Sorafenib and irinotecan (NEXIRI) as second- or later-line treatment for patients with metastatic colorectal cancer and *KRAS*-mutated tumours: a multicentre Phase I/II trial

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Background: This trial evaluated the feasibility and efficacy of combined sorafenib and irinotecan (NEXIRI) as second- or later-line treatment of patients with *KRAS*-mutated metastatic colorectal cancer (mCRC), who had progressed after irinotecan-based chemotherapy.

Methods: In Phase I, in a 3 + 3 dose escalation schedule, patients received irinotecan (125, 150 or 180 mg m^{-2} every 2 weeks), in combination with 400 mg sorafenib b.d. The primary end point was the maximum-tolerated dose of irinotecan. In Phase II, the primary end point was disease control rate (DCR). Secondary end points were progression-free survival (PFS), overall survival (OS) and toxicity.

Results: Phase I included 10 patients (median age 63 (49–73)); no dose-limiting toxicity was seen. In Phase II, 54 patients (median age 60 (43–80) years) received irinotecan 180 mg m^{-2} every 2 weeks with sorafenib 400 mg b.d. Nine patients (17%) remained on full-dose sorafenib. The DCR was 64.9% (95% CI, 51–77). Median PFS and OS were 3.7 (95% CI, 3.2–4.7) and 8.0 (95% CI, 4.8–9.7) months, respectively. Toxicities included Grade 3 diarrhoea (37%), neutropenia (18%), hand-foot syndrome (13%) and Grade 4 neutropenia (17%).

Conclusion: The NEXIRI regimen showed promising activity as second- or later-line treatment in this heavily pretreated mCRC population (ClinicalTrials.gov NCT00989469).

Each year, there are an estimated 1.2 million new cases of colorectal cancer worldwide, with the mortality rate reaching 600 000 (Ferlay et al, 2010). Approximately, half of the patients develop metastases during the course of the disease (Van Cutsem et al, 2010), and for the majority of these patients, treatment is mainly palliative. Over the past 10 years, there has been increasing interest in the combination of chemotherapy (e.g., fluoropyrimidines, irinotecan or oxaliplatin) and targeted therapy (e.g., bevacizumab, cetuximab or panitumumab) in the treatment of metastatic colorectal cancer (mCRC) (Cunningham et al, 2004; Hurwitz et al, 2004; Giantonio et al, 2007; Van Cutsem et al, 2009, 2011; Douillard et al, 2010; Peeters et al, 2010). These combinations have become the standard treatment and resulted in significant improvement in response rate (Cunningham et al, 2004; Hurwitz et al, 2004; Giantonio et al, 2007; Van Cutsem et al, 2009, 2011; Peeters et al, 2010), progression-free survival (PFS) (Cunningham et al, 2004; Hurwitz et al, 2004; Giantonio et al, 2007; Van Cutsem et al, 2009, 2011; Douillard et al, 2010; Peeters et al, 2010) and overall survival (OS) (Hurwitz et al, 2004; Giantonio et al, 2007; Van Cutsem et al, 2009, 2011).

In the era of therapeutic personalisation, the identification of molecular biomarkers has a key role in determining both optimal treatment strategies and clinical outcome in patients with mCRC. The presence of the KRAS gene mutation is a well-established predictive factor for lack of response to anti-epidermal growth factor receptor (EGFR) therapies, regardless of the line of treatment (Benvenuti et al, 2007; Di Fiore et al, 2007; Lièvre et al, 2008). At the time of the trial design, there were no effective therapies targeted specifically against KRAS mutant cancers (Tejpar et al, 2012). Indeed, in patients who have disease progression (DP) after irinotecan- or oxaliplatin-based chemotherapies combined with bevacizumab, the addition of cetuximab to irinotecan (Cunningham et al, 2004), or cetuximab or panitumumab as monotherapy are efficacious only in wild-type KRAS tumours (Amado et al, 2008; Karapetis et al, 2008).

