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LETTER TO THE EDITOR

New use for old drugs: Epirubicin in colorectal cancer

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We have previously published an article describing the protocol for a clinical, non-randomized phase II study (Eudract no. 2013-001648-79) [1], which aimed to provide proof of concept for introducing the anthracycline, epirubicin as an effective, biomarker-guided treatment for patients with metastatic colorectal cancer who are refractory to treatment with oxaliplatin-based chemotherapy and have *TOP2A* gene amplification in their tumor cells.

Introduction

Over the last decades, better selection of patients including assessment by multidisciplinary teams and the use of novel treatment modalities including chemotherapy and novel targeted biological agents have contributed to significant outcome improvements of patients with metastatic colorectal cancer (mCRC). The routine clinical management of patients with mCRC include oxaliplatin-containing therapy followed by irinotecan-containing therapy at progression (or the opposite sequence). Chemotherapy may be combined with the EGF-receptor targeted antibodies cetuximab or panitumumab in patients with RAS wild type tumors, and patients with RAS mutated tumors will often receive the VEGF targeted antibody bevacizumab with first and/or second line therapy.

The objective response rates to first line systemic treatment of mCRC are approximately 50%, but only 10% of mCRC patients will obtain tumor shrinkage during second-line treatment, and in the third line situation tumor regression is rarely observed [2]. Despite progressive disease to fluorouracil (5-FU), irinotecan and oxaliplatin many patients with mCRC maintain an excellent performance status, and further effective therapy is definitely indicated as progression-free survival (PFS) and overall survival (OS) is short without further therapy. In patients pretreated with 5-FU, irinotecan, oxaliplatin and EGFR inhibitor (only RASwt) the median PFS and median OS is short, and no effective treatment is presently available in Denmark.

We propose to assess to test whether drugs already being used in other cancer types might have beneficial effects also in colorectal cancer (CRC). However, due to the unknown response rate when using this approach, such drugs should preferably be used together with predictive biomarkers allowing for a pretreatment selection of patients with the highest likelihood of obtaining benefit from the treatment. Furthermore, the side effect must be acceptable. This approach was the foundation for our clinical phase II study. We aimed to investigate whether epirubicin had a beneficial effect in oxaliplatin-resistant mCRC patients with *TOP2A* gene amplification. We designed the study on the basis of knowledge from patients with breast cancer [3,4] and results from preclinical studies using CRC cell lines [5,6].

Epirubicin is an anthracycline that targets the DNA topoisomerase 2-α (Top2 α) enzyme encoded by the *TOP2A* gene (*TOP2A*). Top2 α has a key role in maintaining the topological status of chromosomes during DNA replication and transcription [7]. Epirubicin is used for treatment of several malignancies, but currently not for mCRC. *TOP2A* gene amplification predicts improved efficacy of epirubicin in patients with breast cancer [3,4] and thus we hypothesized that it also could be an alternative option for patients with mCRC and amplification of the *TOP2A* gene. The aims of the present study were to study the prevalence of *TOP2A* gene amplification in the primary tumor biopsies obtained from patients with oxaliplatin resistant mCRC, and to study the clinical benefit from epirubicin treatment of patients with *TOP2A* gene amplification in their tumor cells.

Methods/design

The present study was an open label, single arm, phase II study, investigating the efficacy of epirubicin in patients with oxaliplatin refractory mCRC and *TOP2A* gene amplification, defined as *TOP2A*/Centromere 17 ratio (*TOP2A*/CEN17) $\geq$ 1.5 in cancer cells. *TOP2A* gene amplification was measured by fluorescence *in situ* hybridization (FISH) in the primary tumor biopsy [8]. A new biopsy from metastatic lesion was not mandatory in the protocol.

