoma. *Oxid Med Cell Longev* 2021; 2021: 9939331.

29. Marquardt A, Solimando AG, Kerscher A, *et al*. Subgroup-independent mapping of renal cell carcinoma-machine learning reveals prognostic mitochondrial gene signature beyond histopathologic boundaries. *Front Oncol* 2021; 11: 621278.

30. Padala SA, Barsouk A, Thandra KC, *et al*. Epidemiology of renal cell carcinoma. *World J Oncol* 2020; 11(3): 79–87.

31. Motzer RJ, Bacik J, Murphy BA, *et al*. Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. *J Clin Oncol* 2002; 20(1): 289–296.

32. Memorial Sloan-Kettering Cancer Center (MSKCC/Motzer) score for metastatic renal cell carcinoma (RCC), https://www.mdcalc.com/memorial-sloan-kettering-cancer-center-mskcc-motzer-score-metastatic-renal-cell-carcinoma-rcc. (accessed 13 December 2018).

33. Mejean A, Ravaud A, Theyenas S, *et al*. Sunitinib alone or after nephrectomy in metastatic renal-cell carcinoma. *N Engl J Med* 2018; 379: 417–427.

34. Van Praet C, Slots C, Vasdev N, *et al*. Current role of cytoreductive nephrectomy in metastatic renal cell carcinoma. *Turk J Urol* 2021; 47(Suppl. 1): S79–S84.

35. Clark DJ, Dhanasekaran SM, Petralia F, *et al*. Integrated proteogenomic characterization of clear cell renal cell carcinoma. *Cell* 2019; 179: 964–983.

36. Courtney KD, Bezwada D, Mashimo T, *et al*. Isotope tracing of human clear cell renal cell carcinomas demonstrates suppressed glucose oxidation in vivo. *Cell Metab* 2018; 28: 793–800.e2.

37. Aggarwal C, Meropol NJ, Punt CJ, *et al*. Relationship among circulating tumor cells, CEA and overall survival in patients with metastatic colorectal cancer. *Ann Oncol* 2013; 24: 420–428.

38. Allard WJ, Matera J, Miller MC, *et al*. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* 2004; 10: 6897–6904.

39. Cristofanilli M, Budd GT, Ellis MJ, *et al*. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004; 351: 781–791.

40. Hou JM, Krebs MG, Lancashire L, *et al*. Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. *J Clin Oncol* 2012; 30: 525–532.

41. Scher HI, Jia X, de Bono JS, *et al*. Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. *Lancet Oncol* 2009; 10: 233–239.

42. Paoletti C and Hayes DF. Circulating tumor cells. *Adv Exp Med Biol* 2016; 882: 235–258.

43. Liu MC, Janni W, Georgoulias V, *et al*. First-line doublet chemotherapy for metastatic triple-negative breast cancer: circulating tumor cell analysis of the tnAcity trial. *Cancer Manag Res* 2019; 11: 10427–10433.

44. Smerage JB, Barlow WE, Hortobagyi GN, *et al*. Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500. *J Clin Oncol* 2014; 32: 3483–3489.

45. Wallwiener M, Riethdorf S, Hartkopf AD, *et al*. Serial enumeration of circulating tumor cells predicts treatment response and prognosis in metastatic breast cancer: a prospective study in 393 patients. *BMC Cancer* 2014; 14: 512.

46. Ring A, Nguyen-Sträuli BD, Wicki A, *et al*. Biology, vulnerabilities and clinical applications of circulating tumour cells. *Nat Rev Cancer* 2023; 23: 95–111.

47. Miyazono Y, Hirashima S, Ishihara N, *et al*. Uncoupled mitochondria quickly shorten along their long axis to form indented spheroids, instead of rings, in a fission-independent manner. *Sci Rep* 2018; 8: 350.

48. Nikolic G, Zivotic M, Cirovic S, *et al*. The utility of mitochondria detection methods applied as an additional tool for the differentiation of renal cell tumours. *Diagnostics* 2023; 13: 2319.

49. Schüffler PJ, Sarungbam J, Muhammad H, *et al*. Mitochondria-based renal cell carcinoma subtyping: learning from deep vs. flat feature representations. *ArXiv:1608.00842*, 2016.