Sorafenib is an oral inhibitor of tumour cell proliferation and angiogenesis, and the lead compound in a series of Raf signalling pathway inhibitors. It has already proved its efficacy in refractory kidney cancer and unresectable hepatocellular carcinoma (Llovet et al, 2008; Escudier et al, 2009). Even in the presence of KRAS mutation, sorafenib potently inhibits activation of the mitogen-activated-protein kinase pathway and extracellular signal-regulated phosphorylation by inhibiting the serine threonine kinases Raf-1 and B-Raf. In addition, sorafenib inhibits the receptor tyrosine kinase activity of vascular endothelial growth factors (VEGFR 1, 2 and 3) and platelet-derived growth factor receptor beta (Wilhelm et al, 2004). In preclinical models, sorafenib has demonstrated antitumour activity in colorectal cancer cell lines, including those with KRAS-mutated tumours (Wilhelm et al, 2004; Martinelli et al, 2010). In humans, only a Phase I trial showed the feasibility of combining a weekly schedule of irinotecan 125 mg m^{-2} (D1,8,15,22 D1 = D42) and a fixed dose of sorafenib 400 mg twice daily in 34 patients with solid tumours (23 of whom had colorectal cancer) (Mross et al, 2007). However, preclinical promising results had already been gathered on the synergistic effect of this combination particularly in KRAS-mutated tumours (personal communication at the time) and were recently published (Mazard et al, 2013).

Novel salvage strategies are needed for improving outcomes in selected KRAS-mutated patients who progress after the failure of all approved standard therapies. In addition, combination schedules need to be evaluated in order to help overcome resistance to chemotherapy. On the basis of the above preclinical and clinical data, we conducted a Phase I/II trial with the aim of assessing the feasibility and efficacy of the combined use of sorafenib and the usual 2-week irinotecan regimen as a second- or later-line treatment of patients with mCRC and KRAS-mutated tumours.

In addition, we investigated sorafenib's pharmacokinetic profile, protein expression on tumour tissues and patient genotypes and their association with efficacy and tolerability.

MATERIALS AND METHODS

This was an open-label, single-arm, multicentre Phase I/II trial. The protocol was approved by the local ethics committee (Comité de Protection des Personnes Sud Méditerranée IV, Montpellier, France, EudraCT number 2008-004285-53) and the French competent authority (Agence Nationale de Sécurité du Médicament et des produits de santé). The trial was registered at ClinicalTrials.gov (NCT00989469).

Patients. Eligible patients had histologically confirmed colorectal cancer with measurable unresectable metastatic lesions and documented DP after irinotecan-based chemotherapy and at least one line of chemotherapy. Patients also had centralised confirmation of KRAS mutation status in codons 12 or 13 in the primary tumour or metastases according to Laurent-Puig et al (2009). Other eligibility criteria included a World Health Organization performance status ⩽2, age ⩾18 years, life expectancy >3 months, adequate organ function, absolute neutrophil count ⩾1500 mm^3, platelet count ⩾100 000 mm^3, haemoglobin >10 g dl^{-1}, total bilirubin ⩽1.5 × institutional upper limit of normal (ULN), AST/ALT ⩽2.5 × ULN (or <5 ULN for patients with metastatic liver involvement), amylase and lipase <1.5 × ULN, and creatinine <1.5 × ULN. Patients were excluded if there was a history of Gilbert's syndrome, prior surgery or radiotherapy within 4 weeks before entering the trial, bone-only metastases, symptomatic brain metastases or carcinomatous meningitis, a history of epileptic seizures requiring long-term anticonvulsant therapy, long-term use of cytochrome P450 enzyme-inducing agents, and simultaneous use of other systemic anticancer treatments. Patients with uncontrolled hypertension, unstable coronary syndrome, cardiac arrhythmia or active infection were also excluded. Written informed consent was obtained from each patient before trial entry.

