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# Results from an *in vitro* and a clinical/pharmacological phase I study with the combination irinotecan and sorafenib

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ABSTRACT

*Purpose:* This single-centre, open-label, phase I dose-escalation study was performed to investigate the safety, pharmacokinetics (PK) and efficacy of sorafenib, a multi-kinase inhibitor, combined with irinotecan, a cytotoxic agent, in patients with advanced, refractory solid tumours.

*Patients and methods:* In an initial dose-escalation phase, patients received irinotecan 125 mg/m$^2$ and sorafenib 100, 200 and 400 mg twice daily (bid) (cohorts 1–3). In an extended phase, colorectal cancer (CRC) patients received fixed-dose irinotecan 140 mg and sorafenib 400 mg bid (cohort 4).

*Results:* Thirty-four patients were treated: 20 in the dose-escalation phase (common tumour types: CRC [45%], ovarian [5%], pancreatic [5%]) and 14 patients in the CRC extension. Frequent drug-related adverse events were gastrointestinal symptoms, dermatological reactions and constitutional symptoms. The maximum tolerated dose was not reached. Generally, concomitant administration of irinotecan had no impact on the PK of sorafenib. Sorafenib 100 or 200 mg bid had no impact on the PK of irinotecan or its metabolite SN38. In contrast, sorafenib 400 mg bid significantly increased irinotecan and SN38 exposures; however, this was not associated with increased toxicities. Stable disease was achieved in 12/20 (60%) evaluable patients in cohorts 1–3, and 10/13 (77%) evaluable patients in cohort 4. A further patient from cohort 4 had a partial response of >200 days. The increase of SN38 exposure might be due to inhibition of formation of the SN38 glucuronide by sorafenib. *In vitro*, sorafenib strongly inhibited SN38 glucuronidation in human liver microsomes as indicated by a $K_i$ value of 2.7 μmol/l.

*Conclusion:* Sorafenib 400 mg bid can be combined with irinotecan 125 mg/m$^2$ or 140 mg for the treatment of patients with advanced, refractory solid tumours, although monitoring for toxicity is recommended.



## 1. Introduction

Sorafenib is a novel, oral multi-kinase inhibitor that targets the Raf/MEK/ERK signalling pathway and receptor tyrosine kinases (RTKs) involved in tumour cell proliferation and angiogenesis.[1] *In vitro*, sorafenib inhibited B-Raf and Raf-1 (C-Raf or C-Raf-1), involved in tumour cell growth, and pro-angiogenic vascular endothelial growth factor receptor (VEGFR)

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and platelet-derived growth factor receptor (PDGFR)[2] Sorafenib prevented tumour cell proliferation *in vitro* and tumour growth in human xenograft models.[1]

Several tumour types have demonstrated mutations and overexpression of factors inhibited by sorafenib. The B-Raf V600E oncogene is present in 63% of melanomas,[3] ⩽50% of papillary thyroid carcinomas,[4] and 40% of sporadic colorectal cancers (CRCs).[5] Although oncogenic Raf-1 mutations have not been detected in human cancers,[6] activating K-Ras mutations resulting in increased signalling through Raf-1 were observed in 45% of CRC cases.[7]

Single-agent sorafenib showed preliminary efficacy in several solid tumour types.[8–11] In metastatic renal cell carcinoma (RCC) patients, sorafenib significantly prolonged progression-free survival versus placebo.[12,13] Sorafenib was also shown to be suitable for long-term administration because of its good safety profile.[8,11–14]

A rationale exists for combining anticancer agents, which have different mechanisms of action, with cytotoxics to encourage additive anti-tumour effects. Cytotoxic irinotecan is widely used alone or in combination with other cytotoxics for the treatment of metastatic CRC.[15] In human cancer cell lines, sorafenib plus irinotecan demonstrated synergistic activity.[16] Studies in colon xenograft models showed that combining sorafenib with irinotecan prolonged tumour growth delay versus either agent alone, without enhanced toxicity.[17]

As both sorafenib and irinotecan are at least partially metabolised through the CYP3A4 pathway,[18] the possibility of interaction requires clinical investigation. Sorafenib undergoes glucuronidation by the UGT1A9 pathway and phase I oxidative metabolism mediated by CYP3A4 .[19,20] Irinotecan is metabolised by various enzymes, and its active metabolite, SN38, is formed by the action of carboxylesterase. SN38 is converted to the inactive SN38 glucuronide by UGT1A1[21] and possibly UGT1A9.[22–24] The inhibitory potency of sorafenib towards SN38 glucuronidation was investigated *in vitro* using human liver microsomes fortified with UDPGA.

This study was undertaken to define the safety and pharmacokinetics of sorafenib in combination with irinotecan, and to assess the anti-tumour activity of this combination in patients with advanced, refractory solid tumours. Based on preclinical efficacy data of sorafenib in models of CRC, and proven clinical activity of irinotecan in this tumour type, an extension part was also conducted in patients with metastatic colon cancer, for whom irinotecan monotherapy would be a reasonable treatment option.

