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Current perspective

Chronomodulated chemotherapy for colorectal cancer: Failing the test of time?

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1. Introduction

The idea that chronopharmacology offers an exploitable advantage for cancer chemotherapy has been espoused for over two decades.[1,2] However, despite our growing understanding of the importance of biological rhythms in human disease, chronopharmacology has had limited influence on current standard treatment recommendations for human tumours. Thus, despite the tireless efforts of its enthusiastic proponents, chronotherapy has not entered the mainstream of clinical oncology. For skeptics, fundamental questions remain including whether the application of chronobiological principles to cancer chemotherapy provides a therapeutic benefit for patients. A more specific question is whether existing clinical trials support the current use of chronomodulated cancer treatments in patients with solid tumours, such as colorectal cancer? Finally, what types of future clinical trials are required to validate or disprove the utility of this approach for the majority of practicing medical oncologists?

In simplest terms, chronotherapy attempts to exploit natural biological rhythms such as 24-h time cycles (circadian rhythms) to optimize cancer therapy by decreasing toxicity and improving antitumour efficacy.[3] The clinical relevance of chronotherapy has been extensively tested in solid tumour patients, with colorectal cancer being the most commonly studied disease type.[4] In this issue of the journal, Garufi and colleagues report their randomized phase II trial of standard or chronomodulated irinotecan infusions in combination with a 4-day chronomodulated infusion of 5-fluorouracil (5-FU) and leucovorin.[5] This study raises important questions about the design of chronotherapy clinical trials and it provides an opportunity to examine the wider impact of this strategy on colorectal cancer treatments.

Preclinical studies demonstrate that chronomodulated treatments may lessen toxicity[6] and improve efficacy in rodent models. In addition, studies in humans show that circadian variation in drug metabolizing enzymes[7,8] can affect the pharmacokinetics of agents such as 5-FU.[7,9–12] Consequently, several strategies have evolved for exploiting chronobiology in cancer chemotherapy. First, assuming that the times of maximal benefit and minimal toxicity are temporally distinct, then the rational selection of the time of drug administration can optimize antitumour efficacy. Second, if chronomodulated chemotherapy can ameliorate drug related toxicities, then further escalation to highest maximally tolerated dose (MTD) may improve the therapeutic benefit of drugs with steep dose response curves. Finally, if circadian variation in

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drug clearance is predictable, then chronomodulated drug infusions could be designed to generate an optimal pharmacokinetic profile that maximizes therapeutic benefit. Clinical trials of chronotherapy in colorectal cancer patients have tested all of these experimental approaches.

2. Literature review

2.1. *Chronomodulation of 5-FU chemotherapy*

Some of the earliest studies of chronomodulated chemotherapy examined temporally modulated prolonged infusions of fluoropyrimidines.[3] In colorectal cancer patients, Bjarnssen and colleagues developed a chronomodulated 14-day continuous infusion regimen of 200 mg/m^2/d of 5-FU and leucovorin at 5–20 mg/m^2/d, administered continuously over 24 h in a circadian infusion pattern, with peak drug delivery occurring at 3:00–4:00 a.m.[13] However, in the six patients that developed $\geqslant$grade 2 toxicities, shifting the infusion time to peak at 9:00–10:00 p.m. improved tolerability allowing for the administration of 5-FU doses that were 25% higher than previously reported. In contrast, Adler did not find an increase in 5-FU tolerability using a shortened chronomodulated infusion of 5-FU and leucovorin administered over 5 days to patients with advanced colorectal cancer.[14] Using an ambulatory infusion system, 75% of the daily 5-FU dose was infused in a non-sinusoidal pattern from midnight to 7:00 a.m. and the remaining 25% of the 5-FU dose was infused from 7:00 a.m. to midnight. In this study, mucositis was dose limiting, leading to the conclusion that chronomodulation of 5-FU did not allow for safe administration of high doses of infusional therapy.[14]