Trial design and treatment

Phase I. Patients were to receive oral sorafenib at a fixed dose of 400 mg twice daily (b.d.) in combination with irinotecan delivered intravenously over 90 min every 2 weeks. There were three dose levels for irinotecan: 125, 150 and 180 mg m^{-2}. A standard 3 + 3 dose escalation design with three to six patients per dose level was used. The Phase I primary end point was the maximum-tolerated dose (MTD) of irinotecan when administered every 2 weeks with 400 mg sorafenib b.d. The MTD was defined as the dose of irinotecan for which at least one dose-limiting toxicity (DLT) occurred for more than 33% of patients during the first four treatment courses. A cycle of therapy was defined as two courses of irinotecan chemotherapy (28 days).

Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Dose-limiting toxicities were defined as any Grade 4 non-haematological toxicity (except for vomiting in the absence of adequate prophylaxis), any toxicity requiring a cycle delay of more than 15 days, any Grade 4 haematological toxicity lasting for more than 7 days, any Grade 3-4 febrile neutropenia, and any concomitant sepsis with Grade 3-4 neutropenia.

Phase II. Patients were to be treated at the dose of irinotecan established in Phase I with 400 mg of oral sorafenib b.d. Trial treatments were given until the development of unacceptable toxicity, patient refusal to continue or DP, and could be discontinued in case of life-threatening toxic event. Sorafenib dose

reductions to 400 mg per day and then every 2 days were permitted for patients experiencing any Grade 3 and 4 haematological and non-haematological toxicities, or any Grade 2 skin toxicity. Subsequent dose re-escalation up to 400 mg b.d. was allowed. In cases of Grade $\geqslant 2$ drug-related toxicity, irinotecan perfusion could be delayed for a maximum of 15 days in the absence of full recovery or resolution to Grade $\leqslant 1$. The Phase II primary end point was the disease control rate (DCR), defined as the rate of objective response and stable disease. Target lesions were assessed every 8 weeks by independent review of thorax-abdomen-pelvis computed tomography according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0. (Therasse *et al*, 2000). Complete or partial response had to be confirmed on two consecutive assessments 4 weeks apart. The secondary end points were toxicity, PFS and OS. A complete blood cell and platelet count were requested weekly, whereas physical examination and routine laboratory tests were performed before each administration of irinotecan. The last three patients enrolled in the Phase I trial were included in the Phase II trial analysis.

Ancillary Phase II investigations

Pharmacokinetic analysis of sorafenib. Peripheral blood samples were taken on Day 1 (before starting treatment) and at each scheduled visit (days 14, 28, 56, months 4, 6). The blood was collected into a lithium heparin tube, and centrifuged at 2800 g for 10 min to separate the plasma. The plasma was transferred to polypropylene tubes and frozen at $-20\,^{\circ}C$ until assayed. Maximum plasma concentration (C_{max}) of sorafenib was evaluated using High-Performance Liquid Chromatography with UV detection. We investigated for any association between sorafenib's pharmacokinetic parameters and response to treatment or toxicity.

Patient genotyping and protein expressions on the tumour tissues. Patients were genotyped for two genes and two polymorphisms which have previously been suspected of having a role in clinical outcomes in similar populations (Zhang *et al*, 2006; Liu *et al*, 2013). Whole blood was collected at enrolment and genomic DNA was extracted from the peripheral lymphocytes using the QIAamp DNA blood maxi kit (Qiagen, Courtaboeuf, France) according to the manufacturer's recommendations. The single-nucleotide polymorphism G870A (c.723G>A, rs9344) in the *CCND1* gene was identified through PCR-high resolution melting (HRM) curve analysis by using previously described cycling conditions and amplification primers (Ho-Pun-Cheung *et al*, 2007). The short tandem (TA) repeat variation within the TATA box of the *UGT1A1* gene (rs8175347) was studied by fragment analysis of fluorescence-labelled PCR products (protocol available on request).

