ASIA PAC J PHARMACOTHER TOXICOL

OPEN ACCESS AMP Asian Medical Press

REVIEW ARTICLE

Received 02 Jan 2025 | Accepted 10 Feb 2025 | Published Online 20 Feb 2025 https://doi.org/10.32948/ajpt.2025.02.10

Time matters: chrono-pharmacotherapy as precision medicine

Samir Arabi[1], Sajjad Ahmad[1]

Cite this article: Arabi S, Ahmad S: Time matters: chrono-pharmacotherapy as precision medicine. Asia Pac J Pharmacother Toxicol 2025, 5: 11-21. https://doi.org/10.32948/ajpt.2025.02.10

Abstract

The circadian clock and its inherent rhythms serve as the foundation for regulating many of the body's vital processes. Recent advances in medicine have leveraged these natural cycles through chronotherapy, the practice of timing drug administration to align with the body's internal clock, to enhance treatment outcomes. Endogenous clocks not only dictate physiological rhythms but also influence how drugs are absorbed, distributed, metabolized, and excreted, thereby affecting both their therapeutic efficacy and potential toxicity. When these circadian rhythms are disrupted, the resulting imbalance has been linked to a range of disorders, such as cancer and cardiovascular diseases, highlighting the growing need for time-sensitive therapeutic strategies. Technological breakthroughs, including wearable sensors, digital health platforms, and sophisticated machine learning techniques, now allow for continuous, real-time monitoring of circadian biomarkers, which in turn helps optimize treatment regimens. In oncology, for example, synchronizing chemotherapy, cardiovascular therapy, immunotherapy and anti-inflammatory drugs with the patient's circadian cycle has been shown to enhance drug effectiveness and improve tolerability. Moreover, observed sex-based differences in circadian drug responses emphasize the importance of tailoring drugs to individual patient profiles. Emerging research is also focusing on directly targeting molecular clock components, showing potential for treating metabolic and oncological conditions. However, fully integrating chronopharmacology into clinical practice will require the development of standardized protocols, regulatory backing, and large-scale trials that incorporate circadian biomarkers. Ultimately, merging circadian biology with therapeutic strategies promises to optimize treatment efficacy, minimize side effects, and enhance patient outcomes. Future studies must address inter-individual variability, sex differences, and circadian misalignment to fully harness chronotherapy's potential in personalized medicine.

Key words circadian rhythms, molecular clock, pharmacodynamics, chrono-pharmacotherapy, precision medicine

1. Department of Biochemistry, Shaheed Salman Building, Islamic Azad University ZIP area 11, Azarshahr Street, Karimkhan-e-Zand Avenue, Tehran 1477893780, Iran.

Correspondence: Sajjad Ahmad (Department of Biochemistry, Shaheed Salman Building, Islamic Azad University ZIP area 11, Azarshahr Street, Karimkhan-e-Zand Avenue, Tehran 1477893780, Iran; E-mail: ahmadsajjad@hotmail.com).

Introduction

Intrinsic biological cycles, termed circadian rhythms, generally align with a 24-hour period, though variations ranging from 20 to 28 hours have been documented [1]. These rhythms originate from genetic molecular clocks operating autonomously within individual cells. Central to their regulation is the hypothalamic pacemaker named suprachiasmatic nuclei (SCN), responsible for synchronizing internal rhythms with external environmental cycles [2]. Significant inter- and intra-individual variability in these rhythms necessitates precise monitoring to determine optimal drug administration windows [3, 4]. Emerging research also underscores sexual dimorphism as a critical modulator of circadian regulation, influencing pharmacokinetics (absorption, distribution, metabolism, excretion) and pharmacodynamics (especially toxicity) processes across molecular, tissue, and systemic levels [5]. While external time cues (e.g., light-dark cycles) help align the circadian timing system, integrating molecular and physiological assessments of the circadian timing system is vital for understanding how circadian phases influence drug responses at the individual level [6]. Notably, disrupted circadian biomarkers independently correlate with adverse outcomes in cancer, highlighting their prognostic value [7]. The integration of wearable devices, digital platforms, and machine learning has revolutionized the real-time tracking of circadian biomarkers, enabling automated analysis of patient data [8, 9]. Breakthroughs in omics-based algorithms now permit the prediction of molecular clock activity in both healthy and diseased tissues using single-timepoint biopsies, propelling chronotherapy towards precision medicine [10]. Systems medicine approaches enable the replication of molecular mechanisms governing drug chronopharmacology across preclinical models and human populations [11, 12]. Here, we explore recent progress in chronopharmacology, particularly in therapies alleviating cancer burden, improving cardiovascular health and promoting immune function. We also discuss sex-specific variations in chronotherapy, a strategy that optimizes treatment timing relative to circadian cycles to enhance therapeutic outcomes and reduce side effects. In addition, we discuss therapeutic targeting of molecular clock mechanisms and its implications for personalized medicine. By harmonizing circadian biology with therapeutic strategies, these advancements pave the way for tailored interventions that align with individual circadian profiles, optimizing efficacy and safety.

Circadian clock and rhythms

At the core of the body's circadian regulation lies the SCN, a cluster of neurons and glial cells within the hypothalamus that functions as the central pacemaker. These cells generate rhythmic oscillations close to 24 hours, yet external environmental factors, such as light-dark transitions and social interactions, fine-tune them to align precisely with a 24-hour cycle (**Figure 1**) [13]. Through its regulation of physiological rhythms, including hormonal release and temperature fluctuations, the SCN synchronizes peripheral circadian clocks throughout the body. Every cell contains its own molecular clock of almost 15 interdependent genes arranged in self-regulating feedback loops that maintain intracellular circadian cycles [14]. However, when external cues or internal coordination are diminished, variations can emerge in the timing, intensity, and duration of circadian oscillations at the cellular level [15, 16]. Disruptions to environmental exposures affecting circadian rhythms, have been implicated in the development of chronic diseases due to the misalignment of molecular clocks with physiological processes (**Figure 1**) [17, 18]. For instance, meal timing influences liver clock activity by affecting insulin and glucagon secretion, while rhythmic food intake independently governs the expression of numerous clock-controlled genes, overriding the liver's endogenous rhythmicity [19]. Extensive genome-wide studies indicate that circadian rhythms are deeply embedded in critical cellular activities, governing processes from chromatin remodeling to gene transcription, and from protein synthesis to their modifications at post-translational level [20]. It is estimated that in male mice, roughly 8000 protein-coding genes exhibit expression patterns aligning with circadian rhythms across at least one of the 12 analyzed organs [21]. Consequently, intracellular mechanisms responsible for metabolism, cell proliferation, repair, and survival operate in a time-dependent manner [1]. Notably, sex-based differences in circadian regulation have been observed, particularly within the SCN, where distinct patterns of sex hormone receptor expression influence overall circadian system function [22]. For example, female rodents tend to exhibit higher-amplitude oscillations in transitioning from rest to activity, and temperature fluctuations compared to men [22]. Furthermore, sex-dependent differences have been documented in circadian gene expression and the downstream pathways regulated by these molecular clocks, with implications for both mice and humans [23, 24].