## 2. Patients and methods

### 2.1. Patient selection

Two different patient populations were recruited. Cohorts 1–3 included patients with advanced, refractory solid tumours. Cohort 4 comprised patients with advanced, histologically confirmed colon cancer, for whom irinotecan was considered a reasonable treatment option. All patients were ⩾18 years, had an Eastern Cooperative Oncology Group performance status of ⩽2 and life expectancy of >12 weeks. Eligible patients had adequate bone marrow function (haemoglobin >90 g/l, absolute neutrophil count [ANC] $\geqslant 1.5 \times 10^9$/l, platelet count $\geqslant 100 \times 10^9$/l), adequate liver function (total bilirubin ⩽1.5× upper limit of normal [ULN], alanine amino transferase (ALT) and aspartate amino transferase (AST) ⩽ 2.5× ULN) and adequate renal function (serum creatinine ⩽ 1.5× ULN).

Patients who were pregnant or breast feeding or had active clinically serious infections, a history of HIV infection or chronic hepatitis B or C, or active brain or meningeal metastasis were excluded. Treatment with chemotherapy or immunotherapy <4 weeks before study entry, or significant surgery or radiotherapy within 3 weeks of the start of the study drug, was not permitted. Hepatocellular carcinoma patients with clinically significant liver function abnormalities were excluded.

Written, informed consent was obtained from all patients who participated in the study, which was conducted according to the Good Clinical Practice guidelines and the principles described in the Declaration of Helsinki.

### 2.2. Study design

This was a single-centre, open-label, non-placebo-controlled, non-randomised, phase I dose-escalation study. Patients in all cohorts received 6-week cycles of sorafenib treatment combined with weekly intravenous (i.v.) irinotecan for 4 weeks followed by 2 weeks without irinotecan.

A reduced starting dose of continuous sorafenib (100 mg bid) was chosen to provide a safety margin. A continuous schedule was then used for sorafenib administration from day 4 of the first cycle. The dose levels of sorafenib were as follows: 100 mg bid in cohort 1 (2 × 50 mg tablets), 200 mg bid in cohort 2 (4 × 50 mg tablets), 400 mg bid in cohort 3 (8 × 50 mg tablets), and 400 mg bid in cohort 4 (2 × 200 mg tablets).

Irinotecan was administered on days 1, 8, 15 and 22 of each cycle; patients in cohorts 1–3 received 125 mg/m$^2$ irinotecan weekly, and patients in cohort 4 received a fixed dose of 140 mg irinotecan weekly. Treatment continued until tumour progression or unacceptable toxicity.

A dose-limiting toxicity (DLT) was defined as any of the following: grade 4 neutropenia and thrombocytopenia (absolute granulocyte count [AGC] $<0.5 \times 10^9$/l for at least 7 days); febrile neutropenia ⩾ grade 3 (AGC $<1.0 \times 10^9$/l and fever >38.5 °C); platelet count <25,000/μl or thrombocytic bleeding; ALT/AST ⩾ grade 3 for >7 days; ⩾ grade 3 non-haematological toxicity (excluding alopecia, and unpremedicated nausea and vomiting); and inability to administer the day 1, cycle 2 dose of both study drugs within 14 days of the planned end of the previous cycle, as a result of an adverse event with possible relationship to study medications.

Because of the toxicities associated with cytotoxics, any clinically significant event that worsened with the addition of sorafenib, or was not generally expected from irinotecan administration and warranted a sorafenib dose interruption, was considered to be a DLT. If a DLT occurred, both sorafenib and irinotecan were withheld until the toxicity resolved to ⩽ grade 2, at which point the investigator could rechallenge the patient with reduced doses of both drugs. For non-haematological and haematological toxicities, the irinotecan dose was reduced by 20–75%. For skin toxicities, the sorafenib dose

was reduced to 200 or 100 mg bid. If the toxicity did not resolve to ⩽ grade 2 within 14 days after treatment interruption, the patient was withdrawn from the study and replaced.

### 2.3. Study outcomes

The primary objective was to define the safety of sorafenib plus irinotecan. Secondary objectives were to evaluate the pharmacokinetics of sorafenib plus irinotecan, and determine the objective tumour response rate.

#### 2.3.1. Safety

All patients who received ⩾ one dose of sorafenib and irinotecan were evaluable for safety. Adverse events were graded according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) v2.0.

A thorough safety evaluation of this combination was performed, with physical examinations (day 1 of each cycle, plus end of study), and analyses of haematology data (days 1, 8, 15, 22, 29, 36 and 43 of cycles 1 and 2, and days 1, 8, 15, 22, 29 and 36 of subsequent cycles, plus end of study) and biochemistry data (days 1, 8, 15 and 22 of each cycle, plus end of study).

#### 2.3.2. Pharmacokinetics

Pharmacokinetics of irinotecan and its active metabolite SN38 were determined on days 1–3 of cycles 1 and 2. Blood samples (8 ml) were collected before the start of the infusion and at 0.5, 1, 1.5 (end of infusion), 1.75, 2, 2.5, 3, 4, 6, 8, 12, 24, 36 and 48 h thereafter. The blood samples were immediately placed in an ice bath and centrifuged within 30 min at 4 °C and approximately 1600 g for 10 min. The plasma was separated, frozen immediately and stored below −70 °C until analysis.

