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ORIGINAL ARTICLE

Thalidomide and celecoxib as potential modulators of irinotecan's activity in cancer patients

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Abstract *Purpose*: Nuclear factor-κB (NF-κB) activation induces resistance to irinotecan. Preclinically, thalidomide and COX-2 inhibitors reduce NF-κB activation. We tested the feasibility of combining irinotecan with thalidomide and thalidomide/celecoxib in patients with refractory malignancies. *Patients/methods*: The study was conducted in two parts. First, the optimal dose of thalidomide (400 or 200 mg daily) in combination with irinotecan 125 mg/m^2 days 1 and 8 every 3 weeks was determined. In the second part, celecoxib 400 mg twice-daily was added to irinotecan/thalidomide. Pharmacokinetics of irinotecan and thalidomide alone or concurrently were evaluated. Tumor necrosis factor alpha, beta-fibroblast growth factor, and NF-κB activation were measured in blood mononuclear cells (PBMC). No CYP450 enzyme inducers/inhibitors were allowed. *Results*: Thirty-six patients were enrolled: Eleven received thalidomide 400 mg, 13 thalidomide 200 mg and 12 thalidomide 400 mg and celecoxib, with irinotecan. For the two-drug combination, there was a higher rate of moderate/severe diarrhea/myelosuppression with thalidomide 200 mg. Thus thalidomide 400 mg was combined with celecoxib. The triple combination resulted in similar toxicity as the doublet with the lower thalidomide dose. Concurrent administration of irinotecan/thalidomide did not influence pharmacokinetics. Anti-tumor responses occurred in two patients and prolonged stabilization in eight others. NF-κB activation increased over time. Patients experiencing tumor response or prolonged stabilization had lower NF-κB activation, albeit not statistically significant ($P = 0.124$). *Conclusions*: The combination of thalidomide/irinotecan is safe and devoid of PK interactions. Thalidomide 400 mg appeared more suitable for combination, whereas the addition of celecoxib did not improve tolerability. Tumor-specific studies in patients with lesser prior treatment will be necessary to establish the therapeutic impact of the combinations.

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Introduction

Irinotecan is a semi-synthetic derivative of the plant camptothecin, which after conversion to its active metabolite SN-38 interacts with complexes formed by cellular topoisomerase I (Topo I) and DNA, leading to irreversible arrest of the replication fork and S-phase-specific cytotoxicity [1]. Irinotecan has demonstrated

anti-tumor activity in a wide spectrum of tumor types [2–4].

De novo or acquired resistance of tumor cells to camptothecins is common. Experimental evidence implies decreased carboxylesterase gene expression, which limits the conversion of irinotecan to SN-38, reduced cellular accumulation of the drug from active drug efflux, and reduced levels of Topo I, as well as alterations in the structure of Topo I and in the cellular response to camptothecin-Topo I-DNA complex formation [5].

Recently, a mechanism of resistance based on the activation of the transcription factor NF-κB has been proposed [6]. In most untransformed cells, NF-κB exists as a heterodimer consisting predominantly of p50 and p65 subunits [7, 8]. These complexes are maintained in an inactive state due to their binding to the inhibitor protein IκB. External stimuli, such as tumor necrosis factor (TNF-α), interleukin-1 and DNA damage, activate NF-κB by stimulating IκB phosphorylation which is mediated through the IκB kinase (IKK) complex [9, 10]. Upon IκB phosphorylation, the protein is ubiquitinated and targeted for degradation via the 26S proteasome complex. NF-κB is then freed to enter the nucleus where it binds to specific sequences in the promoter regions of target genes [11, 12].

Baldwin et al. noted activation of NF-κB in several cell lines following treatment with SN-38 [13, 14]. Protection from irinotecan-induced apoptosis was effectively abrogated by transient inhibition of NF-κB nuclear localization sequences through adenoviral delivery of the super-repressor IκB. Mice xenografted with a human colon cancer cell line experienced a significant decrease in tumor growth by co-administration of irinotecan with IκB [14].

Thalidomide, a teratogenic sedative recognized for its biological effects including inhibition of TNF-α and of vascularization by basic fibroblastic (βFGF) and vascular endothelial (VEGF) growth factors [15–17], has been evaluated in combination with irinotecan in colorectal cancer patients. Despite encouraging antitumor activity, no severe gastrointestinal toxicity occurred [18]. Of interest, thalidomide has been shown to block NF-κB activation through inhibition of IKK [19].

Inhibition of NF-κB activation has also been demonstrated by nonspecific cyclooxygenase (COX) inhibitors [20, 21]. This action is mediated by specific binding of COX inhibitors to IKK, which results in inhibition of its kinase activity, thus preventing nuclear translocation of NF-κB.

The above studies provide evidence that thalidomide and COX inhibitors may inhibit NF-κB activation, and thereby are potential modulators for irinotecan activity. Herein we report a feasibility and pharmacokinetic study with in vivo laboratory correlates of irinotecan combined with thalidomide, or with both thalidomide and celecoxib in patients with refractory solid malignancies.