Circadian rhythm-regulated pharmacokinetics and toxicity

Over the past few decades, research has increasingly emphasized the role of circadian rhythms in pharmacokinetics (absorption, distribution, metabolism, excretion) and toxicity, particularly in relation to drug efficacy and tolerance [25, 26]. Circadian regulation plays a critical role in drug metabolism, particularly through its effects on cytochrome P450 enzymes (CYPs) and ATP-binding cassette (ABC) transporters. Research has demonstrated that the molecular clock governs the rhythmic transcription of these enzymes and transporters in the intestine, leading to significant fluctuations in their activity throughout the day [27, 28]. A meta-analysis has further supported that Abcg2, Abcc1, and Abcc5 exhibit rhythmic mRNA fluctuations Abcc5 across 14 different mouse organs. Strong circadian variations have been observed in SLC drug transporters as well (**Figure 1**) [29]. The liver's uptake of xenobiotics is partly regulated by circadian-controlled SLC transporters. Similarly, xenobiotic uptake into the brain fluctuates throughout the day in male mice, driven by circadian rhythms [30]. Circadian rhythms are fundamental regulators of phase I metabolic processes such as oxidation, reduction, and hydrolysis, which are primarily mediated by CYP enzymes [31, 32]. Additionally, phase II detoxification pathways in the liver and intestines of rodents are influenced by circadian fluctuations, largely controlled by key clock-regulated transcription factors [33]. Despite well-documented sex-based differences in drug metabolism and therapeutic effects, the extent to which sexual dimorphism influences circadian regulation of pharmacokinetics and toxicity remains largely underexplored [34]. Differences in body composition contribute to sex-based variations in drug distribution. Because women generally have a higher proportion of body fat, lipid-soluble drugs, such as benzodiazepines, tend to have a larger volume of distribution in females. Conversely, men, who typically possess greater lean body mass, exhibit a larger volume of distribution for drugs [35]. Sex differences have been observed in the circadian regulation of ABC transporters as well. For example, in the ileum mucosa of mice, both mRNA and protein expression levels of Abcc2 follow distinct circadian rhythms in males and females. These fluctuations influence intestinal tolerability to irinotecan, a topoisomerase I (TOP1) inhibitor [36]. Additionally, sex-based differences have been detected in the circadian regulation of Abcb1a and Abcb1b, where females display significantly greater circadian amplitudes in the mouse ileum [37]. Moreover, in the kidneys, baseline Abcb1a/1b expression is

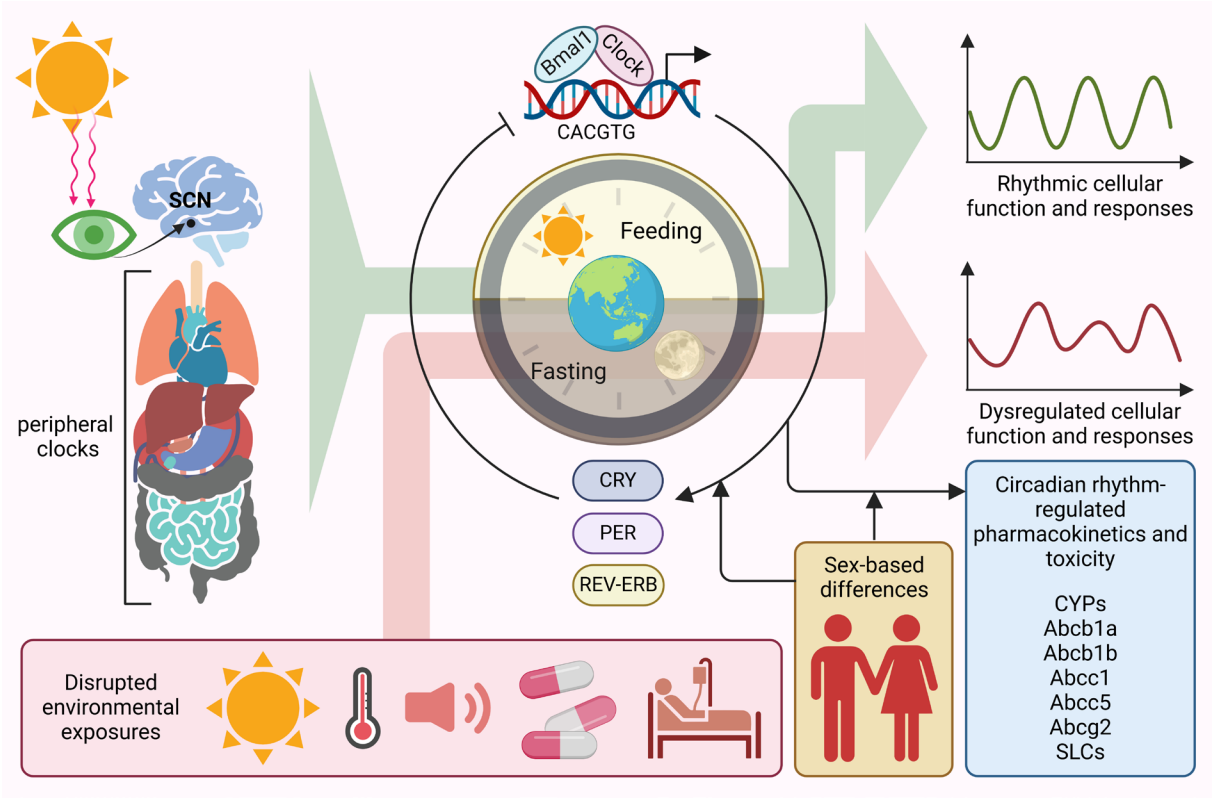

Figure 1. Circadian rhythm-regulated pharmacokinetics and toxicity. Circadian clock in primarily regulated by suprachiasmatic nuclei (SCN), synchronizing the peripheral clocks throughout the body. Bmal1 and Clock are the master regulator of circadian rhythm throughout the body leading rhythmic cellular function and responses. Disrupted environmental exposures such as sunlight, heat, sound, diseases and treatment plans dysregulate circadian rhythms. In addition, sex-based differences also contribute to alterations in circadian rhythms. Different pharmacokinetic and toxicity associated genes are tightly regulated by circadian clock, expression rhythmic expressions.

higher in females than in males [38]. Male mice, however, show no detectable rhythmicity in renal Abcb1a/1b mRNA or P-gp protein levels [39]. In contrast, P-gp protein expression in the liver follows a circadian pattern in females but not in males, suggesting potential differences in the biliary excretion of xenobiotics between sexes [37]. Sex differences are further reflected in variations in drug-induced toxicity. For instance, the toxic effects of acetaminophen and oxaliplatin differ between male and female mice [40, 41]. Notably, female mice exhibit lower susceptibility to acetaminophen toxicity, which has been attributed to their ability to more rapidly replenish mitochondrial GSH following hepatic depletion [42].

Circadian responses to chemotherapy

Cytotoxic drugs, which form the backbone of systemic cancer treatment, belong to multiple classes designed to target rapidly proliferating cells. The importance of synchronizing their administration with circadian rhythms was initially identified in studies on mice and rats treated with arabinofuranosylcytosine, cyclophosphamide, doxorubicin, and cisplatin. These experiments demonstrated that the timing of drug administration significantly affected both its effectiveness and tolerability [3]. Subsequent research has reinforced the idea that circadian fluctuations play a crucial role in the efficacy and/or tolerability of more than 50 anticancer drugs [4]. While variations in blood pharmacokinetics are observed based on when the drugs are administered, this alone does not fully account for differences in tolerance and efficacy [3, 4]. Instead, circadian control over pharmacokinetics and toxicity appears to be a primary factor [11]. The clinical implications of circadian-timed chemotherapy have been evaluated for nearly 20 anticancer drugs across a range of malignancies [43]. A systematic review analyzing data from 18 randomized trials assessed the impact of chrono-modulated chemotherapy, where at least one group received treatment timed according to circadian rhythms [44]. Results indicated that chemotherapy aligned with circadian cycles improved efficacy in three trials (17%) while maintaining the same effectiveness in the remaining 15. Additionally, this approach significantly reduced major toxicity in 11 trials (61%) and partially reduced toxicity in two more (11%), with toxicity increasing in only one study (6%) [45]. Platinum-based chemotherapy agents, such as cisplatin, carboplatin, and oxaliplatin, have been shown to reduce toxicity by up to threefold when administered between ZT15 and ZT19, which corresponds to the midpoint of their nocturnal activity phase (**Figure 2**) [3]. In clinical settings, findings align with these preclinical observations, demonstrating that administering these drugs in the afternoon or early evening enhances tolerability across several cancers. Furthermore, this timing either maintained or improved treatment efficacy [46, 47]. Emerging anticancer drugs are also under investigation for their circadian-dependent effects. The organo-osmium complex which induces apoptosis by generating reactive oxygen species, has shown significant circadian variation in tumor inhibition in preclinical studies. Specifically, hepatocarcinoma (Hepa1–6) tumors in male mice exhibited a 67% reduction when FY26 was administered at ZT18, compared to only a 13% reduction at ZT6 [48].