Using Simon's two stage design [9], we calculated to include 15 patients in the first part of the study. If at least

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one patient achieved tumor control (partial response or stable disease) after 4 months of therapy (at the second CT scan), an additional 10 patients should be accrued in the second stage. In randomized trials on second-line therapy, PFS is around 4 months. A tumor control rate less than 10% after 4 months is not clinically relevant. If 5 out of 25 patients achieved tumor control after six courses of therapy, a tumor control rate of 30% could not be rejected, and we would conclude that the treatment is effective enough to continue with future studies. This design ensures early study termination if there is insufficient effect.

Inclusion criteria were signed written informed consent, age $\geq$ 18 years, WHO performance status 0–2, and a life expectancy of at least 3 months. Furthermore, patients had to have histologically verified, non-resectable, oxaliplatin-resistant mCRC, and available formalin-fixed and paraffin embedded (FFPE) blocks with tumor tissue for FISH analysis of the *TOP2A*/CEN-*17* ratio, which had to be $\geq$ 1.5.

Patients were treated with epirubicin 90 mg/m^2 every three weeks. Treatment continued until a maximum cumulative dose of epirubicin (900 mg/m^2), unacceptable toxicity, progressive disease at CT scan according to RECIST version 1.1 or patients wish of ending treatment.

The study was conducted in compliance with the protocol and in accordance to the ethical principles put forward in the second Declaration of Helsinki and in accordance with good clinical practice (GCP) rules. The trial was approved by the Ethics Committee of Region Syddanmark (2013-001648-79/S-20130042) and by the Danish Medicines Authority (Eudract no. 2013-001648-79).

The primary endpoint of the study was PFS and secondary endpoints were OS and toxicity.

Results

The first patient enrolled in the protocol was a young woman of 36 years. She was diagnosed with *RAS* and *BRAF* wild-type, microsatellite-stable, high-grade adenocarcinoma of the colon (pT3pN2 pV1) in July 2013. She started adjuvant treatment with 5-FU and oxaliplatin (FOLFOX), but during the first cycle of adjuvant treatment, she was diagnosed with metastatic disease (biopsy from cervical lymph node), and cetuximab was added to the chemotherapy. She achieved complete response according to RESCIST 1.1 but despite dose reduction, she experienced severe adverse effects (mainly haematological and skin toxicity), why oxaliplatin and cetuximab was not reinduced. Therapy was paused in January 2014 after 11 cycles of treatment, and in November 2014, CT scan and subsequent biopsy revealed disease recurrence at multiple sites. *TOP2A* amplification (a *TOP2A*/CEN 17 ratio of 1.88) was demonstrated by FISH analysis of FFPE tumor tissue from primary colon cancer. Treatment with epirubicin was initiated according to the protocol. Baseline CT scan was performed in November 2014, 12 days before the first treatment. The first evaluation CT scan was performed after 3 treatment cycles (day 55) and the second scan after 6 cycles of treatment (day 130). The patient had major clinical benefit at the first response evaluation: Improved WHO performance status (from 1 to 0), reduced analgesics consumption, and a decline in serum levels of CEA (from 300 μg/L to 65 μg/L), and only mild and expected side effects. The partial response was maintained until she had received the maximum dose of epirubicin at 6 months. However, after 2 months of treatment break, she had progressive disease.

Because the first patient achieved partial response, we planned to include 25 patients according to the sample size calculation. Unfortunately, we were only able to include 5 additional patients during the following 22 months. We screened archived FFPE tumor tissue from 242 patients for the *TOP2A* amplification and detected a high *TOP2*/CEN17 ratio in additional 17 patients, which corresponds to an amplification rate of 7.0%, a relative frequency slightly lower than the projected amplification rate of 10.5% (6). For various reasons, 12 patients could not be included, mainly because of stable disease and thereby no need for resumption of chemotherapy. Due to the very slow inclusion rate, we invited – without success – other Danish oncology centers, and therefore we finally decided to close the study for further inclusion in September 2016.

