Cancer Chemotherapy and Pharmacology (2018) 81:911–921
https://doi.org/10.1007/s00280-018-3563-2

ORIGINAL ARTICLE

Phase I study of combined indomethacin and platinum-based chemotherapy to reduce platinum-induced fatty acids

Daphne L. van der Velden[1] · Geert A. Cirkel[2] · Julia M. Houthuijzen[3] · E. van Werkhoven[4] · Jeanine M. L. Roodhart[5] · Laura G. M. Daenen[5] · Sovann Kaing[1] · Johan Gerrits[6] · Nanda M. Verhoeven-Duif[6] · Cecile Grootscholten[7] · Henk Boot[7] · Cristisiana Sessa[8] · Haiko J. Bloemendal[2] · Filip Y. De Vos[5] · Emile E. Voest[1]

Received: 24 January 2018 / Accepted: 14 March 2018 / Published online: 24 March 2018

Abstract

Purpose Chemotherapy-resistance remains a major obstacle to effective anti-cancer treatment. We previously showed that platinum analogs cause the release of two fatty acids. These platinum-induced fatty acids (PIFAs) induced complete chemoresistance in mice, whereas co-administration of a COX-1 inhibitor, indomethacin, prevented PIFA release and significantly enhanced chemosensitivity. To assess the safety of combining indomethacin with platinum-based chemotherapy, and to explore its efficacy and associated PIFA levels, a multi-center phase I trial was conducted.

Methods The study was comprised of two arms: oxaliplatin plus capecitabine (CAPOX, arm I) and cisplatin plus gemcitabine, capecitabine or 5FU (arm II) in patients for whom these regimens were indicated as standard care. Indomethacin was escalated from 25 to 75 mg TID, using a standard 3 × 3 design per arm, and was administered orally 8 days around chemo-infusion from cycle two onwards. PIFA levels were measured before and after treatment initiation, with and without indomethacin.

Results Thirteen patients were enrolled, of which ten were evaluable for safety analyses. In arm I, no dose-limiting toxicities were observed, and all indomethacin dose levels were well-tolerated. Partial responses were observed in three patients (30%). Indomethacin lowered plasma levels of 12-*S*-hydroxy-5,8,10-heptadecatrienoic acid (12-*S*-HHT), whereas 4,7,10,13-hexadecatetraenoic acid (16:4(n-3)) levels were not affected. Only one patient was included in arm II; renal toxicity led to closure of this cohort.

Conclusions Combined indomethacin and CAPOX treatment is safe and reduces the concentrations of 12-*S*-HHT, which may be associated with improved chemosensitivity. The recommended phase II dose is 75 mg indomethacin TID given 8 days surrounding standard dosed CAPOX.

Keywords Chemotherapy resistance · Mesenchymal stem cells · Platinum-induced fatty acids · Indomethacin · Oxaliplatin

✉ Emile E. Voest
e.voest@nki.nl

[1] Division of Molecular Oncology and Immunology, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

[2] Division of Medical Oncology, Meander Medical Center, Amersfoort, The Netherlands

[3] Division of Molecular Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands

[4] Biometrics Department, Netherlands Cancer Institute, Amsterdam, The Netherlands

[5] Division of Medical Oncology, University Medical Center Utrecht, Utrecht, The Netherlands

[6] Section Metabolic Diagnostics, Department of Genetics, University Medical Center Utrecht, Utrecht, The Netherlands

[7] Division of Medical Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands

[8] Division of Medical Oncology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland

Introduction

The discovery of the biological properties of platinum salts in 1965 represented a major landmark in the history of systemic anti-cancer treatment [1, 2]. Platinum-containing chemotherapy is prescribed to 10–20% of all cancer patients, and the total number of patients who have benefited from such treatment may easily run in the millions [3]. Unfortunately, the anti-tumor effect of platinum (and other chemotherapies) is often transient and therapy resistance is one of the biggest hurdles to effective cancer therapy.

Understanding and combatting chemoresistance is thus an important area of cancer research. In addition to the various tumor cell-intrinsic resistance-mechanisms that have been identified [2], it is becoming increasingly clear that interaction between tumor cells and their surrounding microenvironment plays a key role in the development of drug resistance [4, 5]. Mesenchymal stem cells (MSCs) have received much interest in this context: MSCs are multi-potent progenitor cells with the ability to home wounded tissues, where they contribute to tissue repair and regeneration. Similarly, they can home developing tumors and interfere with tumor growth and progression [6–10].

We previously identified a novel MSC-characteristic that may benefit tumor cells [11]: when MSCs are exposed to platinum analogs, they secrete two specific and relatively unknown fatty acids: 12-*S*-hydroxy-5,8,10-heptadecatrienoic acid (12-*S*-HHT) and 4,7,10,13-hexadecatetraenoic acid (16:4(n-3)). Both fatty acids proved extremely potent in inducing resistance to any kind of cytotoxic chemotherapy in various tumor-bearing mouse models. Non-platinum-based chemotherapies did not induce the release of these PIFAs. Since the release of these PIFAs is known to be mediated via the cyclooxygenase 1 (COX-1) and thromboxane synthase (TXAS) pathways, it was hypothesized that inhibition of these pathways could prevent PIFA release and (thus) PIFA-mediated chemoresistance. Addition of a COX-1 or TXAS inhibitor, such as indomethacin or ozagrel, respectively, indeed prevented PIFA release and enhanced cisplatin-efficacy in mice, whereas neither indomethacin nor ozagrel alone exerted any anti-tumor effect.

If translatable to cancer patients, inhibition of these pathways could provide a target to prevent PIFA release and to enhance the clinical benefit of chemotherapy by preventing resistance. Several observations support this hypothesis [11, 12]: first, MSCs are present in circulation and tumors of cancer patients, where they may be activated. Second, platinum-stimulated human and mouse MSCs secrete the same, specific PIFAs, both capable of inducing complete chemoresistance in one xenograft- and two mouse models. Third, in cancer patients, an acute rise in plasma-PIFA levels has been observed within hours after platinum-based chemotherapy. And fourth, commonly used food supplements (such as fish oil) may contain both PIFAs [13, 14]. Altogether, these observations suggest that the MSC-mediated mechanism of PIFA-induced chemotherapy-resistance may indeed be translated to the clinic.