To overcome resistance mechanisms, anticancer drugs

are commonly administered in combination regimens. Both experimental studies and clinical data suggest that each drug should be delivered at best effective circadian phase [49]. Current research efforts focus on translational strategies aimed at identifying factors that can stratify patients for chronotherapy, including sex-based differences and circadian biomarkers, while also incorporating systems medicine principles. For instance, synchronized Caco-2 cell culture models have been employed to investigate the molecular and cellular chronopharmacology of irinotecan, a key drug in gastrointestinal cancer treatment [27]. Studies have demonstrated that silencing Bmal1 abolished the circadian fluctuations in irinotecan's bioactivation to SN38, its interaction with TOP1-DNA, and the subsequent induction of apoptosis. Similarly, when Bmal1 and its molecular partner Clock were either suppressed, knocked down, or mutated, cyclophosphamide toxicity remained fixed at its most harmful circadian phase in male mice [50]. These findings emphasize the potential for artificial intelligence-driven models to integrate experimental and clinical data, paving the way for personalized chronotherapy [51]. Despite mounting evidence that chemotherapy tolerability differs between sexes, no formal guidelines exist for sex-specific drug dosing or scheduling [52]. Female patients tend to experience greater adverse effects when receiving time-unspecified doses of 5-FU and irinotecan compared to male patients [53, 54]. Research suggests that sex-related differences in cancer chronotherapy are becoming increasingly apparent, largely due to clinical observations [55]. For instance, studies have revealed that when 5-FU is administered at a constant rate, its plasma concentration exhibits circadian variations, with women showing lower circadian amplitude in 5-FU clearance than men [56]. Moreover, a study involving 210 patients demonstrated that, in cases of diffuse large B-cell lymphoma, female patients, but not male patients, had significantly improved survival rates when rituximab, cyclophosphamide, doxorubicin and vincristine were administered in the afternoon rather than in the morning [57]. Sex-based differences have also been confirmed in preclinical models, particularly regarding irinotecan-induced systemic and organ toxicities, as well as its plasma pharmacokinetics in mice. Statistical modeling has identified the reciprocal transcriptional dynamics of Rev-Erbα and Bmal1 as key regulators of sex-specific variations in response to irinotecan treatment [58]. Although research in this area is still in its early stages, these findings are consistent with clinical observations, highlighting the need for further prospective studies to explore how sex and circadian timing interact to refine cancer chronotherapy.

Circadian responses to cardiovascular therapy

The risk of acute thrombotic cardiovascular events is notably higher in the early morning due to circadian fluctuations in hemostasis and thrombogenicity [59]. Early clinical studies observed significant circadian variations in anticoagulation levels among thrombosis patients receiving continuous unfractionated heparin infusions, with findings suggesting an increased morning time rethrombosis risk and night time bleeding risk [60]. Circadian-dependent variations in both pharmacokinetics and pharmacodynamics have also been observed for anticoagulants. For instance, when rivaroxaban was administered at 8:00 AM, 12

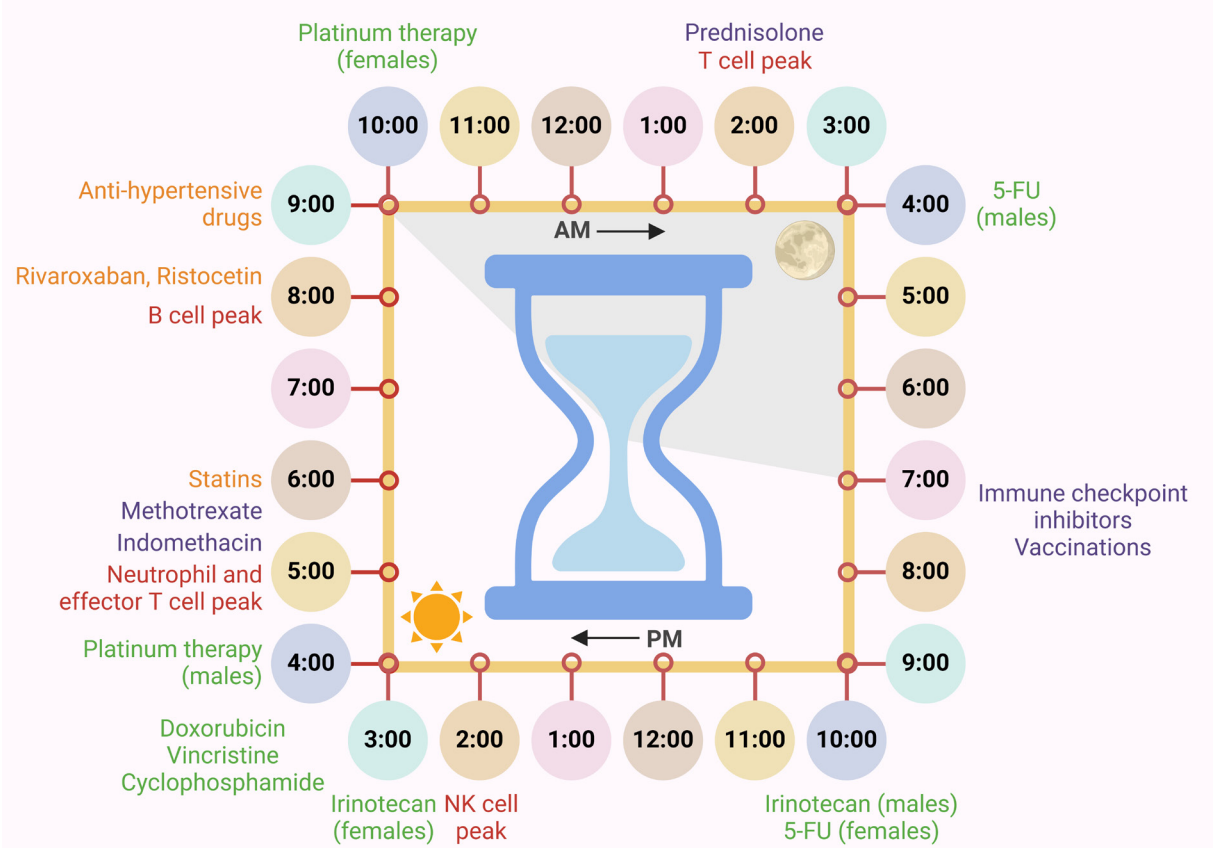

Figure 2. Circadian clock-driven immune system regulation and responses towards treatments. Circadian clock-driven immune system regulations entailing peak timings of various immune responses in the body (red) are listed along with optimized timing for various chemo- (green), cardiovascular (orange) and immunotherapies and anti-inflammatory drugs (purple).

