Prediction of Survival by Neutropenia According To Delivery Schedule of Oxaliplatin-5-Fluorouracil-Leucovorin for Metastatic Colorectal Cancer in a Randomized International Trial (EORTC 05963).

(Article begins on next page)

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PREDICTION OF SURVIVAL BY NEUTROPENIA ACCORDING TO DELIVERY SCHEDULE OF OXALIPLATIN–5-FLUOROURACIL–LEUCOVORIN FOR METASTATIC COLORECTAL CANCER IN A RANDOMIZED INTERNATIONAL TRIAL (EORTC 05963)

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ABSTRACT

Circadian clocks control cellular proliferation and drug metabolism over the 24 h. However, circadian chronomodulated chemotherapy with 5-fluorouracil, leucovorin, and oxaliplatin (chronoFLO4) offered no survival benefit as compared with the non–time-stipulated FOLFOX2, in an international randomized trial involving patients with previously untreated metastatic colorectal cancer (EORTC 05963). The authors hypothesized that treatment near maximum tolerated dose could disrupt circadian clocks thus impairing the efficacy of chronoFLO4 but not of FOLFOX2. Patients with available data (N = 556) were categorized into three subgroups according to the worst grade (G) of neutropenia experienced during treatment. Distinct multivariate models with time-dependent covariates were constructed for each treatment schedule. Neutropenia incidence (all grades) was 33% on chronoFLO4 and 61% on FOLFOX2 (p < .0001), and G3–4 were 7% and 25%, respectively (p < .0001). Neutropenia was significantly more frequent in women than men on either schedule (FOLFOX2, p = .003; chronoFLO4, p = .04). Median survival was 20.7 mo in patients with G3–4 neutropenia versus 12.5 mo in neutropenia-free patients on FOLFOX2 (p < .0001). Corresponding figures were 13.7 and 19.4 mo, respectively, on chronoFLO4 (p = .36). Multivariate analysis confirmed occurrence of severe neutropenia independently predicted for better overall survival on FOLFOX2 (HR = 0.56; p = .015), and worse survival on chronoFLO4 (HR = 1.77, p = .06), with a significant interaction test (p < .0001). Prediction of better survival in neutropenic patients on FOLFOX2 supports the administration of conventional chemotherapy near maximum tolerated dose. The opposite trend shown here for chronoFLO4 supports the novel concept of jointly optimized hematologic tolerability and efficacy through personalized circadian-timed therapy. (Author correspondence: francis.levi@inserm.fr)

INTRODUCTION

Given the documented large interpatient variability in the catabolic and detoxification rates of anticancer drugs (Yang et al., 2010), cytotoxic chemotherapy-induced neutropenia has been advocated as a biological indicator of proper dose intensity in individual patients (Felici et al., 2002; Kvinnsland, 1999). Indeed, the vast majority of studies has reported survival of neutropenic cancer patients was significantly longer than that of patients without such hematologic toxicity, as recently summarized in a meta-analysis (Shitara et al., 2010a). This was also the case for FOLFOX4 or mFOLFOX6 in an Asian cohort of 153 chemo-naïve patients with metastatic colorectal cancer, where neutropenia was positively associated with overall survival, independently of all other known prognostic factors (Shitara et al., 2009). In none of these studies was chemotherapy timing stipulated (Innominato et al., 2010; Lévi et al., 2010; Shitara et al., 2010a).

However, circadian timing was shown to significantly modify the extent of toxicity and efficacy of anticancer drugs, both in experimental models and cancer patients (Granda & Lévi, 2002; Innominato et al., 2010; Lévi & Schibler, 2007; Lévi et al., 2010). Indeed, the ∼3-fold order of magnitude of intrapatient circadian variability in 5-fluorouracil catabolism by dihydropyrimidine dehydrogenase matched that of interpatient variability of the same drug (Gamelin et al., 2008; Harris et al., 1990). Moreover, synchronous 24-h patterns have been shown for both tolerability and efficacy of most anticancer agents, including 5-fluorouracil and oxaliplatin (Granda et al., 2002; Peters et al., 1987). As a result, the administration of chemotherapy outside the optimal timing window can result both in excessive toxicity and poor antitumor efficacy (Lévi et al., 2010).

The joint optimization of tolerability and efficacy through proper circadian timing of anticancer drugs is further supported by proliferation data in human bone marrow and tumor tissue (Abrahamsen et al., 1997; Smaaland et al., 1991, 1992b, 1993, 2002). This concept challenges the current oncology principle that optimal antitumor effects result from treatment near maximum tolerated dose (Frei & Canellos, 1980). Indeed, experimental evidence has now documented that wrongly timed and/or too highly dosed anticancer drugs disrupt circadian physiology and/or the molecular clocks that constitute the circadian timing system (Lévi et al., 2010). This hierarchical network generates ∼24-h (circadian) oscillations in cellular proliferation and metabolism (Lévi et al., 2007a, 2010; Lévi & Schibler, 2007; Liu et al., 2007; Sahar & Sassone-Corsi, 2009; Takahashi et al., 2008). Its disruption impairs the physiologic and molecular bases of treatment delivery according to circadian rhythms, so-called chronotherapy (Lévi et al., 2010).

In the current study, we investigated whether the relevance of chemotherapy-induced neutropenia for overall survival prediction also applied to patients treated with chronotherapy. Thus, in clinical oncology, toxicity of cancer chemotherapy is often positively associated with its efficacy (Frei & Canellos, 1980; Shitara et al., 2010a). This observation has given rise to the general oncologic practice of trying to treat each patient with therapy intensity titrated to near that individual's maximum tolerated dose. Generally positive results of this approach have led oncologists to the acceptance of the opinion that, in order to be effective, some level of cytotoxic drug toxicity must be accepted. However, chronotherapy work on both sides of the Atlantic done over several decades has repeatedly and convincingly shown that when cytotoxic drugs are administered at their optimum times of day in order to avoid or minimize drug toxicity, this expected relationship between toxicity and efficacy is altered. In particular, higher doses can often be given with lower toxicity and concomitant increased or equal efficacy (Hrushesky, 1985; Lévi et al., 1990, 1997). The large randomized controlled multicenter clinical trial analyzed here confirms this dissociation as a unique reality in clinical oncology (Giacchetti et al., 2006). Herein, specifically, we first attempt to validate the positive association between neutropenia and survival in an independent cohort of patients receiving

