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A RANDOMIZED MULTICENTER STUDY OF OPTIMAL CIRCADIAN TIME OF VINORELBINE COMBINED WITH CHRONOMODULATED 5-FLUOROURACIL IN PRETREATED METASTATIC BREAST CANCER PATIENTS: EORTC TRIAL 05971

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Studies in animals synchronized with an alternation of 12 h of light and 12 h of darkness have showed that hematological and systemic toxicities could be reduced if vinorelbine were administered 19 or 23 hours after light onset (HALO), corresponding to 17:00 and 21:00 h in diurnally active humans. This trial aimed to define the least toxic time of vinorelbine administration in metastatic breast cancer patients. Initially, the study treatment consisted of three courses of vinorelbine of 30 mg/m^2/d on D1 and D6 and chronomodulated 5-fluorouracil of 850 mg/m^2 from D2 to D5 every 21 days. Ninety metastatic breast cancer patients were randomized to receive vinorelbine at one of the eight possible dosing times. Further to the recommendations of the Independent Data Monitoring Committee, the vinorelbine dose was reduced to 25 mg/m^2/d midway

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through the study. The primary objective of the study was detection of the least toxic time based on the incidence of grade 3–4 (G3–4) neutropenia. To show a significant result, the 90% confidence interval width of the least toxic time had to be < 6 h. The least toxic time detection based on the incidence of other toxicities was also analyzed. The time of least drug toxic was estimated using a logistic regression model assuming that the logit transformation of the toxicity rate follows a sinusoidal distribution over 24 h. The bootstrap technique was used to obtain the 90% confidence interval. The least toxic time of G3–4 neutropenia was observed at 21:00 h with a non-significant 90% CI. Secondary endpoint analyses indicated the least toxic time could differ when based on other toxicity parameters (e.g., a significant least toxic time of 17:00 h was observed for G3–4 leucopenia), in agreement with animal data. The least toxic time of 10:30 h was estimated for any G3–4 gastrointestinal toxicity. This results of this study do not allow us to recommend an optimal time for vinorelbine administration. It has highlighted, however, the inherent methodological difficulties in the conduct of such a trial in the human setting. It indicates that future optimal time-finding trials should have tolerability and/or activity as the primary endpoint in place of a particular toxicity. The randomized optimal time-finding design may be used to identify the best time of chemotherapy administration. (Author correspondence: bcoudert@dijon.fnclcc.fr)

Keywords Chronotherapy, Vinorelbine, Circadian, Randomized phase II, Breast carcinoma

INTRODUCTION

Metastatic breast cancer (MBC) remains an incurable disease, even if new hormonal, cytotoxic, or targeted therapies improve its outcome. Therapeutic strategies aim at decreasing symptoms, improving quality of life, and prolonging survival (Carlson et al., 2000). The anthracycline-taxane or taxane-antimetabolite combinations remain the most widely used first-line polychemotherapies in MBC (Kataja et al., 2005). However, most patients who receive such medications do so in the adjuvant setting, requiring the development of new combinations with optimal delivery schedules.

The association of vinorelbine with 5-fluorouracil (5-FU) can achieve a 62% objective response rate in MBC, with a median duration of response of 12 months and a median survival of 23 months. Grade 3–4 neutropenia and febrile neutropenia occur in 90% and 8% of patients, respectively. Other toxicities are leucopenia (99.9%; G3–4, 77.8%), thrombocytopenia (19%; G3–4, 5%), mucositis (71%; G3–4, 38%), infection (12.7%), and constipation (9.5%) (Dieras et al., 1996).

Chronotherapeutics aims to determine the most active and least toxic treatment schedule, based on the adjustment of drug delivery to the 24 h rhythms generated by the circadian timing system (Hastings et al., 2003; Mormont & Lévi, 2003). Mammalian cells contain a molecular clock with fine-tuned feedback loops of 12 specific genes (Hastings et al., 2003). This molecular clock regulates cell division cycle, apoptosis, gene expression,

and DNA repair, plus several signaling and metabolic pathways involved in tumor progression and drug sensitivity (Filipski et al., 2003, 2005; Fu & Lee, 2003; Gorbacheva et al., 2005; Granda et al., 2005; You et al., 2005). As a result, anticancer chemotherapy at specific circadian times can reduce the toxicity of host cells and enhance efficacy against cancer cells (Hastings et al., 2003; Mormont et al., 2003). About 30 anticancer drugs have been tested in mice or rats and later found to be better tolerated in cancer patients if chronomodulated according to the circadian system of the patient (Focan, 2002; Lévi, 2001; Lis et al., 2003).

The EORTC Chronotherapy Study Group has developed a chronomodulated 5-FU delivery scheme for the treatment of patients with metastatic colon cancer. The optimal period of 5-FU-infusion with low toxicity and better efficacy was found to occur between 22:00 and 10:00 h, with a peak delivery rate at 04:00 h (Lévi et al., 1992, 1994, 1997).