Subsequent studies explored both chronomodulated and intermittent sequential infusions of 5-FU. For example, Levi conducted a dose escalating trial of an outpatient ambulatory chronomodulated 5-day infusion of 5-FU in colorectal cancer patients.[15] 5-Fluorouracil was infused over 20 h with a sinusoidal peak infusion rate at 4:00 a.m. with no drug infused from 6:00 p.m. to 10:00 p.m. Garufi and colleagues modified this strategy by administering repeated nocturnal 12-h chronomodulated sinusoidal infusions of 5-FU and leucovorin from 10:00 p.m. to 10:00 a.m. with a peak rate at 4:00 a.m.[16] Stomatitis and diarrhoea were dose limiting. The recommended tolerable dose in this phase I study was 900 mg/m^2/d of 5-FU with 150 mg/m^2/d leucovorin infused intermittently for 5 days every 3 weeks. Subsequent phase II testing in 48 chemotherapy-naïve and 28 previously treated advanced colorectal cancer patients showed that further dose escalation was possible in 50% of patients.[17] Mucositis was the most common severe adverse event. In the chemotherapy naïve group, the overall objective response rate was 31% [95% confidence internal (CI) 18–44%] and the median survival was 14 months (95% CI 12–16 month). Further refinement of this chronomodulation strategy by Cure and colleagues,[18,19] shortened the time of intermittent 5-FU infusions to 4 days every 2 weeks and further confirmed the tolerability and efficacy of this approach. In 100 previously untreated patients with metastatic colorectal cancer, the objective response rate was 41% (95% CI 31.5–50.5%) and the median overall survival was 17 months (95% CI 10–28 month).[19] Thus, these single arm studies of intermittent chronomodulated 5-FU infusions were well tolerated and highly active in advanced colorectal cancer.

In attempt to isolate the effect of timing, Falcone randomized 113 patients with gastrointestinal cancers to four different 14-day 5-FU infusion regimens.[20] In the standard infusion arm, 27 patients received a continuous fixed rate 24 h infusion of 5-FU at 200 mg/m^2/d and leucovorin at 5 mg/m^2/d for 14 consecutive days every 4 weeks. In contrast, the three chronotherapy arms administered the same doses of drugs on different chronomodulated schedules. In the "0–8" group ($n = 29$), patients were infused with 67% of the total daily 5-FU and leucovorin dose from midnight to 8:00 a.m. in a fixed non-sinusoidal pattern with the remaining 33% of both drugs infused from 8:00 a.m. to midnight. The "8–16" group ($n = 29$) administered the same fraction of drugs from 8:00 a.m. to 4:00 p.m., and in the final "16–24" chronomodulated group ($n = 28$), drug delivery was maximal from 4:00 p.m. to midnight. Doses were escalated toxicities permitting. Overall, grade 2 or worse mucositis occurred in significantly more patients in the standard infusion group, 44% ($P > 0.01$), compared with 14%, 14%, and 7% in the chronotherapy groups 0–8, 8–16, and 16–24, respectively. Dose escalation resulted in an MTD that was greater than 250 mg/m^2/d in 41%, 46%, and 69% of the chronomodulated patients in groups 0–8, 8–16, and 16–24, respectively, compared with only 11% in the standard infusion arm ($P < 0.01$). Thus, chronomodulated 5-FU infusions could improve tolerability, but the authors questioned the biological rationale underlying chronotherapy. Because all three chronotherapy arms showed comparable toxicity profiles, intermittency and not the absolute timing of therapy was hypothesized to be responsible for the improved therapeutic index. However, the limited power of this study precludes any direct comparison of the chronomodulated arm, and the use of fixed rate chronomodulated infusions differs from the sinusoidal patterns tested in earlier chronotherapy studies.

