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Circadian clocks and drug delivery systems: impact and opportunities in chronotherapeutics

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Chronotherapeutics aims at the adjustment of treatments to ~ 24 h rhythms, which result from the moderation of most biological functions by the circadian timing system (CTS). The integration of CTS-related knowledge in drug delivery concepts challenges most current views, where steady-state constant drug levels are synonymous to enhanced tolerability and efficacy. In contrast, robust molecular clocks rhythmically control Phase I, II and III drug metabolism, as well as pharmacodynamics. Thus, circadian timing of medications predictably modifies drug tolerability and/or efficacy up to several-fold in rodents, as well as in patients. Optimal dosing times indeed complement the recommendations for optimal doses of glucocorticoids, NSAIDs, bronchodilators and so on. Clinically-driven *in vitro* and *in silico* circadian data now provide mechanistic insights for the effective translation of chronotherapeutic delivery, especially for cancer therapies. Programmable-in-time electronic or polymeric drug delivery systems are being used for improving health in patients with cancer or rheumatoid diseases, respectively. Current research aims at the optimization of circadian amplitude and phase of drug delivery according to CTS biomarkers. Intelligent drug delivery systems could then integrate the critical rhythmic information stemming from the individual patient and achieve a critical leap forward in the safe administration of potentially toxic therapeutic agents.

Keywords: cancer, chronic diseases, chronopharmacology, chronotherapeutics, circadian biomarkers, circadian clocks, circadian timing system, drug delivery, drug development, personalized medicine, systems biology, systems pharmacology

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1. Introduction

Current concepts of drug delivery mostly consider the need to achieve constant drug exposure at the sites of drug effects over days, weeks or months in order to optimize treatment efficacy. Typically, this involves the delivery of drugs as intravenous infusions at a constant rate for several days or weeks, or as specific pharmaceutical formulations, which results in prolonged drug release in the circulation [1]. The oral, subcutaneous or intramuscular administration of sustained release preparations is also used for providing constant drug exposure for 24 h or more. However, such concepts of drug delivery are being challenged with our advancing knowledge of the circadian timing system (CTS). Indeed, drug effects predictably vary not only as a function of dose but also of administration timing. In some experimental models, the relevance of timing may even exceed that of dose, as recently shown for seliciclib, a cyclin-dependent kinase inhibitor, among several other agents [2].

2. The circadian timing system

Circadian rhythms (with an ~ 24 h period) have been shown for most biological variables in many living organisms, including cyanobacteria, plants, flies, rodents

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and humans [3]. Rhythms along other time scales also characterize biological functions. This is the case for ultradian hourly rhythms in pituitary hormonal secretions or NF-κB cellular signaling pathways and for yearly rhythms in the reproductive behavior of mammals. Circadian rhythms are especially relevant for pharmacotherapy, as they alter drug metabolism and control most drug targets at cellular and molecular levels over 24 h. Circadian rhythms are generated within each cell by molecular clocks, consisting of interwoven transcription/translation feedback loops involving 15 clock genes [3,4]. The molecular clocks are coordinated along the 24 h time scale by an array of physiological rhythms, which are generated by the suprachiasmatic nuclei. This latter circadian pacemaker is located in the hypothalamus. It receives daily inputs from environmental cycles, and generates rhythmic physiological outputs, such as rest–activity, body temperature and hormonal secretions. The CTS encompasses these molecular, cellular, physiologic and pacemaker components. The main function of the CTS is to coordinate bodily and cellular functions, down to the main pathways that are responsible for drug pharmacokinetics (PK) and drug metabolism over the 24 h. Chronopharmacology investigates the circadian dependencies in drug PK, pharmacodynamics (PD) and PK–PD relationships and their mechanisms. Indeed, circadian changes modulate Phase I, II and III drug metabolism, detoxification and disposition processes at cell, tissue and whole organism levels [4,5]. As a result, the CTS determines optimal times of day or night when medications are best tolerated and/or most effective. Moreover, drugs can also modify the CTS, with consequences on quality of life and symptoms. Chronotherapeutics integrates the CTS control of biological functions into the design of circadian drug delivery patterns in order to optimize treatment effects (**Figure 1**).