Patients and methods

Eligibility

Patients with solid malignancies refractory to conventional therapy or for whom no effective therapy existed were eligible. Eligibility criteria also included: (1) age $\geq$ 18 years; (2) Eastern Oncology Group performance status 0–2; (3) life expectancy $\geq$ 3 months; (4) no major surgery, radiotherapy, or chemotherapy within 28 days of study entry; (5) no prior irinotecan or thalidomide; (6) adequate organ function: hematopoietic (neutrophil count [ANC] of $\geq$ 1,500/μL, platelets $\geq$ 100,000/μL, and hemoglobin [Hgb] $\geq$ 9.0 g/dL); hepatic (total bilirubin level $<$ 1.5 mg/dL, aspartate-amino [AST] and alanine-amino [ALT] transaminases $<$ 3 times upper normal limits [UNL]); and renal (serum creatinine $<$ 1.5 mg/dL or calculated creatinine clearance $\geq$ 60 mL/min); (7) no brain metastases, unless previously irradiated, stable, asymptomatic and required no treatment with corticosteroids; (8) absence of uncontrolled infections or psychiatric disorders that would interfere with consent or follow up; (9) no pre-existing grade $\geq$ 2 peripheral neuropathy and (10) no history of myocardial infarction within the previous 6 months, unstable angina or congestive heart failure requiring therapy. Patients receiving phenytoin, phenobarbital, valproic acid or other antiepileptic prophylaxis, as well as celecoxib and other COX-2 or COX-1 inhibitors, were not eligible and a negative blood test for pregnancy was mandatory for premenopausal women. Both men and women with reproductive potential were required to use an effective contraceptive method and to comply with the FDA-mandated System for Thalidomide Education and Prescribing Safety (S.T.E.P.S.®) program. Patients gave written informed consent according to federal/institutional guidelines before treatment.

Study design, drug administration and treatment schedule

The study was conducted in two parts. In the first part, patients were allocated to one of two groups: (1) Cohort A - irinotecan 125 mg/m^2 in combination with 400 mg per day of thalidomide or (2) Cohort B - irinotecan 125 mg/m^2 in combination with 200 mg per

day of thalidomide. Since 350 mg/m^2 of irinotecan every 3 weeks in combination with thalidomide 400 mg daily had been previously reported as well tolerated in colorectal cancer patients [18], it was felt that this will be a safe starting dose to confirm previous reports and evaluate the pharmacological and biological questions of the study. The lower dose of thalidomide (200 mg) was studied to help assessing dose-related effect on the putative toxicity protection effect by thalidomide. In order to better assess the toxicity profile of the combinations, ten patients completing a full cycle of therapy were required in each cohort.

In the second part, celecoxib 400 mg twice-daily was added in subsequent patients (Cohort C) to the recommended irinotecan/ thalidomide dose from the first part of the study.

Irinotecan was administered intravenously over 90 min on day 1 and 8 of each 3-week cycle. Both thalidomide (daily) and celecoxib (twice-daily) were administered orally starting on day 3 and continued throughout the study. Both oral medications were administered at least 1 h prior to irinotecan.

Dose limiting toxicities and dosage modifications

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0 [22]. Dose limiting toxicity (DLT) was defined as one of the following: (1) an ANC $< 500/\mu$L for > 5 days, or associated with fever; (2) platelets $< 25{,}000/\mu$L; (3) $\geq$ grade 3 nonhematologic toxicity (except diarrhea, nausea/ vomiting and somnolence); (4) grade 4 diarrhea (needing parenteral support, grade 3 on Toxicity criteria version 3.0) despite optimal antidiarrheal medications; and (5) grade 4 vomiting despite $5HT_3$ antiemetics

Planned treatment with irinotecan within a cycle of therapy was held in the presence of grade ≥ 3 nonhematologic (except somnolence) or grade ≥ 3 hematologic toxicity, whereas thalidomide was held for grade 4 hematologic and grade ≥ 3 non-hematologic. Treatment was resumed when hematologic or non-hematologic toxicity resolved to grade 0–2. For grade 2 somnolence, the thalidomide dose was decreased by 25%, and by 50% if there was no improvement within 48 h. Grade 3 somnolence dictated holding thalidomide. Missed doses of irinotecan were not to be made up.

A new cycle of treatment was to begin only when granulocytes were $\geq 1{,}500/\text{mm}^3$ and platelets were $\geq 100{,}000/\text{mm}^3$ and any other treatment-related toxicities were $\leq$ grade 1, otherwise treatment was held for up to 2 weeks. If the toxicity had not resolved to grade 0–1 at the end of this period, the patient was withdrawn from study unless clinical benefit had been documented, in which case a treatment delay of up to one additional week to allow recovery was permitted. Irinotecan was reduced by 25 mg/m^2 from the prior dose based on dose limiting myelosuppression, or any $\geq$ grade 3 nonhematologic toxicity during the previous cycle, whereas thalidomide was not reduced for hematologic toxicity, but reduced (25%) for grade 4 nonhematologic toxicity. No dose modifications for celecoxib were to be performed.

Pretreatment and follow-up assessments

Histories, physicals, and routine laboratory studies were performed pretreatment and weekly. For women of childbearing potential, a pregnancy test in blood or urine was performed within 24 h of starting thalidomide. Contraceptive counseling was performed prior to treatment and two methods of birth control were started at least 4 weeks prior to the first administration of thalidomide. Tumors were measured after every other cycle, and treatment was continued in the absence of progressive disease or intolerable toxicity. A complete response (CR) was defined as the disappearance of all disease on two measurements separated by a minimum of 4 weeks. A partial response (PR) required more than 50% reduction in the sum of the products of the bidimensional measurements of all measurable lesions documented by two measurements separated by at least 4 weeks, and progressive disease required an increase in 25% in the sum of the products of all measurable lesions or the appearance of new lesions.