2.2. *Chronomodulation of oxaliplatin chemotherapy*

Chronotherapy designs were frequently utilized in European clinical studies of oxaliplatin. For example, an early phase I study of a 5-day continuous infusion of single-agent oxaliplatin randomized patients to a circadian rhythm-modulated infusion with a peak rate occurring at 4:00 p.m. or to a standard fixed rate infusion.[21] Doses were escalated as tolerated, and cycles were repeated every 3 weeks. Overall, less neutropenia and peripheral neuropathy occurred on the chronomodulated arm allowing for a 15% higher oxaliplatin MTD. Obviously, the power of this small 23 patient study is insufficient for a formal comparison of the two treatment arms. A subsequent phase II study of this regimen administered a sinusoidal 24-h infusion of oxaliplatin to 29 patients with advanced colorectal cancer, the majority of whom had progressed on prior 5-FU-based chemotherapy.[22] The overall objective response rate was 10%. Diarrhoea and peripheral neuropathy were the dose limiting toxicities.

Levi and colleagues conducted an outpatient phase II study of chronomodulated oxaliplatin combined with chronomodulated 5-FU and leucovorin in 93 colorectal

cancer patients.[23] In this study, 5-FU was administered over 5-days at 700 mg/m^2/d, leucovorin at 300 mg/m^2/d and oxaliplatin at 25 mg/m^2/d repeated every 21 days. Oxaliplatin was infused in a sinusoidal pattern over 11.5 h from 10:15 a.m. to 9:45 p.m. with a peak infusion rate at 4:00 p.m. In contrast, 5-FU and leucovorin were infused together from 10:15 p.m. to 9:45 a.m. with a peak infusion rate at 4:00 a.m. Doses were escalated in individual patients and diarrhoea, nausea, and vomiting were the most common dose limiting toxicities. Two drug-related toxic deaths were observed. Overall, the objective response was 58% (95% CI 48–68%) and the median overall survival was 15 months. Nearly one-half of the patients had prior chemotherapy and 15 had previously received oxaliplatin. Thus, these combinations are highly actively in advanced colorectal cancer; however, the exact contribution of chronomodulation is impossible to ascertain from these single arm studies.

Levi and colleagues further examined the importance of chronotherapy in a series of randomized studies utilizing a non-chronomodulated control arm.[24,25] In a multicenter randomized trial conducted in 92 colorectal cancer patients, the activity of a chronomodulated oxaliplatin, 5-FU and leucovorin regimen was compared to a fixed rate continuous infusion of all three agents infused over 5 days repeated every 3 weeks.[24] In the chronomodulated arm, oxaliplatin at 20 mg/m^2/d, 5-FU at 600 mg/m^2/d, and leucovorin at 300 mg/m^2/d were infused over 11.5 h in the same sinusoidal pattern used in the earlier phase II study.[23] Both treatment arms used identical starting doses, and intrapatient dose escalation was permitted. Substantially more $\geqslant$grade 3 stomatitis occurred with the fixed rate infusion, 89%, compared with 18% on the chronomodulated arm. Because of superior tolerability, the median dose intensity on the chronotherapy arm was 700 mg/m^2/d compared with 500 mg/m^2/d on the fixed rate infusion. The objective response on the chronomodulated arm ($n = 47$) was 53% (95% CI 38–68%) compared with 32% (95% CI 18–46%) ($P = 0.038$) on the fixed rate infusion ($n = 45$). Median survival was also significantly better for the chronomodulated arm, 19 vs. 14.9 months, respectively ($P = 0.03$). Unfortunately, this trial was stopped prematurely after enrolling only 92 out of a planned 210 patients because of oxaliplatin stability problems. A potential chemical interaction with 5-FU in the fixed rate infusion line could theoretically inactivate 10–50% of the oxaliplatin, but was not relevant for the chronomodulated arm.

A second, essentially identical subsequent study was initiated using a double lumen infusion system that precluded any drug interactions.[25] This follow-up phase III trial, randomized 186 completely new untreated patients with advanced colorectal cancer, 93 to the fixed infusion arm and to on the chronomodulated arm. Severe stomatitis was significantly less common on the chronotherapy arm, 14% vs. 76% ($P < 0.001$), and peripheral neuropathy was reduced, 16% vs. 31% ($P < 0.01$). Intrapatient escalation of the 5-FU dose led to a 40% higher dose intensity on the chronomodulated arm, 3500 mg/m^2 vs. 2500 mg/m^2 on the fixed rate arm. The chronotherapy objective response rate was also improved, 51% (95% CI 40.0–61.0%) vs. 29% (95% CI 19.6–38.4%) in the constant infusion arm ($P = 0.003$); however, median survival was similar, 16.9 vs. 14.9 months, respectively. Thus, no survival advantage was observed in this study; however, 22% of the patients on the constant infusion arm subsequently crossed over to chronotherapy, potentially obscuring a survival benefit. Based on both of these randomized studies, the authors concluded that chronomodulation was more effective and generated less toxicity than the same drugs administered as a constant rate infusion.