Given the central role of platinum analogs in many chemotherapeutic regimens, and the fact that virtually all patients with advanced disease eventually develop resistance to chemotherapy, the potential impact of preventing chemoresistance by inhibiting PIFA release is significant. No clinical trials have tested the combination of platinum-based chemotherapy and COX-1 or TXAS-inhibitors. Since indomethacin and ozagrel seemed equally capable of enhancing chemotherapy efficacy in our preclinical study, indomethacin may provide a logical candidate for further clinical exploration, given that it is a well-established non-selective COX inhibitor, which recommended dosage for analgesic, anti-flogistic purposes (75–200 mg/day) results in adequate plasma concentrations to inhibit COX-1 [15, 16]. Inhibition of COX-1, however, can reduce renal blood flow and may thereby exacerbate platinum-associated nephrotoxicity [17–20]. For this reason, concomittant use of non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, during platinum- (and especially cisplatin-) based treatment is generally contra-indicated. A phase I study, to evaluate the safety of combining indomethacin with platinum-based treatment and to explore its efficacy and associated PIFA levels, was therefore, conducted.

Materials and methods

Study design

The PIFA-01 study was a prospective, multi-center, open-label, phase I dose-escalation trial, conducted at three hospitals in the Netherlands and one in Switzerland (NCT01719926). The study aimed to assess the safety of indomethacin combined with cisplatin- or oxaliplatin-based regimens. Secondary and exploratory endpoints included efficacy and pharmacodynamics of such treatment, as assessed by best overall response, progression free- and overall survival and treatment-induced PIFA levels, respectively. The study consisted of two arms, based on two commonly used platinum-containing regimens: patients treated with oxaliplatin-plus capecitabine (CAPOX) were enrolled in arm I, and patients treated with cisplatin plus gemcitabine, capecitabine or 5FU were enrolled in arm II. The study was approved by the Medical Ethical Committee of the University Medical Center in Utrecht and conducted in accordance

with GCP guidelines and the Declaration of Helsinki's ethical principles for medical research [21]. Written informed consent was obtained from all study participants. Patients were included from August 2013 to June 2017 and have been observed until 1 month after end of study treatment (or death) for safety analyses, and until the 1st of October, 2017 (or death) for progression free- and overall survival analyses.

Patient selection

Main inclusion criteria included age ≥ 18 years, World Health Organisation (WHO) performance status ≤ 1 and normal organ functions, defined as glomerular filtration rate (GFR) ≥ 60 ml/min, absolute neutrophil count (ANC) ≥ 1.5×10^9/l, platelets ≥ 100×10^9/l, hemoglobin (Hb) ≥ 6.0 mmol/l, partial thromboplastin time (PTT) ≤ 1,5 × upper limit of normal (ULN), bilirubin ≤ 1.5 ULN, and aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 3.0 × ULN, or ≤ 5 × ULN if liver metastases were present. All patients were required to have a histologically proven and radiologically evaluable advanced carcinoma, for which palliative treatment with one of the above-described chemotherapy regimens was indicated. Prior chemotherapy was allowed, as long as patients were platinum naïve for ≥ 6 months.

Study treatment

Patients received their first chemotherapy cycle according to standard of care (Fig. 1), to maximize safety and to provide an internal control for biomarker analyses. If GFR remained ≥ 60 ml/min and no grade 4 toxicity was observed by the end of cycle one, indomethacin was added from cycle two onwards. Indomethacin was taken orally, 8 days per treatment cycle, starting 2 days before chemotherapy infusion and continued for 5 days thereafter for steady-state purpose, reaching C_{max} at time of platinum administration. Proton pump inhibiters (PPI) were prescribed for all patients from day 15 of the 1st cycle onwards and continued till the end of study treatment to prevent gastric ulcers. Patients were asked to refrain from using comedication expected to increase toxicity or change indomethacin plasma levels, such as selective serotonin reuptake inhibitors (SSRIs), angiotensin-converting enzyme (ACE) inhibitors, antithrombotics, diuretics, et cetera. All concurrent medications were recorded at each interim visit (i.e., ≥ 1× per treatment cycle). The first and lowest indomethacin dose level was 25 mg three times daily (total daily dose 75 mg). This was escalated to 50 mg three times daily for the second dose level (total daily dose 150 mg), and 75 mg three times daily for the third and highest dose level (total daily dose 225 mg).

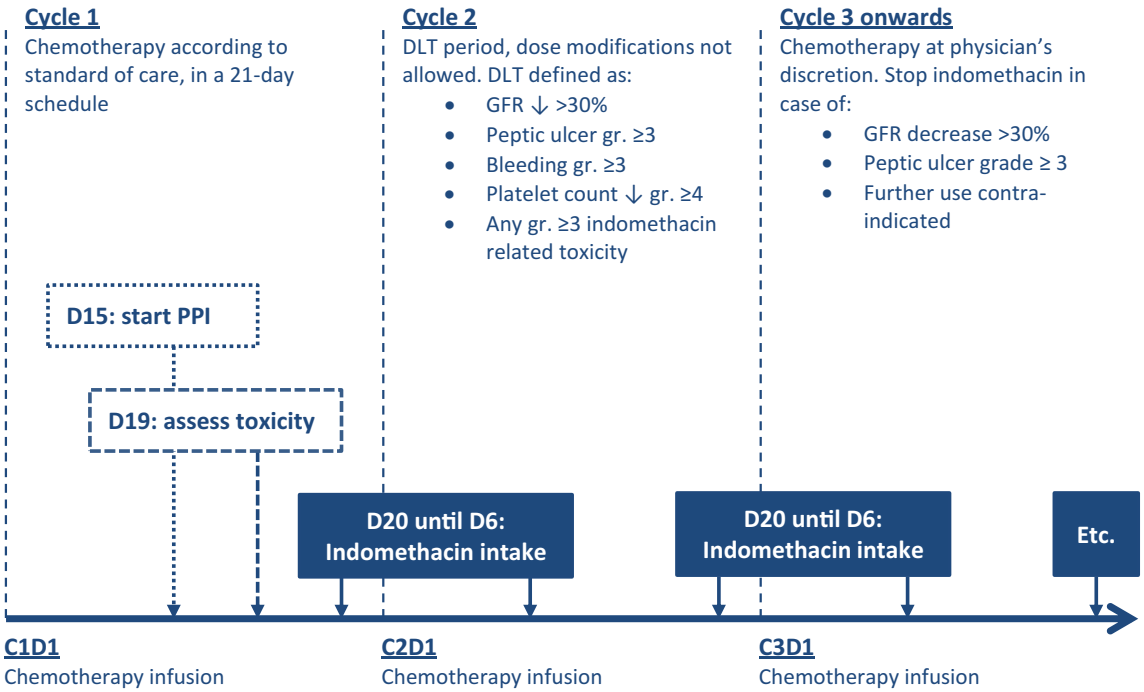


Fig. 1 Study flowchart. Schematic overview of study design. Treatment cycle one was administered according to standard of care for oxaliplatin plus capecitabine (arm I) or cisplatin plus gemcitabine, capecitabine or 5FU (arm II). From day 15 onwards, proton pump inhibiters were prescribed to prevent gastric ulcers. On day 19, GFR and toxicity were evaluated. If GFR remained ≥ 60 ml/min and no grade 4 toxicity was observed, indomethacin was added from day 20 until day 6 of the next treatment cycle. *C* treatment cycle, *D* day, *DLT* dose-limiting toxicity, *PPI* proton pump inhibitor