Expression of EGFR, HER2, PTEN, NF-kB and cyclin D1 was evaluated by immunohistochemistry on 3-μm tissue sections of paraffin-embedded specimens as previously performed in previous studies (Chung *et al*, 2005; Frattini *et al*, 2007; Scartozzi *et al*, 2007; Cascinu *et al*, 2008; Loupakis *et al*, 2009). The antibody clones, suppliers, antigen-retrieval procedures, dilutions, staining protocols and cut-point scoring are available on request.

We investigated for any association between genotypes or protein expression and response to treatment or toxicity.

Statistical analysis. In the Phase II trial, 45 patients were required to determine the DCR assuming a Simon two-stage minimax design with $\alpha = 10\%$ and $\beta = 5\%$. To anticipate potential exclusions or losses, nine more patients were included, giving a 20% leeway. A DCR of 20% (p0) or less would indicate that the treatment combination lacked antitumour activity; a DCR of 40% (p1) or more would be considered promising. Patients were evaluable for tolerability and efficacy if they had received at least one and four courses of treatment, respectively. To proceed to second-stage accrual,

four or more patients with disease control among the first 21 patients were required. Promising activity was defined as a partial response or a stable disease observed for 14 patients or more on interim analysis at the end of the second stage. Progression-free survival and OS were estimated using the Kaplan–Meier method.

Associations between response to treatment or toxicity and sorafenib's pharmacokinetic parameters, patient genotypes or protein expression on tumour tissues were investigated by the χ^2 test (or the Fisher test, if applicable). A *P*-value of 0.05 was considered to indicate statistical significance.

RESULTS

Phase I. Ten patients with a median age of 63 years (range, 49–73 years) were enrolled between February and June 2009 from five French centres. All patients had a good performance status (0 to 1). The primary tumour was located in the colon (90%) or rectum (10%). The experimental regimen was administered as a third- (60%) or later-line treatment. Three, four and three patients received irinotecan at 125, 150 and 180 mg m^{-2}, respectively. A median of 6 (range, 1–12) courses of treatment were delivered. No DLT was seen in any patient. Grade 3 skin toxicity included rash ($n=2$), hand-foot syndrome ($n=2$), cutaneous abdominal abscess ($n=1$) and phlyctena ($n=1$). Of the patients treated with irinotecan 150 mg m^{-2}, three had Grade 3 diarrhoea, one withdrew consent and one had Grade 4 neutropenia lasting less than 7 days; of the patients treated with irinotecan 180 mg m^{-2}, two had Grade 3 diarrhoea. In total, six patients (67%) required sorafenib dose reduction to 400 mg per day after two courses of treatment because of toxicity. Seven (78%) of nine evaluable patients had stable disease after four courses of treatment. The recommended Phase II dose of irinotecan every 2 weeks was therefore defined to be 180 mg m^{-2} when combined with 400 mg sorafenib b.d.

Phase II

Patients. Fifty-four patients were recruited between June and December 2009 from 10 French centres (Figure 1). Baseline demographics and disease characteristics are listed in Table 1. The majority of patients had liver metastases (72%) and nearly half of the patients presented with lung metastases. Surgical resection of primary tumour and/or distant metastases had been performed in 47 (87%) and 17 (31%) patients, respectively. See Table 2 for details of prior therapy. All patients had previously received palliative fluorouracil- and irinotecan-based chemotherapy with a median of three lines of treatment per patient (maximum eight lines).

Treatment compliance and treatment-related toxicity. The median number of cycles received per patient was four (range, 1–8). Thirteen patients (24%) completed six or more cycles of treatment. Relative dose intensity was 89% for irinotecan and 61% for sorafenib. Nine patients (17%) received sorafenib at the full dose of 400 mg b.d. throughout the treatment schedule. Forty patients (74%) discontinued treatment for the following reasons: DP ($n=26$), toxicity ($n=6$), investigator's decision ($n=5$) and withdrawal of consent ($n=3$).