Experiments involving nearly 900 mice, synchronized with an alternation of 12 h of light and 12 h of darkness, have demonstrated that hematological and systemic toxicities (body weight loss, lethal toxicity) were halved when vinorelbine was administered at 19 hours after light onset (HALO) or at 23 HALO (Tampellini et al., 1995). These circadian times coincide with the second half of the activity span in relation to the 24 h rest-activity cycle, and would respectively correspond to 17:00 and 21:00 h in humans. The most toxic times occurred in the second half of the rest span (7 or 11 HALO), corresponding respectively to 04:00 and 08:00 h in humans. The administration of vinorelbine at its least toxic dosing time (19 HALO) safely increased its tolerable dose in mice-bearing P388 leukemia and significantly improved survival, as compared to drug administration at 7 or 11 HALO. Cure rate was increased five-fold by administering vinorelbine at 19 HALO (Filipski et al., 1999). Moreover, it has also been demonstrated that vinorelbine can suppress the rest-activity and temperature rhythms of the animals (Li et al., 2002).

The aim of EORTC trial 05971 was to define in humans the least toxic time (LTT) of vinorelbine administration, using a regimen in which this medication was combined with chronomodulated 5-FU in MBC patients. The main objective was the LTT detection based on the incidence of G 3–4 neutropenia per patient over three courses of treatment. LTT detection based on the incidence of other toxicities was also analyzed.

PATIENTS AND METHODS

Candidates for this study were women presenting with histologically proven MBC. Eligible patients had a WHO performance status of two or better; prior administration of at least one line of chemotherapy, either for MBC or as adjuvant/neoadjuvant treatment within the last year, with a minimum of 4 wks between the last sequence of chemotherapy or

radiotherapy and inclusion in the study; and adequate hematological, renal, and hepatic function (absolute neutrophil counts [PNN] $\geq 1.5.10^9$/L, platelet count $\geq 100.10^9$/l, serum creatinine ≤ 140 μmol/l, ASAT, ALAT, and total bilirubin $\leq 2.5 \times$ upper limit of normal range). Prior high-dose chemotherapy, prior radiation therapy except to the primary tumor, axillary or mammary chain treatment, concurrent anti-tumor or steroid therapy, pregnancy or nursing, and biology contraindicating chemotherapy were study exclusion criteria. Patients diagnosed with patent cardiac insufficiency, ischemic disease, cerebral metastases, bowel obstruction, bronchoconstriction outside pulmonary lymphangitis, or any serious chronic disease were also ineligible to enter the study. All patients provided written informed consent according to GCP, and the experimental protocol conforms to international ethical standards (Touitou et al., 2006).

Treatment consisted of three chemotherapy courses, each comprising six administration days every 3 wks. The length of the treatment administration was six days because vinorelbine and 5-FU could not be administered concomitantly through the same catheter because of precipitation of the drug. Vinorelbine had to be administered alone, before and after 5-FU, on day l and on day 6 as a 20 min venous infusion at a randomized time in a fixed dose of 30 mg/m^2/d (later reduced to 25 mg/m^2/d by the independent data monitoring committee or IDMC), diluted in 30 ml of physiological serum (see Figure 1). Therefore, in order to keep the 5-FU dose intensity similar to that of the study by Dieras et al. (1996), 5-FU was chronomodulated as a sinusoidally modulated delivery infusion rate from 22:00 to 10:00 h, with the maximum rate at 04:00 h, from day 1 through day 5 at a fixed dose of 850 mg/m^2/d.

Dose reduction was made in the case of occurrence of G3 constipation; G1–2 neuropathy; G3 leucopenia, neutropenia, or thrombocytopenia

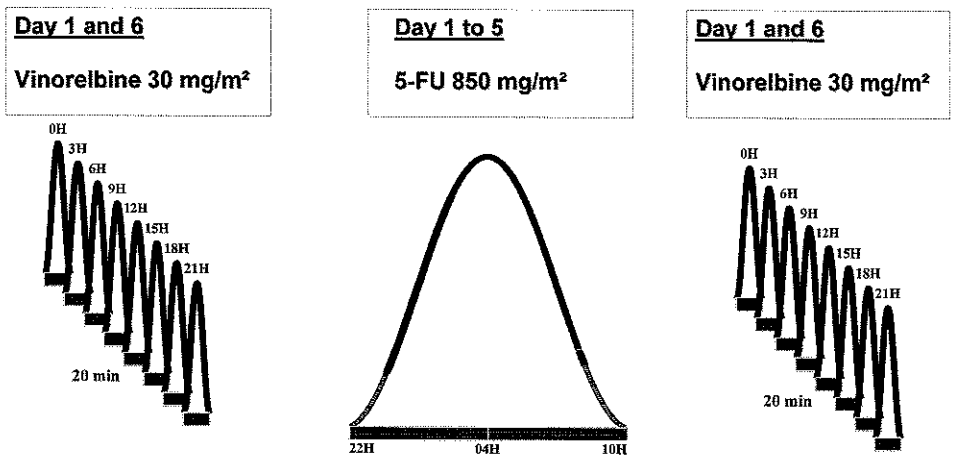


FIGURE 1 Administration scheme of vinorelbine and 5-FU.