The pharmacokinetics of sorafenib were determined on day 42 of cycle 1, and on day 1 of cycle 2. Blood samples (5 ml) were collected prior to dosing and 0.5, 1, 2, 4, 8, 10 and 12 h thereafter. The blood samples were placed in a refrigerator (4 °C) and centrifuged within 30 min at 4 °C and approximately 1600 g for 10 min. The plasma was separated, frozen immediately and stored below −15 °C until analysis.

Sorafenib plasma concentrations were assessed using a fully validated, specific LC/MS-MS assay with a lower limit of quantification (LLOQ) of 0.1 mg/l. Mean inter-assay precision and accuracy were 3.5–7.1% and 95.9–97.2%, respectively.

Plasma irinotecan and SN38 were quantified using a fully validated HPLC assay with fluorescence detection. The LLOQs for irinotecan and SN38 were 9.99 μg/l and 0.5 μg/l, respectively. Mean inter-assay precision was 1.7–9.6% for irinotecan, and 2.2–8.4% for SN38; mean inter-assay accuracy was 89.7–103.0% for irinotecan, and 91.0–111.0% for SN38.

The pharmacokinetic parameters were calculated using the KINCALC (Bayer) programme applying non-compartmental evaluation. Area under the concentration curve (AUC) was calculated according to the linear/logarithmic trapezoidal procedure, and $C_{max}$ and $t_{max}$ were read directly from the data.

Although $AUC_{0-12}$ was the original target parameter for sorafenib, several samples at 12 h had a negative time deviation, thereby necessitating an extrapolation. As this extrapolation was not always possible due to fluctuating plasma concentrations, $AUC_{0-10}$ was calculated instead. Furthermore, the original target parameter for SN38, $AUC_{0-\infty}$, could not be calculated reliably, as the extrapolated part of the AUC exceeded 20% in several cases. Therefore, $AUC_{0-48}$ was calculated instead.

#### 2.3.3. Efficacy

All patients who completed ⩾ one cycle of treatment were evaluable for response, according to World Heath Organisation (WHO) criteria. Patients who showed tumour progression were replaced. Tumour response was measured at baseline, after cycle 1, and every 3 months thereafter (up to 2 years) until progressive disease or death.

#### 2.3.4. In vitro assay for SN38 glucuronidation

Pooled human liver microsomes were purchased from BD Gentest (Woburn, USA) and SN38 was obtained from Abatra Technology (Xi'an, China). 17α-Ethinylestradiol, saccharolactone, camptothecin, alamethicin and UDPGA were supplied by Sigma-Aldrich (Steinheim, FRG).

The incubation mixture contained SN38 (5 μmol/l), $MgCl_2$ (5 mmol/l), 0.5 mg/ml microsomal protein, alamethicin (50 μg/mg protein) and 100 mmol/l phosphate buffer and was placed on ice for 15 min. Saccharolactone (5 mmol/l) and sorafenib (1–10 μmol/l) or 17α-ethinylestradiol (10–100 μmol/l), as a known UGT1A1 inhibitor, were added. After preincubation at 37 °C for 5 min, the reaction was started with the addition of UDPGA (5 mmol/l). Reaction mixtures were incubated for 30 min and stopped by addition of acetonitrile/phosphoric acid (20%) 5:1 containing camptothecin as internal standard. Precipitated proteins were removed by centrifugation (8000 rpm, 3 min) and the supernatant was analysed by HPLC with a Prodigy ODS column (3 μm, 150 × 3.0 mm i.d., Phenomenex, Aschaffenburg, FRG). The mobile phase A consisted of 3 mmol/l heptanesulfonic acid in 50 mmol/l potassium phosphate buffer (pH 4.0) and mobile phase B was acetonitrile. Elution was conducted at a flow rate of 0.5 ml/min using the following gradient: 0 min, 10% B; from 0 to 10 min, 35% B; from 10 to 15 min, 40% B; from 15 to 20 min, 70% B; and from 20 to 23 min, 10% B. Fluorescence detection was performed using an excitation of 355 nm and emission at 515 nm. The retention times of SN38 glucuronide, SN38 and camptothecin were 6.7, 12.4 and 13.1 minutes, respectively.

### 2.4. Statistical analysis

No formal sample-size estimation was performed, as this was primarily a descriptive phase I trial of safety and tolerability.

## 3. Results

### 3.1. Patients' characteristics

Thirty-four patients with advanced, refractory solid tumours were enrolled (cohort 1, $n = 6$; cohort 2, $n = 7$; cohort 3, $n = 7$). Fourteen patients with advanced, histologically confirmed CRC were enrolled in the extension part (cohort 4) (Table 1).