The most important data regarding the six included patients is shown in Table 1. Except for from the first included patient, no other patients achieved tumor shrinkage and time to progressive disease was 2 months or less in 4/6

Table 1. Patient characteristics.

RAS/MMR status	*TOP2*/CEN17	Heracles IHC score/CISH	Age/sex	Previous therapy	PS	No. of epirubicin cycles	Best respon-se	PFS (months)	OS (months)	Subsequent therapy
*RAS*wt, pMMR	1.88	1+	37 F	FOLFOX-cet × 12	1	9	PR	6.4	11.0	None
*KRAS*mut, pMMR	1.55	2+ / no amplification	63 M	FOLFOX-bev × 12	1	3	PD	1.6	23.5	FOLFIRI × 12 S1 – bev × 9 IRIS × 3
*KRAS*mut, pMMR	1.91	2+ / amplification	56 F	Adjuvant FOLFOX × 9	1	2	PD	2.1	2.4	None
*RAS*wt, pMMR	1.77	0	63 F	FOLFOX – pan × 12	0	1	PD	1.0	2.8	None
*NRAS*mut, pMMR	1.79	1+	59 F	Peri-operative CapOx × 6	0	6	NC	4.4	43.5	IRIS – bev × 8 TemCap × 8 FTD/TPI-bev × 12
*KRAS*mut, pMMR	1.80	0	70 F	FOLFOX-bev × 21 (Atezo after 8)	1	2	PD	1.7	2.4	None

PS: performance status; cet: cetuximab; pan: panitumumab; ateza: atezolizumab; FOLFOX: 5FU and oxaliplatin; CapOx: capecitabine and oxaliplatin; FOLFIRI: 5FU and irinotecan; bev: bevacizumab; IRIS: irinotecan ans S1; TemCap: temozolomide and capecitabine; FTD/TPI: trifluridine and tipiracil; PR: partial response; PD: progressive disease; NC: no changes.

patients. Three of six patients did not receive any further line of therapy due to fast clinical deterioration.

Subsequently, we examined whether some of the patients harbor co-amplifications of HER-2 to see if there was association with treatment effect. We found only HER2+ amplification in one patient. It was not in the patient with response to the treatment.

Discussion

This phase II study evaluating the efficacy of epirubicin in patients with oxaliplatin refractory mCRC and *TOP2A* gene amplification, we were only able to include six patients in the study. Unfortunatly, only one of the patients obtain tumor control and clinical benefit from the treatment.

Despite the recent approvals of trifluridine/tipiracil and regorafenib, the number of useful cytotoxic drugs in patients with mCRC is still very limited and response rates decrease dramatically with each new line of therapy. New and non-cross resistant treatment options are urgently needed, and biomarkers predictive of response to chemotherapeutic treatments are still lacking. Data on *TOP2A* aberration in CRC are sparse, with reported *TOP2A* amplification rates ranging from 2.2 to 46.6% depending on the analytical methods [6,10,11]. Previous studies found that epirubicin had limited efficacy in unselected patients with mCRC [12–15] but we anticipated that re-purposing epirubicin to patients with mCRC and *TOP2A* gene amplification may represent a valid treatment option.

Hence, the aim of this clinical phase II study was to take a further step toward personalized mCRC treatment. Unfortunately, we did not succeed to include a sufficient number of patients to complete the study, and thus to be able to confirm or disprove our hypothesis. The time to progressive disease with modern systemic second line therapy is 5–7 months [16]. We included and treated six patients in the protocol but except for the first patient, who had a major tumor shrinkage, no patient seemed to benefit from therapy with epirubicin monotherapy. Combination chemotherapy might have improved the response, but in this phase II protocol, we choose epirubicin as monotherapy to see if there was a signal. Our hypothesis of biomarker-guided therapy for this small group of patients with mCRC and *TOP2A* amplification could not be sanctioned and we cannot recommend using epirubicin even in highly selected mCRC outside of clinical trials.

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