non–time-stipulated chemotherapy, with intrapatient dose escalation up to maximum tolerated dose. The hypothesis that chronotherapy achieves best efficacy if tolerability is good is explored here clinically, using a data set obtained in a multicenter, randomized, controlled, phase III trial. The study compared chronomodulated (chronoFLO4) versus conventional (FOLFOX2) flat infusion schedules combining oxaliplatin (l-OHP), 5-fluorouracil (5-FU), and leucovorin (LV), as first-line treatment for patients with metastatic colorectal cancer (EORTC 05963) (Giacchetti et al., 2006). Thus, in this trial, the patients received the same starting doses of the same drugs at the same frequency, though with pharmacologically different delivery schedules (Figure 1).
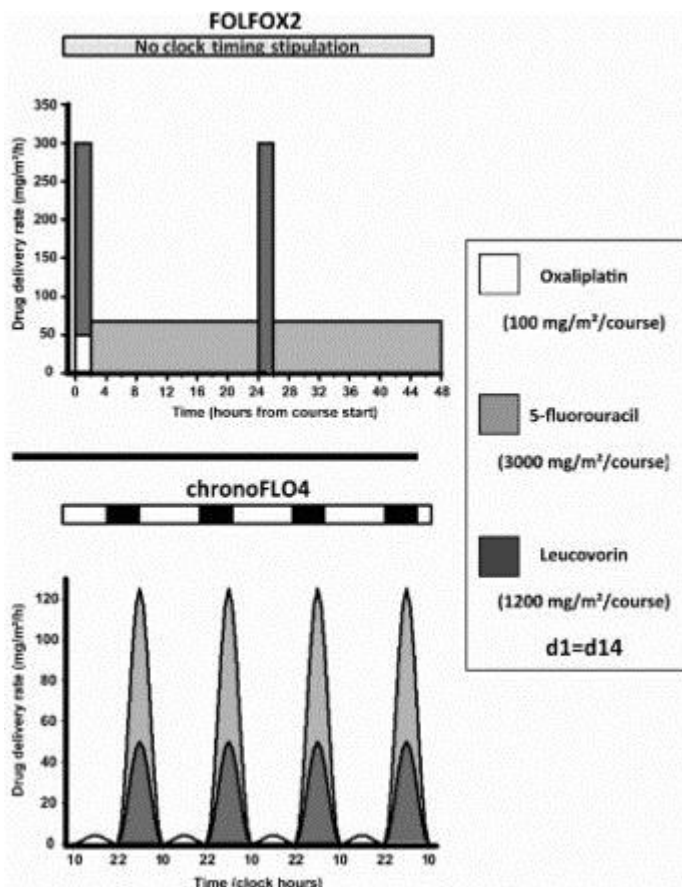
FIGURE 1. Chemotherapy delivery schedules. Top panel: conventional 2-day schedule FOLFOX2, with no stipulation of drug-administration time. Bottom panel: chronomodulated 4-day schedule chronoFLO4, with defined drug-administration time. Each schedule associated oxaliplatin (white), 5-fluorouracil (light gray), and leucovorin (dark gray) infusions were given fortnightly. 5-Fluorouracil dose was increased up to 20% if no Grade ≥2 toxicity occurred.

MATERIALS AND METHODS

Study Population

This study describes the results of an unplanned analysis of prospectively collected data from the international EORTC 05963 phase III trial, in previously untreated patients with metastatic colorectal cancer, randomized to receive either a conventional flat FOLFOX2 or a chronomodulated chronoFLO4 schedule (Giacchetti et al., 2006). From October 1998 to February 2002, this trial enrolled 564 patients from 36 institutions in 10 countries (Giacchetti et al., 2006). The study, conducted in accordance with the Helsinki Declaration, was approved by the respective national ethics review boards involved, and all patients provided a signed informed consent (Portaluppi et al., 2010). Admission criteria in this trial included histologically proven, previously untreated colorectal adenocarcinoma with measurable and surgically unresectable metastatic lesions outside the brain, written informed consent according to national laws, adequate hematologic, renal, and hepatic functions, performance status (PS) ≤ 2 (World Health Organization [WHO] scale), age between 18 and 76 yrs, and no concomitant other cancer nor overt and uncontrolled cardiac, respiratory, chronic, or infectious diseases (Giacchetti et al., 2006).

Chemotherapy Schedules

FOLFOX2 involved the administration of l-OHP and LV as 2-h flat (non-varying-in-time constant) infusions on day 1, followed by constant-rate infusional 5-FU for 22 h. LV was administered again on day 2 after 5-FU, and it was followed by constant-rate 5-FU infusion for 22 h (de Gramont et al., 1997; Giacchetti et al., 2006) (Figure 1, top panel).

ChronoFLO4 consisted of a 4-day course of alternating chronomodulated infusions of 5-FU–LV from 22:15 to 09:45 h, with a peak flow rate at 04:00 h, and l-OHP from 10:15 to 21:45 h, with a peak flow rate at 16:00 h (Giacchetti et al., 2006; Lévi et al., 1999) (Figure 1, bottom panel).

Courses were repeated in both arms every 14 days. Drug starting doses, 5-FU dose-escalation scheme, and drug-dose reductions followed the same guidelines in both arms. The starting doses per course were 100 mg/m2 for l-OHP, 1200 mg/m2 for LV, and 3000 mg/m2 for 5-FU. This latter dose could be increased by 400 mg/m2 on the second and by an additional 200 mg/m2 on the third course, if no Grade ≥ 2 toxicity occurred. Dose reductions (200 and 400 mg/m2/course for 5-FU and 10 and 20 mg/m2/course for l-OHP for Grades 3 and 4, respectively) were planned for patients experiencing clinical (diarrhea, stomatitis, hand-foot syndrome) and/or hematological (neutropenia, thrombocytopenia) toxicities. The subsequent course was delayed until every toxic event recovered to Grade 0 or 1 (National Cancer Institute Common Toxicity Criteria, version 2) (Giacchetti et al., 2006). Delivered dose intensities for l-OHP and 5-FU were calculated during the whole treatment duration as the actual total dose administered/m2/wk between day 1 of the first course until day 14 of the last course of chemotherapy received.