These two trials are the largest positive randomized studies of chronotherapy in colorectal cancer patients reported to date, and they have been embraced by some researchers as providing definitive support for the routine use of this approach.[4] However, several problems remain. First, the potential chemical instability of oxaliplatin in the fixed-rate infusion arm of the earlier 92 patient study makes it difficult to assess the reliability of the efficacy outcomes.[24] In addition, although both studies were designed with response rate and not survival as the primary endpoint, only the earlier smaller study showed a significant survival advantage. In the larger follow-up trial with 186 patients, no survival benefit was seen. If we hold to the accepted regulatory standards established in the United States for the evaluation of first-line treatments for metastatic colorectal cancer, this study does not meet the level of proven efficacy.

A second issue is that the two treatment arms differ by more than just the circadian timing of drug administration. Sequential chronomodulated 11.5-h infusions of oxaliplatin followed by 5-FU and leucovorin were compared with continuous 5-day fixed rate infusions of all three agents. Thus, these two treatment arms differ substantially in the duration of drug administration (11.5 h vs. 24 h infusions) and in the sequence of drug exposure (sequential vs. concurrent). As suggested by Sandor,[26] a better control would be to use pharmacologically equivalent, but differently timed treatments to address the specific role of circadian chronotherapy. Thus, these trials fail to provide clear and convincing evidence supporting the routine use of chronomodulated chemotherapy in patients with advanced colorectal cancer.

Currently, several follow-up phase II studies have intensified this chronomodulated oxaliplatin, 5-FU and leucovorin combination by shortening the treatment interval to 4 days repeated every 2 weeks.[27,28] These single arm studies have largely confirmed the efficacy and tolerability of these regimens. More recently, a randomized multicenter trial with 200 advanced colorectal cancer patients showed the value of adding a 6-h constant rate infusion of oxaliplatin to a 5-day chronomodulated infusion of 5-FU and leucovorin every 21 days.[29] However, this study was not designed to assess the value of chronomodulation. Currently, the standard of care for treating newly diagnosed patients with advanced colorectal cancer in Europe and N. America is to use fixed rate infusions of 5-FU, leucovorin, and oxaliplatin on a bi-weekly schedule without chronomodulation, with or without targeted therapies.

A large multicenter randomized trial performed by the Chronotherapy Group of the European Organization for Research and Treatment of Cancer (EORTC 05963) was presented in preliminary form in 2004.[30] This study of 564 newly diagnosed patients with advanced colorectal cancer compared standard FOLFOX chemotherapy ($n = 282$) with a chronomodulated bi-weekly regimen ($n = 282$) of oxaliplatin, 5-FU and

leucovorin developed by Bertheault-Cvitkovic and colleagues.[27] Chronomodulation generated significantly less ⩾grade 3 neutropenia (7.0% vs. 25%, $P < 0.001$), but ⩾grade 3 diarrhoea (28.3% vs. 10.8%, $P < 0.001$) and mucositis (13.8% vs. 6.8%, $P = 0.002$) were significantly increased. No difference in efficacy was seen. The externally confirmed response rate for the chronotherapy arm was 42.2% vs. 45.3% for the FOLFOX arm. Similarly, no significant differences were seen between the chronotherapy and FOLFOX arms in terms of the median progression-free survival, 8.3 months (95% CI of 7.4–9.4 months) vs. 8.3 months (95% CI of 7.9–9.3 months), respectively, nor in median overall survival, 19.6 months (95% CI of 18.2–21.2 months) vs. 18.7 months (95% CI of 17.7–21.0 months), respectively. Full publication of this trial is still forthcoming. Nonetheless, the largest randomized study of chronotherapy in advanced colorectal cancer patients performed to date shows no improvement in therapeutic benefit.