Pharmacokinetic sampling and assay

Blood was sampled to assess the PK of irinotecan alone (day 1), thalidomide alone (day 12), or the combination (day 22). For irinotecan, heparinized blood samples (5 mL) were drawn via venipuncture in the contralateral arm to the infusion at the following times: before drug, at the end of infusion, then 2, 4, 6, 24 and 48 h after the start of infusion. Blood samples were immediately centrifuged, plasma removed, and frozen at –20°C for subsequent analysis. For thalidomide, heparinized blood samples (6 mL) were collected at baseline, then 1, 2, 4, 6, 24 and 48 h post administration. Blood samples were immediately centrifuged and an equal amount of cold Sorensen's citrate buffer (pH 1.5) added to each plasma sample. The samples were then flash-frozen and stored at –70°C to prevent nonenzymatic degradation of thalidomide.

Samples were obtained for TNF-α, βFGF and NF-κB analyses, at baseline, 24 and 48 h during cycle 1

(days 1–3, 12–14) and cycle 2 (days 22–24). Serum was obtained from 3 mL of non-heparized blood for TNF-α and βFGF, whereas NF-κB was analyzed in peripheral mononuclear cells (PBMCs). Serum and PBMCs samples were stored at –70°C until assayed.

Irinotecan and SN-38 samples were analyzed using a validated and sensitive high-performance liquid chromatography (HPLC) method previously described [23]. Phenacetin was added as an internal standard to the plasma samples containing thalidomide and extracted with diethyl-ether and evaporated to dryness under a gentle stream of nitrogen [24]. The samples were reconstituted with 200 µl of 30% acetonitrite and analyzed by HPLC as previously described [25].

βFGF and TNF-α levels were measured using a quantitative sandwich enzyme immunoassay technique (Quantikine®, R&D Systems Inc., Minneapolis, MN), whereas the relative amount NF-κB_{P65} in PBMC was determined by an ELISA assay (Trans-AM NF-κB_{P65}, Active Motif, Carlsbad, CA). All samples were normalized to the patient's baseline value.

Pharmacokinetic analyses

Irinotecan, SN-38, SN-38G and thalidomide plasma concentrations were analyzed by non-compartment methods. Peak plasma concentrations (Cp_{max}) for irinotecan, SN-38, SN-38G and thalidomide were determined by visual inspection of each individual's plasma concentration-versus-time profile. Elimination rate constants were estimated by linear regression of the last two data points on the terminal log linear portion of the concentration-time curve. The terminal half-life ($t_{½}$) was calculated by dividing 0.693 by the elimination rate constant and the area under the plasma concentration (AUC) by using the linear trapezoidal rule up to the last measurable data point, then extrapolated to infinity. The systemic clearance (CL) for irinotecan was determined by dividing the dose by the AUC. A metabolic ratio, estimated as the ratio of $AUC_{SN\text{-}38}$ or $AUC_{SN\text{-}38} + AUC_{SN\text{-}38G}$ to $AUC_{CPT\text{-}11}$, was used as a measure of the relative extent of the conversion of irinotecan to SN-38. The relative extent of glucuronidation of SN-38 was estimated as the ratio of $AUC_{SN\text{-}38G}$ to $AUC_{SN\text{-}38}$.

Statistical methods

Descriptive statistics and Spearman rank correlations were used to analyze the PK data. A paired *t* test was used to compare PK parameters following dosing on day 1 (irinotecan alone), day 12 (thalidomide alone) and day 22 (combination). The relationships between systemic exposure and toxicity were explored. Dose, C_{max} and $AUC_{0–24}$ for irinotecan, SN-38 and thalidomide were related to categorical grades of toxicities.

To look at the changes in the response variable for the laboratory correlates over time and treatment groups, we used the generalized estimating equations (Stata's® XTGEE) [26], using robust standard errors (or the Huber/White/sandwich estimator of variance) to produce valid standard errors when there is with-in subject correlation. The model includes terms for treatment group, time, clinical benefit, and a treatment by time interaction. Statistical analysis used the statistical software program Stata®, Version 8.2, StataCorp, College Station, TX.

Results

General

Table 1 depicts the number of patients at each of the three cohorts and the number of cycles administered at the starting doses, as well as at modified doses. Thirty-six patients received 100 cycles. Eleven, 13 and 12 patients were required in cohorts A, B and C, respectively to assure adequate toxicity assessment in ten patients per cohort completing at least one full cycle. Patient characteristics are listed in Table 2. Thirty-two patients had received prior chemotherapy, including eight who had received three or more regimens. A pulmonary embolism was the only DLT observed during the first cycle of treatment in Cohort A, although this patient had a previous history of hypercoagulability (previous DVT). One other patient developed a DVT. Despite the lower thalidomide dose in Cohort B, two patients developed febrile neutropenia. In view of the above and a relatively lower rate of grades 2/3 diarrhea for patients in cohort A, this dose-schedule (irinotecan 125 mg/m^2 on days 1 and 8 and thalidomide 400 mg daily) was combined with celecoxib 400 mg twice daily in the second part of the study (Cohort C). One DLT (bowel obstruction) occurred in this last group, in addition to one episode of DVT.