Dose-limiting toxicities (DLTs) were assessed during cycle two. DLTs were defined as (1) a decrease in GFR > 30%, (2) peptic ulcer disease grade ≥ 3, (3) bleeding grade ≥ 3 or platelet count decrease grade ≥ 4, or (4) any relevant grade ≥ 3 toxicity considered to be related to-, or exacerbated by the addition of indomethacin. Dose reductions were not allowed in the second treatment cycle as toxicity-defined DLT; in that case, patients were taken off-study and considered non-evaluable. Maximum tolerable dose (MTD) assessment was based on the second treatment cycle-safety evaluations (i.e., DLT-period).

From treatment cycle three onwards, indomethacin was discontinued in case of (1) peptic ulcer disease grade ≥ 3, (2) GFR decrease > 30%, or if (3) the treating physician deemed further use of indomethacin contra-indicated. Chemotherapy continuation or dose modification was at the physician's discretion.

Safety assessments

All patients underwent a complete medical history, physical examination, assessment of vital signs and WHO performance status, and laboratory analysis of hematology, coagulation, clinical chemistry and urine before treatment initiation. These assessments were repeated throughout study participation (before start of every new treatment cycle) and/or as clinically indicated. Adverse events (AEs) were classified and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Efficacy assessments

Baseline tumor measurements were performed within 28 days of treatment initiation and every 9 weeks thereafter, or more frequently at the physician's discretion. Overall response rate was evaluated by the investigators according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [22].

Biomarker analyses

To characterize biologic activity of indomethacin, patients were asked to refrain from using other non-steroidal anti-inflammatory drugs (NSAIDs), prostaglandin-synthethase inhibitors and /or omega-3/omega-6 containing products [13, 14] from 2 weeks before treatment initiation onwards. Plasma 12-*S*-HHT and 16:4(n-3) levels were measured as described and validated previously [13, 23]. Plasma samples were obtained before and at 1, 2 and 4 h after start of chemotherapy infusion ($T=0$, $T=1$, $T=2$ and $T=4$, respectively; duration cisplatin and oxaliplatin infusion was 4 and 2 h, respectively), on day one of treatment cycle one and two (before and after start of indomethacin-treatment, respectively). Plasma was stored at − 80 °C until further analysis. All samples were processed within 2 h after blood withdrawal.

Statistical analyses

Since this was a phase I safety and tolerability trial, analyses were descriptive. Indomethacin dosage was escalated using a standard 3 × 3 design per study arm: per dose level, three patients were enrolled. If no DLT was observed, indomethacin dose would be escalated to the next dose level/cohort. If one out of three subjects experienced a DLT, the cohort would be expanded with three additional subjects. If two or more subjects experienced a DLT, that dose would be considered to be the toxic dose for the respective chemotherapy-indomethacin combination. A DLT rate of > 17% (i.e., > 1 out of six subjects) was considered unacceptable.

All patients who received ≥ 1 dose of indomethacin during on-study chemotherapy were included in the safety analyses (primary study endpoint). Adverse events (AEs) that were or could have been related to indomethacin were summarized by counting the number ofgrade ≥ 1, grade ≥ 2, and grade ≥ 3 AEs per patient, and determining the maximum grade per patient. These numbers were compared between indomethacin dose levels using the Kruskal–Wallis test. The percentages of patients with grade ≥ 1, grade ≥ 2 and grade ≥ 3 were compared using Fisher's exact test.

All patients who received ≥ 2 cycles of on-study chemotherapy were included in efficacy evaluations (secondary study endpoint). Best overall response was analyzed per patient. Median progression free- and overall survival were estimated using the Kaplan–Meier method, overall and for each indomethacin dose level individually. Estimated means were compared between indomethacin dose levels using the log rank test.

All patients with valid and complete biomarker data were included for biomarker analyses (exploratory study endpoint). Linear mixed-effects models were used to assess (1) whether plasma PIFA levels increase during platinum-contain chemotherapy (cycle one only, no other fixed effects), (2) whether addition of indomethacin lowers plasma PIFA levels (cycle one vs. two as fixed effect, time as fixed-effect covariate), (3) whether the indomethacin dose level as continuous (fixed) effect was associated with PIFA (time as fixed-effect covariate) and (4) whether PIFA levels are associated with chemotherapy efficacy (cycle one and two, no other fixed effects). From this last model, only the difference between the two most extreme observed response levels was reported, i.e., partial response vs. progressive disease. In none of the models, a random slope seemed required on the basis of the (restricted) log-likelihood. PIFA measurements below the limit of detection were treated as zeros.

Results

Patient characteristics and treatment details

Between September 2013 and June 2017, 13 patients were enrolled: 12 in arm I and one in arm II. Accrual took longer than expected, partly due to Ethical Committee-imposed age-restrictions (≤ 70 years) at the beginning of the study, and partly due to the fact that the CAPOX is typically administered as first-line treatment rather than in the advanced, metastatic setting that this study focused on.

Ten patients were evaluable for safety- and efficacy analyses; baseline characteristics are shown in Table 1. The median number of prior treatment regimens was 2 (range 0–4). Evaluable patients received on-study chemotherapy for a median of 4 cycles (range 2–6). Median follow-up duration was 3.2 months (range 1.6–8.5) for safety analyses, and 7.9 months (range 3.4–24.3) for progression-free and overall survival analyses. Chemotherapy regimen was adjusted in six patients: two patients had 25% dose reductions from cycle one onwards (one due to DPD deficiency, the other due to hand–foot syndrome and neutropenia later on). Three patients had dose delays from cycle three onwards (two due to bone marrow toxicity, the other due to unforeseen dental surgery). One patient experienced an oxaliplatin-related infusion reaction in cycle three and did, therefore, not receive the full, planned dose.