2.3. *Irinotecan based regimens*

Building on their prior experience with chronomodulated 5-FU and leucovorin,[16] Garufi conducted a phase I trial of irinotecan in combination with chronomodulated 5-FU and leucovorin.[31] In this dose escalation trial in advanced colorectal cancer patients, the recommended regimen was 325 mg/m^2 of irinotecan infused on day 1 in combination with 700 mg/m^2/d and leucovorin at 150 mg/m^2/d for five days repeated every 3 weeks. The chronomodulated 5-FU was infused in same 11.5 h sinusoidal pattern used in previous studies.[16] This regimen was active with a response rate of 23% in previously treated patients with colorectal cancer. However, the addition of irinotecan resulted in dose limiting diarrhoea ultimately necessitating a reduction in 5-FU starting doses.

A follow-up study by the same group published in this issue of the journal, analyzed the value of irinotecan chronomodulation using a randomized phase II trial design.[5] All patients received a chronomodulated 5-FU and leucovorin infusion from 10:00 p.m. to 10:00 a.m. with a peak infusion rate at 4:00 a.m. for 4 days every 2 weeks. Patients were randomized to a standard 1-h fixed rate infusion ($n = 33$) of irinotecan at 180 mg/m^2 or to a chronomodulated 6-h sinusoidal irinotecan infusion ($n = 35$) administered from 2:00 a.m. to 8:00 a.m. with a peak infusion rate at 5:00 a.m. The most common serious side effect was diarrhoea; however, haematological toxicities were minimal and serious toxicities did not differ in the two treatment arms. The primary study endpoint, objective response, occurred in 18.2% (95% CI 5.0–31.3%) of patients receiving the standard infusion and in 25.7% (95% CI 11.2–40.2%) on the chronomodulated arm. This randomized phase II study was statistically designed to detect at least seven responses in 33 evaluated patients to have a 90% probability of detecting a baseline response of 10% when the true response rate is 30% in each arm individually. By these criteria, the standard treatment arm narrowly missed this endpoint, while the chronomodulated arm narrowly satisfied it. Median progression-free survival in the standard arm was 6.0 months (95% CI 4.0–7.0 months) and 8.0 months (95% CI 4.0–12.0 months) on the chronomodulated arm, and the median overall survival was 18 months (95% CI 10–26 months) and 28 months (95% CI 13–42 months), respectively. The authors stated that the chronomodulated irinotecan arm showed a benefit in efficacy based upon a longer duration of response, a prolonged progression-free survival, and longer overall survival.

However, given the limitations of randomized phase II study designs, what can be concluded from this study? The dangers inherent in the interpretation of randomized phase II trials are well described by Liu and colleagues.[32] First is that randomized phase II results are preliminary pilot trials conducted prior to confirmatory phase III studies. They should never be regarded as conclusive. A second issue is that it is inappropriate to include a control arm in these designs because of the tendency and temptation to make erroneous inferences. Even when significant *P*-values (i.e., less than 0.05) are detected post hoc, the conclusions may still be inappropriate because of the limited sample sizes. For this same reason, when "early stoppage" rules are invoked in appropriately designed phase III studies, more stringent tests for statistical significance than a *P*-value of 0.05 are required[32] To their credit, Garufi and colleagues acknowledge most of these shortcomings in their thorough discussion where they recognize the need for further larger, well-designed phase III trials[5] However, a less sophisticated readership, eager to embrace a "winner" in a randomized phase II trial design, may be less critical. Thus, we can only conclude that further testing of this regimen is warranted.

2.4. *Other chronomodulated combinations*

Additional studies have combined chronomodulated 5-FU with other agents such as gemcitabine[33] cisplatin,[34] and carboplatin.[35] These single arm phase I/II studies have generally confirmed the tolerability and general ease in combining chronomodulated 5-FU infusions with other chemotherapeutic agents for the treatment of gastrointestinal tumours. However, none of these studies provides additional insight as to the potential benefit of chronomodulated vs. fixed rate drug treatments.