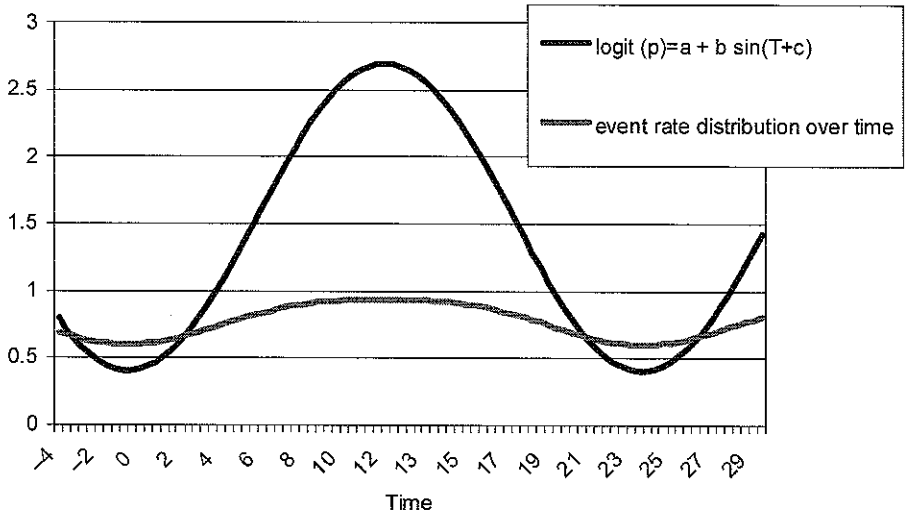
lasting seven days or more; G4 leucopenia; G4 neutropenia; G4 thrombocytopenia; G4 febrile neutropenia; G3–4 mucositis or diarrhea; G3–4 vomiting (if uncontrolled with setrons); or for any other G3–4 toxic symptom. The first dose reduction level was 5-FU to 650 mg/m^2/d and vinorelbine to 25 mg/m^2/d. If needed, a second level of dose reduction was 5-FU to 500 mg/m^2/d and vinorelbine to 20 mg/m^2/d.

Toxicity and adverse events were recorded according to the NCIC-CTC v2.0. In addition to baseline, blood samples for hematological counts were taken on days 8, 11, 14, 17, and 20 ($\pm$1 day) of each 21-day course. Other examinations were completed before the initiation of each course and 21 days after the last course. An independent data monitoring committee (IDMC) was planned per protocol to carefully monitor toxicity and development of the study.

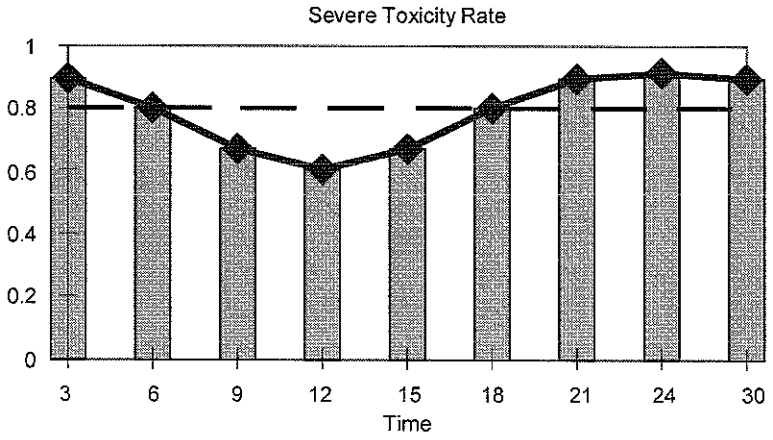
Statistical Considerations

A statistical design was especially developed to address the aim of this study. The number of patients to be randomized was calculated based on the assumption that the overall average rate of G3–4 neutropenia over three cycles was 80% but could be reduced to 60% when vinorelbine is administered at the optimal time, assuming that the toxicity rate induced by the vinorelbine administration follows a 24 h rhythm with the maximum and minimum 12 h apart. It was shown through simulations that a minimum of 80 patients (ten eligible patients in eight groups, with drug delivery times staggered by 3 h over the 24 h) were required to estimate the LTT with an associated 90% confidence interval (CI) of $<$6 h. Patients were, therefore, randomly assigned to receive the vinorelbine administration at one of the eight possible dosing times: 00:00, 03:00, 06:00, 09:00, 12:00, 15:00, 18:00, or 21:00 h. The randomization between arms was done using the minimization technique. Patients were stratified by institution and the number of previous chemotherapy regimens (second- vs. third-line treatment).