3. Circadian clocks challenge conventional drug delivery concepts

3.1 Findings in experimental models

Circadian timing largely modifies the extent of toxicity of 40 anticancer drugs among agents in all pharmacological classes in mice or rats. These findings led to the concept of chronotoxicity, for example, the occurrence of periodic and predictable changes in the toxic effects of medications. More specifically, circadian timing improved anticancer treatment tolerability up to fivefold and could nearly double efficacy in experimental studies. Such large differences occur irrespective of oral, intravenous, intraperitoneal, intra-arterial or the number of daily or weekly administrations [4,6]. Drug chronopharmacology usually displays reciprocal 24 h patterns in mice or rats and in humans, whose circadian physiology and clock gene expression patterns differ by nearly 12 h with reference to the light–dark schedule. In rodents and humans, the daytime-dependent toxicity and/or efficacy of medications reflect circadian drug uptake and/or metabolism, circadian drug sensitivity of target cells and tissues, or both. Because preclinical data do show that therapy timing is an important determinant of drug success, Phase I, II and III clinical trials should incorporate issues related to therapy timing in the development of new drugs for cancer as well as for the chronic diseases that represent the main health issues in our ageing society [4,7].

3.2 Drug timing recommendations based on clinical Phase III trials

While many clinical studies have shown dosing time dependencies in drug effects in humans, a coordinated chronopharmacologic drug development has seldom been performed from preclinical to clinical situations, with final assessments in randomized Phase III trials. The need for a consistent methodology to identify the optimal time from experimental to clinical situations is essential for chronotherapeutics.

Several randomized Phase III trials have validated the relevance of an optimal circadian schedule for outcome with large benefits in primary end point in patients with rheumatologic, respiratory or malignant diseases. Yet, several trials failed to do so (**Table 1**). Reasons for failures have seldom been fully disclosed. Our experience based on multiple Phase I, II and III trials of chronotherapeutic delivery in nearly 2000 cancer patients has revealed prominent differences in benefits from a fixed chronotherapy schedule, yet with differences according to sex and circadian function [4,8-9]. While the conventional principle for chemotherapy administration can be summarized as 'the more the toxicity, the better the outcome', recent data regarding cancer chronotherapy reveal the following opposite relationship: 'the least the toxicity, the better the outcome' [10]! This new paradigm supports the development of novel circadian drug delivery schedules jointly aiming at minimizing toxicity and enhancing efficacy in an individual patient. Such a strategy in cancer could serve as a model for driving the development of optimal therapies with a narrow therapeutic index in patients with other severe chronic diseases.

4. Circadian biomarkers for the personalization of chronotherapeutic delivery in cancer

Rhythms in hormonal secretions including cortisol, catecholamines, melatonin, autonomic nervous system activity, core body temperature and psychomotor performance form a dynamic physiological network which resets and coordinates the peripheral molecular clocks. These physiological rhythms can be monitored and serve as biomarkers of the CTS (**Figure 2**). For instance, the rest–activity rhythm has been monitored noninvasively using a wrist actigraph in large cohorts of cancer patients. Statistically significant relations were found between rest–activity rhythm parameters and patient symptoms and quality of life. Most importantly, rest–activity parameters also displayed a statistically significant and independent prognostic value for patient survival [11]. Core body temperature is a circadian biomarker which robustly reflects