Neutropenia Evaluation

A complete hematologic assessment was performed fortnightly, prior to each chemotherapy administration. Neutropenia was graded after each course according to the Common Terminology Criteria

for Adverse Events, version 3.0. Each patient was classified into one of three categories according to the most severe grade (G) of neutropenia experienced throughout treatment: G0, none; G1–2, moderate; G3–4 or febrile neutropenia, severe. Primary prophylaxis with granulocyte-colony stimulating factors was not permitted.

Statistical Methods

The primary endpoint of this study was the association between neutropenia and overall survival, defined as the interval between the date of randomization and the date of death, due to any cause. Patients who were lost to follow-up (n = 12) before death were censored at the last contact date. The database was locked on November 9th, 2007; at this date, after a median follow-up of 87 mo (range: 68–108 mo), 507 events (89.9%) had occurred, and 45 patients were still alive. Given the rationale behind this study, the subsequent analyses were separately performed in each treatment arm. As preliminary analysis, the overall survival functions according to the severity of neutropenia were estimated and compared in the three groups of patients defined by the worst neutropenia experienced by each patient during follow-up. Kaplan-Meier's method and log-rank test were used for estimation and comparison, respectively. This approach was used simply for graphical representation but not for drawing conclusive results, since neutropenia was not a baseline feature and varied over time (Yamanaka et al., 2007). Therefore, the main analysis consisted of using Cox's regression models, including a time-dependent covariate (TDC) defined from the updated measurements of the chemotherapy-induced neutropenia. For each patient, the worst grade of neutropenia occurring between randomization and time T > 0 was defined as the value of the TDC at T (Shitara et al., 2009, 2010b; Yamanaka et al., 2007). The value of the variable for each individual patient, therefore, was not decreasing over time and could change over time according to the severity of neutropenia occurring by that time. The effect of the TDC on survival was adjusted for the following fixed covariates (Kohne et al., 2002; Sanoff et al., 2008): sex, age, PS at inclusion, number of metastatic sites, percentage of liver involvement with tumor, dose intensities of 5-FU and l-OHP, prior adjuvant chemotherapy, Dukes' stage at diagnosis, site of primary tumor, surgical resection of the primary tumor, prior surgery of metastases, baseline circulating leukocyte count, plasma alkaline phosphatase activity, and concentrations of carcinoembryonic antigen (CEA) and CA19.9.

The difference in the effect of worst neutropenia (as TDC) on overall survival between treatment arms was further explored by comparing the coefficients of the neutropenia variables obtained in the Cox models for each treatment arm. More precisely, the square of the difference between the natural logarithms of the hazard ratios (HRs) was divided by the sum of their variances; under the null hypothesis of equality between the HRs, the above statistic is asymptotically distributed as a chi-square with 1 degree of freedom.

Neutropenia incidence and severity per patient was calculated in each treatment arm, and these rates were compared with a two-sided chi-square test. Comparisons among the three categories of patients (absent, moderate, or severe neutropenia) were performed using nonparametric Mann-Whitney or Jonckheere-Terpstra tests for continuous measurements and Pearson's chi-square or Fisher's exact two-sided tests for proportions of categorical variables. The prognostic value of neutropenia categories on objective response rate (WHO criteria) was assessed using a binary logistic regression model. Univariate Cox modeling was used to assess the effect of worst neutropenia experienced (as TDC) on progression-free survival. In order to avoid possible selection and lead-time bias due to higher risk of developing neutropenia according to the increasing number of cycles of chemotherapy received (Yamanaka et al., 2007), further sensitivity analysis was performed with a landmark that restricted neutropenia data for patient categorization to the first eight

courses (Shitara et al., 2009). This analysis only included patients receiving at least eight treatment courses. The worst neutropenia encountered during the initial eight courses of chemotherapy was considered as a fixed covariate. Survival time was then computed from the completion of the eighth treatment course. Exploratory subgroup analyses were performed according to sex and baseline PS, in each treatment arm separately, to test the prognostic value of worst neutropenia (as TDC) on overall survival. Finally, associations between randomized treatment and progression-free survival, objective response rate, and overall survival were explored in the three categories of most severe neutropenia experienced (absent, moderate, or severe), using univariate Cox or binary logistic regressions. The threshold for statistical significance was set at $p < .05$.

All analyses were performed using PASW Statistics 18 software (SPSS, Chicago, IL, USA).