The statistical analysis was performed with SAS (version 9). The LTT was estimated using a logistic regression model assuming that the logit transformation of the toxicity rate follows a sinusoidal distribution over time (i.e., over the eight different arms; see Figure 2). The associated 90% CIs were obtained by the bootstrap technique (Baron et al., 2001). A 90% CI $<$ 6 h width for the LTT based on G3–4 neutropenia on three cycles was considered statistically and clinically significant. It would imply that vinorelbine administered at the optimal time would be worth implementing in clinical practice. The 90% CIs for the LTT based on other toxicities were also computed. To take into account the change in vinorelbine dose midway through the study, logistic regression analyses were performed separately in each of the two dose groups



(a)



(b)

FIGURE 2 A: The logistic regression is used to fit the sinusoidal curve joining the "logit" of the event rates of the K arms. The statistical model corresponds to Logit (P) = a + b sin (T + c). The optimal time corresponds to the time where the minimum or maximum is observed on this sinusoidal curve relative to the 24 h scale. B: Example theoretical event rate for Grade 3–4 neutropenia (for example) and the estimated shape using a logistic regression model. The time T = 12, which corresponds to the least toxic time, is the parameter to be estimated such that an associated 90% confidence interval has a range of <6 h (Baron et al., 2001).

(vinorelbine 30 and vinorelbine 25 groups; patients were analyzed in the dose group corresponding to the dose they actually received on day 1 of cycle 1). In addition, all patients together were analyzed using logistic regression stratified by vinorelbine dose. The latter was considered the main analysis.

RESULTS

From January 1999 to June 2004, 90 MBC patients were entered. Ten more patients than scheduled were randomized in order to have a minimum of ten patients in each of the eight treatment arms (see Figure 3). After the inclusion of 46 patients, based on the IDMC report of excessive toxicity, the starting dosage of vinorelbine was reduced to 25 mg/m^2/d. Accordingly, in case of toxicity, the vinorelbine doses at the first and the second levels of reduction were lowered to 20 mg/m^2/d and 15 mg/m^2/d, respectively. Five patients were found to be ineligible, one due to cerebral metastasis, two because of prior radiotherapy to bone, and two because of insufficient delay since the last treatment. Two patients in whom the allocated treatment was not started after randomization were also excluded, leading to the analysis of 83 patients (see Table 1). Some 40 patients were treated with 30 mg/m^2/d vinorelbine and 43 with 25 mg/m^2/d. Vinorelbine was administered at 00:00, 03:00, 06:00, 09:00, 12:00, 15:00, 18:00, and 21:00 h in 12, 11, 10, 10, 9, 10, 10, and 11 patients, respectively. Treatment violations were encountered that led to the under-treatment of 30 patients (technical pump problem occurred in 24 patients, dosage mistakes in two patients, and miscellaneous reasons in four other patients) and over-treatment of nine patients, essentially due to not applying the dose-reduction per protocol. Sixty-seven patients (81%) completed the three cycles planned per protocol, seven (8%) received two cycles, and one cycle only was administered to nine (11%) patients. Ten patients (12%) prematurely withdrew from study due to toxicity, four patients (5%) for progressive disease, one patient (1%) for patient refusal, and one patient (1%) for intercurrent death not related to treatment. Overall, a total of 224 cycles were administered (40, 33, and 27, respectively, of the first, second, and third cycles in the vinorelbine 30 group and 43, 41, 40, respectively, of the first, second, and third cycles in the vinorelbine 25

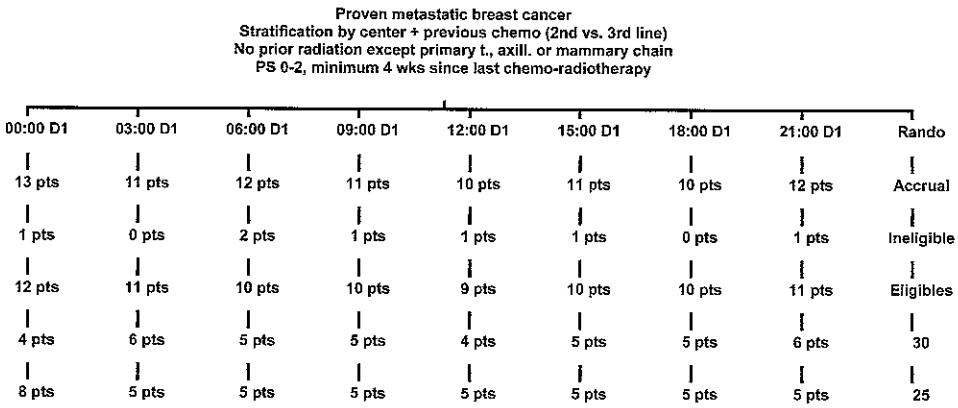


FIGURE 3 CONSORT flow chart of the trial.