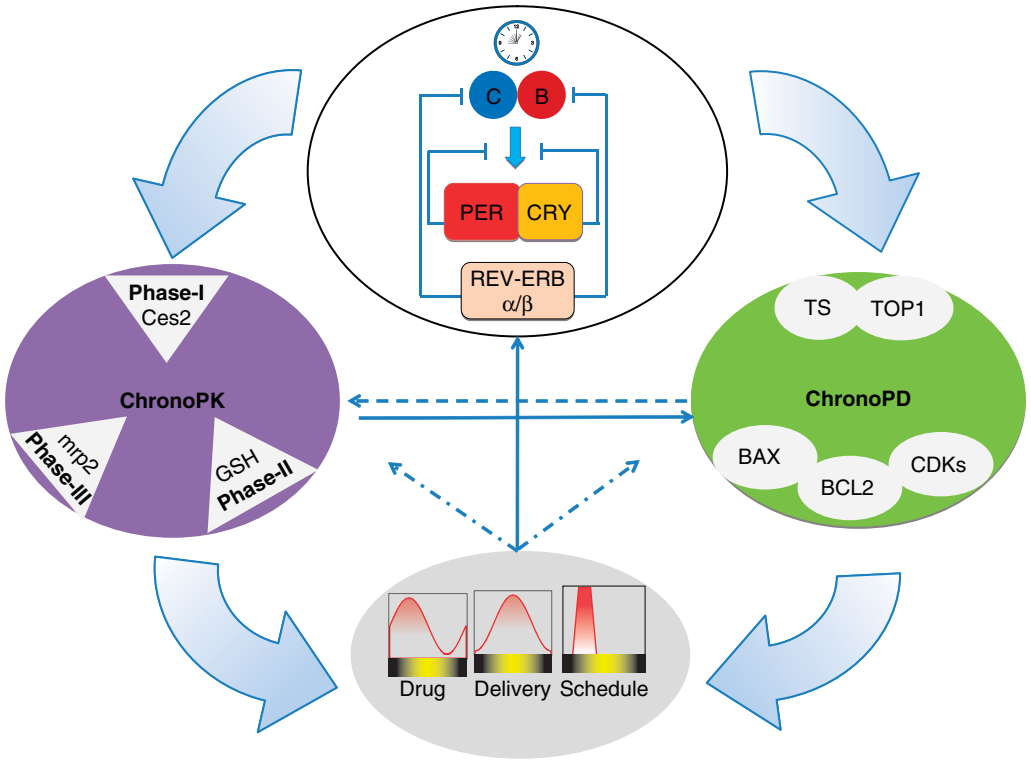

Figure 1. Circadian clock control of PK and PD for designing circadian drug delivery schedules. The circadian clock involves activating molecular loop with dimers of CLOCK (C)- BMAL1 (B) proteins and negative feedback loops through PER, CRY and REV-ERB α/β proteins (top panel). The clock controls drug transport, bioactivation, detoxification, metabolism and elimination (left panel). This results in chronoPK at cellular and whole organism levels. Circadian clocks also control several drug targets including cell cycle, DNA repair and apoptosis proteins, which account for chronoPD in particular for anticancer drugs (right panel). The study of the relations between chronoPK and chronoPD helps in constructing and personalizing optimal drug delivery schedules (lower panel).

BMAL1:Brain and muscle aryl hydrocarbon receptor nuclear translocator; CLOCK:Circadian locomotor output cycles kaput; CRY: Cryptochrome; PD: Pharmacodynamics; PER: Period; PK: Pharmacokinetics.

the CTS pacemaker and provides relevant information on phase and amplitude, both in experimental models and in humans [12,13]. Salivary cortisol and plasma melatonin rhythms are also minimally invasive biomarkers of the CTS, which convey relevant prognostic information for cancer patients [14]. Rest–activity, body temperature, plasma cortisol and melatonin concentrations display statistically validated and consistent 24 h rhythms in groups of patients with early- or late-stage cancers of the breast, lung, colon, prostate, ovary, or head and neck, yet with inter-patient variability. These findings support the administration of fixed chronotherapy schedules for the well-synchronized patients. For those patients with altered CTS, personalized drug delivery patterns or chronotherapy strategies ought to be determined. Ongoing approaches to these issues involve systems chronopharmacology approaches based on *in vitro*, *in silico* and *in vivo* studies [2,15]. These investigations could further guide novel chronotherapeutic approaches, where the host CTS is modified with pharmacological agents in order to enhance central circadian coordination, reset timing or modify period length, shield the molecular clocks or their signaling pathways from treatment- or disease-related disruption, or force desynchronization between tissues. Such chronobiotic drugs could target the central pacemaker, its output signals, specific genes in the molecular clock or downstream pathways. Lithium salts, melatonin agonists or antagonists, as well as glucocorticoid-related medications, display chronobiotic properties. Yet, these agents deserve prospective testing in patients whose disease is associated with circadian disruption. Indeed, pharmacologic targeting at molecular clock components could unravel new effective drugs for improving the outcome of patients with different diseases.