Hematological toxicity

Hematologic toxicity was of mild to moderate severity (Table 3). Grade 4 neutropenia occurred in two patients and both events were associated with fever. Interestingly, these two instances occurred in cohort B. Despite the higher dose of thalidomide in both Cohorts A and C, and the addition of celecoxib in Cohort C, no

Table 1 Dosing scheme

Cohort	Dose level			Number of patients			Total courses
	Irinotecan (mg/m^2)	Thalidomide (mg daily)	Celecoxib (mg daily)	New	Reduced to this dose	Total	
A	**125**	**400**	**NA**	**11**	**0**	**11**	**30**
	100	400	NA	0	1	1	3
	125	300	NA	0	3	3	3
B	**125**	**200**	**NA**	**13**	**2**	**15**	**30**
	100	200	NA	0	2	2	2
	75	200	NA	0	1	1	1
C	**125**	**400**	**800**	**12**	**0**	**12**	**19**
	125	300	800	0	5	5	8
	100	300	800	0	2	2	2
	75	300	800	0	1	1	1
	56	200	800	0	1	1	1
Total				36			100

Bold reflects starting doses

episodes of grade 4 neutropenia occurred in these patients. Anemia and thrombocytopenia were infrequent, with one episode each of grade 4 toxicity (cohort B).

Non-hematologic toxicity

Diarrhea and somnolence were the most common non-hematologic toxicities (Table 3). Overall, 36 cycles in 26 patients were complicated by diarrhea. Eight patients develop grade 3 or 4 toxicity. All episodes of diarrhea were successfully managed with loperamide and dose modifications due to diarrhea were only required in three patients. Of interest, some differences in the rate of diarrhea for Cohort A (6 patients; 18% of cycles; two grade 3, no grade 4) compared to Cohort B (11 patients; 43% of cycles; four grade 3, one grade 4) were observed (P value for number of cycles differences = 0.032, two-sided Fisher's exact test), suggesting a dose-dependent protective effect of thalidomide on irinotecan induced diarrhea. However, nine patients receiving celecoxib in combination with thalidomide 400 mg had diarrheic episodes (52% of cycles, one grade 3).

As expected after treatment with thalidomide, somnolence was common. This effect was observed in 56 cycles, being of higher grade and significance during the first 22 days of treatment during which, as per PK requirement, the thalidomide was given in the morning. Modifications in the dose of thalidomide were required in 15 cycles due to this toxicity. Other toxicities include paresthesias, nausea/vomiting, constipation, skin-rash and fatigue. Three episodes of venous thrombosis, including a pulmonary embolism, occurred in patients receiving 400 mg of thalidomide (cohorts A and C). No cardiac related events occurred.

Antitumor activity

Two patients, one each in cohorts A and B, demonstrated more than 50% decrease in their tumors. A 57-year-old with NSCLC with progressive disease after two taxane-containing regimens had a 53% reduction in bilateral lung masses after receiving two cycles of irinotecan/thalidomide. Unfortunately, a computer

Table 2 Patient characteristics

Characteristic	Number of patients
Patients treated	36
Male	21
Female	15
Median age (range), years	59 (35–78)
ECOG performance status	
0	8
1	17
2	11
Previous radiotherapy	20
Previous chemotherapy	
None	4
1 line	15
2 lines	9
≥ 3 lines	8
Disease site:	
Lung (non-small cell)	10
Colorectal	5
Thyroid	4
Pancreas	3
Malignant Carcinoid	3
Gastric	2
Esophagus	2
Cholangiocarcinoma	2
Unknown primary, anal, renal, head and neck, duodenal	1 each

Table 3 Toxicities of irinotecan in combination with thalidomide and celecoxib

Toxicity[a]	Irinotecan/Thalidomide 400 (A)				Irinotecan/Thalidomide 200 (B)				Irinotecan/Thalidomide/ Celecoxib (C)			
	1	2	3	4	1	2	3	4	1	2	3	4
Hematologic												
Neutropenia	NA	3 (27)	3 (27)	0	NA	2 (15)	3 (23)	2 (15)	NA	2 (17)	3 (25)	0
Anemia	NA	4 (36)	1 (9)	0	NA	7 (54)	1 (8)	1 (8)	NA	6 (50)	0	0
Thrombocytopenia	1 (9)	0	0	0	0	0	1 (8)	1 (8)	4 (33)	0	0	0
Non-Hematologic												
Nausea/vomiting	3 (27)	1 (9)	3 (27)	0	5 (38)	3 (23)	0	0	1 (8)	3 (25)	3 (25)	0
Diarrhea	3 (27)	1 (9)	2 (18)	0	3 (23)	3 (23)	4 (31)	1 (8)	3 (25)	5 (42)	1 (8)	0
Constipation	2 (18)	2 (18)	2 (18)	0	2 (15)	3 (23)	0	0	1 (8)	4 (33)	0	0
Somnolence	2 (18)	4 (36)	3 (27)	0	3 (23)	3 (23)	1 (8)	0	3 (25)	6 (50)	1 (8)	0
Fatigue/asthenia	5 (45)	2 (18)	2 (18)	0	5 (38)	1 (8)	1 (8)	0	2 (17)	2 (17)	3 (25)	0
Paresthesias	1 (9)	1 (9)	2 (18)	0	3 (23)	1 (8)	0	0	5 (42)	1 (8)	0	0
Skin rash	2 (18)	1 (9)	0	0	2 (15)	0	0	0	1 (8)	2 (17)	0	0
Venous thrombosis	NA	0	1 (9)	1 (9)	NA	0	0	0	NA	0	1 (9)	0

Numbers are numbers of patients experiencing toxicities; percentages are in parentheses. 100 cycles were administered: Cohort A, 11 pts (39 cycles); Cohort B, 13 pts (30 cycles); Cohort C, 12 pts (31 cycles)