In arm I, three patients went off-study during cycle one due to rapid disease progression ($n = 2$) and worsening of pre-existing gastro-intestinal complaints ($n = 1$), and were, therefore, not evaluable. In the remaining nine patients, no DLTs were observed. Reasons to discontinue study treatment for these patients included disease progression ($n = 5$), patient preference to stop despite ongoing response ($n = 2$; one patient decided to participate in another trial, the other decided to continue CAPOX off-study due to increasing reluctance against oral medication), worsening of pre-existent neuropathy ($n = 1$), and indomethacin non-compatible co-medication (carbasalate calcium because of capecitabine-related cardiovascular toxicity ($n = 1$)).

The patient in arm II developed a DLT (acute kidney injury grade one) after completion of the first 8 days of combined cisplatin-indomethacin treatment, and went off-study during cycle two. According to the cohort rules, five more patients could enroll. Due to high probability of additional DLTs as estimated by the local investigators,

Table 1 Baseline characteristics

	Arm I			Arm II	Overall
	Dose level 1 Indomethacin 25 mg TID	Dose level 2 Indomethacin 50 mg TID	Dose level 3 Indomethacin 75 mg TID	Dose level 1 Indomethacin 25 mg TID	
Number of evaluable patients	3	3	3	1	10
Age (in years)	61 (60–67)	66 (51–59)	43 (30–72)	60	61 (30–72)
Gender (male)	3 (100%)	2 (67%)	1 (33%)	1	7 (70%)
ECOG performance status					
0	1 (33%)	1 (33%)	3 (100%)	1	6 (60%)
1	2 (67%)	2 (67%)	0 (0%)	0	4 (40%)
Primary tumor type					
Colorectal adeno ca.	3 (100%)	3 (100%)	2 (67%)	0	8 (80%)
Esophageal adeno ca.	0 (0%)	0 (0%)	1 (33%)	1	2 (20%)
Previous therapies	2 (0–2)	2 (1–4)	0 (0–2)	1	2 (0–4)
Creatinine (mmol/l)	64 (64–94)	60 (49–91)	73 (57–88)	57	64 (49–94)
Creatinine clearance (ml/min)	60 (60–90)	90 (79–116)	82 (71–108)	107	83 (60–116)
Proteinuria					
Negative	2 (67%)	1 (33%)	0 (0%)	1	4 (40%)
Trace	1 (33%)	1 (33%)	2 (67%)	0	4 (40%)
Positive	0 (0%)	1 (33%)	0 (0%)	0	1 (10%)
Missing	0 (0%)	0 (0%)	1 (33%)	0	1 (10%)

Baseline characteristics of all study subjects who were evaluable for safety and efficacy analyses. All values for arm I (oxaliplatin plus capecitabine) and for arm I and II combined ('overall') are displayed as median (and range), or as number (and percentage). Since only one patient was enrolled in arm II (cisplatin plus gemcitabine, capecitabine or 5FU), baseline characteristics of this patient are displayed as absolute values

adeno ca. adenocarcinoma, *ECOG* Eastern Cooperative Oncology Group

however, inclusion came to a stop and arm II was closed prematurely due to non-accrual.

Safety

All AEs that were or could have been related to the addition of indomethacin are shown in Table 2. Nausea was the most common [observed in four patients (40%)], followed by fatigue, malaise, vomiting, ALAT-, ASAT-increase and white blood cell decrease [each observed in two patients (20%)]. Every other AE was observed in one patient only (10%). Out of the 33 AEs that were observed, 24 were grade one (73%), eight were grade two (24%), and one was grade three (3%). Grade four or worse AEs were not observed. Indomethacin-relatedness was classified by local investigators as 'unlikely', 'possibly', 'probably' or 'definitively' in twelve, seventeen, one and three cases (36, 52, 3 and 9%, respectively). The number of grade ≥ 1, grade ≥ 2 or grade ≥ 3 AEs per patient did not differ between indomethacin dose levels ($p = 0.09$, 0.5, and 0.5, respectively), nor did the maximum grade AE per patient ($p = 0.6$), nor did the number of patients with grade ≥ 1, grade ≥ 2 or grade ≥ 3 AEs ($p = 1$, 0.7 and 1, respectively).

Two serious AEs (SAEs) were observed, both in patients who went off-study before indomethacin initiation: one patient was hospitalized due to a liver abscess, caused by primary disease progression, which ultimately led to the patient's death. Another patient was hospitalized due to worsening of pre-existent gastro-intestinal complaints, which resolved without sequela. To minimize the risk of recurrent gastro-intestinal complaints, however, it was decided to continue chemotherapy off-study (without addition on indomethacin). No other serious adverse events were observed and no unexpected or treatment-related deaths occurred; all deaths were caused by primary disease progression.

Efficacy

Response data are summarized in Table 2. No complete responses were observed. Three patients (30%) experienced a partial response; two at dose level one (one in each arm) and one at dose level two, arm I. Stable disease was observed in three patients (30%), one at each dose level in arm I. The remaining four patients (40%) had progressive disease at first response evaluation. Median progression free- and overall survival were 5.5 months (95% CI 0.0–11.9) and 15.1 months (95% CI 1.8–28.4), respectively; differences between indomethacin dose levels were non-significant ($p = 0.4$ and 0.9, respectively).

Biomarkers

Plasma PIFA levels of all biomarker-evaluable patients ($n = 9$) and change therein upon chemotherapy infusion and indomethacin administration, as well as association with chemotherapy efficacy, are shown in Fig. 2 and Table 3. During the first cycle of chemotherapy, before addition of indomethacin, both PIFA levels seemed to increase in the 4 h after chemotherapy infusion, but differences did not reach significance ($p = 0.09$ for 12-*S*-HHT, and $p = 0.2$ for 16:4(n-3)). Upon addition of indomethacin, however, 12-*S*-HHT levels were significantly reduced across all time points (i.e., before start of chemotherapy up to 4 h thereafter: −0.81 nmol/l, 95% CI −1.20 to −0.43, $p = 0.0001$). Furthermore, each indomethacin dose level-increase was associated with a significant drop in 12-*S*-HHT level (−0.33 nmol/l, 95% CI −0.51 to −0.15, $p = 0.0005$). For 16:4(n-3), no effect of indomethacin was observed between treatment cycles ($p = 0.2$), nor between indomethacin dose levels (as a continuous variable, $p = 0.5$).

No association was found between PIFA levels and response in the three-category partial response (PR), stable disease (SD) and progressive disease (PD) ($p = 0.3$ for 12-*S*-HHT and $p = 0.2$ for 16:4(n-3) on three degrees of freedom), neither after combining PR and SD into a group with 'clinical benefit' (results not shown). Only the difference between the two most extreme responses observed (PR vs. PD) was reported (Table 3).