5. Chronoprogrammable drug delivery systems

The concept and the industrial development of external multichannel programmable pumps have promoted the clinical development of cancer chronotherapeutics. Non-hospitalized patients have been receiving chronomodulated infusions of

Table 1. Results of Phase III clinical trials reporting effects from circadian-based treatments.

	Drugs	No. of patients	Design	Main findings	Ref.
Rheumatology					
Osteoarthritis	Indomethacin-SR 0 8.00 vs 13.00 vs 20.00 h	517	4 crossover multi-center trials (3 randomized, 1 double-blind placebo-controlled)	*Adverse events:* 08.00 h dose: 30% 20.00 h dose: 7% ($p < 0.001$) *Efficacy:* Individual pain pattern determines best drug timing	[17]
Rheumatoid arthritis	Prednisone c hronomodulated (chrono)-release vs morning tablet	288	Double-blind, randomized, multi-center	*Morning stiffness* Chrono-release: - 22% Morning tablet: -0.4% ($p = 0.045$) *IL-6* Chrono-release: -28.6% Morning tablet: 0% ($p = 0.03$)	[18]
Pneumology–allergology					
Asthma	Montelukast e vening vs b.i.d. different dose levels	343 children	Double-blind, randomized, multi-center	Once a day evening dose of 10 mg as effective as 10 mg b.i.d. for FEV1 (+11.4 vs +12.6 l), daily symptoms and β-agonist use	[19]
Cancer					
Colorectal metastases	5-Fluorouracil-leucovorin-oxaliplatin Chrono vs flat rate	278	2 randomized European trials	*Severe mucositis* Chrono: 14% Constant: 76% ($p < 0.001$) *Sensory neuropathy* Chrono: 16% Constant: 31% ($p < 0.01$) *Efficacy (tumor response)* Chrono: 51% Constant: 29% ($p = 0.003$)	[20]
	5-Fluorouracil-leucovorin-oxaliplatin Chrono over 4 days vs conventional over 2 days (treatment near maximum tolerated dose in each patient)	564	Randomized, international	*Median survival* Chrono: 19.6 m Conventional: 18.7 m (NS) Gender determines optimal schedule ($p < 0.001$) *Women* Chrono: 16.3 m Conventional: 19.1 m *Men* Chrono: 21.4 m Conventional: 18.3 m	[8]
Endometrial cancer	Doxorubicin-cisplatin f ixed time (doxo, 06.00 h; CisPt, 18.00 h) vs convenient	342	Randomized, multi-center	*Median survival* Fixed time: 13.2 m Convenient time: 11.2 m (NS)	[21]

b.i.d.: Twice a day; FEV1: Forced expiratory volume in 1 s; m: Months.

chemotherapy using a chronoprogrammable electronic pump (Melodie™) or a more recent elastomeric pump with programmed rhythmic electronic control of infusion flow rate (CIP™). Both of these chronopumps allow the chronomodulated delivery of up to four drugs over one to several days in out-patients and with minimal nursing intervention [16]. Recently, novel drug loaded nanocarriers have also been investigated for chronotherapeutics. Indeed, the circadian administration of drug-containing nanoformulations appears to be a new therapeutic strategy which could increase cancer curability without added side effects, costs and risks for the patients [1]. On the other hand, controlled release erodible polymers and novel floating pulsatile oral drug delivery systems could be amenable to chronotherapeutic delivery for many drugs including fluoropyrimidines as well as targeted non-cytostatic therapeutic agents [1,16]. These systems could