hours plasma concentrations were nearly half of those when taken at 8:00 PM [61]. Similarly, ristocetin-driven platelet aggregation, was significantly lower when taken at 8:00 AM, compared to its effects when taken at 8:00 PM (**Figure 2**) [62]. These clinical findings align with experimental evidence from male rats, where factor X activity peaks during the early rest phase, specifically at ZT4 [63]. Since blood pressure regulation follows a circadian rhythm, antihypertensive drugs act on physiological pathways that naturally fluctuate over a 24-hour period [11]. Disruptions in this cycle have been associated with negative health consequences [64]. Many antihypertensive drug targets are deeply integrated into the mechanisms governing blood pressure oscillations [65]. These medications are generally prescribed once daily to ensure stable blood pressure control throughout the day [66]. While no specific guidelines dictate an optimal time for administration, morning dosing is the most common approach [67]. However, a systematic review evaluating 153 randomized trials comparing morning versus evening administration consistently reported better safety and efficacy outcomes when antihypertensives were taken at night [65]. Similar patterns have been observed in oncology, where dual therapy for blood pressure control is more effective and better tolerated when administered in the evening [65]. Individuals exhibiting a nondipping or riser blood pressure pattern, where nocturnal blood pressure fails to decrease as expected, may particularly benefit from nighttime antihypertensive intake [68]. A notable exception to this trend is diuretics, which increase urine output and, if taken before bedtime, may lead to frequent nighttime urination, disrupting sleep and negatively affecting overall health [69]. Given the widespread prevalence of hypertension and its associated risks, a more personalized, integrative treatment approach has been increasingly emphasized. This involves considering factors such as lifestyle, coexisting medical conditions, and organ-specific physiological differences [70]. Statins are widely used to lower low-density lipoprotein (LDL) cholesterol and reduce the risk of cardiovascular events [71]. Research in rats has shown that hepatic expression of HMG-CoA mRNA follows a circadian pattern [72], although additional factors such as hormonal fluctuations and feeding-fasting cycles also influence this process [73]. In humans, variations in circadian timing systems and individual lifestyle habits may affect statin efficacy at different times of the day. While evening administration is generally recommended [66], no strong evidence indicating significant clinical benefits based on dosage timing has been found [74].

Circadian immune system

Circadian clocks are not limited to the central pacemaker and peripheral tissues; immune cells themselves possess intrinsic circadian rhythms [75]. Both innate and adaptive immune cells harbor functional circadian machinery and their proliferation, migration, and functional activity follows a circadian pattern. For example, in healthy men, the total count of circulating lymphocytes is nearly twice as high at 2:00 AM compared to 8:00 AM. In contrast, circadian variations in NK and effector T cells follow a different rhythm, peaking in the afternoon, whereas B cell levels reach their highest concentration around 8:00 PM (**Figure 2**) [76, 77]. The SCN exerts control over immune cell rhythms through neuronal and hormonal pathways, particularly via cortisol and catecholamines, which regulate immune activity based on the time of day [78]. Current investigations are focused on understanding how sex differences, microbiome composition, and circadian rhythms collectively shape immune responses and influence pharmacological effects. A well-documented sex bias exists in autoimmune disease prevalence [79] and susceptibility to severe infections (49). Interestingly, studies in germ-free mice suggest that microbiome depletion can diminish this sex-based disparity, modifying the incidence of conditions such as type 1 diabetes and liver cancer [80]. Additionally, research indicates that circadian rhythms in microbiota-host interactions are more pronounced in females than in males [81]. The interaction between the circadian timing system and the microbiome appears to be crucial in shaping immune system development and function across the lifespan, with potential applications in optimizing therapeutic strategies [82, 83].

Circadian responses to immunotherapy and anti-inflammatory drugs

Medication timing plays a pivotal role in determining both its therapeutic benefits and potential side effects, a concept particularly evident in the use of immunotherapy and anti-inflammatory drugs in both human patients and laboratory rodents [84]. Since their regulatory approval in 2014, immune checkpoint inhibitors have become a fundamental component of cancer treatment [85]. Findings from several independent retrospective studies have consistently revealed that patients experience enhanced progression-free and overall survival when immune checkpoint inhibitor infusions are scheduled in the morning or early in the day [86, 87], with infusions given later in the day correlating with poorer outcomes [88]. These observations highlight the importance of precisely scheduling treatments according to optimal circadian timings. Despite immune checkpoint inhibitors possessing relatively long plasma half-lives [89], their pronounced time-dependent efficacy underscores the significance of circadian pharmacodynamics within tumors and their associated lymph nodes. Experimental studies support this notion, revealing that T lymphocytes fail to migrate efficiently from the bloodstream to lymph nodes during the latter half of the nocturnal activity period in mice, corresponding to the afternoon and evening in humans. This suggests that immune checkpoint inhibitors administered during these hours may exhibit reduced effectiveness due to impaired immune cell trafficking. Although emerging evidence indicates that efficacy of immune checkpoint inhibitor may vary by sex [90], the interaction between sex and administration timing remains unexplored. Recent research on everolimus has uncovered sexually dimorphic circadian immune responses in mice with males exhibiting a nearly threefold higher susceptibility compared to females [91]. These results underscore the necessity for sex-specific preclinical studies incorporating mechanistic insights to refine immunotherapy strategies. Circadian rhythms also modulate the immune response following vaccination [92]. Research has demonstrated that vaccines administered in the morning elicit more robust adaptive and trained immune responses, including heightened antibody production, compared to those given in the evening. This pattern has been observed with vaccines for Bacillus Calmette-Guérin, influenza, and SARS-CoV-2 [93]. Similarly, the pharmacokinetics of immunosuppressive medications fluctuate according to circadian rhythms. In recipients of solid organ transplants, morning administration of mycophenolate mofetil leads to higher peak plasma concentrations (C_max) and increased area under the curve (AUC_0–12h) values compared to evening dosing. These findings suggest that adjusting the timing of immunosuppressive therapy could enhance its efficacy [94, 95].

Chronopharmacological research has also reinforced the beneficial impact of medication timing for the treatment of both corticosteroids and nonsteroidal anti-inflammatory drugs. For instance, in individuals with osteoarthritis, taking the sustained-release version of indomethacin in the evening rather than in the morning led to nearly four times lower toxicity and twice the therapeutic efficacy [96]. Likewise, in patients with rheumatoid arthritis, administering short-acting prednisolone around 2:00 AM