Patients in cohorts 1–3 received sorafenib for a median of 11.4 weeks (range 1.7–40.3). The median duration of treatment with sorafenib in cohort 4 was 21.9 weeks (range 6.1–41.6). Eight patients (57%) in cohort 4 received sorafenib for >20 weeks, compared with just four (20%) in cohorts 1–3. Six

**Table 1 – Patients' baseline characteristics (population valid for safety analysis) (n = 34)**

| Characteristic | Cohorts 1–3 ($n$ = 20) | Cohort 4 ($n$ = 14) |
|---|---|---|
| Gender, male:female [$n$(%)] | 10 (50):10 (50) | 11 (79):3 (21) |
| Median age, years (range) | 61 (36–76) | 61 (44–76) |
| Median BMI, kg/m$^2$ (range) | 25 (18–32) | 25 (19–32) |
| Median body surface area, m$^2$ (range) | 1.9 (1.5–2.2) | 1.9 (1.5–2.2) |
| ECOG PS, 0:1:2 [$n$(%)] | 4 (20):15 (75):1 (5) | 7 (50):7 (50):0 (0) |
| Site of primary tumour lesion [$n$ (%)] | | |
| Colon carcinoma | 9 (45) | 14 (100) |
| Ovarian carcinoma | 1 (5) | 0 (0) |
| Pancreatic carcinoma | 1 (5) | 0 (0) |
| Other | 9 (45) | 0 (0) |
| Clinical/radiographic tumour status at entry [$n$(%)] | | |
| Progressive disease | 20 (100) | 13 (93) |
| NA | 0 (0) | 1 (7) |
| Prior surgery [$n$ (%)] | | |
| Diagnostic test | 7 (35) | 3 (25) |
| Cancer surgery | 15 (75) | 14 (100) |
| Other procedures | 2 (10) | 0 (0) |
| Prior radiotherapy | 3 (15) | 3 (21) |
| Prior systemic therapy | 18 (90) | 14 (100) |

BMI: body mass index; ECOG PS: Eastern Cooperative Oncology Group performance status; NA: not assessable.

of 20 (30%) patients in cohorts 1–3 and five of 14 (36%) in cohort 4 had sorafenib dose reductions or interruptions. Overall, all patients from cohorts 1–3 and 11/14 (79%) from cohort 4 received at least 90% of the planned dose of sorafenib.

Irinotecan was administered for a median of 2 cycles (range 1–5) in cohorts 1–3, and 2.5 cycles (range 1–4) in cohort 4. Four patients (20%) in cohorts 1–3 received $\geqslant$ three cycles of irinotecan, compared with seven (50%) in cohort 4. Irinotecan dose reductions were necessary for 11/20 patients (55%) in cohorts 1–3 and 5/14 (36%) in cohort 4; only nine patients (27%) from all cohorts received at least 90% of the planned dose.

Twelve patients from cohorts 1–3 (60%) and 12 from cohort 4 (86%) discontinued treatment due to disease progression. Six patients (30%) from cohorts 1–3 and one (7%) from cohort 4 withdrew from the study due to adverse events. The remaining three patients withdrew due to protocol violation, non-compliance to study medication, or withdrawal of consent.

### 3.2. *Safety*

All 34 patients were evaluated for safety. The most frequently occurring drug-related adverse events were gastrointestinal, dermatological and constitutional symptoms (Table 2). The incidence of drug-related toxicities was similar between low doses (100 and 200 mg bid) of sorafenib in cohorts 1 and 2 and the higher dose (400 mg bid) in cohort 3. Hand–foot skin reaction (HFSR) was only observed in cohort 4.

The most common drug-related adverse events of grade 3/4 were diarrhoea, decreased ANC/AGC, and decreased leukocytes (total white blood cells) (Table 3).

Eleven patients (32.4%) required a sorafenib dose reduction or interruption. One patient in cohort 1 experienced toxicity that necessitated a dose reduction or interruption. Four patients each in cohorts 3 and 4 required a dose reduction or interruption due to adverse events.

Seven patients from cohorts 1–3 withdrew due to adverse events, of which three toxicities were determined to be unrelated to either of the study drugs.

The maximum tolerated dose (MTD) of sorafenib plus irinotecan was not reached at the highest dose level. DLTs related to sorafenib were experienced by three patients, all in cohorts 3 and 4 (cerebellar haemorrhage, $n$ = 1; HFSR, $n$ = 2).

Laboratory abnormalities were typical for the patients selected; most experienced decreases in haematology parameters, including ANC/AGC (61.8%), leukocytes (76.7%), and haemoglobin (94.1%). Elevated laboratory values, such as alkaline phosphatase (AP; 88.2%), serum glutamic oxaloacetic transaminase (100%), serum glutamic pyruvic transaminase (76.5%) and lipase (47.1%), occurred frequently. Increases in serum lipase in 30% of patients in cohorts 1–3 and 71% of patients in cohort 4 were possibly related to sorafenib, and were predominantly grade 1/2 in severity.

### 3.3. *Pharmacokinetics*

#### 3.3.1. *Sorafenib*

Pharmacokinetics of sorafenib were available from 24 patients (cohort 1–3, $n$ = 18; cohort 4, $n$ = 6). Two patients discontinued the study during cycle 1. As the corresponding profiles from cycle 2 were not available, results from these patients were excluded from the overall pharmacokinetic evaluation.