Discussion

The primary aim of this study was to evaluate the safety of combined indomethacin and oxaliplatin (arm I)- or cisplatin (arm II)-based chemotherapy. In addition, we aimed to explore the effect of indomethacin on efficacy of chemotherapy and PIFA levels, and the relation between PIFA levels and chemotherapy efficacy.

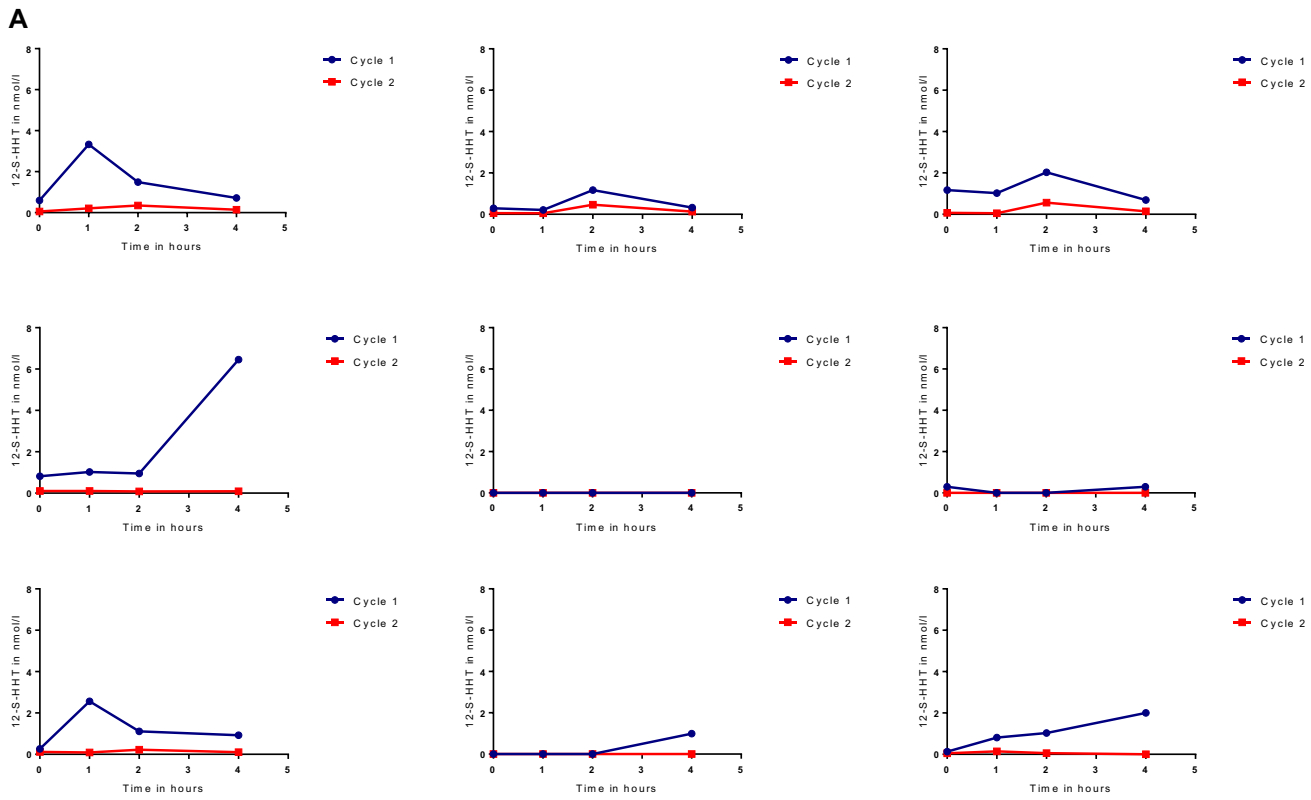
First of all, combined indomethacin and cisplatin/oxaliplatin treatment was generally well tolerated. The vast majority of AEs was mild (73% grade one, 24% grade two, 3% grade three), and 'unlikely' (36%) or 'possibly' (52%) related to indomethacin. Definitive indomethacin relatedness was rare (9%). One patient in arm II experienced a DLT, due to > 30% decrease in GFR after completion of the first 8 days of combined indomethacin-cisplatin treatment. This was in line with expectations, given the impact of NSAIDs on renal clearance of cisplatin [17–20]. Although the effect on renal function was mild (acute kidney injury grade one) and although the patient recovered without sequela, the reluctance amongst the investigators to include more patients in this arm was substantial. Therefore, we can not draw conclusions regarding the safety of combined