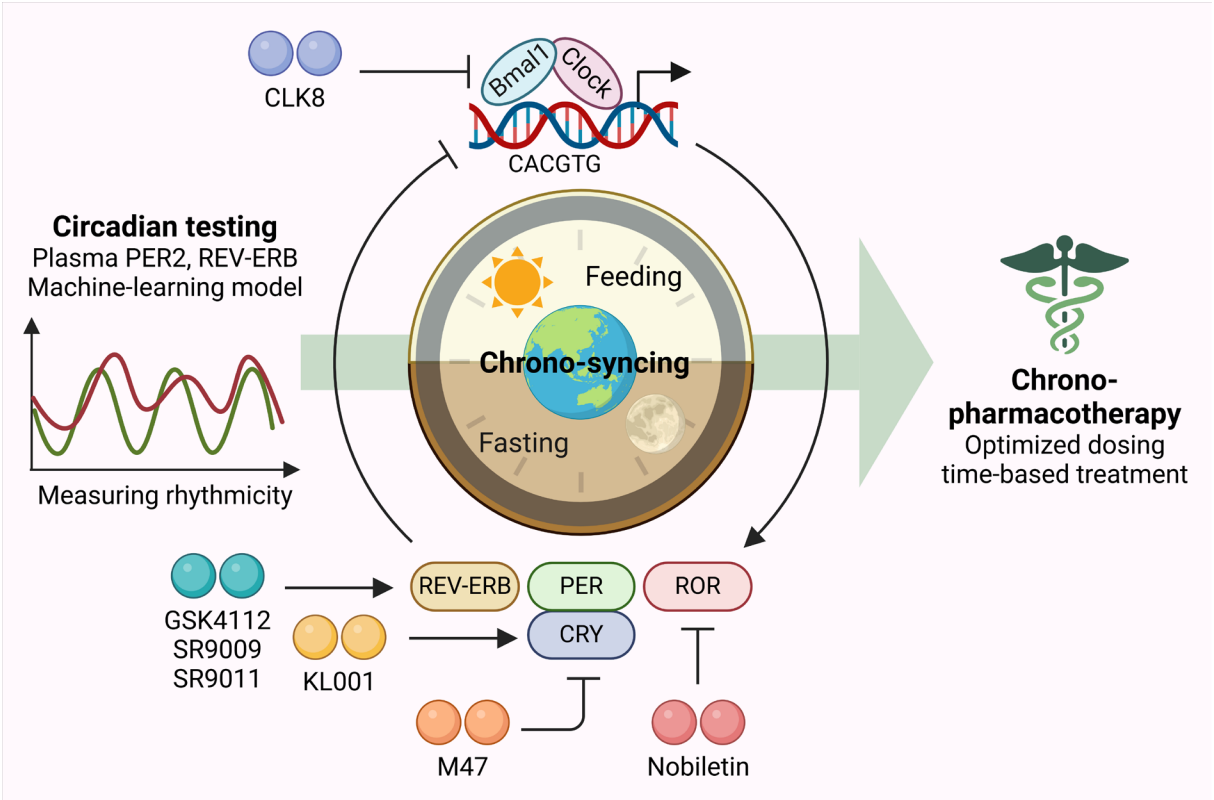

Figure 3. Chrono-pharmacotherapy as precision medicine. Plasma PER2, REV-ERB levels along with machine-learning models can serve as circadian testing tools to measure rhythmicity. Based on which, chrono-syncing can be performed using different therapeutic agents targeting genes involved in regulating circadian clock. This can help in devising optimized dosing time-based treatment plans as precision medicine.

was found to be most effective in reducing morning stiffness [97]. To improve adherence to treatment without requiring patients to wake up for nighttime dosing, researchers developed a modified-release formulation of prednisone. When taken at 10:00 PM, this formulation delayed drug release by approximately four hours, synchronizing with peak levels of circulating cytokines responsible for joint stiffness [98, 99]. Similarly, methotrexate, an anti-inflammatory dihydrofolate reductase inhibitor, showed superior effectiveness in rheumatoid arthritis patients when taken in the evening compared to the conventional thrice-daily regimen [100]. Moreover, the relevance of circadian-based drug administration is not limited to inflammatory diseases. Studies indicate that aligning the timing of central nervous system–acting medications with circadian rhythms can improve therapeutic outcomes in both psychiatric and neurological disorders [101, 102].

Diagnosing circadian rhythms to tailor precision chronopharmacotherapy

Given the substantial variation in circadian timing function among individuals, shaped by factors such as sex, lifestyle, genetic predisposition, and medical history, there is growing recognition that personalized treatment strategies based on circadian cycles may be more effective than generalized chronotherapy. Achieving precision medicine in this context requires reliable diagnostic tools capable of assessing circadian rhythm parameters at an individual level. Chronotype questionnaires, which assess personal preferences for activity and sleep timing, have revealed significant differences based on age and sex [103]. One of the most commonly used biomarkers for circadian phase is the dim light melatonin onset (DLMO) test (**Figure 3**) [104]. However, a comprehensive meta-analysis of 152 studies involving 4,397 healthy participants showed substantial variability in DLMO timing between individuals, with no significant differences detected between sexes [105]. Despite its reliability, practical constraints, such as environmental, dietary, and pharmacological factors, often limit the feasibility of DLMO testing in clinical settings [106]. To overcome these challenges, alternative methods for assessing circadian timing system are under development. Machine learning algorithms have been employed to predict circadian phase based on mRNA expression levels of core clock genes such as Period homolog 2 (PER2) and REV-ERBβ from a single blood sample [107]. Moreover, transcriptomic models driven by artificial intelligence can estimate molecular clock function by analyzing RNA sequencing data from tissue biopsies [108]. Recent advancements in wearable sensor technology and remote monitoring platforms have made it possible to continuously track circadian biomarkers in real-world conditions. Devices that record rest-activity patterns and body positioning have shown striking inter-individual differences, sometimes as large as 12 hours, in peak activity or body temperature rhythms, observed in both healthy and diseased individual [109].

Targeting circadian clock as precision chronopharmacotherapy

The field of chronopharmacology is progressing rapidly, particularly with the introduction of innovative pharmacological agents designed to target molecular clock proteins [110]. These compounds have the potential to optimize the timing of drug administration by synchronizing it with the body's natural

circadian rhythms or restoring disrupted clock functions, ultimately reducing disease susceptibility and improving chronotherapy. The discovery of KL001 marked a significant milestone in this field, as it was the first molecule found to modulate the molecular clock by preventing CRY's ubiquitin-dependent degradation, thereby prolonging the circadian cycle [111]. This compound has demonstrated antitumor effects by inhibiting the CLOCK-BMAL1 complex, leading to reduced glioblastoma proliferation in cell cultures [112]. Additionally, KL001's ability to stabilize CRY has been linked to the suppression of gluconeogenesis in hepatocytes, suggesting its potential therapeutic application for diabetes (**Figure 3**) [110, 113]. Since then, several CRY modulators have exhibited hypoglycemic effects in diabetic mice [114]. More recently, M47, a newly identified selective CRY1 destabilizer, has shown promising pharmacokinetic properties and the ability to cross the blood-brain barrier in mice [115]. In p53−/− cancer-prone mice, M47 treatment extended lifespan by 25% compared to control groups, highlighting its potential as a therapeutic candidate for cancers with mutations in p53 gene. CLK8 has emerged as the only known candidate capable of modulating the interaction between CLOCK and BMAL1 [116]. By disrupting the nuclear translocation of CLOCK, CLK8 enhances the amplitude of circadian rhythms without altering the overall length of the cycle. Given that reduced circadian amplitude is associated with aging and various diseases, compounds like CLK8, which strengthen rhythmic oscillations, could offer significant therapeutic advantages. A retinoic acid–related orphan receptor (ROR) agonist, nobiletin, has also been found to enhance circadian amplitude while extending the clock period. This compound has also demonstrated beneficial effects on metabolism and longevity in mice [117]. In the realm of REV-ERB targeting compounds, GSK4112 was the first synthetic agonist identified. This molecule has been shown to suppress Bmal1, inhibit gluconeogenesis, and decrease the secretion of the pro-inflammatory cytokines. Additionally, SR9009 and SR9011, originally developed as stenabolics, have been recognized as potent REV-ERB agonists (**Figure 3**) [118]. Due to their widespread impact on metabolic gene expression in various tissues, REV-ERB agonists hold promise for treating sleep disorders, jet lag, and metabolic conditions. Moreover, SR9009 and SR9011 have exhibited selective cytotoxic effects against cancer cells. They have also been shown to slow glioblastoma progression [119]. However, a notable limitation of these compounds is their off-target affinity for the liver X receptor α (LXRα), a key regulator of inflammation and energy metabolism. To address this issue, newer compounds have been developed, demonstrating greater selectivity for REV-ERBα. Overall, despite the progress in developing molecules that target the circadian clock and rhythms, many of these compounds still face significant challenges in transitioning from in vitro research to in vivo applications due to suboptimal pharmacokinetic properties.