[a]Toxicities are reported as per NCI common toxicity criteria 2; grade 1 anemia/neutropenia not reported (allowed at study entry and retreatments)

tomographic scan obtained due to persistent lightheadedness disclosed brain metastases. Following brain irradiation, further reduction in the lung masses followed four more cycles of the same regimen off-protocol. The second individual was a 78-year-old with cholangiocarcinoma metastatic to the liver who had disease progression after treatment with gemcitabine/capecitabine. Prolonged disease stabilizations (range 4–9 months) occurred in two patients with NSCLC (including a patient with five prior chemotherapy regimens); two patients with metastatic thyroid carcinoma; a patient with malignant carcinoid; as well as patients (one each) with medullary thyroid cancer; esophageal cancer and metastatic anal carcinoma.

Pharmacokinetic analyses

Evaluable pharmacokinetic data were available from 30 patients. Seventeen of the 24 patients receiving irinotecan/thalidomide had evaluable paired samples after irinotecan alone and again while receiving steady daily thalidomide 200 mg (N = 8) or 400 mg (N = 9) in Cycle 2 (day 22). Thalidomide PK assessment permitted paired comparisons in 16 patients. Seven of the 12 patients receiving the triple drug combination had irinotecan PKs assessable both when given alone and while receiving steady daily thalidomide and celecoxib.

Mean ± SD PK parameters for irinotecan and thalidomide are shown in Tables 4 and 5. Irinotecan and thalidomide PK parameters were consistent with previously reported values [25, 27, 28]. Mean differences in irinotecan and thalidomide PKs when given alone or in combination were small. The most relevant parameters, SN-38, SN-38G and irinotecan AUCs, as well as the various AUC ratios showed no significant differences, albeit some reductions were observed in irinotecan and SN-38 C_{max}, when given in combination with thalidomide. No clinically relevant PK interaction occurred when irinotecan is administered concurrently with thalidomide plus celecoxib (Table 6), and no clear differences in systemic exposure were observed among patients experiencing the most severe toxicities (Fig. 1).

Correlative studies

Table 7 lists PBMC mean NF-κB_{p65} ratios (normalized to baseline), as well as for βFGF expression. TNF-α was in all cases below limits of detection. Figure 2 illustrates the variation for the means of NF-κB_{p65} values over time across the different cohorts.

To evaluate a possible effect of irinotecan on NF-κB_{p65} or βFGF, XTGEE models were fit with all the data generated over time. For the three treatment groups NF-κB_{p65} expression increased 74.9% over the first 14 days; and 61.2% over all 24 days ($P = 0.008$), whereas no significant variation was detected for βFGF. However, differences of NF-κB activation over time among treatment cohorts was not significant. Patients experiencing clinical benefit, defined as antitumor response or prolonged (≥ 4 months) disease stabilization, had lower (36%) NF-κB_{p65} expression (Fig. 3) compared to the rest of patients. However, this was not statistically significant ($P = 0.124$). The same analysis for βFGF expression showed that neither type of treatment or clinical benefit predicted for differences.

Table 4 Pharmacokinetics of irinotecan and metabolites when administered alone and in combination with thalidomide

PK parameter	All patients with PK assessment on day 1[a] (N = 23)	PK assessments performed in the same patients on both day 1 and day 22 (N = 17)			
		Irinotecan alone[a]	Irinotecan thalidomide[b]	Mean % change[c]	Paired t test P value
Irinotecan					
C_{max} (ng/mL)[d]	1,409 ± 526	1,291 ± 286	1,090 ± 283	–11.8	0.05
CL (L/h/m^2)	12.8 ± 4.3	14.4 ± 3.7	15.6 ± 5.1	10.0	0.25
$T_{½}$ (h)	11.1 ± 1.4	10.7 ± 1.2	11.3 ± 2.5	6.9	0.39
SN-38					
C_{max} (ng/mL)[d]	18.7 ± 7.2	16.9 ± 5.6	14.7 ± 4.6	–11.5	0.02
$AUC_{0-\infty}$ (ng h/mL)[d]	333 ± 208	265 ± 99.7	246 ± 107	–3.3	0.38
$t_{½}$ (h)	18.7 ± 5.9	17.0 ± 4.4	18.7 ± 8.0	17.4	0.47
SN-38 glucuronide					
C_{max} (ng/mL)[d]	68.4 ± 41.0	64.9 ± 40.1	61.3 ± 28.2	4.0	0.46
$AUC_{0-\infty}$ (ng h/mL)[§]	1,720 ± 1,821	1,277 ± 1,016	1,165 ± 636	6.4	0.38
$t_{½}$ (h)	16.0 ± 4.4	15.2 ± 3.6	16.4 ± 4.2	11.4	0.28
$AUC_{0-\infty}$ ratio					
SN-38/CPT-11	0.034 ± 0.011	0.033 ± 0.012	0.032 ± 0.011	2.1	0.68
SN-38 + SN-38G/CPT-11	0.194 ± 0.109	0.182 ± 0.100	0.185 ± 0.083	8.7	0.84
SN-38G/SN-38	4.86 ± 3.21	4.78 ± 3.53	5.15 ± 3.81	12.6	0.24

C_{max} Peak plasma concentration, *CL* clearance, $t_{½}$ half-life, $AUC_{0-\infty}$ area under the plasma concentration time curve from time zero to infinity, *SN-38G* SN-38 glucuronide

[a]Irinotecan given alone on day 1

[b]Irinotecan + thalidomide given concurrently on day 22

[c]Mean of individual patient change in pharmacokinetics parameter (combination vs. given alone) expressed as a percent

[d]Normalized to an irinotecan dose of 125 mg/m^2 since the dose of CPT-11 may have been reduced on day 22

Spearman rank correlation showed no significant association between irinotecan or thalidomide PK and NF-κB_{p65} or βFGF expression.