TABLE 1 Baseline Characteristics of Patients

	Vinorelbine 00:00 h	Vinorelbine 03:00 h	Vinorelbine 06:00 h	Vinorelbine 09:00 h	Vinorelbine 12:00 h	Vinorelbine 15:00 h	Vinorelbine 18:00 h	Vinorelbine 21:00 h
N	12	11	10	10	9	10	10	11
Age								
Median (yrs)	57.4	53.3	55.7	57.7	63	62.4	60.9	50.2
Range (yrs)	41.2–71.5	34.9–66.0	34.3–80.1	42.3–76.0	30.0–75.9	34.1–67.8	36.8–81.2	35.5–81.2
WHO performance status								
PS 0	9 (75.0)	8 (72.7)	7 (70.0)	4 (40.0)	7 (77.8)	5 (50.0)	4 (40.0)	4 (36.4)
PS 1	3 (25.0)	3 (27.3)	3 (30.0)	5 (50.0)	2 (22.2)	5 (50.0)	5 (50.0)	6 (54.5)
PS 2	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	1 (10.0)	1 (9.1)
Prior surgery								
No	2 (16.7)	0 (0.0)	2 (20.0)	0 (0.0)	1 (11.1)	4 (40.0)	0 (0.0)	0 (0.0)
Curative	9 (75.0)	11 (100.0)	6 (60.0)	9 (90.0)	7 (77.8)	5 (50.0)	10 (100.0)	10 (90.9)
Palliative	1 (8.3)	0 (0.0)	2 (20.0)	1 (10.0)	1 (11.1)	1 (10.0)	0 (0.0)	1 (9.1)
Prior radiotherapy								
No	4 (33.3)	3 (27.3)	3 (30.0)	2 (20.0)	2 (22.2)	5 (50.0)	0 (0.0)	3 (27.3)
Primary	8 (66.7)	8 (72.7)	7 (70.0)	8 (80.0)	7 (77.8)	5 (50.0)	10 (100.)	8 (72.7)
Prior (neo)adjuvant chemotherapy								
No	4 (33.3)	4 (36.4)	6 (60.0)	3 (30.0)	6 (66.7)	5 (50.0)	4 (40.0)	3 (27.3)
≥1 yr*	6 (50.0)	6 (54.5)	3 (30.0)	4 (40.0)	1 (11.1)	3 (30.0)	6 (60.0)	6 (54.5)
<1 yr†	2 (16.7)	1 (9.1)	1 (10.0)	3 (30.0)	2 (22.2)	2 (20.0)	0 (0.0)	2 (18.2)
Prior palliative CT								
No	1 (8.3)	1 (9.1)	0 (0.0)	1 (10.0)	1 (11.1)	0 (0.0)	0 (0.0)	1 (9.1)

(*continued*)

TABLE 1 Continued

	Vinorelbine 00:00 h	Vinorelbine 03:00 h	Vinorelbine 06:00 h	Vinorelbine 09:00 h	Vinorelbine 12:00 h	Vinorelbine 15:00 h	Vinorelbine 18:00 h	Vinorelbine 21:00 h
One line	7 (58.3)	7 (63.6)	8 (80.0)	8 (80.0)	7 (77.8)	6 (60.0)	7 (70.0)	7 (63.6)
Two lines	4 (33.3)	3 (27.3)	2 (20.0)	1 (10.0)	1 (11.1)	4 (40.0)	3 (30.0)	3 (27.3)
Prior hormonal therapy								
No	2 (16.7)	1 (9.1)	4 (40.0)	4 (40.0)	3 (33.3)	4 (40.0)	1 (10.0)	4 (36.4)
Yes	10 (83.3)	10 (90.9)	6 (60.0)	6 (60.0)	6 (66.7)	6 (60.0)	9 (90.0)	7 (63.6)
Prior immunotherapy or BRM								
No	12 (100.0)	11 (100.0)	10 (100.0)	8 (80.0)	8 (88.9)	10 (100.0)	10 (100.0)	11 (100.0)
Yes	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
Associated chronic disease								
No	10 (83.3)	5 (45.5)	8 (80.0)	7 (70.0)	5 (55.6)	5 (50.0)	8 (80.0)	7 (63.6)
Yes	2 (16.7)	6 (54.5)	2 (20.0)	3 (30.0)	3 (33.3)	5 (50.0)	2 (20.0)	4 (36.4)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
Analgesics at entry								
No	9 (75.0)	7 (63.6)	6 (60.0)	7 (70.0)	5 (55.6)	8 (80.0)	5 (50.0)	8 (72.7)
Opiates	0 (0.0)	0 (0.0)	2 (20.0)	1 (10.0)	1 (11.1)	0 (0.0)	0 (0.0)	1 (9.1)
Opiates + other	0 (0.0)	1 (9.1)	1 (10.0)	0 (0.0)	1 (11.1)	1 (10.0)	1 (10.0)	0 (0.0)
Other	3 (25.0)	3 (27.3)	1 (10.0)	2 (20.0)	1 (11.1)	1 (10.0)	2 (20.0)	2 (18.2)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	0 (0.0)	2 (20.0)	0 (0.0)

*Interval superior or equal to one year between (neo)adjuvant and palliative chemotherapy.
†Interval inferior to one year between (neo)adjuvant and palliative chemotherapy.
Abbreviations: V = vinorelbine, WHO = World Health Organization, PS = performance status, CT = chemotherapy, BRM = biological response modifier.

group). Vinorelbine and 5-FU relative dose intensities were respectively 79.4% and 78.2% in the vinorelbine 30 group, while they were 88.1% and 87.4% in the vinorelbine 25 group. There was no specific time when dose reduction was statistically more frequent.