RESULTS

Neutropenia Incidence and Patient Features

Of the 564 patients randomized, 556 (98.6%) are included in the current analysis (279 in the FOLFOX2 arm and 277 in the chronoFLO4 arm). Six of the eight remaining patients did not start allocated treatment, whereas two received a single course without any available data on hematological toxicity. Nearly twice as many patients experienced neutropenia on FOLFOX2 (n = 168, 60.6%) than chronoFLO4 (n = 91, 32.6%) (p < .0001). Moderate (G1–2) or severe (G3–4) neutropenia occurred, respectively, in 119 (42.7%) and 69 (24.7%) patients on FOLFOX2 and in 91 (32.9%) and 18 (6.5%) patients on chronoFLO4 (p < .0001) (Figure 2A). The most severe episode of neutropenia occurred over the four initial courses for 20.2% of the patients, and over the eight initial ones for 61.6% of the patients. Only 7.4% of the patients displayed worst neutropenia after the 12th course (Figure 2B). Except for the very first course, moderate or severe neutropenia consistently occurred more frequently in patients on FOLFOX2 than chronoFLO4 (Figure 2B). Neutropenia occurred more frequently in females than males, both on FOLFOX2 (77.5% vs. 60.7%, respectively; p = .003) and on chronoFLO4 (46.5% vs. 34.4%, respectively; p = .04). Similarly, a trend toward a higher incidence of G3–4 neutropenia was found in women as compared to men, both on FOLFOX2 (30.6% vs. 20.8%; p = .06) and on chronoFLO4 (9.6% vs. 4.3%; p = .08). Irrespective of sex, neutropenia was more frequent and more severe in patients receiving FOLFOX2 than chronoFLO4 (p < .0001).
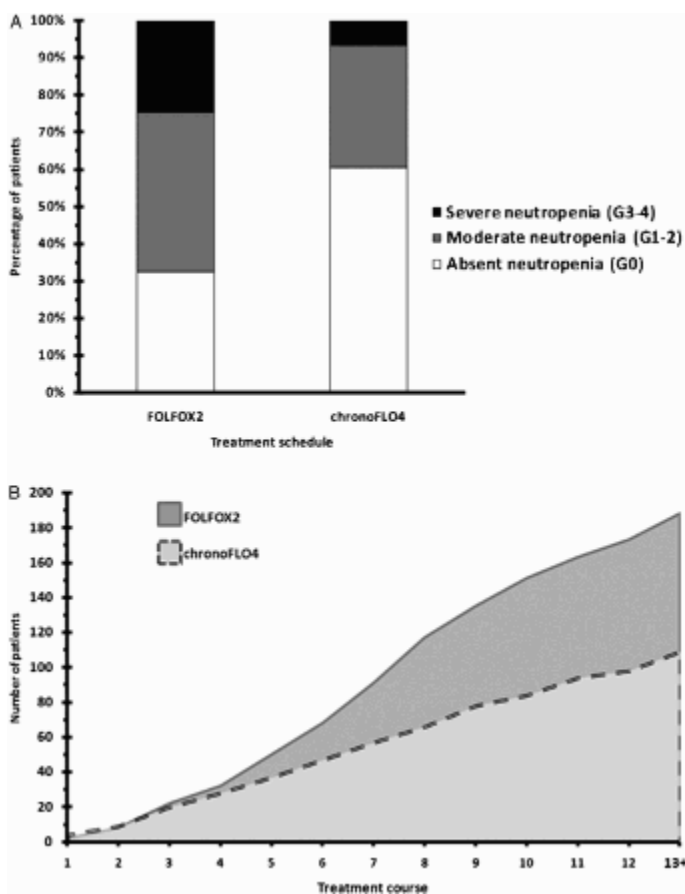
FIGURE 2. (A) Distribution of patients according to the most severe neutropenia experienced in each treatment arm. White boxes: no neutropenia (G0). Gray boxes: moderate neutropenia (G1–2). Black boxes: severe neutropenia (G3–4). (B) Treatment cycle corresponding to the occurrence of the first worst neutropenia episode, in each treatment arm. Continuous line: FOLFOX2. Dashed line: chronoFLO4.

Actual Dose Intensities

The dose intensities of l-OHP and 5-FU varied significantly according to neutropenia category in each treatment arm (p ≤ .011) (Table 1 and Figure 3). Two-by-two comparisons with a threshold p value of .001 are tagged in Figure 3. Moderate or severe neutropenia was associated with comparable dose intensities in both schedules (Table 1 and Figure 3). Conversely, in neutropenia-free patients, the actual dose intensities of l-OHP and 5-FU were lower on chronoFLO4 as compared with FOLFOX2 (p < .0001 and p = .004, respectively) (Table 1 and Figure 3).
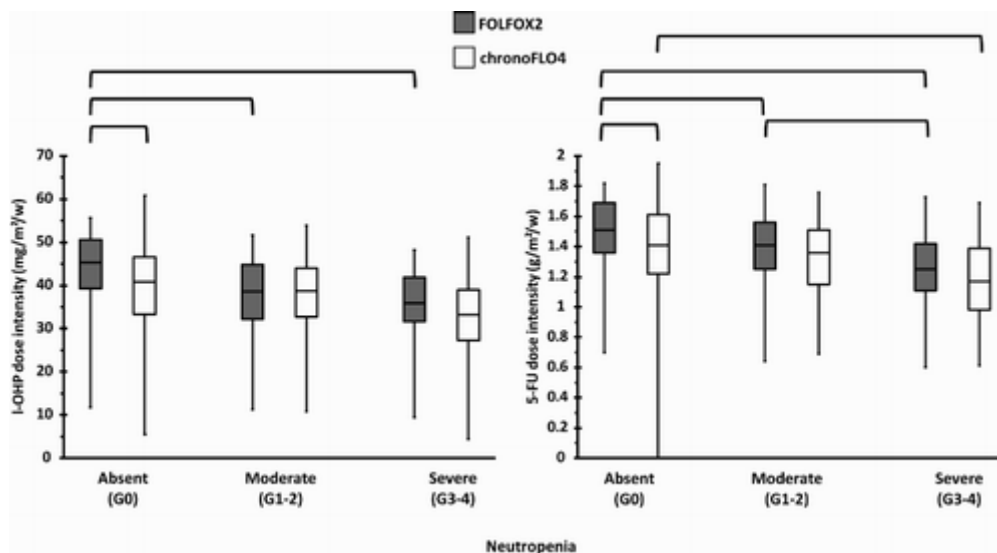
FIGURE 3. Boxplots representing the actual dose intensity of oxaliplatin (l-OHP, mg/m2/wk; left panel) and 5-fluorouracil (5-FU, g/m2/wk; right panel), according to the categories of worst neutropenia, in each treatment arm (FOLFOX2, grey boxes; chronoFLO4, white boxes). Boxes: 1st and 3rd quartiles; middle line: median; bars: range. Highly statistically significant (p ≤ .001) two-by-two differences are tagged.

TABLE 1. Patient characteristics

Characteristic		FOLFOX2				chronoFLO4			
		G0 (absent) (n = 91)	G1–2 (moderate) (n = 119)	G3–4 (severe) (n = 69)	All (n = 279)	G0 (absent) (n = 168)	G1–2 (moderate) (n = 91)	G3–4 (severe) (n = 18)	All (n = 277)
Age (yrs)	Median	62	62	64	62	62	62	60	62
	Range	33–76	32–76	37–75	32–76	24–76	22–76	37–71	22–76
Sex (%)	Female/Male	28/73	44/56	49/51	40/60	36/64	46/54	61/39	41/59