Conclusion and future prospects

Over the past 50 years, extensive research using laboratory rodents has highlighted the critical role of circadian rhythms in shaping drug responses across multiple pharmacological categories. Moreover, growing evidence indicates that the timing of drug administration significantly impacts both the efficacy and tolerability of various medications in human patients. Chronopharmacology is rapidly emerging as a key component of precision circadian medicine and personalized chronotherapy. One of the most significant advancements in this field has been the recognition of sex-based differences in circadian regulation, challenging the long-standing belief that the central timekeeping system operates uniformly in both male and female mammals [104]. Despite these compelling findings, further large-scale randomized clinical trials are necessary to establish standardized guidelines for incorporating circadian timing into routine medical practice. Notably, despite the well-documented interplay between cell cycle regulation and circadian clock, circadian-based evaluations have not been conducted for the emerging class of cyclin-dependent kinase 4/6 inhibitors [120]. This research gap presents a major challenge in advancing the fields of chronopharmacology and chronotherapy, hindering the clinical application of molecular clock discoveries. The collective evidence emphasizes the urgency for regulatory agencies, research institutions, funding bodies, and the pharmaceutical and biomedical sectors to prioritize the integration of chronopharmacology into clinical practice. Utilizing physiological, hormonal, and molecular circadian timing system biomarkers could enhance drug safety and efficacy by enabling personalized medication schedules tailored to patients who possess a well-regulated circadian timing system. In contrast, individuals with a dysfunctional circadian timing system tend to experience worse clinical outcomes and may require behavioral, environmental, or pharmacological interventions to restore circadian function and optimize chronotherapy results [51]. Although preclinical studies have demonstrated circadian influences on nearly all drug classes, additional clinical research is required to investigate sex-based and inter- and intra-patient variability in circadian timing system. Fully harnessing the therapeutic potential of circadian drug response mechanisms will likely depend on coordinated chronotherapeutic strategies spanning in vitro experiments, rodent models, and large-scale human clinical trials, including Phase III randomized trials. These studies must incorporate sex differences and circadian timing system biomarkers in patient stratification. Ultimately, the goal of chronopharmacology is to develop personalized drug administration schedules that maximize therapeutic benefits, ushering in a new era of precision medicine.

Acknowledgments

No applicable.

Ethics approval

No applicable.

Data availability

The data will be available upon request.

Funding

None.

Authors' contribution

Samir Arabi contributed to draft, critical revision of the article, table making, and figure drawing; Sajjad Ahmad checked and revised the manuscript and approved the final submission.

Competing interests

The authors declare no competing interests.

References

1. Fagiani F, Di Marino D, Romagnoli A, Travelli C, Voltan D, Di Cesare Mannelli L, Racchi M, Govoni S, Lanni C: Molecular regulations of circadian rhythm and implications for physiology and diseases. Sig Transduct Target Ther 2022, 7(1): 41.

2. Hastings MH, Reddy AB, Maywood ES: A clockwork web: circadian timing in brain and periphery, in health and disease. Nat Rev Neurosci 2003, 4(8): 649-661.
3. Lévi F, Okyar A, Dulong S, Innominato PF, Clairambault J: Circadian timing in cancer treatments. Annu Rev Pharmacol Toxicol 2010, 50: 377-421.
4. Dallmann R, Okyar A, Lévi F: Dosing-Time Makes the Poison: Circadian Regulation and Pharmacotherapy. Trends Mol Med 2016, 22(5): 430-445.
5. Lévi FA, Okyar A, Hadadi E, Innominato PF, Ballesta A: Circadian Regulation of Drug Responses: Toward Sex-Specific and Personalized Chronotherapy. Annu Rev Pharmacol Toxicol 2024, 64: 89-114.
6. Ayyar VS, Sukumaran S: Circadian rhythms: influence on physiology, pharmacology, and therapeutic interventions. J Pharmacokinet Pharmacodyn 2021, 48(3): 321-338.
7. Amorós-Figueras G, Jorge E, Alonso-Martin C, Traver D, Ballesta M, Bragós R, Rosell-Ferrer J, Cinca J: Endocardial infarct scar recognition by myocardial electrical impedance is not influenced by changes in cardiac activation sequence. Heart Rhythm 2018, 15(4): 589-596.
8. Hesse J, Malhan D, Yalçin M, Aboumanify O, Basti A, Relógio A: An Optimal Time for Treatment-Predicting Circadian Time by Machine Learning and Mathematical Modelling. Cancers (Basel) 2020, 12(11): 3103.
9. Talamanca L, Naef F: How to tell time: advances in decoding circadian phase from omics snapshots. F1000Res 2020, 9: F1000 Faculty Rev-1150.
10. Dose B, Yalçin M, Dries SPM, Relógio A: TimeTeller for timing health: The potential of circadian medicine to improve performance, prevent disease and optimize treatment. Front Digit Health 2023, 5: 1157654.
11. Cederroth CR, Albrecht U, Bass J, Brown SA, Dyhrfjeld-Johnsen J, Gachon F, Green CB, Hastings MH, Helfrich-Förster C, Hogenesch JB et al: Medicine in the Fourth Dimension. Cell Metab 2019, 30(2): 238-250.
12. Schwartzberg L, Kim ES, Liu D, Schrag D: Precision Oncology: Who, How, What, When, and When Not? Am Soc Clin Oncol Educ Book 2017, 37: 160-169.
13. Levi F, Schibler U: Circadian rhythms: mechanisms and therapeutic implications. Annu Rev Pharmacol Toxicol 2007, 47: 593-628.
14. Hesse J, Martinelli J, Aboumanify O, Ballesta A, Relógio A: A mathematical model of the circadian clock and drug pharmacology to optimize irinotecan administration timing in colorectal cancer. Comput Struct Biotechnol J 2021, 19: 5170-5183.
15. Filipski E, King VM, Etienne MC, Li X, Claustrat B, Granda TG, Milano G, Hastings MH, Lévi F: Persistent twenty-four hour changes in liver and bone marrow despite suprachiasmatic nuclei ablation in mice. Am J Physiol Regul Integr Comp Physiol 2004, 287(4): R844-851.
16. Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Siepka SM, Hong HK, Oh WJ, Yoo OJ et al: PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci U S A 2004, 101(15): 5339-5346.
17. Finger AM, Kramer A: Mammalian circadian systems: Organization and modern life challenges. Acta Physiol (Oxf) 2021, 231(3): e13548.
18. Daiber A, Frenis K, Kuntic M, Li H, Wolf E, Kilgallen AB, Lecour S, Van Laake LW, Schulz R, Hahad O et al: Redox Regulatory Changes of Circadian Rhythm by the Environmental Risk Factors Traffic Noise and Air Pollution. Antioxid Redox Signal 2022, 37(10-12): 679-703.
19. Fougeray T, Polizzi A, Régnier M, Fougerat A, Ellero-Simatos S, Lippi Y, Smati S, Lasserre F, Tramunt B, Huillet M et al: The hepatocyte insulin receptor is required to program the liver clock and rhythmic gene expression. Cell Rep 2022, 39(2): 110674.
20. Takahashi JS: Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet 2017, 18(3): 164-179.
21. Robles MS, Humphrey SJ, Mann M: Phosphorylation Is a Central Mechanism for Circadian Control of Metabolism and Physiology. Cell Metab 2017, 25(1): 118-127.
22. Anderson ST, FitzGerald GA: Sexual dimorphism in body clocks. Science 2020, 369(6508): 1164-1165.
23. Talamanca L, Gobet C: Sex-dimorphic and age-dependent organization of 24-hour gene expression rhythms in humans. Science 2023, 379(6631): 478-483.
24. Wucher V, Sodaei R, Amador R, Irimia M: Day-night and seasonal variation of human gene expression across tissues. PLoS Biol 2023, 21(2): e3001986.
25. Dobrek L: Chronopharmacology in Therapeutic Drug Monitoring-Dependencies between the Rhythmics of Pharmacokinetic Processes and Drug Concentration in Blood. Pharmaceutics 2021, 13(11): 1915.
26. Dong D, Yang D, Lin L, Wang S, Wu B: Circadian rhythm in pharmacokinetics and its relevance to chronotherapy. Biochem Pharmacol 2020, 178: 114045.
27. Dulong S, Ballesta A, Okyar A, Lévi F: Identification of Circadian Determinants of Cancer Chronotherapy through In Vitro Chronopharmacology and Mathematical Modeling. Mol Cancer Ther 2015, 14(9): 2154-2164.
28. Wada E, Koyanagi S, Kusunose N, Akamine T, Masui H, Hashimoto H, Matsunaga N, Ohdo S: Modulation of peroxisome proliferator-activated receptor-α activity by bile acids causes circadian changes in the intestinal expression of Octn1/Slc22a4 in mice. Mol Pharmacol 2015, 87(2): 314-322.
29. Pácha J, Balounová K, Soták M: Circadian regulation of transporter expression and implications for drug disposition. Expert Opin Drug Metab Toxicol 2021, 17(4): 425-439.
30. Zhang YK, Yeager RL, Klaassen CD: Circadian expression profiles of drug-processing genes and transcription factors in mouse liver. Drug Metab Dispos 2009, 37(1): 106-115.
31. Radzialowski FM, Bousquet WF: Daily rhythmic variation in hepatic drug metabolism in the rat and mouse. J Pharmacol Exp Ther 1968, 163(1): 229-238.
32. Nair V, Casper R: The influence of light on daily rhythm in hepatic drug metabolizing enzymes in rat. Life Sci 1969, 8(23): 1291-1298.
33. Gachon F, Olela FF, Schaad O, Descombes P, Schibler U: The circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification. Cell Metab 2006, 4(1): 25-36.
34. Nicolaides NC, Chrousos GP: Sex differences in circadian endocrine rhythms: Clinical implications. Eur J Neurosci 2020, 52(1): 2575-2585.
35. Soldin OP, Chung SH, Mattison DR: Sex differences in drug disposition. J Biomed Biotechnol 2011, 2011: 187103.
36. Okyar A, Piccolo E, Ahowesso C, Filipski E, Hossard V, Guettier C, La Sorda R, Tinari N, Iacobelli S, Lévi F: Strain- and sex-dependent circadian changes in abcc2 transporter expression: implications for irinotecan chronotolerance in mouse ileum. PLoS One 2011, 6(6): e20393.
37. Okyar A, Kumar SA, Filipski E, Piccolo E, Ozturk N, Xandri-Monje H, Pala Z, Abraham K, Gomes A, Orman MN et al: Sex-, feeding-, and circadian time-dependency of P-glycoprotein expression and activity - implications for mechanistic pharmacokinetics modeling. Sci Rep 2019, 9(1): 10505.
38. Cui YJ, Cheng X, Weaver YM, Klaassen CD: Tissue distribution, gender-divergent expression, ontogeny, and chemical induction of multidrug resistance transporter genes (Mdr1a, Mdr1b, Mdr2) in mice. Drug Metab Dispos 2009, 37(1): 203-210.
39. Ando H, Yanagihara H, Sugimoto K, Hayashi Y, Tsuruoka S, Takamura T, Kaneko S, Fujimura A: Daily rhythms of P-glycoprotein expression in mice. Chronobiol Int 2005, 22(4): 655-665.
40. Du K, Williams CD, McGill MR, Jaeschke H: Lower susceptibility