Discussion

The broad spectrum of antitumor activity of irinotecan, as well as its complex metabolism and mechanism of action, render irinotecan one of the most interesting anticancer agents in clinical use. Preclinical studies show that activation of NF-κB is one of the most compelling mechanisms of tumor resistance to this agent.

Based on the demonstration in preclinical studies of inhibition of NF-κB activation by thalidomide and COX inhibitors, and the reported amelioration of irinotecan-mediated toxicity by these agents [18, 29], we performed this feasibility and pharmacological study of weekly irinotecan in combination with thalidomide with or without celecoxib. The results of this study

Table 5 Pharmacokinetics of thalidomide when administered alone and in combination with irinotecan

PK parameter	All patients with PK assessment on day 12[a] (N = 20)	Patients with PK assessments performed in the same patients on both day 12 and day 22 (N = 9 at 400 mg and N = 7 at 200 mg)			
		Thalidomide alone[a]	Thalidomide irinotecan[b]	Mean % change[c]	Paired t test P Value
C_{max} (ng/mL)					
200 mg	1,769 ± 756	1,968 ± 735	2,042 ± 689	9.1	0.73
400 mg	3,700 ± 1,384	3,700 ± 1,384	4,653 ± 2,038	27	0.03
AUC_{0-24} (mcg h/mL)					
200 mg	22.8 ± 8.4	25.2 ± 8.6	24.4 ± 6.6	–0.2	0.72
400 mg	50.2 ± 19.8	50.2 ± 19.8	52.5 ± 24.5	4.7	0.58

C_{max} Peak plasma concentration, AUC_{0-24} area under the plasma concentration time curve from time 0 to 24 h

[a]Thalidomide given alone on day 12

[b]Thalidomide + Irinotecan given concurrently on day 22

[c]Mean of individual patient change in pharmacokinetics parameter (combination vs. given alone) expressed as a percent

Table 6 Pharmacokinetics of irinotecan when given alone or in combination with thalidomide and celecoxib

PK parameter	All patients with PK assessment during course 1[a] (N = 11)	PK assessments performed in the same patients when (N = 7)			
		Course 1[a]	Course 2[b]	Mean % change[c]	Paired t test P value
Irinotecan					
C_{max} (ng/mL)[d]	1,511 ± 687	1,601 ± 848	1,185 ± 175	–14.2	0.25
CL (L/h/m^2)	12.3 ± 3.3	12.1 ± 2.1	13.6 ± 3.2	13.5	0.27
$t_{½}$ (h)	10.9 ± 2.0	11.5 ± 2.2	11.5 ± 2.8	1.3	0.99
SN-38					
C_{max} (ng/mL)[d]	23.7 ± 12.6	20.1 ± 8.1	18.7 ± 5.0	9.0	0.71
$AUC_{0-\infty}$ (ng h/mL)[§]	353 ± 225	307 ± 109	337 ± 106	14.1	0.46
$t_{½}$ (h)	22.0 ± 13.8	24.0 ± 17.4	29.9 ± 25.8	50.1	0.63
SN-38 glucuronide					
C_{max} (ng/mL)[d]	66.3 ± 37.9	64.9 ± 41.5	59.8 ± 38.0	–5.6	0.30
$AUC_{0-\infty}$ (ng h/mL)[§]	1,060 ± 540	1,015 ± 536	1,281 ± 880	19.8	0.20
$t_{½}$ (h)	16.7 ± 3.5	15.9 ± 1.8	23.0 ± 13.7	39.4	0.26
$AUC_{0-\infty}$ ratio					
SN-38/CPT-11	0.037 ± 0.020	0.034 ± 0.016	0.043 ± 0.006	40.9	0.16
SN-38 ± SN-38G/CPT-11	0.152 ± 0.035	0.147 ± 0.044	0.193 ± 0.082	30.0	0.07
SN-38G/SN-38	4.86 ± 3.21	3.80 ± 2.03	3.49 ± 1.64	–2.7	0.22

C_{max} Peak plasma concentration, *CL* clearance, $t_{½}$ half-life, $AUC_{0-\infty}$ area under the plasma concentration time curve from time zero to infinity, *SN-38G* SN-38 glucuronide

[a]Irinotecan given alone on day 1

[b]Irinotecan + thalidomide + celecoxib given concurrently on day 22

[c]Mean of individual patient change in pharmacokinetic parameter (Course 2 vs. Course 1) expressed as a percent

[d]Normalized to an irinotecan dose of 125 mg/m^2

showed that the combinations are feasible, with acceptable myelosuppression and gastro-intestinal toxicity, as well as manageable somnolence and neuropathy. In addition, antitumor activity was also observed in this treatment-refractory patient population. Recommended doses for the irinotecan/thalidomide doublet and for use in combination with celecoxib are 125 mg/m^2 on days 1 and 8 every 3 weeks for irinotecan and 400 mg daily for oral thalidomide. Although intra-patient escalation in the dose of thalidomide in subsequent cycles may be possible, as it is frequently done in the therapy of multiple myeloma [30], this concept was not tested in this study.