Finally, Price and colleagues recently conducted a large randomized phase III study of chronotherapy with 5-FU and mitomycin-C in 320 advanced colorectal cancer patients.[36] Patients were randomized to a prolonged continuous fixed rate infusion of 5-FU at 300 mg/m^2/d continuously or a chronomodulated infusion of 450–600 mg/m^2/d of 5-FU infused at a constant rate from 10:15 p.m. to 9:45 a.m. every 6 weeks. Both treatment arms also included mitomycin-C at 7 mg/m^2. The objective response rate for the prolonged infusion was 38% vs. 30.3% for the chronomodulated arm ($P = 0.176$) and the median survival was 15.8 months vs. 16.3 months ($P = 0.275$), respectively. Quality of life assessments were comparable on both arms, but the chronomodulated infusion generated more ⩾grade 3 diarrhoea, 19.8% vs. 6.5%, than the prolonged infusion ($P < 0.001$). Nonetheless, the overall median dose intensity was 28.8% higher for the chronomodulated infusion arm ($P < 0.001$). Thus, chronomodulation of 5-FU did appear to improve the tolerability of the combination; however, there was no significant response or survival benefit. A potential criticism of this study is that it utilized fixed-rate

Table 1 – Randomized studies of chronomodulated vs. standard infusions of chemotherapy in colorectal cancer patients

Regimen	Parameter	Fixed rate arm	Chronomodulated arm	*P*-value	Comments	Reference
Fixed rate: 5-FU 200–250 mg/m^2/d LCV 5 mg/m^2/d × 14 d q4 wks with dose escalation	Patient number	27	29, 29, 28 in groups 0–8, 8–16, 16–24, respectively	NA	Fixed rate infusion over 24 h. Response rates comparable in all treatment groups but significantly higher MTD with chronomodulation. Not powered for comparison of chronotherapy arms. Constant non-sinusoidal infusions used on chronotherapy arms.	[20]
Chronomodulated 0–8 group: 5-FU 200–350 mg/m^2/d LCV 5 mg/m^2/d × 14 d q4 wks 67% of 5-FU/LCV infused at flat rate from midnight to 8:00 a.m.	ORR	12%	19%, 17%, 22% in groups 0–8, 8–16, 16–24, respectively	NA		
Chronomodulated 8–16 group: Same doses but 67% of 5-FU/LCV infused at flat rate from 8:00 a.m. to 4:00 p.m.	MTD ⩾250 mg/m^2/d	11%	41%, 46%, 69% in groups 0–8, 8–16, 16–24, respectively	$P < 0.01$ vs. all chronotherapy arms		
Chronomodulated 16–24 group: Same doses but 67% of 5-FU/LCV infused at flat rate from midnight to 8:00 a.m.						
Fixed rate: Oxaliplatin 20 mg/m^2/d 5-FU 600 mg/m^2/d LCV 300 mg/m^2/d × 5 d q3 wks	Patient number	45	47	NA	Early termination of study due to problem with potential chemical inactivation of oxaliplatin in fixed rate infusion precludes comparison treatment arms	[24]
	ORR	32% (95% CI 18–46%)	53% (95% CI 38–68%)	$P = 0.038$		
	OS	14.9 mo	19.0 mo	$P = 0.03$		
Chronomodulated (11.5 h): Oxaliplatin 20 mg/m^2/d 5-FU 600 mg/m^2/d LCV 300 mg/m^2/d × 5 d q3 wks	Grade 3/4 stomatitis	40%	8%	$P < 0.0001$		
	Median 5-FU dose intensity	500 mg/m^2/d	700 mg/m^2/d	$P < 0.0001$		
Fixed rate: Oxaliplatin 20 mg/m^2/d 5-FU 600 mg/m^2/d LCV 300 mg/m^2/d × 5 d q3 wks	Patient number	93	93	NA	Improved ORR and tolerability with chronomodulated infusion. No survival benefit but 22% of pts crossed over to chronomodulated arm	[25]
	ORR	29% (95% CI 19.6–38.4%)	51% (95% CI 40–61%)	$P = 0.003$		
	PFS	7.9 mo	9.8 mo	$P = 0.2$		
	OS	16.9 mo	14.9 mo	$P > 0.05$		
Chronomodulated (11.5 h): Oxaliplatin 20 mg/m^2/d	Grade 3 stomatitis	76%	14%	$P < 0.001$		