PS (%)	0/1/2	41/44/16	54/40/7	54/41/6	50/41/9	49/40/11	48/41/11	33/50/17	48/41/12
# metastatic sites (%)	1/2 / ≥ 3	45/35/20	53/31/16	54/30/16	51/32/17	48/36/17	58/36/6	50/33/17	51/36/13
% liver tumor involvement	None/≤ 25/25%	17/37/46	8/50/43	23/48/29	14/45/41	14/44/42	15/58/26	11/44/44	14/49/37
Prior adjuvant chemotherapy (%)	Yes	22	12	19	17	16	22	28	18
Synchronous metastases (%)	Yes	73	81	64	74	76	73	72	75
primary tumor Site (%)	Colon/rectum	79/21	76/24	71/29	76/24	79/21	72/28	78/22	77/23
Prior surgery: primary tumor (%)	Yes	86	87	84	86	89	88	89	89
Prior surgery: metastases (%)	Yes	3	7	4	5	5	3	17	5
Baseline WBC 10.0×10^9/L (%)		41	19	26	28	31	11	28	24
Baseline ALP (%)	≥300 IU/L	34	25	19	27	33	23	22	29
Baseline CEA (%)	≥10 ng/mL	66	70	62	67	68	57	78	65
Baseline CA19.9 (%)	≥37 IU/L	50	48	45	48	48	42	44	46
Cycles: total (%)	Median	8	12	11	10	9	11	8	10
	1st and 3rd quartiles	4–10	9–15	8–12	8–12	4–12	8–13	6–11	6–12
	Range	1–21	4–40	2–49	1–49	1–44	1–28	2–22	1–44
Dose intensity of oxaliplatin (mg/m^2/wk)	Median	45.3	38.6	35.9	40.1	40.8	38.8	33.2	39.4
	1st and 3rd quartiles	39.3–50.6	32.2–44.8	31.6–41.9	33.8–46.4	33.3–46.6	32.7–44.0	27.3–38.9	32.5–44.7
	Range	11.7–55.7	11.2–51.7	9.4–48.3	9.4–55.7	5.5–60.9	10.8–53.9	4.4–51.2	4.4–60.9
Dose intensity of 5-fluorouracil (g/m^2/wk)	Median	1.51	1.41	1.25	1.41	1.41	1.36	1.17	1.38
	1st and 3rd quartiles	1.36–1.69	1.25–1.56	1.11–1.42	1.24–1.60	1.22–1.61	1.15–1.51	0.98–1.39	1.19–1.56
	Range	0.70–1.82	0.64–1.81	0.60–1.73	0.60–1.82	0–1.95	0.69–1.76	0.61–1.69	0–1.95
Objective response rate (%)	Value	16.5	60.5	56.5	45.2	36.3	58.2	44.4	44.0
	95% CI	8.2–25.8	52.2–69.8	44.7–69.3	38.7–51.3	29.7–42.3	27.2–48.8	20.9–67.1	37.7–50.3
Median time to progression (mo)	Value	4.5	9.7	9.7	8.5	7.7	9.3	9.2	8.3
	95% CI	2.9–6.1	8.5–10.9	8.6–10.8	7.8–9.3	6.8–8.7	7.8–10.8	4.5–13.9	7.4–9.`2
Incidence of other G3–4 toxicities*	% of patients	55.6	75.6	70.1	67.8	68.3	75.6	82.4	71.5

Patient characteristics in each treatment arm according to the categories of the worst neutropenia experienced.

PS = performance status (WHO scale); WBC = white blood cells; ALP = alkaline phosphatases;
IU = international units.
*Excluding leukopenia and neutropenia

Prognostic Value of Neutropenia on Objective Response and Progression-Free Survival

Objective response rate varied significantly according to the severity of the worst neutropenia in each treatment arm. Thus, response rate was lowest in the neutropenia-free patients than the neutropenic ones, both on FOLFOX2 (p < .0001) and on chronoFLO4 (p = .004) (Table 1). The occurrence of neutropenia on FOLFOX2 was significantly associated with longer progression-free survival (Table 1) (p = .034). Conversely, an opposite trend was noted for chronoFLO4 (p = .061), with patients experiencing severe neutropenia displaying a higher risk of earlier progression than neutropenia-free patients (HR: 1.71; 95% confidence interval [CI]: 1.08–2.71; p = .023).

Prognostic Value of Neutropenia on Overall Survival

Median (95% CI) overall survival was 18.5 (17.0–19.9) mo in the FOLFOX2 arm and 19.3 (18.1–20.6) mo in the chronoFLO4 arm.

Preliminary analysis showed that overall survival functions varied significantly according to neutropenia category in the FOLFOX2 arm (p < .0001), but not in the chronoFLO4 arm (p = .36) (Figure 4). Overall survival was similar in patients receiving FOLFOX2 with moderate or severe neutropenia (Figure 4 and Table 2).

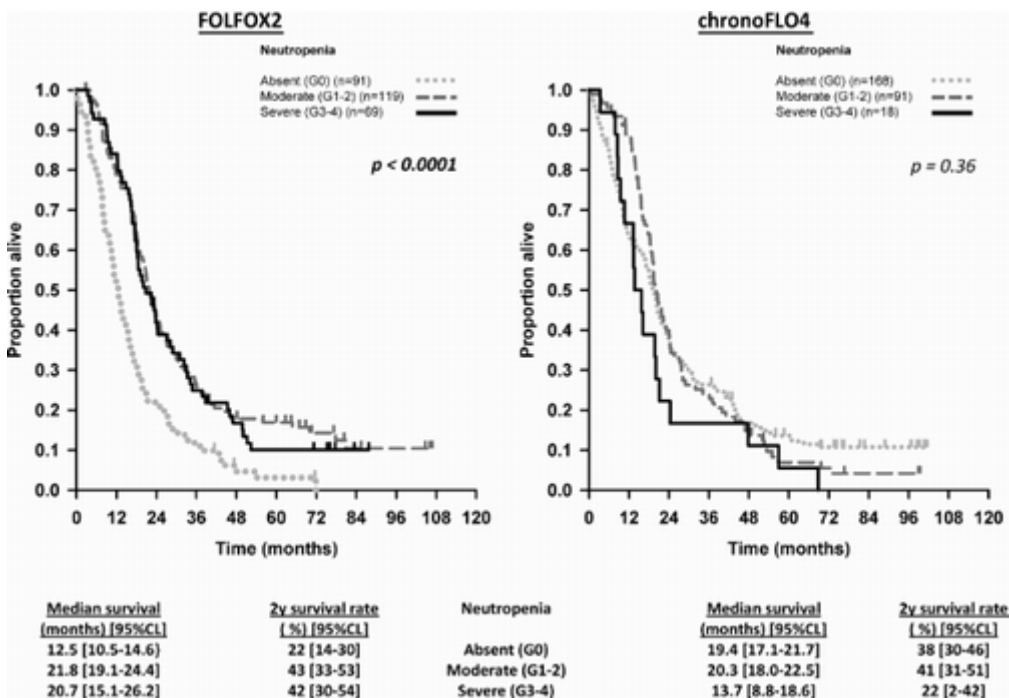| FOLFOX2		Neutropenia	chronoFLO4	
Median survival (months) [95%CL]	2y survival rate [%] [95%CL]		Median survival (months) [95%CL]	2y survival rate [%] [95%CL]
12.5 [10.5-14.6]	22 [14-30]	Absent (G0)	19.4 [17.1-21.7]	38 [30-46]
21.8 [19.1-24.4]	43 [33-53]	Moderate (G1-2)	20.3 [18.0-22.5]	41 [31-51]
20.7 [15.1-26.2]	42 [30-54]	Severe (G3-4)	13.7 [8.8-18.6]	22 [2-42]