The reported potential protective effect on irinotecan-induced toxicity for thalidomide 400 mg is not yet understood, stressing the importance of pharmacological studies demonstrating that thalidomide does not alter the pharmacokinetics of irinotecan or its active metabolite. This study showed that thalidomide did not interfere with irinotecan or SN-38 AUC either at 200 or 400 mg daily. Similarly, the addition of celecoxib produced no appreciable change on the PK of either irinotecan or thalidomide. Given the influence of the CYP450 system on irinotecan's metabolism [31, 32] we specifically avoided CYP450 enzyme inducing or inhibiting agents, which could produce spurious results on the PK interaction assessment.

Review of the literature shows one other study assessing the impact of thalidomide on the pharmacokinetics of irinotecan [33]. In contrast to our study, alterations by thalidomide in the clearance of irinotecan were reported in a small number of patients (n = 7). Unfortunately, the results of the referenced study are only in abstract form, and no discussion was provided regarding CYP450 enzyme inducing or inhibiting agents use; a precaution underscored by the high number of patients (n = 5) with central nervous system tumors treated.

In agreement with previous reports, the rate of moderate to severe gastrointestinal toxicity and notably of myelosuppression in the small number of patients treated in this trial appeared lower than expected for irinotecan as a single agent at the dose-schedule tested [34], and it was lower for thalidomide 400 mg compared to 200 mg, suggesting dose-dependency for this toxicity-protective effect. Fujita et al. [35] showed that thalidomide, could inhibit the lipopolysaccharide-mediated induction of COX-2 in murine macrophages. The same mechanism could be involved in the observation that thalidomide may reduce the severity of irinotecan-induced diarrhea in human patients. Govindarajan et al. [18] reported their experience with 17 patients with metastatic colorectal cancer who had progressed after receiving 5-fluorouracil (5-FU) who

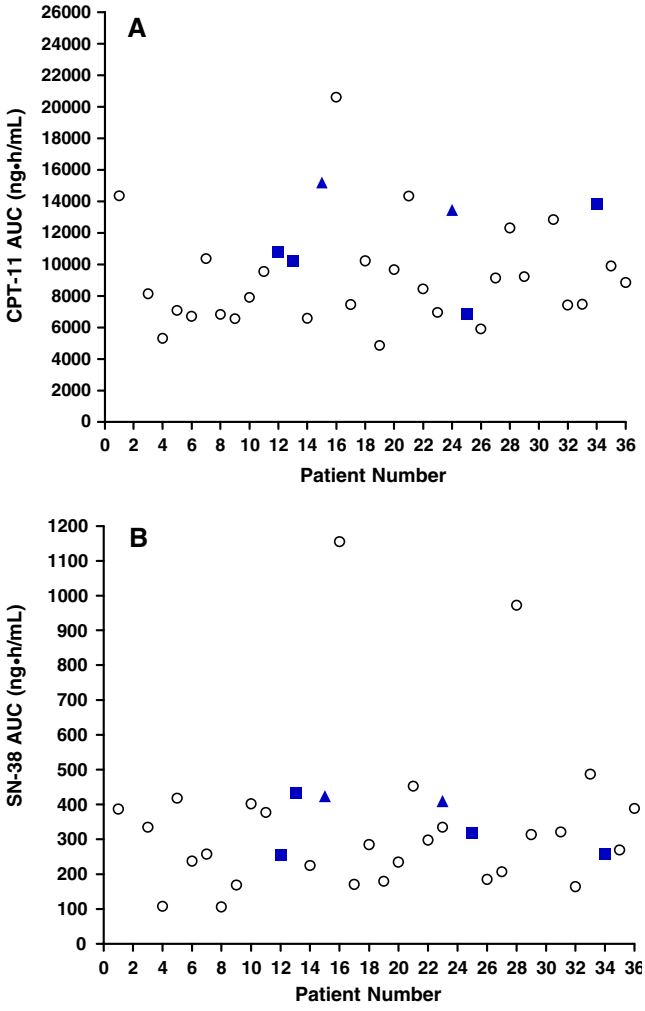

Fig. 1 Comparison of irinotecan (**a**) and SN-38 (**b**) AUC values in patients experiencing grade 3 diarrhea (*filled square*) or grade 3 diarrhea and febrile neutropenia (*filled triangle*) versus patients not experiencing these toxicities

were treated with irinotecan (300–350 mg/m^2) every 21 days and thalidomide 400 mg/day. There was a remarkable absence of gastrointestinal (GI) toxicities in this group (nausea $P < 0.001$, vomiting $P < 0.001$, and diarrhea $P < 0.001$). Thus, the remarkable decrease in the GI toxicities observed with a 400 mg dose in their study was consistent with the results we observed at the higher thalidomide dose level in the present study.

However, the addition of celecoxib, another agent that putatively results in amelioration of irinotecan-mediated toxicity [29], did not result in further protective effect, showing a similar rate of gastrointestinal toxicities as for the group receiving the lower dose of thalidomide.

Although recent labeling changes for irinotecan points to a 10% prevalence of UGT1A1 enzyme polymorphism and an increase in the risk of neutropenia for patients with decreased UGT1A1 activity due to reduced glucuronidation of SN-38, it is unlikely that the differences observed among the groups treated in this study are due to this factor, since levels of SN-38 were similar in patients experiencing DLT (Fig. 1b), and the pharmacokinetic comparisons were performed in the same patients and not between patients.