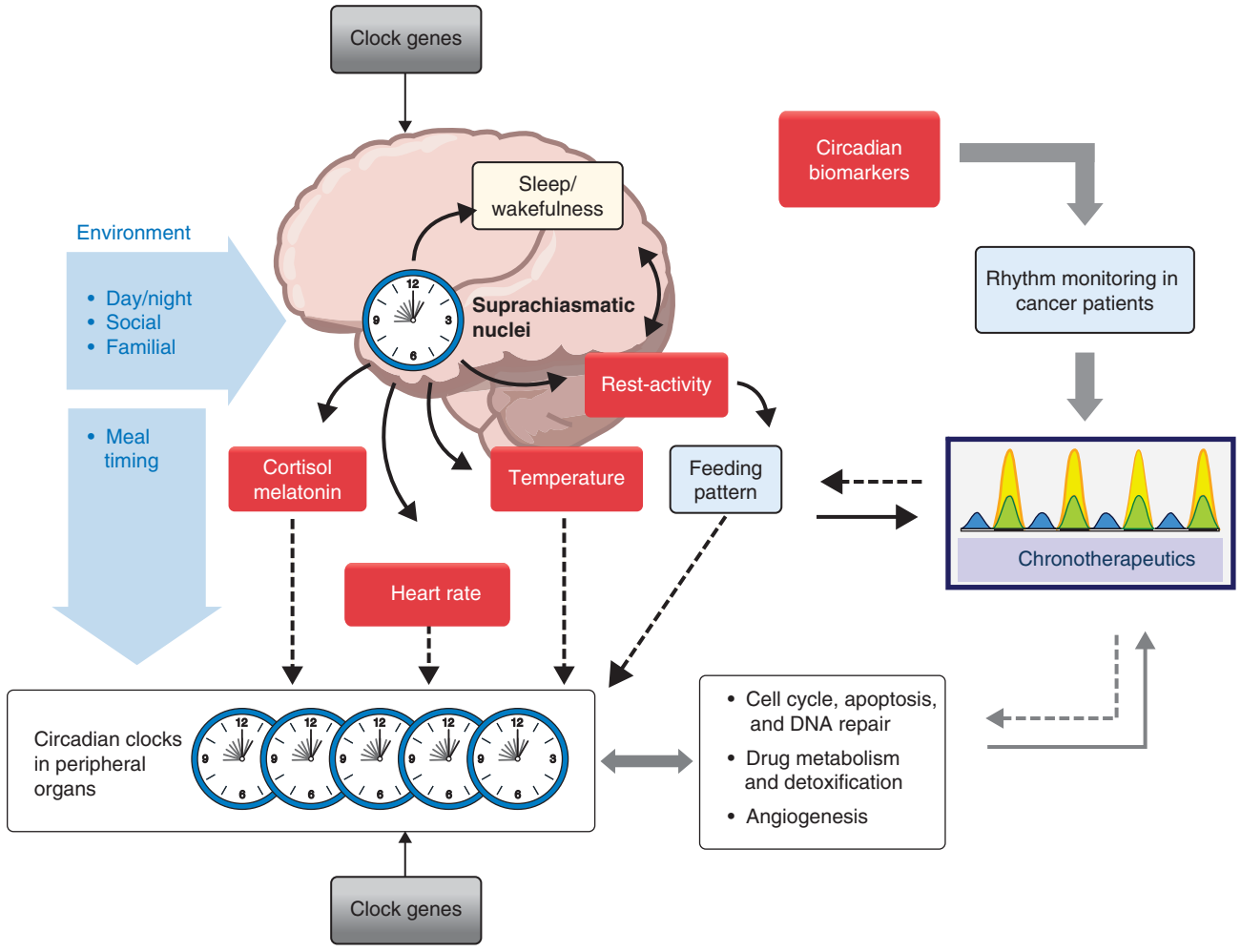

Figure 2. A comprehensive appraisal of the CTS for designing chronotherapy schedules. The CTS is composed of a hypothalamic pacemaker the SCN, an array of SCN-generated circadian physiology outputs and molecular clocks in cells of all the peripheral tissues. The SCN generates behavioral rhythms and synchronizes ubiquitous clocks in peripheral organs through neuronal, physiological and endocrine output signals, resulting in measurable and therapeutically exploitable circadian variations. Thus, rhythms in circulating cortisol, catecholamines and melatonin as well as in autonomic nervous system activity (i.e., heart rate), body temperature and rest-activity form a dynamic physiological network which resets and coordinates the peripheral molecular clocks. These rhythms can be monitored and serve as biomarkers of the CTS. Chronotherapeutics aims at improving treatment tolerability and efficacy through the adjustment of drug delivery to the CTS, which can in turn be influenced by the treatment regimen. This is particularly relevant for anticancer agents.

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CTS: Circadian timing system; SCN: Suprachiasmatic nuclei.

also be taken up by the patients according to relevant self-assessed symptoms, for example, pain, or information derived from relevant physiological biomarkers (temperature, hormones and rest–activity).

6. Conclusions

Dosing time has long been known to influence drug exposure, adverse events and efficacy. Rhythmic changes over 24 h or other timescales also characterize many disease processes. An extensive knowledge has been gathered over the past 20 years regarding the generation of circadian rhythms within each cell and the circadian control of drug metabolism, detoxification and efficacy. This knowledge has to be now fully incorporated into the current concepts in drug delivery in order to jointly improve tolerability and efficacy of medications. This is especially the case for cancer among