All patients received at least two courses of treatment and were therefore evaluable for tolerance. There was no toxicity-related death, and most adverse events were Grade 1 or 2. Grade 3 toxicities included diarrhoea (37%), asthenia (22%), neutropenia (18%) and hand-foot syndrome (13%). The main Grade 4 treatment-related toxicity was neutropenia, observed in 16.7% of patients (Table 3). The irinotecan dose was adjusted in 19 patients (35%), and a treatment delay was required in 38 patients (70%).
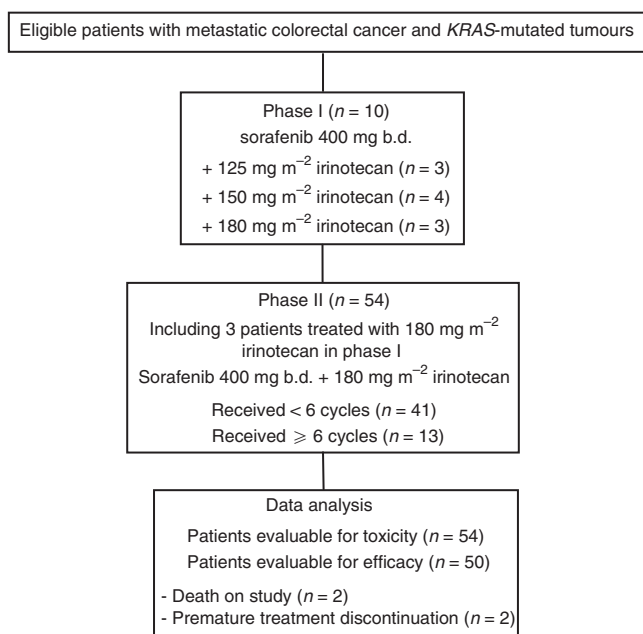
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│ Eligible patients with metastatic colorectal cancer and  │
│ KRAS-mutated tumours                                      │
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│ Phase I (n = 10)                                          │
│ sorafenib 400 mg b.d.                                     │
│ + 125 mg m⁻² irinotecan (n = 3)                           │
│ + 150 mg m⁻² irinotecan (n = 4)                           │
│ + 180 mg m⁻² irinotecan (n = 3)                           │
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│ Phase II (n = 54)                                         │
│ Including 3 patients treated with 180 mg m⁻²              │
│ irinotecan in phase I                                     │
│ Sorafenib 400 mg b.d. + 180 mg m⁻² irinotecan            │
│ Received < 6 cycles (n = 41)                              │
│ Received ⩾ 6 cycles (n = 13)                              │
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│ Data analysis                                             │
│ Patients evaluable for toxicity (n = 54)                  │
│ Patients evaluable for efficacy (n = 50)                  │
│ - Death on study (n = 2)                                  │
│ - Premature treatment discontinuation (n = 2)             │
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Figure 1. Trial flowchart.

Table 1. Patient baseline demographics and clinical characteristics (Phase II)

	Patients, N = 54	%
Median age, years (range)	60 (43–80)	
Gender		
Male	32	59.3
Female	22	40.7
WHO performance status[a]		
0	23	46.0
1	26	52.0
2	1	2.0
Site of primary tumour		
Colon	28	51.9
Rectum	20	37.0
Rectosigmoid junction	6	11.1
Number of metastatic sites		
1	28	51.8
2	19	35.2
⩾ 3	7	13.0

Abbreviation: WHO = World Health Organization.
[a]Data not available for four patients.

Table 2. Details of prior therapy (Phase II patients)

	Patients, N = 54	%
Type of prior therapy		
Surgery for primary tumour	47	87.0
Surgery for metastases	17	31.5
Adjuvant chemotherapy	24	44.4
Palliative chemotherapy	54	100.0
1 Line	7	12.9
2 Lines	11	20.4
3 Lines	15	27.8
⩾ 4 Lines	21	38.9
Radiotherapy	25	46.3
Failure of previous medication		
5-FU	54	100.0
Irinotecan	54	100.0
Oxaliplatin	52	96.3
Bevacizumab	49	90.7
Cetuximab	9	16.7

Table 3. Toxicity per patient (Phase II)