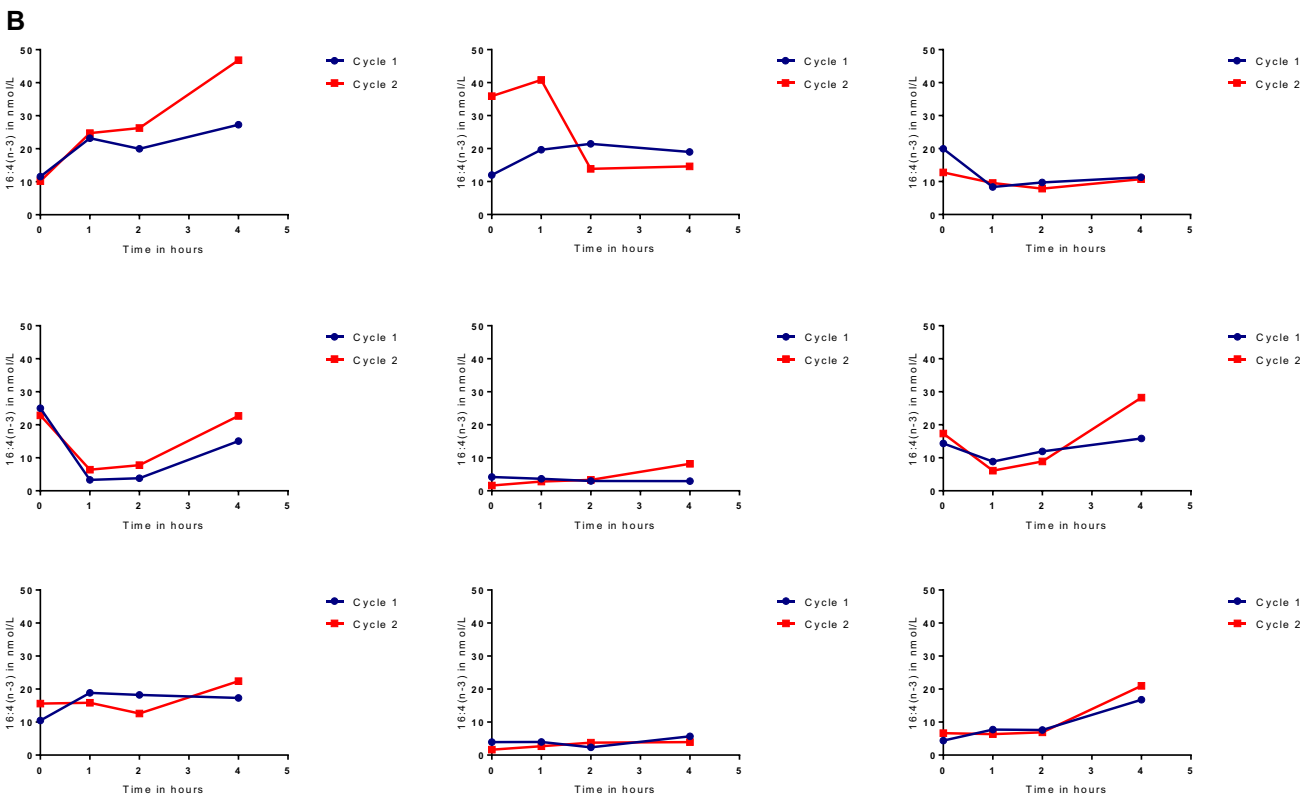
Table 2 Toxicity and efficacy

	Arm I									Arm II			Overall (10 patients)		
	Dose level 1 Indomethacin 25 mg TID (3 patients)			Dose level 2 Indomethacin 50 mg TID (3 patients)			Dose level 3 Indomethacin 75 mg TID (3 patients)			Dose level 1 Indomethacin 25 mg TID (1 patient)					
	Patient:														
	2	3	4	5	7	8	10	11	13	1			G 1	G 2	G 3
(A) Safety															
Renal AEs															
Acute kidney injury										1**			1×		
Gastro-intestinal AEs															
Diarrhea					1								1×		
Nausea	1*					1		2*		1			3×	1×	
Vomiting			1***	1*									2×		
Hematologic AEs															
Anemia						1							1×		
Platelet count ↓					1*								1×		
White blood cells ↓	1*				1								2×		
Hepatic AEs															
Alkaline phosphatase ↑	1*												1×		
ALAT ↑	1*				1*								2×		
ASAT ↑	2*				1*								1×	1×	
GGT ↑	3*														1×
Other AEs															
Dizziness						1*							1×		
Dry mouth			1***										1×		
Dry skin	1*												1×		
Dysesthesia					2									1×	
Edema ankles				1***									1×		
Fasciculation	1*												1×		
Fatigue				1*									1×		
Fever									1				1×		
Hyperkalemia					1								1×		
Injection site reaction					2									1×	
Lethargy	1*												1×		
Malaise					2	2								2×	
Muscle cramp	2*													1×	
Palm.-plant. eryth. syndr					2									1×	
Total number of patients with grade 1, 2 or 3 AE per dose level	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3
	2	1	1	3	2		1	1		1			24×	8×	1×
(B) Efficacy															
Complete response															
Partial response	PR				PR					PR			3×		
Stable disease		SD		SD				SD					3×		
Progressive disease			PD			PD	PD		PD				4×		

Part A displays all adverse events that were or could have been treatment related, per patient, per indomethacin dose level and overall. Relatedness to indomethacin was classified as unlikely, possibly (*), probably (**) or definitively (***). Data per arm show maximum grade per patient in that arm; each patient could be counted under more than one preferred term. Part B displays best overall response according to RECIST, per indomethacin dose level and overall

AE adverse event, *CTCAE* National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03, *G* grade, *Palm.-plant. eryth. syndr.* palmar-plantar erythrodysesthesia syndrome, *RECIST* Response Evaluation Criteria in Solid Tumors version 1.1, *TID* three times daily, ↑ increase, ↓ decrease, *n* × observed in *n* patients


A
12-S-HHT in nmol/l
Time in hours
Cycle 1
Cycle 2


B
16:4(n-3) in nmol/L
Time in hours
Cycle 1
Cycle 2

◂**Fig. 2** PIFA levels on day one of treatment cycle one and two. **a** Plasma 12-*S*-hydroxy-5,8,10-heptadecatrienoic acid (12-*S*-HHT) levels in nmol/l per patient (*y*-axis), measured before and on 1, 2 and 4 h after oxaliplatin infusion (*x*-axis). *top row* indomethacin 25 mg TID, *middle row* 50 mg TID, *lower row* 75 mg TID, *cycle 1* without indomethacin, *cycle 2* with indomethacin. **b** Plasma hexadeca-4,7,10,13-tetraenoic acid (16:4(n-3)) levels in nmol/l per patient (*y*-axis), measured before and on 1, 2 and 4 h after oxaliplatin infusion (*x*-axis). *top row* indomethacin 25 mg TID, *middle row* 50 mg TID, *lower row* 75 mg TID, *cycle 1* without indomethacin, *cycle 2* with indomethacin

indomethacin-cisplatin treatment. Combined indomethacin-CAPOX treatment, however, proved to be safe: no DLTs were observed in any of the enrolled patients, the MTD was not reached, and no significant difference in grade one, two or three AEs were observed between indomethacin dose levels. Thus, indomethacin 75 mg TID administered 8 days around chemotherapy infusion is safe in patients treated with CAPOX, and forms the recommended phase II dose.

Another study aim was to explore the effects of platinum-containing chemotherapy (with or without indomethacin) on plasma PIFA levels, and to explore PIFA levels in relation to response to chemotherapy. Based on our previous findings [11, 12], platinum-containing chemotherapy was expected to cause a rise in PIFA levels. Addition of indomethacin was expected to counteract this effect, thereby enhancing chemotherapy efficacy. In line with our preclinical studies, 12-*S*-HHT was indeed effectively blocked by indomethacin. Unexpectedly, however, 16:4(n-3) levels in patients did not reflect the preclinical findings. A potential explanation could be that 16:4(n-3), a fatty acid for which limited studies are available, is not exclusively produced via COX-1, or that it can be found in foods other than fish oil as well [24].

This prompts the question whether reduction of 12-*S*-HHT alone is sufficient to prevent PIFA-induced chemotherapy resistance, given that both PIFAs affected chemotherapy efficacy in mice. In the present study, however, no significant associations were found between PIFA levels and response to chemotherapy. Yet, our study was not designed (and thus underpowered) to test for differences in chemotherapy efficacy. In addition, the fact that five out of nine biomarker-evaluable patients discontinued study treatment for other reasons than progressive disease, may have limited our ability to correlate PIFA levels with chemotherapy efficacy.