other chronic diseases where treatments display narrow therapeutic index. A vast amount of critical molecular, physiologic and pharmacologic data support the expectation that electronically-controlled and chronopharmaceutical drug delivery systems will profoundly improve treatment efficacy and tolerability, as

well as compliance, and further enhance the quality of life of patients with chronic diseases.

7. Expert opinion

Circadian timing modifies up to fivefold the extent of tolerability and doubles that of efficacy for anticancer medications in experimental models and in patients. Inter-subject differences in chronotherapeutic effects are related to differences in sex, CTS characteristics and possibly disease stage. Circadian clocks involve 15 specific genes and operate in each cell so that they moderate most pharmacology effects in a coordinated fashion, through identified molecular mechanisms. Recent *in vitro* and *in silico* circadian data, as well as results from Phase III clinical trials of chronotherapeutics, now provide mechanistic insights into the effective translation of the chronotherapeutic delivery paradigm, especially for cancer therapies.

A main challenge is the identification of those parameters which critically alter the optimal drug delivery schedule in an individual patient. Dynamic assessments of the CTS are currently combining minimally invasive technologies and data-based mathematical models, so as to personalize chronotherapeutics.

Programmable-in-time electronic or polymeric drug delivery systems are currently improving health in patients with cancer or rheumatoid diseases. An ongoing step involves the personalization of the circadian amplitude or phase of rhythmic drug delivery according to the CTS biomarkers.

Intelligent drug delivery systems could then integrate critical rhythmic and genomic information stemming from the individual patient thus providing a critical leap in the safe administration of potentially toxic therapeutic agents, especially in oncology.