of female mice to acetaminophen hepatotoxicity: Role of mitochondrial glutathione, oxidant stress and c-jun N-terminal kinase. Toxicol Appl Pharmacol 2014, 281(1): 58-66.
41. Dulong S, de Souza LEB: Sex and Circadian Timing Modulate Oxaliplatin Hematological and Hematopoietic Toxicities. Pharmaceutics 2022, 14(11): 2465.
42. Masubuchi Y, Nakayama J, Watanabe Y: Sex difference in susceptibility to acetaminophen hepatotoxicity is reversed by buthionine sulfoximine. Toxicology 2011, 287(1-3): 54-60.
43. Marcu LG: Developments on tumour site-specific chrono-oncology towards personalised treatment. Crit Rev Oncol Hematol 2022, 179: 103803.
44. Printezi MI, Kilgallen AB, Bond MJG, Štibler U, Putker M, Teske AJ, Cramer MJ, Punt CJA, Sluijter JPG, Huitema ADR et al: Toxicity and efficacy of chronomodulated chemotherapy: a systematic review. Lancet Oncol 2022, 23(3): e129-e143.
45. Siegel RL, Wagle NS: Colorectal cancer statistics, 2023. CA Cancer J Clin 2023, 73(3): 233-254.
46. Zhang PX, Jin F, Li ZL, Wu WL, Li YY, Long JH, Chen GY, Chen XX, Gan JY, Gong XY et al: A randomized phase II trial of induction chemotherapy followed by cisplatin chronotherapy versus constant rate delivery combined with radiotherapy. Chronobiol Int 2018, 35(2): 240-248.
47. Li J, Chen R, Ji M, Zou SL, Zhu LN: Cisplatin-based chronotherapy for advanced non-small cell lung cancer patients: a randomized controlled study and its pharmacokinetics analysis. Cancer Chemother Pharmacol 2015, 76(3): 651-655.
48. Kumar SA, Needham RJ, Abraham K, Bridgewater HE, Garbutt LA, Xandri-Monje H, Dallmann R, Perrier S, Sadler PJ, Lévi F: Dose- and time-dependent tolerability and efficacy of organo-osmium complex FY26 and its tissue pharmacokinetics in hepatocarcinoma-bearing mice. Metallomics 2021, 13(2): mfaa003.
49. Innominato PF, Lévi FA, Bjarnason GA: Chronotherapy and the molecular clock: Clinical implications in oncology. Adv Drug Deliv Rev 2010, 62(9-10): 979-1001.
50. Gorbacheva VY, Kondratov RV, Zhang R, Cherukuri S, Gudkov AV, Takahashi JS, Antoch MP: Circadian sensitivity to the chemotherapeutic agent cyclophosphamide depends on the functional status of the CLOCK/BMAL1 transactivation complex. Proc Natl Acad Sci U S A 2005, 102(9): 3407-3412.
51. Ballesta A, Innominato PF, Dallmann R, Rand DA, Lévi FA: Systems Chronotherapeutics. Pharmacol Rev 2017, 69(2): 161-199.
52. Wagner AD, Oertelt-Prigione S, Adjei A, Buclin T, Cristina V, Csajka C, Coukos G, Dafni U, Dotto GP, Ducreux M et al: Gender medicine and oncology: report and consensus of an ESMO workshop. Ann Oncol 2019, 30(12): 1914-1924.
53. Cristina V, Mahachie J, Mauer M, Buclin T, Van Cutsem E, Roth A, Wagner AD: Association of Patient Sex With Chemotherapy-Related Toxic Effects: A Retrospective Analysis of the PETACC-3 Trial Conducted by the EORTC Gastrointestinal Group. JAMA Oncol 2018, 4(7): 1003-1006.
54. Chansky K, Benedetti J, Macdonald JS: Differences in toxicity between men and women treated with 5-fluorouracil therapy for colorectal carcinoma. Cancer 2005, 103(6): 1165-1171.
55. Spitschan M, Santhi N: Sex differences and sex bias in human circadian and sleep physiology research. Elife 2022, 11: e65419.
56. Bressolle F, Joulia JM, Pinguet F, Ychou M, Astre C, Duffour J, Gomeni R: Circadian rhythm of 5-fluorouracil population pharmacokinetics in patients with metastatic colorectal cancer. Cancer Chemother Pharmacol 1999, 44(4): 295-302.
57. Kim DW, Byun JM, Lee JO, Kim JK, Koh Y: Chemotherapy delivery time affects treatment outcomes of female patients with diffuse large B cell lymphoma. JCI Insight 2023, 8(2): e164767.
58. Ahowesso C, Li XM, Zampera S, Peteri-Brunbäck B, Dulong S, Beau J, Hossard V, Filipski E, Delaunay F, Claustrat B et al: Sex and dosing-time dependencies in irinotecan-induced circadian disruption. Chronobiol Int 2011, 28(5): 458-470.
59. Chaudhary R, Sharma T, Tantry US, Asgar JA, Kundan P, Duhan S, Gill H, Singh A, Alasadi Y, Gurbel PA: Serial assessment of thrombogenicity and hemodynamics in patients with type II diabetes in a clinical research unit: Evidence for circadian variations in clot formation. J Thromb Thrombolysis 2022, 54(3): 393-400.
60. Schved JF, Gris JC, Eledjam JJ: Circadian changes in anticoagulant effect of heparin infused at a constant rate. Br Med J (Clin Res Ed) 1985, 290(6477): 1286.
61. Brunner-Ziegler S, Jilma B, Schörgenhofer C, Winkler F, Jilma-Stohlawetz P, Koppensteiner R, Quehenberger P, Seger C, Weigel G, Griesmacher A et al: Comparison between the impact of morning and evening doses of rivaroxaban on the circadian endogenous coagulation rhythm in healthy subjects. J Thromb Haemost 2016, 14(2): 316-323.
62. Schoergenhofer C, Schwameis M, Brunner M, Zeitlinger M, Winkler F, Jilma B, Brunner-Ziegler S: Assessing the influence of diurnal variations and selective Xa inhibition on whole blood aggregometry. Scand J Clin Lab Invest 2015, 75(6): 531-536.
63. Soulban G, Labrecque G: Circadian rhythms of blood clotting time and coagulation factors II, VII, IX and X in rats. Life Sci 1989, 45(25): 2485-2489.
64. Gumz ML, Shimbo D: Toward Precision Medicine: Circadian Rhythm of Blood Pressure and Chronotherapy for Hypertension - 2021 NHLBI Workshop Report. Hypertension 2023, 80(3): 503-522.
65. Hermida-Ayala RG, Mojón A, Fernández JR, Smolensky MH, Hermida RC: Ingestion-time differences in the pharmacodynamics of dual-combination hypertension therapies: Systematic review and meta-analysis of published human trials. Chronobiol Int 2022, 39(4): 493-512.
66. Ruben MD, Smith DF, FitzGerald GA, Hogenesch JB: Dosing time matters. Science 2019, 365(6453): 547-549.
67. Hermida RC, Smolensky MH, Balan H, Castriotta RJ, Crespo JJ, Dagan Y, El-Toukhy S, Fernández JR, FitzGerald GA, Fujimura A et al: Guidelines for the design and conduct of human clinical trials on ingestion-time differences - chronopharmacology and chronotherapy - of hypertension medications. Chronobiol Int 2021, 38(1): 1-26.
68. Hermida RC, Mojón A, Fernández JR, Hermida-Ayala RG, Crespo JJ, Ríos MT, Domínguez-Sardiña M, Otero A, Smolensky MH: Elevated asleep blood pressure and non-dipper 24h patterning best predict risk for heart failure that can be averted by bedtime hypertension chronotherapy: A review of the published literature. Chronobiol Int 2023, 40(1): 63-82.
69. Irwin MR: Sleep and inflammation: partners in sickness and in health. Nat Rev Immunol 2019, 19(11): 702-715.
70. Valenzuela PL, Carrera-Bastos P: Lifestyle interventions for the prevention and treatment of hypertension. Nat Rev Cardiol 2021, 18(4): 251-275.
71. Chou R, Dana T, Blazina I, Daeges M, Jeanne TL: Statins for Prevention of Cardiovascular Disease in Adults: Evidence Report and Systematic Review for the US Preventive Services Task Force. Jama 2016, 316(19): 2008-2024.
72. Hamprecht B, Nüssler C, Lynen F: Rhythmic changes of hydroxymethylglutaryl coenzyme a reductase activity in livers of fed and fasted rats. FEBS Lett 1969, 4(2): 117-121.
73. Ness GC, Chambers CM: Feedback and hormonal regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase: the concept of cholesterol buffering capacity. Proc Soc Exp Biol Med 2000, 224(1): 8-19.
74. Izquierdo-Palomares JM, Fernandez-Tabera JM, Plana MN, Añino Alba A, Gómez Álvarez P, Fernandez-Esteban I, Saiz LC, Martin-Carrillo P, Pinar López Ó: Chronotherapy versus conventional statins therapy for the treatment of hyperlipidaemia. Cochrane Database Syst Rev 2016, 11(11): Cd009462.
75. Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk HD, Kramer A, Maier B: A circadian clock in macrophages controls