In the search for mechanisms at the molecular level that could validate the rationale of combining these agents, we evaluated in PBMC the expression of TNF-α and βFGF as well as activation of NF-κB. TNF-α levels were below limits of detection and no changes in βFGF from baseline were observed throughout treatment. PBMC NF-κB activation increased with time, however, suggesting either an irinotecan-exposure related event or events associated with tumor progression (cytokines produced by tumor, including TNF have been shown to result in increased NF-κB activation, [36]). Patients experiencing clinical benefit had lower increases in NF-κB activation, although this difference did not reach statistical significance in this small group of patients.

Limitations of this study include the absence of an irinotecan single-agent control group that would

Table 7 NF-κB activation and bFGF expression in blood mononuclear cells

	Days								
	1[a]	2	3	12	13	14	22	23	24
NF-κB_{p65}									
Iri/Thal 400	1	1.87 ± 0.50	1.9 ± 0.53	1.68 ± 0.38	1.49 ± 0.51	0.98 ± 0.22	2.65 ± 0.82	1.37 ± 0.27	1.86 ± 0.48
Iri/Thal 200	1	1.06 ± 0.18	1.23 ± 0.19	1.83 ± 0.31	1.48 ± 0.23	1.62 ± 0.25	1.57 ± 0.32	1.48 ± 0.33	1.42 ± 0.34
Iri/Thal/Cel	1	0.88 ± 0.16	0.9 ± 0.15	1.78 ± 0.52	1.32 ± 0.38	0.91 ± 0.18	1.21 ± 0.59	0.98 ± 0.30	1.47 ± 0.52
BFGF									
Iri/Thal 400	1	1 ± 0.10	0.96 ± 0.07	1.15 ± 0.22	0.96 ± 0.11	0.93 ± 0.10	1.02 ± 0.07	0.96 ± 0.10	0.9 ± 0.08
Iri/Thal 200	1	0.98 ± 0.07	0.95 ± 0.06	1.02 ± 0.08	0.89 ± 0.09	0.96 ± 0.10	1.07 ± 0.21	1.08 ± 0.12	0.99 ± 0.10
Iri/Thal/Cel	1	0.85 ± 0.07	0.95 ± 0.07	0.99 ± 0.09	0.93 ± 0.08	0.85 ± 0.07	0.95 ± 0.10	1.04 ± 0.13	1.04 ± 0.12

Values are means ± SD

Iri irinotecan, *Thal* thalidomide, *Cel* celecoxib

[a]Values were normalized to each patient's baseline value on day 1

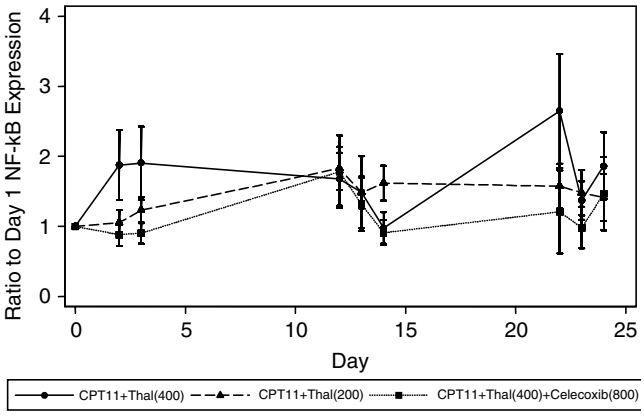

Fig. 2 Variation for the means of NF-κB_{p65} ratios over time across the different treatment cohorts. Error bars are ± 1 standard error. Ratios are to day 1 NF-κB_{p65} in each patient

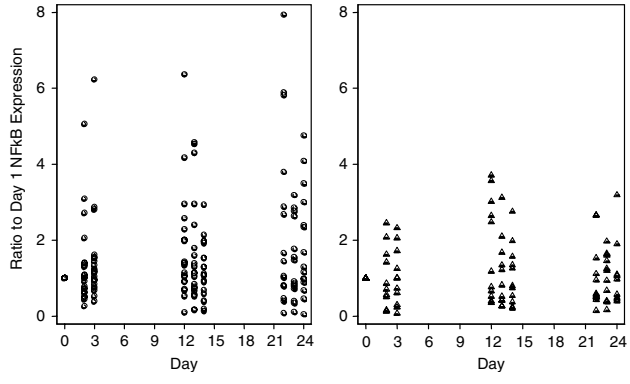

Fig. 3 Scatterplots depicting NF-κB activation ratios to day 1. **a** Patients experiencing no clinical benefit; **b** patients experiencing clinical benefit. Clinical benefit is antitumor response or prolonged (≥ 4 months) disease stabilization

permit evaluation of a potential effect of thalidomide or thalidomide/celecoxib on the increase over time of NF-κB activation and the absence of pre and post-treatment tumor samples that can establish PBMC as an adequate surrogate of intratumoral events. Similarly, the antitumor activity observed could have been due to the effect of irinotecan by itself.

In conclusion, the combinations of thalidomide/irinotecan and thalidomide/celecoxib/ irinotecan are safe and devoid of PK interactions. The protective effect of celecoxib in irinotecan-induced gastrointestinal toxicity previously reported was not confirmed. Although the potential effect of thalidomide and/or celecoxib on irinotecan-induced NF-κB activation remains speculative, the results of this study supports tumor-specific efficacy and mechanistic studies.

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