Over the three cycles, G3–4 neutropenia was observed in 90% and 88% of the vinorelbine 30 and 25 patients, respectively, G3–4 leucopenia in 83% and 70%, G4 febrile neutropenia in 50% and 19%, and G3 thrombopenia and anemia in 4% each. Drug-related G3–4 mucositis occurred in 15% and 9%, and G3–4 diarrhea in 8% and 2%. The main other G3–4 adverse events occurring with a frequency in the range of 1% to 5% were cardiovascular, gastrointestinal (vomiting, constipation, or other), infection, sensory, pulmonary, and alopecia (see Table 2).

The estimate of the LTT and its associated 90% CI for neutropenia G3–4 and several other toxicities are given in Table 3. The overall average G3–4 neutropenia rate was 90%, higher than what was expected per design (80%). G3–4 neutropenia (see Figure 4a) was not found significantly modified by the time of vinorelbine administration. The LTT was estimated at about 21:00 h with a CI width of more than 20 h, far greater than the 6 h required for significance (LTT 20:57 h, 90% CI [10:48–07:03 h]). Secondary analyses showed that the LTT differed when based on different toxicity parameters. G4 neutropenia alone showed a non-significant LTT estimated at 07:40 h (90% CI [22:28–15:56 h]). Based on G3–4 leucopenia, with an overall average toxicity rate of 76%, the LTT was estimated at 17:15 h, with the 90% CI width just < 6 h (90%CI [14:12–20:08 h]; see Figure 4b). This time of vinorelbine administration almost corresponds to the least toxic and most efficient time of treatment in the animal model (19 HALO). The drug-related LTT for G3–4 mucositis or diarrhea was estimated at 13:12 h (90% CI: [08:39–16:05 h], and that for all G3–4 gastrointestinal toxicities was estimated at 13:42 h (90% CI: [09:13–16:55 h]). The LTT for receiving at least one dose reduction or at least one cycle delay or discontinuing vinorelbine treatment for any toxicity was estimated at about 08:15 h with the 90% CI width < 6 h (08:13 h, 90% CI [06:07–10:39 h]). Unfortunately, severe protocol violations biased the interpretation of this endpoint—indeed, 60% of the patients in the 09:00 h arm did not have per protocol dose-delays or dose-reductions for toxicity, as compared to about 20% in the other arms. Dose-intensity was also studied. By its nature, the dose-intensity of the administered dosage reflects dose-reduction, dose-delays, and toxicity impact. Dose-intensity was not related to the time of vinorelbine administration.

DISCUSSION

This study could not identify an optimal time of vinorelbine administration that significantly reduced the occurrence of G3–4 neutropenia,

TABLE 2 Distribution of Main Severe Toxicities over Three Cycles per Time of Administration and Dose in mg/m^2/d of Vinorelbine

	Vinorelbine 00:00 h		Vinorelbine 03:00 h		Vinorelbine 06:00 h		Vinorelbine 09:00 h		Vinorelbine 12:00 h		Vinorelbine 15:00 h		Vinorelbine 18:00 h		Vinorelbine 21:00 h	
Vinorelbine dose (mg/m^2/d)	30	25	30	25	30	25	30	25	30	25	30	25	30	25	30	25
Number of patients (N)	4	8	6	5	5	5	5	5	4	5	5	5	5	5	6	5
G3–4, any hematological toxicity, n (%)	4 (100)	6 (75)	5 (83)	5 (100)	5 (100)	5 (100)	4 (80)	4 (80)	4 (100)	4 (80)	5 (100)	5 (100)	4 (80)	5 (100)	6 (100)	4 (80)
G3–4, neutropenia, n (%)	4 (100)	6 (75)	5 (83)	5 (100)	5 (100)	5 (100)	4 (80)	4 (80)	4 (100)	4 (80)	5 (100)	5 (100)	3 (60)	5 (100)	6 (100)	4 (80)
G3–4, febrile neutropenia, n (%)	3 (75)	1 (13)	4 (67)	1 (20)	3 (60)	1 (20)	2 (40)	0 (0)	3 (75)	1 (20)	3 (60)	2 (40)	0 (0)	1 (20)	2 (33)	1 (20)
G4, neutropenia, n (%)	4 (100)	5 (63)	5 (83)	4 (80)	5 (100)	4 (80)	3 (60)	2 (40)	4 (100)	3 (60)	5 (100)	5 (100)	2 (40)	5 (100)	5 (83)	3 (100)
G3–4, leucopenia, n (%)	4 (100)	5 (63)	5 (83)	5 (100)	5 (100)	5 (100)	4 (80)	2 (40)	3 (75)	4 (80)	4 (80)	2 (40)	3 (60)	4 (80)	5 (83)	3 (60)
G3–4, thrombocytopenia, n (%)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)
G3–4, cardiovascular, n (%)	1 (25)	0 (0)	0 (0)	1 (20)	1 (20)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)
G3–4, any gastrointestinal toxicity, n (%)	3 (75)	2 (25)	3 (50)	0 (0)	2 (40)	0 (0)	0 (0)	1 (20)	1 (25)	0 (0)	0 (0)	1 (20)	1 (20)	1 (20)	0 (0)	1 (20)
G3–4, diarrhea, n (%)	1 (25)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	1 (20)	0 (0)	0 (0)
G3–4, vomiting, n (%)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
G3–4, stomatitis, n (%)	1 (25)	2 (25)	3 (50)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	1 (20)
G3–4, constipation, n (%)	1 (25)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	2 (50)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
G3–4, other gastrointestinal toxicity, n (%)	0 (0)	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)
G3–4, infection, n (%)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)
G3–4, sensory, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
G3–4, pulmonary, n (%)	2 (50)	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
G3, alopecia, n (%)	0 (0)	2 (25)	0 (0)	1 (20)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	1 (20)	0 (0)	0 (0)	0 (0)
Toxicities inducing dose reduction or delay or discontinuation, n (%)	4 (100)	5 (63)	5 (83)	3 (60)	5 (100)	2 (40)	2 (40)	0 (0)	4 (100)	3 (60)	5 (100)	4 (80)	3 (60)	5 (100)	4 (67)	3 (60)