Meanwhile, synergy between indomethacin- and platinum-based chemotherapy has been described before: in mice-bearing murine colorectal cancer tumors, for example, synergy was observed with cisplatin, but not with adriamycin [25]. In human lung cancer xenografts, combined indomethacin-oxaliplatin treatment increased tumor-inhibition rates in one study [26] and reduced expressions of lymph node metastasis related factors in another [27]. Further, indomethacin enhanced cisplatin efficacy in human uterine cervical and ovarian cancer cells [28, 29]. In addition, in a randomized trial including 22 pet dogs with naturally occuring invasive bladder cancer, cisplatin plus piroxicam (a COX inhibitor, similar to indomethacin) led to a remission rate of 71%, compared to 0% for cisplatin alone ($p < 0.002$) [30]. Altogether, these observations suggest that indomethacin can indeed enhance chemosensitivity; possibly by reduction of baseline 12-*S*-HHT levels alone. In addition, the fact that indomethacin is a known COX-1 inhibitor, whereas other trials to date have mainly focused on (and found little effect of) adding COX-2 inhibitors to chemotherapy [31, 32], supports the notion that indomethacin in anti-cancer treatment merits further research: present study provides evidence that it would be safe to do so. Furthermore, it proofs that patients using indomethacin for analgesic or anti-flogistic purposes can safely continue indomethacin during CAPOX treatment.

In summary, combined CAPOX- and indomethacin treatment was well-tolerated. Compared to regular CAPOX, addition of indomethacin up to 75 mg TID hardly increased overall toxicity, nor renal toxicity at any grade. Combined cisplatin-indomethacin treatment, however, did cause renal toxicity in the one patient receiving such treatment. Indomethacin significantly reduced plasma 12-*S*-HHT levels, but had no effect on 16:4(n-3) levels; additional research is needed to assess the effects on chemotherapy efficacy. The recommended phase II dose was established at 75 mg, taken three times daily, 8 days per treatment cycle, starting 2 days before chemotherapy infusion and continued for 5 days thereafter.

Table 3 Biomarker analyses

	12-*S*-HHT		16:4(n-3)	
	Change	95% CI, *p*	Change	95% CI, *p*
(i) Change in PIFA levels upon infusion of platinum-containing chemotherapy, before addition of indomethacin (cycle 1 only)	0.21	(−0.04 to 0.47, $p=0.09$)	0.78	(−0.43 to 1.99, $p=0.2$)
(ii) Change in PIFA levels upon infusion of platinum-containing chemotherapy, after addition of indomethacin (cycle 1 vs. cycle 2)	−0.81	(−1.20 to −0.43, $p=0.0001$)*	2.14	(−1.05 to 5.33, $p=0.2$)
(iii) Change in PIFA levels upon each indomethacin dose level increase (cycle 1 and 2)	−0.33	(−0.51 to −0.15, $p=0.0005$)*	0.47	(−1.00 to 1.95, $p=0.5$)
(iv) Difference in PIFA levels between patients with PR vs. patients with PD at 1st response evaluation (cycle 1 and 2)	−0.50	(−1.60 to 0.61, $p=0.3$)	−9.12	(−24.55 to 6.33, $p=0.2$)

Association between plasma PIFA levels (in nmol/l) and four different variables. The first three rows display the estimated mean change in respective PIFA level, upon (i) chemotherapy infusion, (ii) indomethacin addition, and (iii) indomethacin dose level increase. Row (iv) displays the difference in PIFA levels between patients with PR vs. patients with PD at first response evaluation

PIFA platinum-induced fatty acids, *PD* progressive disease, *PR* partial response, *T* time in hours, relative to start chemotherapy infusion, *12-S-HHT* 12-*S*-hydroxy-5,8,10-heptadecatrienoic acid, *16:4(n-3)* hexadeca-4,7,10,13-tetraenoic acid

*Significant, $p \leq 0.05$

Acknowledgements This work was supported by a Grant from the Dutch Cancer Society (KWF), Grant number UU 2012-5712, and received financial support rom the Swiss Advisory Board for Research EOC for study monitoring. The authors gratefully acknowledge the support of all four participating sites, and all study participants.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Rosenberg B, VanCamp L, Trosko JE, Mansour VH (1969) Platinum compounds: a new class of potent antitumour agents. Nature 222(5191):385–386
2. Kelland L (2007) The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer 7(8):573–584. https://doi.org/10.1038/nrc2167
3. The "Accidental" Cure—Platinum-based Treatment for Cancer: The Discovery of Cisplatin (2014) National Cancer Institute. https://www.cancer.gov/research/progress/discovery/cisplatin. Accessed 8 Aug 2017
4. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013
5. Meads MB, Gatenby RA, Dalton WS (2009) Environment-mediated drug resistance: a major contributor to minimal residual disease. Nat Rev Cancer 9(9):665–674. https://doi.org/10.1038/nrc2714
6. Bianchi G, Borgonovo G, Pistoia V, Raffaghello L (2011) Immunosuppressive cells and tumour microenvironment: focus on mesenchymal stem cells and myeloid derived suppressor cells. Histol Histopathol 26(7):941–951
7. Cuiffo BG, Karnoub AE (2012) Mesenchymal stem cells in tumor development: emerging roles and concepts. Cell Adhes Migr 6(3):220–230. https://doi.org/10.4161/cam.20875
8. Droujinine IA, Eckert MA, Zhao W (2013) To grab the stroma by the horns: from biology to cancer therapy with mesenchymal stem cells. Oncotarget 4(5):651–664. https://doi.org/10.18632/oncotarget.1040
9. Klopp AH, Gupta A, Spaeth E, Andreeff M, Marini F 3rd (2011) Concise review: dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? Stem Cells (Dayton Ohio) 29(1):11–19. https://doi.org/10.1002/stem.559
10. Sun Z, Wang S, Zhao RC (2014) The roles of mesenchymal stem cells in tumor inflammatory microenvironment. J Hematol Oncol 7:14. https://doi.org/10.1186/1756-8722-7-14
11. Roodhart JM, Daenen LG, Stigter EC, Prins HJ, Gerrits J, Houthuijzen JM, Gerritsen MG, Schipper HS, Backer MJ, van Amersfoort M, Vermaat JS, Moerer P, Ishihara K, Kalkhoven E, Beijnen JH, Derksen PW, Medema RH, Martens AC, Brenkman AB, Voest EE (2011) Mesenchymal stem cells induce resistance to chemotherapy through the release of platinum-induced fatty acids. Cancer Cell 20(3):370–383. https://doi.org/10.1016/j.ccr.2011.08.010
12. Roodhart JM, Daenen LG, Stigter EC, Medema RH, Martens AC, Brenkman AB, Voest EE (2011) Clinical relevance of mesenchymal stem cell-induced resistance to chemotherapy. J Clin Oncol 29(15_suppl):10557
13. Daenen LG, Cirkel GA, Houthuijzen JM, Gerrits J, Oosterom I, Roodhart JM, van Tinteren H, Ishihara K, Huitema AD, Verhoeven-Duif NM, Voest EE (2015) Increased plasma levels of chemoresistance-inducing fatty acid 16:4(n-3) after consumption of fish and fish oil. JAMA Oncol 1(3):350–358. https://doi.org/10.1001/jamaoncol.2015.0388
14. Daenen LG, Roodhart JM, Stigter EC, Gerritsen MG, Medema RH, Brenkman AB, Voest EE (2011) The effect of fish oil on