Overall, concomitant administration of irinotecan 125 mg/m$^2$ and sorafenib 100 or 200 mg bid had no impact on the pharmacokinetics of sorafenib (Table 4). However, concomitant administration of irinotecan 125 mg/m$^2$ with sorafenib 400 mg bid increased mean $AUC_{0-10}$ of sorafenib by 68% and $C_{max}$ of sorafenib by 78%. The reduced fixed dose of 140 mg irinotecan (cohort 4) had no effect on the pharmacokinetics of sorafenib.

**Table 2 – Incidence of drug-related, treatment-emergent adverse events occurring in ⩾10% of all patients [n (%)]**

| Sorafenib dosage | Cohort 1($n$ = 6) 100 mg bid | Cohort 2($n$ = 7) 200 mg bid | Cohort 3($n$ = 7) 400 mg bid | Total (cohorts 1–3) ($n$ = 20) 100–400 mg bid | Cohort 4($n$ = 14) 400 mg bid |
|---|---|---|---|---|---|
| Blood/bone marrow | 2 (33) | 4 (57) | 3 (43) | 9 (45) | 4 (29) |
| ANC/AGC | 2 (33) | 0 (0) | 1 (14) | 3 (15) | 3 (21) |
| Leukocytes (total WBC) | 2 (33) | 3 (43) | 3 (43) | 8 (40) | 2 (14) |
| Constitutional symptoms | 3 (50) | 5 (71) | 6 (86) | 14 (70) | 8 (57) |
| Fatigue | 3 (50) | 3 (43) | 5 (71) | 11 (55) | 7 (50) |
| Weight loss | 0 (0) | 3 (43) | 3 (43) | 6 (30) | 3 (21) |
| Gastrointestinal symptoms | 6 (100) | 7 (100) | 7 (100) | 20 (100) | 13 (93) |
| Anorexia | 0 (0) | 4 (57) | 2 (29) | 6 (30) | 2 (14) |
| Diarrhoea[a] | 6 (100) | 5 (71) | 6 (86) | 17 (85) | 11 (79) |
| Nausea | 3 (50) | 5 (71) | 4 (57) | 12 (60) | 5 (36) |
| Stomatitis/pharyngitis | 0 (0) | 1 (14) | 2 (29) | 3 (15) | 2 (14) |
| Vomiting | 4 (67) | 4 (57) | 4 (57) | 12 (60) | 3 (21) |
| Metabolic/laboratory | 2 (33) | 1 (14) | 0 (0) | 3 (15) | 0 (0) |
| Other | 1 (17) | 1 (14) | 0 (0) | 2 (10) | 0 (0) |
| Pain | 1 (17) | 0 (0) | 2 (29) | 3 (15) | 4 (29) |
| Abdominal pain/cramping | 1 (17) | 0 (0) | 2 (29) | 3 (15) | 3 (21) |
| Dermatology/skin | 4 (67) | 2 (29) | 7 (100) | 13 (65) | 12 (86) |
| Alopecia | 4 (67) | 2 (29) | 6 (86) | 12 (60) | 9 (64) |
| Hand–foot skin reaction | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 8 (57) |
| Pruritus | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 4 (29) |
| Other | 0 (0) | 1 (14) | 1 (14) | 2 (10) | 7 (50) |

Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0).
ANC: absolute neutrophil count; AGC: absolute granulocyte count; WBC: white blood cell.
a Patients without colostomy.

**Table 3 – Incidence of drug-related, treatment-emergent adverse events with National Cancer Institute Common Toxicity Criteria grade ⩾3 occurring in two or more of 20 patients in cohorts 1–3 or any of 14 patients in cohort 4 [n(%)]**

| | Cohort 1($n$ = 6) | Cohort 2($n$ = 7) | Cohort 3($n$ = 7) | Total (cohorts 1–3)($n$ = 20) | Cohort 4($n$ = 14) |
|---|---|---|---|---|---|
| All events | 3 (50) | 4 (57) | 5 (71) | 12 (60) | 7 (50) |
| Blood/bone marrow | 1 (17) | 1 (14) | 1 (14) | 3 (15) | 3 (21) |
| ANC/AGC | 0 (0) | 0 (0) | 1 (14) | 1 (5) | 3 (21) |
| Leukocytes (total WBC) | 1 (17) | 0 (0) | 1 (14) | 2 (10) | 1 (7) |
| Gastrointestinal | 2 (33) | 3 (43) | 3 (43) | 8 (40) | 4 (29) |
| Diarrhoea[a] | 2 (33) | 3 (43) | 3 (43) | 8 (40) | 3 (21) |
| Vomiting | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (7) |
| Infection/febrile neutropenia | 4 (67) | 1 (14) | 2 (29) | 7 (35) | 7 (50) |
| Febrile neutropenia | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (7) |
| Dermatology/skin | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 (14) |
| Hand–foot skin reaction | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 (14) |

ANC: absolute neutrophil count; AGC: absolute granulocyte count; WBC: white blood cell.
a Patients without colostomy.