TABLE 3 Time (Clock Hour and Minutes) Estimations of Least Vinorelbine Toxicity

Toxicity, over three cycles	Vinorelbine 30 LTT [90% CI], n = 40	Vinorelbine 25 LTT [90% CI], n = 43	Stratification by dose LTT [90% CI], n = 83
Neutropenia G3–4	20:02 [07:15–03:07]	24:00 [15:09–12:00]	20:57 [10:48–07:03]
Neutropenia G4	19:51 [07:31–01:43]	07:33 [02:17–11:14]	07:40 [22:28–15:56]
Febrile neutropenia G3–4	20:13 [15:07–02:02]	06:28 [20:13–14:55]	21:57 [13:06–07:03]
Leucopenia G3–4	17:36 [10:40–22:37]	17:03 [13:26–20:49]	17:15 [14:12–20:08]
Mucositis or diarrhea G3–4	16:08 [13:26–21:07]	09:05 [05:09–12:00]	13:12 [08:39–16:05]
Any gastrointestinal G3–4	15:28 [12:26–17:43]	08:32 [02:41–15:10]	13:42 [09:13–16:55]
Dose reduction or cycle delay or treatment discontinuation for toxicity reasons*	07:30 [19:53–17:41]	08:09 [06:29–10:28]	08:13 [06:07–10:39]

*No dose-reduction or cycle-delay if patients recovered from G3–4 hematological toxicity during the cycle.

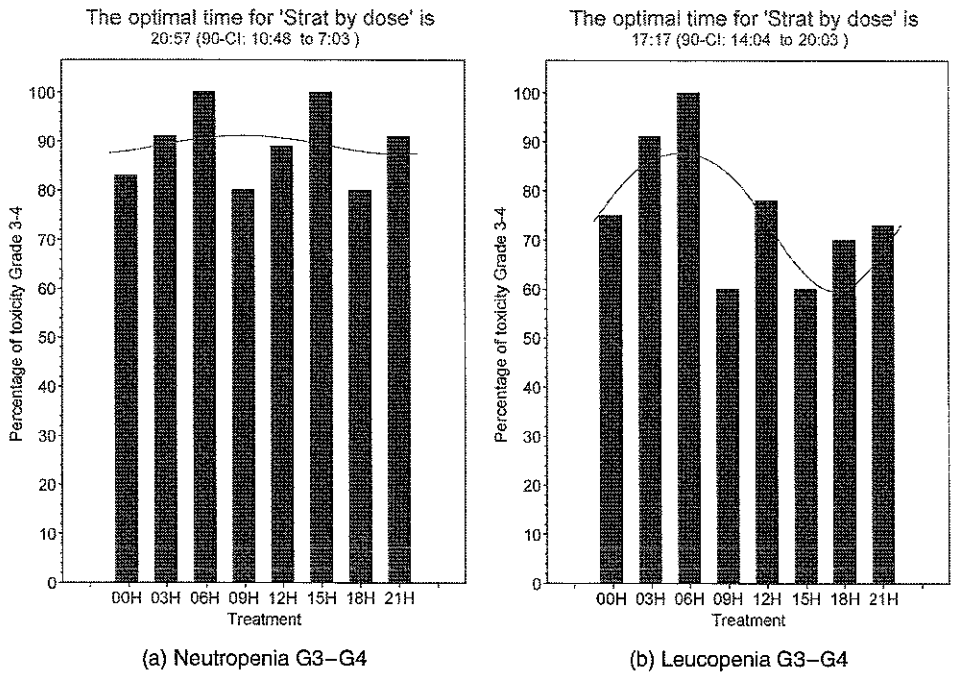


FIGURE 4 Percentage of grade 3–4 neutropenia and leucopenia per time of administration of vinorelbine.

the selected primary endpoint. The estimated LTT was 20:57 h with a non-significant 90% CI width of about 20 h. In this group of patients, the overall average rate of G3–4 neutropenia was higher than the 80% planned in the design of the study, and it was comparable to that seen in the study of Dieras et al. (1996). The toxicity experienced with vinorelbine and 5-FU in the EORTC group studies parallels the international experience of other groups that developed in response much less toxic associations of vinorelbine with 5-FU during the same period (Berruti et al., 2000; Delcambre et al., 2004). The high incidence of toxicity, which led to the reduction of the vinorelbine dosage after the inclusion of 46 patients, had many consequences. On the one hand, the vinorelbine relative dose-intensity was increased in the vinorelbine 25 regimen as compared to the initial vinorelbine 30 regimen. On the other hand, this change certainly introduced additional variability. The benefit of the randomization was certainly also decreased because of the small number of patients per treatment arm. Consequently, LTT estimation was done within each vinorelbine dose level and within the entire group, with stratification according to the two vinorelbine dose levels. The only consistent result concerned leucopenia.