5-FU 600 mg/m^2/d LCV 300 mg/m^2/d × 5 d q3 wks	Grade 3 peripheral neuropathy	31%	16%	$P < 0.01$		
	Median 5-FU dose intensity	2500 mg/m^2	3500 mg/m^2/d	$P < 0.0001$		
Fixed rate: Irinotecan 185 mg/m^2 over 1 h 5-FU 600 mg/m^2/d LCV 300 mg/m^2/d × 4 d q2 wks	Patient number	33	35	NA	Randomized phase II study, 1 h vs. 6 h irinotecan infusion. Not powered for direct comparisons.	[5]
	ORR	18.2% (95% CI 5.0–31.3%)	25.7% (95% CI 11.2–40.2%)	NA		
Chronomodulated: Irinotecan 185 mg/m^2 over 6 h 5-FU 600 mg/m^2/d LCV 300 mg/m^2/d × 4 d q2 wks	PFS	6.0 months (95% CI 4.0–7.0)	8.0 months (95% CI 4.0–12.0)	NA		
	OS	18 months (95% CI 10–26 mo)	28 months (95% CI 13–42 mo	NA		
Fixed rate: 5-FU 300 mg/m^2/d CIV × 6 wks Mitomycin-c 7 mg/m^2 q6 wks	Patient number	158	160	NA	Flat rate not sinusoidal infusion on chronomodulated arm. Dose reduced from 600 to 450 mg/m^2/d due to toxicity on chronomodulated arm. No difference in efficacy endpoints, higher 5-FU dose intensity tolerability for chronomodulated arms	[36]
	ORR	30.3% (95% CI 22.7–37.8%)	38% (95% CI 30–46%)	$P = 0.176$		
	FFS	8.0 mo	9.0 mo	$P = 0.131$		
	OS	15.8 mo	16.3 mo	$P = 0.275$		
Chronomodulated: 5-FU 450–600 mg/m^2/d over 11.5 h from 10:15 p.m. to 9:45 a.m. × 6 wks Mitomycin-C 7 mg/m^2 q6 wks	Grade 3/4 diarrhoea	6.5%	19.8%	$P < 0.001$		
	Median 5-FU dose intensity	277 mg/m^2/d	357 mg/m^2/d	$P < 0.001$		
Fixed rate FOLFOX2: Oxaliplatin 100 mg/m^2 over 2 h LCV 500 mg/m^2 over 2 h 5-FU 24-h infusion 1.5–2 g/m^2 over 2 d q2 wks	Patient number	282	282	NA	EORTC 05011. No differences in efficacy endpoints	[30]
	ORR	45.3%	42.2%	$P = 0.45$		
	PFS	8.3 mo (95% CI 7.9–9.3 mo)	8.3 mo (95% CI 7.4–9.4 mo)	$P = 0.88$		
	OS	18.7 mo (95% CI 17.7–21.0 mo)	19.6 mo (95% CI 18.2–21.2 mo)	$P = 0.53$		
Chronomodulated: Oxaliplatin 20 mg/m^2/d 5-FU 600 mg/m^2/d LCV 300 mg/m^2/d × 4 d q2 wks	Grade 3/4 neutropenia	25.0%	7.0%	$P = 0.001$		
	Grade 3/4 diarrhoea	10.8%	28.3%	$P < 0.001$		
	Grade 3/4 mucositis	6.8%	13.8%	$P = 0.002$		

Abbreviations. 5-FU, 5-fluorouracil; LCV, leucovorin; wks, weeks; ORR, objective response rates; 95% CI, 95% confidence interval; NA, not applicable; PFS, progression-free survival; OS, overall survival; MTD, maximally tolerated dose; FOLFOX2, oxaliplatin + folinic acid + 5-fluorouracil; FFS, failure free survival; mo, months.

and not sinusoidally modulated infusions on the chronotherapy arm.