FIGURE 4.   Overall survival curves in each treatment arm (FOLFOX2, left panel; chronoFLO4, right panel) according to the occurrence of the most severe neutropenia. Dotted light gray lines: no neutropenia (G0). Dashed darker gray lines: moderate neutropenia (G1–2). Solid black lines: severe neutropenia (G3–4). p values derived from log-rank test. This represents the graphical display of preliminary analyses.

TABLE 2.   Cox proportional hazard models for overall survival

Treatment schedule		FOLFOX2				ChronoFLO4			
Parameter		Univariate		Multivariate with TDC		Univariate		Multivariate with TDC	
		HR	p	HR	p	HR	p	HR	p
Neutropenia (TDC)	Absent (G0)	1	<0.0001	1	0.04	1	0.025	1	0.07
	Moderate (G2-3)	0.58 [0.43-0.78]		0.67 [0.44-1.00]		1.17 [0.89-1.54]		1.36 [0.96-1.92]	
	Severe (G3-4)	0.57 [0.40-0.79]		0.56 [0.35-0.90]		1.86 [1.18-2.92]		1.77 [0.98-3.20]	
Sex	Female	1	0.065	1	0.32	1	<0.0001	1	0.07
	Male	1.28 [0.99-1.65]		1.20 [0.84-1.71]		0.63 [0.49-0.81]		0.73 [0.52-1.03]	
Baseline PS	0	1	<0.0001	1	0.009	1	<0.0001	1	0.43
	1	1.56 [1.20-2.03]		1.13 [0.79-1.61]		1.70 [1.30-2.23]		1.29 [0.87-1.91]	
	2	4.80 [3.07-7.51]		2.58 [1.40-4.74]		1.63 [1.09-2.46]		1.23 [0.72-2.12]	
Number of metastatic sites	1	1	<0.0001	1	0.009	1	0.008	1	0.007
	2	1.87 [1.41-2.48]		1.46 [1.03-2.07]		1.47 [1.12-1.92]		1.73 [1.22-2.44]	
	≥3	2.18 [1.54-3.08]		1.96 [1.19-3.22]		1.56 [1.06-2.28]		1.50 [0.87-2.59]	
Percentage of liver involvement by tumor	None	1	0.049	1	0.35	1	0.006	1	0.23
	≤25%	0.93 [0.64-1.35]		0.68 [0.40-1.15]		0.80 [0.55-1.16]		0.67 [0.38-1.17]	
	>25%	1.29 [0.89-1.89]		0.73 [0.41-1.29]		1.24 [0.85-1.83]		0.84 [0.46-1.54]	
Surgery of primary tumor	Yes	0.66 [0.46-0.93]	0.017	0.75 [0.48-1.18]	0.22	0.86 [0.58-1.26]	0.43	1.15 [0.70-1.87]	0.59
Baseline WBC	≥10.0 × 10⁹/L	1.86 [1.42-2.45]	<0.0001	1.82 [1.24-2.68]	0.002	1.45 [1.09-1.93]	0.037	0.93 [0.61-1.43]	0.74
Baseline ALP	≥300 IU/L	1.91 [1.44-2.53]	<0.0001	1.66 [1.11-2.49]	0.014	1.64 [1.24-2.16]	0.001	1.33 [0.91-1.97]	0.15
Baseline CA19.9	≥37 IU/L	1.71 [1.24-2.36]	0.001	1.46 [0.99-2.13]	0.055	1.94 [1.41-2.67]	<0.0001	1.51 [1.05-2.18]	0.027
Dose intensity of oxaliplatin (mg/m²/wk)	Quantitative	1.05 [1.03-1.06]	<0.0001	1.05 [1.02-1.08]	0.002	1.04 [1.02-1.05]	<0.0001	1.07 [1.04-1.11]	<0.0001
Dose intensity of 5-fluorouracil (g/m²/wk)	Quantitative	3.10 [1.81-5.29]	<0.0001	0.36 [0.13-1.05]	0.06	1.56 [0.94-2.59]	0.083	0.34 [0.12-1.01]	0.052

Univariate and multivariate Cox proportional hazard models for overall survival in each treatment arm. Neutropenia is a time-dependent covariate (TDC). Other adjustment factors, which were never significant, included age, prior adjuvant chemotherapy, Dukes' stage at diagnosis, site of primary tumor, prior surgery of metastases, and baseline CEA. HR = hazard ratio [95% confidence interval]; PS = performance status (WHO scale); WBC = white blood cells; ALP = alkaline phosphatases; IU: international units.

Cox proportional hazard modeling with neutropenia as the time-dependent covariate (TDC) confirmed that the decreased risk of an earlier death conferred by occurrence of neutropenia (moderate or severe) on FOLFOX2 remained statistically significant following adjustment for other known prognostic factors (p = .04) (Table 2). On the contrary, although the risk of earlier death associated with the occurrence neutropenia (as TDC) on chronoFLO4 significantly increased when this toxicity arose (p = .025), multivariate Cox proportional hazard modeling found no significant prognostic value for neutropenia on this treatment modality, even though a consistently opposite trend to that observed for FOLFOX2 was found (p = .07) (Table 2), with patients with severe neutropenia on chronoFLO4 displaying poorest survival. Thus, the occurrence of severe neutropenia was independently associated with survival, yet with an opposite relative risk of an earlier death, according to treatment modality: a significantly lower risk on FOLFOX2 (HR: 0.56; 95% CI: 0.35–0.90; p = .015) and a nonsignificant trend toward a higher risk on chronoFLO4 (HR: 1.77; 95% CI: 0.98–3.20; p = .06). Indeed, highly significant difference in the effect of the occurrence of neutropenia between treatment arms was observed concerning both moderate and severe neutropenia (all p < .001). These results remained similar if the worst neutropenia observed for each patient was considered as a simple baseline prognostic covariate (not shown).