Leucopenia was frequent, ranging from 60% to 100%, and was comparable to that seen in the study of Dieras et al. (1996). The LTT based

on G3–4 leucopenia was observed at about 17:00 h with a 90% CI of <6 h. This almost corresponds to the least toxic and most efficient time of administration in animal model (19 HALO) (Filipski et al., 1999; Tampellini et al., 1995). The LTT of G3–4 stomatitis or diarrhea was in the early afternoon (13:00 h) and that of the gastrointestinal reactions in the late morning (10:30 h). Unplanned additional analysis were performed to try to better utilize the database. Grouping circadian times (i.e., 00:00–06:00 h vs. 09:00–21:00 h) did not add any significant result. Even though the study design was not made to detect and monitor the blood cell nadir, the maximum duration of recovery from any nadir was studied; however, it did not demonstrate circadian dependencies or any relationship with the time of vinorelbine administration.

Another point to be discussed is the interval between the doses of vinorelbine and the daily 5-FU peak, which were different for each of the eight experimental subgroups. In many publications with pharmacokinetics studies, no interactions between 5-FU and vinorelbine have been demonstrated (Fety et al., 2001; Fumoleau et al., 2000; Nole et al., 2006; Sano et al., 2006). The interval between the first dose of vinorelbine and the start of 5-FU administration ranged between 1 h and 22 h. The interval between the termination of 5-FU infusion and the last dose of vinorelbine ranged between 14 h and 35 h (see Figure 2). The long vinorelbine half-life (38 h) suggests that a significant amount of vinorelbine, whatever the time of its administration, was present during 5-FU administration, giving rise to the expectation that varying the time of vinorelbine administration could not induce pharmacokinetic interactions by itself, and that possible differences in toxicities could be due only to the time of vinorelbine administration. The short half-life of 5-FU (6 min) suggests that no significant 5-FU remained at the time of the second dosage of vinorelbine. In the study of Dieras et al. (1996), continuous 5-FU infusion was given immediately after and before the vinorelbine administrations. Continuous 5-FU infusion represented a first attempt to reduce 5-FU bolus toxicity (Lokich, 1985). Moreover, the chronodelivery could reduce stomatitis (Grade 3 or 4), the dose-limiting toxic effect of 5-FU (Lévi et al., 1994), by five-fold. Oral mucositis, diarrhea, and/or hand-foot syndrome limited 5-FU dose escalation, and their incidence was dose-dependent, while hematologic events were not of concern (Lévi et al., 1995). Despite the differences between the current study and that by Dieras et al. concerning scheme administration, the toxicities appeared quite similar. However, drug-drug interactions at a pharmacokinetic level are only the tip of the chronopharmacodynamic iceberg. Disturbing a set of proliferation-related targets and then giving a second antiproliferative drug is a setup for drug-target-time interactions. Therefore, without pharmacokinetic data, drug interactions cannot be definitively ruled out.

This trial did not allow us to recommend an optimal time for vinorelbine administration. Joint analyses of all the toxicity endpoints, including both hematological and non-hematological toxicities, did not allow us to identify a common LTT, probably because of different underlying chronobiological mechanisms. This indicates that the design of such a study should not be aimed at significantly reducing the incidence of one particular toxicity, but more pragmatically at optimizing the delivery of the drug and its tolerability, defined as the proportion of patients with at least one dose-reduction, one cycle delay, or discontinued treatment for toxicity. Unfortunately, in this trial, severe protocol violations at the 09:00 h administration time biased the interpretation of this endpoint.

This study also highlighted the inherent difficulties in the conduct of such a trial in the human setting, such as the necessity for good study design, and particularly the choice of the most appropriate endpoint, variability in outcomes, and effect of dose change during study. The randomized optimal time finding design, used in the present study, nevertheless gave the community a useful tool for identifying the best time to administer chemotherapy. It can be used for any other drug where toxicity could hamper clinical utility. Moreover, drug activity is also thought to be chronodependent and might be increased by chronomodulation. The determination of the best time for drug administration to achieve activity in humans can also be determined the same way.

To establish the proof of concept in cancer chronotherapy, most of the published literature (Giacchetti et al., 2006; Natoli et al., 1996) use a chronobiological vs. non-chronobiological design or compares two times of administration as a means of improving the activity/toxicity ratio of the chemotherapy (Barrett et al., 1993; Hrushesky, 1985; Lévi et al., 1990). Nevertheless, cancer chronotherapy is still waiting definite acceptance. With the increasing genomic data on the role of the human circadian system on cancer, translational studies are now mandatory and must be implemented at the same time as the clinical studies.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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