	Grade 2	Grade 3	Grade 4
	N (%)	N (%)	N (%)
Haematologic toxicity			
Anaemia	8 (14.8)	2 (3.7)	0 (0.0)
Thrombocytopenia	2 (3.7)	0 (0.0)	0 (0.0)
Neutropenia	6 (11.1)	10 (18.5)	9 (16.7)
Febrile neutropenia	0 (0.0)	2 (3.7)	1 (1.9)
Lipase	2 (3.7)	0 (0.0)	1 (1.9)
Cutaneous toxicity			
Hand-foot syndrome	5 (9.3)	7 (13.0)	0 (0.0)
Rash, erythema	4 (7.4)	0 (0.0)	0 (0.0)
Gastrointestinal toxicity			
Anorexia	18 (33.3)	7 (13.0)	0 (0.0)
Diarrhoea	19 (35.2)	20 (37.0)	0 (0.0)
Nausea	12 (22.2)	3 (5.6)	0 (0.0)
Vomiting	8 (14.8)	2 (3.7)	0 (0.0)
Mucositis	4 (7.4)	4 (7.4)	0 (0.0)
Other			
Asthenia	20 (37.0)	12 (22.2)	0 (0.0)

was 3.7 months (95% CI, 3.2–4.7) and the median OS was 8.0 months (95% CI, 4.8–9.7).

Ancillary Phase II investigations

Sorafenib pharmacokinetics. No relationship was found between treatment response or toxicity and sorafenib's pharmacokinetic parameters.

Patient genotyping and protein expression on tumour tissues. Among the 48 genomic DNA analysed, 23 displayed the homozygous wild-type UGT1A1 (*1/*1) genotype, 23 the heterozygous UGT1A1 (*1/*28) genotype and two the homozygous polymorphic UGT1A1 (*28/*28) genotype. However,

Response and survival. See Table 4 and Figure 2. Tumour response was not evaluable in two patients (3.7%) due to premature treatment discontinuation. The patient who partially responded had previously received 12 cycles of adjuvant chemotherapy combining bevacizumab and FOLFIRI regimen, first-line chemotherapy combining bevacizumab and FOLFOX regimen and second-line treatment with oral capecitabine. The DCR was 64.9% (95% CI, 51–77%) in the intention-to-treat population. Of the nine patients treated with full-dose sorafenib for the whole-treatment schedule, six patients had a stable disease (66.7%). At a median follow-up of 7.0 months (range, 1.2–21.9 months), the median PFS

Table 4. Treatment response and survival in the Phase II intention-to-treat population

	Patients, $N = 54$	% (95% CI)
Overall response rate		
CR	0	—
PR	1	1.8
SD	34	63.0
PD	17	31.5
NE	2	3.7
DCR (PR + SD)	35	64.9 (51–77)
OS		
Median, months (95% CI)		8 (4.8–9.7)
Rates	6 Months	58 (44–70)
	12 Months	26 (14–40)
	18 Months	13 (4–26)
PFS		
Median, months (95% CI)		3.7 (3.2–4.7)
Rates	6 Months	28 (17–40)
	12 Months	3 (0–13)

Abbreviations: CI = confidence interval; CR = complete response; DCR = disease control rate; NE = not evaluable; OS = overall survival; PD = progressive disease; PFS = progression-free survival; PR = partial response; SD = stable disease.

Figure 2. Kaplan–Meier curves of (**A**) progression-free survival and (**B**) overall survival.

no significant difference in toxicity was observed according to UGT1A1*28 genotype. Analysis of the CCND1 (rs9344) single-nucleotide polymorphism showed that 27.1% of patients were homozygous for the A allele (A/A genotype), 20.8% were homozygous for the G allele (G/G genotype) and 52.1% were heterozygous (A/G genotype). All those variants were distributed according to the Hardy–Weinberg equilibrium. A significant association was found between the CCND1 G870A polymorphism and tumour response in the patients evaluable for efficacy; patients harbouring the A/A variant were significantly associated with stable disease (12/13 patients, $P = 0.003$). We did not detect any association between EGFR, HER2, PTEN, NF-kB or cyclin D1 expression and treatment efficacy or toxicity. CCND1 G870A polymorphisms were not associated with any particular cyclin D1 expression.