**Table 4 – Ratios of $AUC_{0-10}$ and $C_{max}$ for sorafenib and corresponding 90% confidence intervals following oral sorafenib 100–400 mg bid with (cycle 2) or without (cycle 1) concomitant irinotecan 125 mg/m$^2$ or 140 mg i.v**

| Cohort | Dose of sorafenib | Dose of irinotecan | $n$ | Ratio of sorafenib of cycle 2 (combined sorafenib and irinotecan) versus cycle 1 (sorafenib alone)[a] | |
|---|---|---|---|---|---|
| | | | | $AUC_{0-10}$ (90% CI) | $C_{max}$ (90% CI) |
| 1 | 100 mg bid | 125 mg/m$^2$ | 6 | 1.07 (0.94–1.22) | 1.09 (0.81–1.48) |
| 2 | 200 mg bid | 125 mg/m$^2$ | 7 | 1.11 (0.83–1.50) | 1.02 (0.69–1.52) |
| 3 | 400 mg bid | 125 mg/m$^2$ | 5 | 1.68 (1.27–2.23) | 1.78 (1.27–2.49) |
| 4 | 400 mg bid | 140 mg | 6 | 1.05 (0.83–1.33) | 0.93 (0.69–1.27) |

AUC: area under curve; $C_{max}$: maximum concentration; CI: confidence interval.
a Ratio of geometric means of $C_{max}$ or $AUC_{0-10}$ to assess the effect of plasma exposure to irinotecan on sorafenib plasma PK parameters.

#### 3.3.2. *Irinotecan and SN38*

Sorafenib 100 or 200 mg bid in combination with irinotecan 125 mg/m$^2$ (cohorts 1 and 2) did not significantly change the pharmacokinetics of irinotecan or SN38 (Table 5). Contrastingly, concomitant administration of sorafenib 400 mg bid and irinotecan 125 mg/m$^2$ or 140 mg (cohorts 3 and 4) significantly increased plasma exposure to irinotecan and SN38.

In patients receiving irinotecan 125 mg/m$^2$ and sorafenib 400 mg bid (cohort 3), mean $AUC_{0-\infty}$ and $C_{max}$ of irinotecan increased by 26% and 36%, respectively, upon simultaneous administration of both drugs, and by 42% and 73%, respectively, with concomitant administration of irinotecan 140 mg and sorafenib 400 mg bid (cohort 4) (Table 5). Mean $AUC_{0-48}$ and $C_{max}$ of SN38 increased by approximately 120% with concomitant irinotecan 125 mg/m$^2$, and approximately 70% with concomitant irinotecan 140 mg (Fig. 1).

Although administration of the 400 mg sorafenib dose concomitantly with irinotecan (125 mg/m$^2$ or 140 mg) significantly increased exposure to irinotecan and its metabolite SN38, this was not associated with increased toxicities (no increase in the incidence of fatigue, diarrhoea or decreased leucocyte count).

#### 3.3.3. *Effect of sorafenib on SN38 glucuronidation in pooled human liver microsomes*

Sorafenib strongly inhibited formation of the SN38 glucuronide as indicated by the $K_i$ value of 2.7 μmol/l estimated from a Dixon plot (Fig. 2). A Lineweaver-Burk plot showed a mixed-type inhibition of the SN38 glucuronidation by sorafenib (Figure not shown). 17α-Ethinylestradiol was applied as a positive control and moderately affected this conjugation reaction ($IC_{50}$: 39 μmol/l) in accordance with previous results.[25]

### 3.4. *Efficacy*

Tumour response was evaluated in all 20 patients in cohorts 1–3, and 13 of 14 patients in cohort 4. Only one of 13 patients (8%) in cohort 4 achieved partial response, which was observed in cycle 2 and lasted > 200 days (Table 6). This patient had CRC with multiple liver metastases, and had previously

**Table 5 – Ratios of AUC and $C_{max}$ for irinotecan and SN38 and corresponding 90% confidence intervals following irinotecan 125 mg/m$^2$ or 140 mg i.v. with (cycle 2) or without (cycle 1) concomitant oral sorafenib 100–400 mg bid**

| Cohort | Dose of irinotecan | Dose of sorafenib | *n* | Ratio of irinotecan of cycle 2 (combined irinotecan and sorafenib) versus cycle 1 (irinotecan alone)[a] | | Ratio of SN38 of cycle 2 (combined irinotecan and sorafenib) versus cycle 1 (irinotecan alone)[a] | |
|---|---|---|---|---|---|---|---|
| | | | | AUC (90% CI) | $C_{max}$ (90% CI) | AUC (90% CI) | $C_{max}$ (90% CI) |
| 1 | 125 mg/m$^2$ | 100 mg bid | 6 | 1.02 (0.71–1.46) | 0.97 (0.80–1.18) | 0.99 (0.72–1.36) | 0.98 (0.74–1.29) |
| 2 | 125 mg/m$^2$ | 200 mg bid | 7 | 1.09 (0.67–1.75) | 0.98 (0.81–1.17) | 1.23 (0.82–1.84) | 1.12 (0.74–1.70) |
| 3 | 125 mg/m$^2$ | 400 mg bid | 5 | 1.26 (0.99–1.59) | 1.36 (1.10–1.69) | 2.20 (1.40–3.46) | 2.22 (1.01–4.88) |
| 4 | 140 mg | 400 mg bid | 6 | 1.42 (1.14–1.78) | 1.73 (1.57–1.90) | 1.67 (1.27–2.19) | 1.67 (1.30–2.14) |

AUC: area under curve; $C_{max}$: maximum concentration; CI: confidence interval.
a Ratio of geometric means of $C_{max}$ or AUC to assess the effect of plasma exposure to sorafenib on irinotecan and SN38 plasma pharmacokinetic parameters.