Technologies can involve electronic devices, electrokinetic nanochannel drug delivery systems and new pharmaceutical approaches adapted to rhythmic drug delivery and their automatic control by relevant biomarkers.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Youan BC. Chronopharmaceutical drug delivery systems: hurdles, hype, or hope? Adv Drug Deliv Rev 2010;62:898-903
• **A good overview of drug delivery systems adapted to chronotherapeutic issues.**
2. Iurisci I, Filipski E, Sallam H, et al. Liver circadian clock, a pharmacological target of cycline-dependent kinase inhibitor seliciclib.. Chronobiol Int 2009;26:1169-88
3. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: Organisation and coordination of central and peripheral clocks. Annu Rev Physiol 2010;72:517-49
4. Levi F, Okyar A, Dulong S, et al. Circadian timing in cancer treatments. Annu Rev Pharmacol Toxicol 2010;50:377-421
•• **This extensive review describes the CTS and summarizes experimental and clinical data concerning chronopharmacology of anticancer drugs and cancer chronotherapeutics.**
5. Okyar A, Levi F. Circadian control of cell cycle pathways: relevance of cancer chronotherapeutics. In: Yoshida K. editor. Trends in Cell Cycle Research. India: Research Signpost; Kerala: 2008. p. 293-317
6. Levi F, Schibler U. Circadian rhythms: mechanisms and therapeutic implications. Annu Rev Pharmacol Toxicol 2007;47:593-628
•• **A first review linking the molecular clock to therapeutics, including drug metabolism and detoxification mechanisms.**
7. Levi F, Focan C, Karaboue A, et al. Implications of circadian clocks for the rhythmic delivery of cancer therapeutics. Adv Drug Deliv Rev 2007;59:1015-35
8. Giacchetti S, Bjarnason G, Garufi C, et al. Phase III trial comparing 4-day chronomodulated therapy versus 2-day conventional delivery of fluorouracil, leucovorin, and oxaliplatin as first-line chemotherapy of metastatic colorectal cancer: the European Organisation. J Clin Oncol 2006;24:3562-9
9. Innominato PF, Levi FA, Bjarnason GA. Chronotherapy and the molecular clock: clinical implications in oncology. Adv Drug Deliv Rev 2010;62:979-1001
10. Innominato PF, Giacchetti S, Moreau T, et al. Prediction of survival by neutropenia according to delivery schedule of oxaliplatin-5-fluorouracil-leucovorin for metastatic colorectal cancer in a randomized international trial. Chronobiol Int 2011; In press
11. Innominato PF, Focan C, Gorlia T. Circadian rhythm in rest and activity: a biological correlate of quality of life and a predictor of survival in patients with metastatic colorectal cancer. Chronobiol Int 2011;28:586-600
12. Li XM, Delaunay F, Dulong S, et al. Cancer inhibition through circadian reprogramming of tumor transcriptome with meal timing. Cancer Res 2010;70:3351-60
13. Scully CG, Karaboue A, Liu WM, et al. Skin surface temparature rhythms as potential circadian biomarkers for personalized chronotherapeutics in cancer patients. Interface Focus 2011;1:48-60
14. Sephton SE, Sapolsky RM, Kraemer HC, Spiegel D. Diurnal cortisol rhythm as a predictor of breast cancer survival. J Natl Cancer Inst 2000;92:994-1000
15. Ballesta A, Dulong S, Abbara C, et al. A Combined experimental and mathematical approach for molecular-based optimization of irinotecan circadian delivery. PLoS Comput Biol 2011; In press
16. Levi F, Altinok A, Goldbeter A. Circadian rhythms and cancer

chronotherapeutics. Chapter 15. In: Cesario A, Marcus FB. editors. Cancer Systems Biology, Bioinformatics and Medicine: Research and Clinical Applications. Springer Dordrecht Heidelberg; London, New York: 2011

17. Levi F, Le Louarn C, Reinberg A. Timing optimizes sustained-release indomethacin treatment of osteoarthritis. Clin Pharmacol Ther 1985;37:77-84

18. Buttgereit F, Doering G, Schaeffler A, et al. Efficacy of modified-release versus standard prednisone to reduce duration of morning stiffness of the joints in rheumatoid arthritis (CAPRA-1): a double-blind, randomized controlled trial. Lancet 2008;371:205-14

• **The first randomized trial validating the concept of oral chronomodulated delivery of prednisone in patients with rheumatoid arthritis.**

19. Altman LC, Munk Z, Seltzer J, et al. A placebo-controlled, dose-ranging study of montelukast, a cysteinyl leukotriene-receptor antagonist. Montelukast Asthma Study Group. J Allergy Clin Immunol 1998;102:50-6

20. Levi F. Circadian chronotherapy for human cancers. Lancet Oncol 2001;2:307-15

21. Gallion HH, Brunetto VL, Cibull M, et al. Randomized phase III trial of standard timed doxorubicin plus cisplatin versus circadian timed doxorubicin plus cisplatin in stage III and IV or recurrent endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol 21:3808-13

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