DISCUSSION

The results of this Phase I/II trial confirmed the feasibility of combining sorafenib and irinotecan in extensively pretreated patients with mCRC and KRAS mutation. In the Phase I part of the trial, dose escalation of irinotecan was achieved without any DLT, and the recommended Phase II dose was irinotecan 180 mg m^{-2} once every 2 weeks with fixed-dose sorafenib 400 mg b.d. Principal toxicities included hand-foot syndrome, diarrhoea, asthenia and neutropenia. This treatment combination led to a DCR of 65% (95% CI, 51–77%). Median PFS and OS were 3.7 months and 8.0 months, respectively.

When compared with previously published data in the same kind of heavily pretreated mCRC population with KRAS mutation, our results appear promising. Two Phase III trials assessed the use of the anti-EGFR antibodies cetuximab and panitumumab alone in patients who failed on standard chemotherapy. Both of these studies reported median PFS and OS of 1.9 months and ∼5 months in KRAS-mutated patients, respectively (Amado et al, 2008;
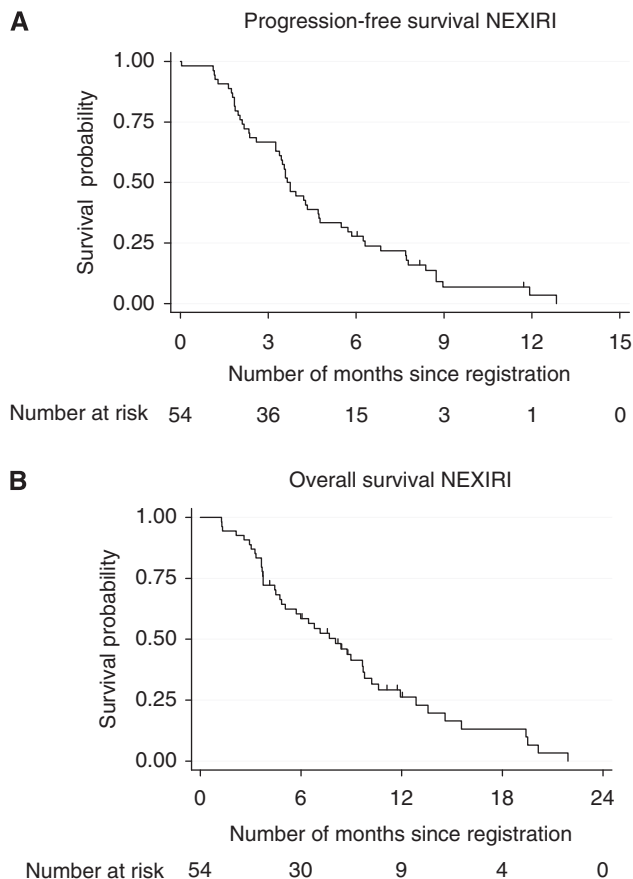
Karapetis et al, 2008). More recently, the multitarget drug regorafenib has been evaluated in a randomized Phase III trial as second- or later-line treatment of mCRC with KRAS-mutated or wild-type tumours (Grothey et al, 2013). A total of 760 patients were to receive best supportive care with either oral regorafenib (160 mg daily) or placebo. In this study, 48% of patients had been pretreated by four or more lines for metastatic disease, all had received bevacizumab and 54% had KRAS mutation. The DCR was 41% vs 15% ($P < 0.001$), median PFS was 1.9 vs 1.7 months (HR = 0.49; 95% CI, 0.42–0.58, $P < 0.001$) and median OS was 6.4 vs 5 months (HR = 0.77; 95% CI, 0.64–0.94, $P = 0.0052$) in the regorafenib arm as compared with placebo, respectively. Subgroup analyses revealed that, among patients treated by regorafenib, KRAS wild-type patients seem to have longer OS than those with KRAS-mutated tumours (HR = 0.65; 95% CI, 0.48–0.90 vs HR = 0.87; 95% CI, 0.67–1.12; $P = 0.0038$, respectively). However, subsequent analysis demonstrated that there is no statistically significant interaction between OS and KRAS status (Van Cutsem and Grothey, 2013).