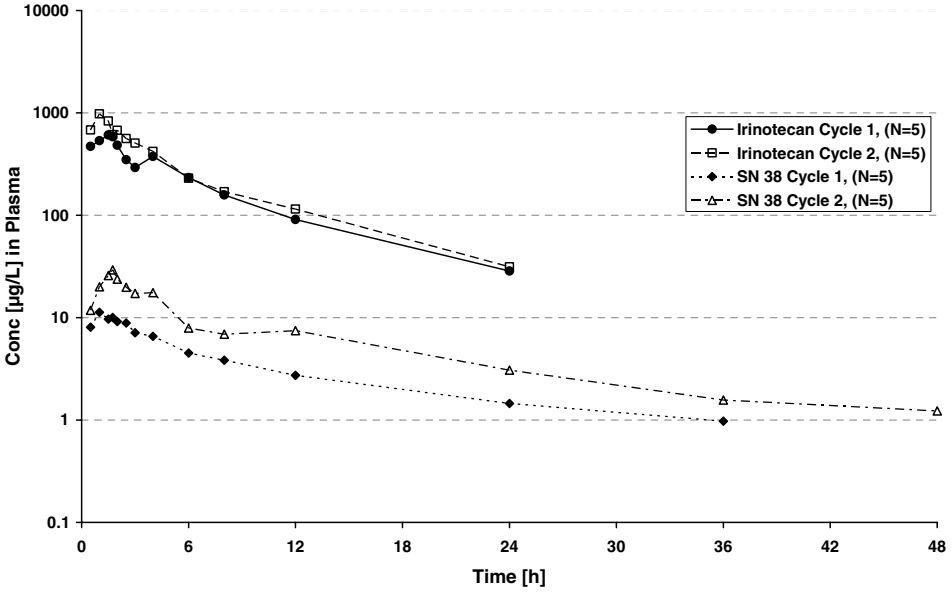


**Fig. 1 – Concentration–time curves of irinotecan and SN38 following 125 mg/m$^2$ irinotecan in cohort 3, showing the effect of sorafenib on the pharmacokinetics of SN38 (increase in exposure).**

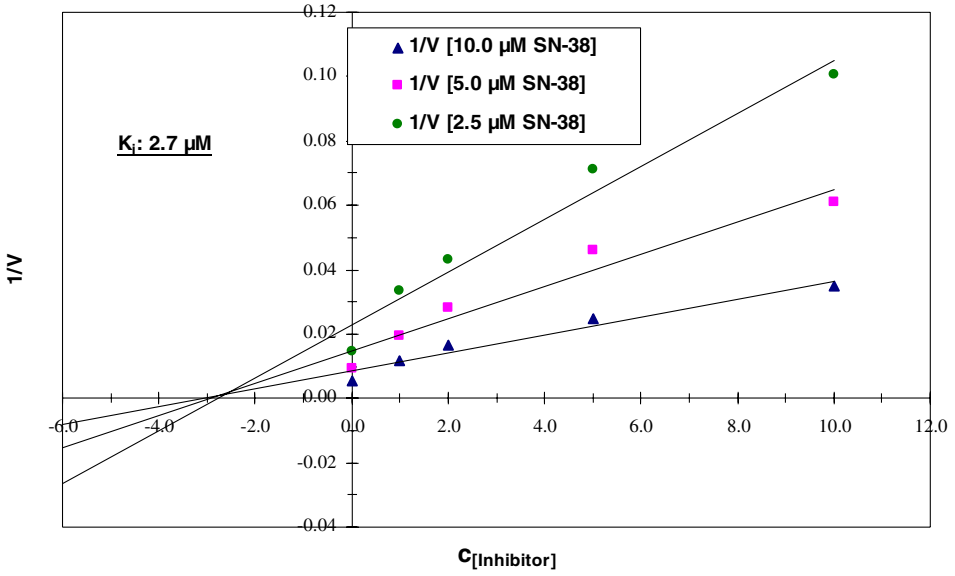


**Fig. 2 – Inhibition of SN38 glucuronidation by sorafenib in human liver microsomes (Dixon plot).**

**Table 6 – Tumour response in patients receiving twice-daily sorafenib in combination with weekly irinotecan (patients valid for tumour response analysis n = 33) [n (%)]**

| | Cohort 1 100 mg bid (*n* = 6) | Cohort 2 200 mg bid (*n* = 7) | Cohort 3 400 mg bid (*n* = 7) | Total (cohorts 1–3) 100–400 mg bid (*n* = 20) | Cohort 4 400 mg bid (*n* = 13) |
|---|---|---|---|---|---|
| Best response | | | | | |
| Partial response | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (8) |
| Stable disease | 5 (83) | 2 (29) | 5 (71) | 12 (60) | 10 (77) |
| Progressive disease[a] | 0 (0) | 5 (71) | 2 (29) | 7 (35) | 2 (15) |
| Progressive disease[b] | 1 (17) | 0 (0) | 0 (0) | 1 (5) | 0 (0) |

a Measurement proven.
b Based on clinical judgement.

received a combination of 5-fluorouracil and oxaliplatin. This patient was later diagnosed with progressive disease due to new liver lesions.