Subgroup analyses according to sex or baseline PS confirmed the better prognosis in patients with moderate or severe neutropenia on FOLFOX2 in both women and men and in each PS subgroup (p ≤ .18; not shown). Conversely, patients with moderate or severe neutropenia on chronoFLO4 tended to display an

increased risk of earlier death in each subgroup analyzed, although never with statistical significance (p ≥ .09; not shown). Thus, the opposite trend of the association between neutropenia and overall survival according to the pharmacological schedule found in the whole cohort was recapitulated in every subgroup assessed.

The landmark analysis limited neutropenia data to the first eight courses, and included the 404 patients who received at least eight treatment courses (212 patients on FOLFOX2 and 192 patients on chronoFLO4). Thus, the relative proportion of patients with moderate or severe neutropenia remained significantly higher on FOLFOX2 than chronoFLO4 (p < .0001). Furthermore, the survival of neutropenia-free patients was significantly shorter than that of patients with moderate or severe neutropenia on FOLFOX2 (p = .051). Conversely, neutropenia-free patients lived longer than the neutropenic ones on chronoFLO4 (p = .002). In this landmark group as well, the interaction between the randomized treatment schedule and worst neutropenia grade significantly influenced overall survival (p = .005); this association persisted independently from the other prognostic factors in a multivariate analysis (p = .001). Further exploratory analyses showed that the chances of obtaining a better outcome, i.e., higher objective response rate, longer progression-free survival, and longer survival, were significantly increased in neutropenia-free patients receiving chronoFLO4 and in severely neutropenic patients treated with FOLFOX2 (Figure 5).
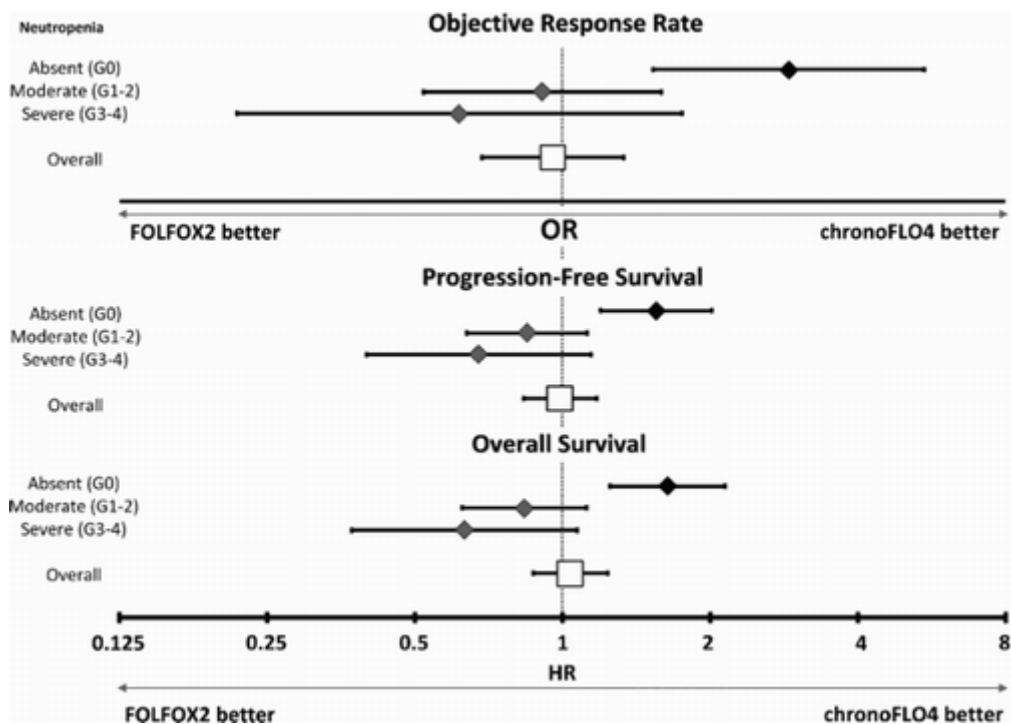

FIGURE 5.   Forrest plot of the effect of treatment in each subgroup of patients defined by the most severe neutropenia developed. OR = odds ratio; HR = hazard ratio.

DISCUSSION

This international study confirmed, in an independent cohort of patients, that the occurrence of chemotherapy-induced neutropenia is associated with improved survival. This was here the case for patients receiving conventional FOLFOX for previously untreated metastatic colorectal cancer (Shitara et al., 2009). The clinical trial design involved higher than usual starting doses and intrapatient dose escalation so as to administer the maximum tolerated dose in each individual patient (Colucci et al., 2005; Cunningham et al., 2009; de Gramont et al., 2000; Giacchetti et al., 2006, 2000; Maindrault-Goebel et al., 2001). The timing of drug administration was not stipulated and varied both among patients and between courses for FOLFOX2 (Giacchetti et al., 2006; Innominato et al., 2010; Lévi et al., 2010). Conversely, chronoFLO4 delivered the three same drugs at defined times so as to take into account the circadian timing system (Giacchetti, 2002; Hrushesky & Bjarnason, 1993; Innominato et al., 2010; Lévi, 2002; Lévi & Schibler, 2007; Lévi et al., 2007b, 2010). This approach was based on data showing that predictable 24-h changes characterize the detoxification of anticancer drugs, as well as tissue-specific replication rates, especially in human bone marrow (Abrahamsen et al., 1997; Bjarnason & Jordan, 2002; Bjarnason et al., 1999, 2001; Buchi et al., 1991; Harris et al., 1990; Lévi & Schibler, 2007; Lévi et al., 2010; Smaaland et al., 1991, 1992a, 2002).