Our study is limited by the fact that our Phase I definition of DLT was relatively generous, and that dose reductions in sorafenib had been initially planned for cases of Grade 3 diarrhoea. In a trial such as ours using drug combinations, there is the potential for overlapping toxicities, and some of the toxicities reported in Phase I (such as the Grade 3 diarrhoea) would normally have qualified as DLTs. Possibly due to this limitation, only 17% of Phase II patients received full-dose sorafenib 400 mg b.d. Treatment-related toxicities including Grade 3 diarrhoea (37%) and hand-foot syndrome (13%) and Grade 3-4 neutropenia (35%) were primarily responsible for the high frequency of sorafenib dose reductions to 400 mg. This explains the relative dose intensity of 89% for irinotecan and

61% for sorafenib. For future trials investigating the same drug combination, we therefore recommend that diarrhoea is initially managed by reducing the irinotecan dose, starting at 120 mg m^{-2} and progressively increasing in case of good tolerance. Compliance to sorafenib 400 mg b.d. could therefore be improved.

Although there are currently no specific biomarkers predicting response to sorafenib, our exploratory studies demonstrated a significant association between the common G870A polymorphism of CCND1 and stabilising of the disease under the therapy. In particular, the A/A genotype was associated with better disease control on univariate analyses, suggesting cyclin D1 as a potential biomarker of the combination.

Our trial supports preclinical data indicating synergistic anti-tumour effects of combined sorafenib and irinotecan. Sorafenib appears to retain NF-kappa-B in the cytoplasm, and the drug combination may inhibit NF-kappa-B activation, resulting in an enhanced cytotoxicity of irinotecan (Azad *et al*, 2013). Other studies have shown that multi-tyrosine kinase inhibitors such as sorafenib may also reverse irinotecan resistance (Mross *et al*, 2007; Azad *et al*, 2013). Indeed, sorafenib has been identified both *in vitro* and *in vivo* as an inhibitor of the drug-efflux pump ABCG2, favouring irinotecan intracellular accumulation thereby enhancing its toxicity (Mazard *et al*, 2013). This chemosensitizing property previously described by Wei *et al* (2012) has also been ascribed to the inhibition of the irinotecan-mediated p38 and ERK activation (Mazard *et al*, 2013).

Possible pharmacokinetic interactions between irinotecan and sorafenib also need to be considered. Our pharmacokinetic investigation was limited to sorafenib, and did not reveal any correlation between treatment-related toxicity and efficacy. However, Mross *et al* (2007) showed that sorafenib administered at 400 mg b.d. significantly increases irinotecan and SN38 exposure. Another pharmacokinetic study suggested a correlation between sorafenib and exposure to its metabolites with OS and DLTs in 18 patients with CRC, who had been treated with irinotecan at a recommended dose of 100 mg m^{-2} IV D1, D8 (D1 = D42), cetuximab according to the standard weekly schedule, and sorafenib 400 mg b.d. (Azad *et al*, 2013). Whatever the mechanisms involved, further investigation of this combination appears to be warranted to confirm its efficacy. Pharmacokinetic studies are particularly needed to better characterise any synergy between the two agents. Supported by the preclinical data and this trial's results, the efficacy of the combination is being further tested in an ongoing multicentre randomized Phase II trial (ClinicalTrials.gov NCT01715441).

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CONFLICT OF INTEREST

Marc Ychou has served on the Bayer board, Olivier Bouché has received honoraria from Bayer and served on the Pfizer board, and Pierre Laurent-Puig has served on the Amgen, Pfizer and Merck boards. The remaining authors declare no conflict of interest.

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