Stable disease (SD) was the best response in 12/20 patients (60%) in cohorts 1–3, and 10/13 patients (77%) in cohort 4. Three out of nine patients with CRC in cohorts 1–3 achieved SD. Three of 20 patients (15%) in cohorts 1–3 and one of 13 patients (8%) in cohort 4 showed no tumour progression throughout the study (i.e. for 37–84 days and 174 days, respectively).

Median time to progression was 156.5 days (range 44–279 days) for patients with CRC in cohort 4, and 61 days (range 41–229 days) for the nine patients with CRC in cohorts 1–3.

## 4. Discussion

Sorafenib can be combined safely with irinotecan to treat advanced solid tumours. The most frequent drug-related adverse events observed in this study were gastrointestinal symptoms, dermatological and constitutional symptoms. Diarrhoea is a commonly reported side-effect with single-agent sorafenib,[11,8] and a well-known non-haematological DLT with irinotecan[25] Although haematological abnormalities were common, these may be attributable to irinotecan, as neutropenia, anaemia and thrombocytopenia are associated with irinotecan.

The MTD for this combination was not reached, even at the highest doses of sorafenib plus irinotecan. There were more dose reductions due to toxicity with high-dose irinotecan (125 mg/m$^2$) than with the lower, fixed dose (140 mg). However, increasing the dose of sorafenib from 100 to 400 mg bid was not associated with an increase in toxicity in patients who received the same dosage of irinotecan (cohorts 1–3).

Concomitant administration of sorafenib 400 mg bid with irinotecan 125 mg/m$^2$ or 140 mg significantly increased exposure to irinotecan and its metabolite SN38, which was not associated with increased toxicities. *In vitro*, applying human liver microsomes sorafenib considerably inhibited SN38 glucuronide formation as indicated by a $K_i$ value of 2.7 µmol/l. The inhibition of the uridine diphosphate glucuronosyltransferases responsible for SN38 glucuronidation might be a likely explanation of the increase in SN38 exposure in patients coadministered with sorafenib 400 mg bid. Due to the small number of patients analysed, the clinical significance of these interactions remains unclear. Although irinotecan 140 mg is not a standard recommended dose, it provided an advantage over the standard 125 mg/m$^2$ regimen, as shown by the reduced

number of adverse events, the absence of dosing errors, less pharmacokinetic interaction and lower treatment costs. Body surface area does not predict either the clearance of irinotecan or the pharmacokinetics of SN38,[26] and therefore supports the use of a fixed-dose of irinotecan to reduce toxicity and pharmacoeconomic costs.

Encouraging preliminary anti-tumour results were obtained; disease control rate was 60% in cohorts 1–3, and 85% (metastatic CRC patients in cohort 4). However, only two of nine CRC patients assigned to cohorts 1–3 were in cohort 3, which received the higher dose of both sorafenib and irinotecan. Therefore, the efficacy benefits of sorafenib 400 mg bid with irinotecan 140 mg to treat advanced CRC have yet to be firmly established. These findings are noteworthy, as 16/34 treated patients had already failed irinotecan-containing regimens. Furthermore, in four patients, this combination induced sustained SD. These responses may reflect re-sensitisation of patients to irinotecan treatment, or a direct effect of sorafenib.

Sorafenib is safely combinable with other chemotherapeutic agents, including gemcitabine, oxaliplatin, docetaxel and carboplatin/paclitaxel, in patients with a variety of tumour types.[27–31] As in the present study, the adverse events were mainly mild to moderate in severity, and manageable. DLTs included HFSR, diarrhoea, mucositis and febrile neutropenia.[27–31]

The combination of other targeted agents with irinotecan has shown promise. Cetuximab plus irinotecan, for example, showed clinically significant activity in patients with irinotecan-refractory CRC, and the response rate in the combination therapy group (22.9%) was higher than the monotherapy group (10.8%).[32]

In summary, sorafenib 400 mg bid is combinable with irinotecan 125 mg/m$^2$ or 140 mg for the treatment of patients with advanced, refractory solid tumours. However, careful monitoring of toxicity is recommended for patients treated with sorafenib 400 mg bid plus irinotecan 125 mg/m$^2$ due to the observed pharmacokinetic interaction. Further evaluations of the combination of sorafenib with irinotecan – either at 125 mg/m$^2$ or preferably at a fixed dose of 140 mg – are warranted. In light of the disappointing results with FOLFIRI after FOLFOX failure in CRC,[33] these studies should be performed in patients with irinotecan-naive metastatic CRC after FOLFOX failure.

## Conflict of interest statement

None declared.

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