3. Summary and conclusions

In summary, no chronotherapy study conducted to date provides sufficiently compelling conclusive proof of concept to change current treatment practices for colorectal cancer. The two largest randomized chronotherapy studies in this disease show no benefit in antitumour efficacy as assessed by objective response, overall survival, and progression or failure-free survival.[30,36] These include the 320 patient trial conducted by Price and colleagues[36] and 554 patients studied under the auspices of the EORTC.[30] However, the majority of randomized studies do show a difference in tolerability or dose intensity that generally favours chronomodulation (Table 1). However, this interpretation is complicated by the multiple differences between the chronotherapy and control arms that extend beyond the timing of drug administration. These include differences in sequence, dose, and duration that may affect clinical outcomes independent of chronobiology. Thus, chronotherapy is not as yet established as a useful strategy for cancer chemotherapy in colorectal cancer.

If chronotherapy remains an experimental approach, what studies are required to highlight its potential utility? The ideal chronotherapy trial should have several characteristics. First, the control and test arms should utilize the same duration of treatment. Differences between treatment arms should be limited to the maximum extent possible to the timing of therapy, ideally selected by chronobiological principles. Currently, the EORTC is conducting a trial that meets these specifications in which colorectal cancer patients are randomized to receive irinotecan at one of six different times of day (EORTC 05011). This trial, which recently completed accrual, uses a complex combination regimen containing irinotecan, oxaliplatin, and 5-FU plus leucovorin developed previously by Garufi and colleagues.[37] A second more controversial point is to restrict the treatment arms to the same doses of therapy, with careful monitoring of the toxicity profiles. Although using fixed dose regimens may obscure the theoretical benefit of greater dose intense therapy with chronomodulation, it also avoids a potential source of bias. Use of equivalent drug doses for the chronotherapy and control arms minimizes investigator input and is likely necessary to provide unequivocal proof of concept. Even better would be a blinded study where the programmed infusion schedule is unknown to the investigating physician. Finally, the optimal endpoints for a randomized study should clearly and unambiguously define any therapeutic benefit in terms of response rate, progression-free survival and, optimally, overall survival. Overall survival is the traditional endpoint of choice for evaluating new first-line therapies for advanced colorectal cancer; however, the recent development of multiple active chemotherapeutic combination regimens and newer targeted therapies may make this assessment more problematic for a newly initiated chronotherapy study.

It is much easier to recommend the types of chronotherapy clinical trials to avoid. Small, randomized phase II studies incorporating standard control arms are harmful because they can obscure meaningful evaluations. These should be avoided at all cost. Patients are better served by enrollment into larger well-designed studies that will ultimately support definitive conclusions. Also to be discouraged are multiple small studies that recapitulate the same or very similar chronotherapy principles. Furthermore, randomly testing different times of drug administration for all regimens active in colorectal cancer is not a sound research strategy. New chronotherapy trials should be designed based upon strong preclinical or pharmacologically based rationales. Understandably, large trials are hard to organize and require substantial resources. Such studies may become more difficult to implement as mainstream enthusiasm for chronomodulated approaches wanes, especially given the length of time this debate has raged. Nonetheless, the simplest and only true means to solve this dilemma is to conduct well designed and definitive clinical trials.

Thus, although definitive studies are lacking, the fundamental principles of chronobiology are still worthy of further clinical testing. However, because of the rapid pace of progress in developmental therapeutics in colorectal cancer and the limitations of resources, patients, and time, pressure is growing to satisfy the burden of proof for the utility of this approach. Thus, chronotherapy in the treatment of colorectal cancer is a therapeutic strategy for which the clock is ticking.

Conflict of interest statement

None declared.

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