chemotherapy activity in mice: clinical implications. J Clin Oncol 29(15_suppl):10532
15. de Groot DJA, de Vries EGE, Groen HJM, de Jong S (2007) Non-steroidal anti-inflammatory drugs to potentiate chemotherapy effects: from lab to clinic. Crit Rev Oncol Hematol 61(1):52–69. https://doi.org/10.1016/j.critrevonc.2006.07.001
16. Huntjens DR, Danhof M, Della Pasqua OE (2005) Pharmacokinetic-pharmacodynamic correlations and biomarkers in the development of COX-2 inhibitors. Rheumatology 44(7):846–859. https://doi.org/10.1093/rheumatology/keh627
17. Greene SN, Ramos-Vara JA, Craig BA, Hooser SB, Anderson C, Fourez LM, Johnson BM, Stewart JC, Knapp DW (2010) Effects of cyclooxygenase inhibitor treatment on the renal toxicity of cisplatin in rats. Cancer Chemother Pharmacol 65(3):549–556. https://doi.org/10.1007/s00280-009-1061-2
18. Gambaro G, Perazella MA (2003) Adverse renal effects of anti-inflammatory agents: evaluation of selective and nonselective cyclooxygenase inhibitors. J Intern Med 253(6):643–652
19. Pabla N, Dong Z (2008) Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int 73(9):994–1007. https://doi.org/10.1038/sj.ki.5002786
20. Madias NE, Harrington JT (1978) Platinum nephrotoxicity. Am J Med 65(2):307–314
21. World Medical Association (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. Jama 310 (20):2191–2194. https://doi.org/10.1001/jama.2013.281053
22. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer (Oxf 1990) 45(2):228–247. https://doi.org/10.1016/j.ejca.2008.10.026
23. Stigter EC, Letsiou S, vd Broek NJ, Gerrits J, Ishihara K, Voest EE, Verhoeven-Duif NM, Brenkman AB (2013) Development and validation of a quantitative LC-tandem MS assay for hexadeca-4,7,10,13-tetraenoic acid in human and mouse plasma. J Chromatogr B Anal Technol Biomed Life Sci 925:16–19. https://doi.org/10.1016/j.jchromb.2013.01.012
24. Ishihara K, Murata M, Kaneniwa M, Saito H, Komatsu W, Shinohara K (2000) Purification of stearidonic acid (18:4(n-3)) and hexadecatetraenoic acid (16:4(n-3)) from algal fatty acid with lipase and medium pressure liquid chromatography. Biosci Biotechnol Biochem 64(11):2454–2457
25. Hattori K, Matsushita R, Kimura K, Abe Y, Nakashima E (2001) Synergistic effect of indomethacin with adriamycin and cisplatin on tumor growth. Biol Pharm Bull 24(10):1214–1217
26. Sun W, Chen G (2016) Impact and mechanism of non-steroidal anti-inflammatory drugs combined with chemotherapeutic drugs on human lung cancer-nude mouse transplanted tumors. Oncol Lett 11(6):4193–4199. https://doi.org/10.3892/ol.2016.4493
27. Xing D, Chen YQ, Wang DC, Zhao YX, Chen G (2016) Combined effects off indomethacin and oxaliplatin on lymph node metastasis related factors in human lung cancerxenografts in nude mice. Pak J Pharm Sci 29(6):2083–2088
28. Barnes AP, Miller BE, Kucera GL (2007) Cyclooxygenase inhibition and hyperthermia for the potentiation of the cytotoxic response in ovarian cancer cells. Gynecol Oncol 104(2):443–450. https://doi.org/10.1016/j.ygyno.2006.08.008
29. Ogino M, Minoura S (2001) Indomethacin increases the cytotoxicity of cis-platinum and 5-fluorouracil in the human uterine cervical cancer cell lines SKG-2 and HKUS by increasing the intracellular uptake of the agents. Int J Clin Oncol 6(2):84–89
30. Knapp W, Glickman DW, Widmer NR, Denicola W, Adams DG, Kuczek L, Bonney TL, DeGortari PE, Han A, Glickman C L (2000) Cisplatin versus cisplatin combined with piroxicam in a canine model of human invasive urinary bladder cancer. Cancer Chemother Pharmacol 46(3):221–226. https://doi.org/10.1007/s002800000147
31. Kohne CH, De Greve J, Hartmann JT, Lang I, Vergauwe P, Becker K, Braumann D, Joosens E, Muller L, Janssens J, Bokemeyer C, Reimer P, Link H, Spath-Schwalbe E, Wilke HJ, Bleiberg H, Van Den Brande J, Debois M, Bethe U, Van Cutsem E (2008) Irinotecan combined with infusional 5-fluorouracil/folinic acid or capecitabine plus celecoxib or placebo in the first-line treatment of patients with metastatic colorectal cancer. EORTC study 40015. Ann Oncol 19(5):920–926. https://doi.org/10.1093/annonc/mdm544
32. Pierga J, Delaloge S, Giacchetti S, Brain E, Savignoni A, Sigal-Zafrani B, Mathieu M, Bertheau P, Guinebretière J, De Cremoux P, Spyratos F, Marty M (2009) A multicenter randomized phase ii study of sequential epirubicin/cyclophosphamide followed by docetaxel with or without celecoxib or trastuzumab according to HER2 status, as primary chemotherapy for localized invasive breast cancer patient. Cancer Res 69(24 Supplement):5054–5054. https://doi.org/10.1158/0008-5472.sabcs-09-5054