Circadian rhythms in therapeutic effects are generated by molecular clocks residing within each cell and coordinated by a hypothalamic pacemaker (Dibner et al., 2010; Lévi & Schibler, 2007; Lévi et al., 2010; Paschos et al., 2010). Wrongly timed or excessively dosed cancer medications, in turn, profoundly disrupt circadian physiology and molecular clocks in experimental models (Lévi et al., 2010). This results in severe alterations of the circadian coordination of both drug-detoxification pathways and cell-cycle gating. This circadian disruption interferes with the beneficial control exerted by the circadian timing system on tumor progression, and can concurrently hamper both treatment tolerability and anticancer efficacy (Filipski et al., 2002, 2004, 2005; Lévi et al., 2010). Indeed, robust circadian host physiology predicted for prolonged survival, independently of other known prognostic factors in patients with breast or colorectal metastatic cancer (Innominato et al., 2009; Mormont et al., 2000; Sephton et al., 2000). Furthermore, this concept contrasts chronotherapeutics with the principle that governs conventional cancer treatments: the larger the treatment toxicity, the better the antitumor efficacy (Frei & Canellos, 1980; Shitara et al., 2010a). However, 2 studies out of 13 reported no positive association between the occurrence of Grade 3–4 leukoneutropenia and survival (Kim et al., 2010; Miyoshi et al., 2009; Shitara et al., 2010a). In our and most other prior studies, no difference in survival has been found between patients with moderate or severe neutropenia receiving non–time-stipulated chemotherapy (Figure 4) (Shitara et al., 2010a). These findings suggest that in comparing severe to absent or moderate neutropenia, one could blunt existing survival differences, mostly related to the occurrence and not the severity of neutropenia (Figure 4) (Shitara et al., 2010a). The current study provides the first clinical evidence of a substantial difference between non–time-stipulated and circadian-based chemotherapy for the toxicity-efficacy relationship; despite the same drug doses and course frequency being used, the overall observed outcomes were comparable (Giacchetti et al., 2006). Neutropenic patients on FOLFOX2 displayed best efficacy outcomes independently of other known prognostic factors, in accordance with prior reports (Shitara et al., 2009, 2010a; Yamanaka et al., 2007). Conversely, the occurrence of neutropenia with chronoFLO4 was not associated with any improvement in survival, and was even associated with impaired efficacy outcomes. Despite the low incidence of Grade 3 or 4 hematologic toxicity on chronoFLO4 (6.5%; Figure 2, Table 1), the negative trend that characterized the relationship between neutropenia and survival was almost statistically significant ($p = .07$; Table 2).

Moreover, the earlier the occurrence of neutropenia on chronoFLO4, the poorer the survival on this schedule, according to the sensitivity analyses in the landmark group (p = .002). The present report indicates that wrongly timed and/or excessively dosed chronomodulated treatments could simultaneously produce undue toxicity and achieve poor efficacy, in good agreement with experimental data (Lévi et al., 2010). Thus, the opposite relationships between hematologic toxicity and efficacy outcomes according to chronomodulated or conventional drug delivery likely explain the observed similar overall survival of patients on either schedule (Giacchetti et al., 2006). Indeed, a specific feature of this trial was that chemotherapy doses were escalated from one course to the next until reaching the maximum tolerated dose for each individual patient (Giacchetti et al., 2006). The occurrence of neutropenia resulted in comparable reductions in the actual dose intensities of l-OHP and 5-FU in both treatment schedules (Figure 3, Table 1). However, drug doses were reduced also in case of severe clinical toxicities that were slightly more frequent in patients on chronoFLO4 (Table 1). No relationship was found between any efficacy endpoint and diarrhea on either delivery schedule, even though this symptom was the main dose-limiting toxicity on chronoFLO4 (Giacchetti et al., 2006) (data not shown). This finding suggests that bone marrow toxicity could induce a peculiar effect on the circadian timing system, possibly through the release of proinflammatory cytokines with circadian disruptive effects. Thus, elevated serum levels of transforming growth factor-α (TGF-α), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were associated with circadian disruption and poor survival in patients with metastatic colorectal cancer (Rich et al., 2005).

Altogether, female patients were more likely than men to experience neutropenia (Table 1), in agreement with prior reports (Chansky et al., 2005; Diasio & Lu, 1994; Schwab et al., 2008). Each patient on chronoFLO4 received the same chronomodulated schedule with respective peak times of l-OHP and 5-FU-LV delivery rates at 16:00 h and at 04:00 h, selected according to preclinical data from male mice, human tissue proliferation studies, and early clinical trials (Abrahamsen et al., 1997; Bjarnason & Jordan, 2002; Bjarnason et al., 1999, 2001; Buchi et al., 1991; Giacchetti, 2002; Giacchetti et al., 2006; Lévi et al., 2010; Smaaland et al., 1991, 1992a, 2002). A phase I–II clinical trial confirmed the better tolerability of the current chronoFLO4 in male patients, through exploring schedules with peak times of drug delivery staggered by ±3, ±6, ±9, or ±12 h in separate cohorts of patients with metastatic colorectal cancer (Lévi et al., 2007b). In female patients, however, a large interpatient variability in optimal timing was apparent, with an ~6-h phase advance of optimal circadian timing for the peak delivery rates of l-OHP and 5-FU–LV (Lévi et al., 2007b).

CONCLUSION

In conclusion, we confirm here the principle that is currently advocated to guide non–circadian-based chemotherapy, i.e., lack of any neutropenia reflects insufficient drug dosing and, therefore, impaired efficacy. We show, however, that this principle does not apply to circadian-based delivery. Furthermore, severe neutropenia appears to be detrimental to the efficacy of chronomodulated chemotherapy. These results support the policy of dose escalation until maximum tolerated dose is reached in each patient for non–circadian-based chemotherapy (Frei & Canellos, 1980). Conversely, chronomodulated chemotherapy provides a novel treatment paradigm, where good tolerability is required for achieving optimal efficacy (Bross et al., 1966). Cancer chronotherapeutics has thus entered a new era: the current main goal consists of the joint improvement of both the safety and efficacy of any chemotherapy regimen, through the adjustment of drug doses and circadian delivery patterns according to sex and other individual patient

characteristics. Obviously, this novel strategy of chronopharmacological refinement and personalization requires prospective clinical validation.

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