inflammatory immune responses. Proc Natl Acad Sci U S A 2009, 106(50): 21407-21412.
76. Lange T, Luebber F: The contribution of sleep to the neuroendocrine regulation of rhythms in human leukocyte traffic. Semin Immunopathol 2022, 44(2) :239-254.
77. Ackermann K, Revell VL, Lao O, Rombouts EJ, Skene DJ, Kayser M: Diurnal rhythms in blood cell populations and the effect of acute sleep deprivation in healthy young men. Sleep 2012, 35(7): 933-940.
78. Downton P, Early JO: Circadian rhythms in adaptive immunity. Immunology 2020, 161(4): 268-277.
79. Lasrado N, Jia T, Massilamany C, Franco R, Illes Z, Reddy J: Mechanisms of sex hormones in autoimmunity: focus on EAE. Biol Sex Differ 2020, 11(1): 50.
80. Xie G, Wang X, Zhao A, Yan J, Chen W, Jiang R, Ji J, Huang F, Zhang Y, Lei S et al: Sex-dependent effects on gut microbiota regulate hepatic carcinogenic outcomes. Sci Rep 2017, 7: 45232.
81. Elderman M, de Vos P, Faas M: Role of Microbiota in Sexually Dimorphic Immunity. Front Immunol 2018, 9: 1018.
82. Zheng D, Liwinski T, Elinav E: Interaction between microbiota and immunity in health and disease. Cell Res 2020, 30(6): 492-506.
83. Thaiss CA, Zmora N, Levy M, Elinav E: The microbiome and innate immunity. Nature 2016, 535(7610): 65-74.
84. Ursini F, De Giorgi A, D'Onghia M, De Giorgio R: Chronobiology and Chronotherapy in Inflammatory Joint Diseases. Pharmaceutics 2021, 13(11): 1832.
85. Guha P, Heatherton KR, O'Connell KP, Alexander IS: Assessing the Future of Solid Tumor Immunotherapy. Biomedicines 2022, 10(3): 655.
86. Qian DC, Kleber T, Brammer B, Xu KM, Switchenko JM, Janopaul-Naylor JR, Zhong J, Yushak ML, Harvey RD, Paulos CM et al: Effect of immunotherapy time-of-day infusion on overall survival among patients with advanced melanoma in the USA (MEMOIR): a propensity score-matched analysis of a single-centre, longitudinal study. Lancet Oncol 2021, 22(12): 1777-1786.
87. Patel JS, Woo Y, Draper A, Jansen CS: Impact of immunotherapy time-of-day infusion on survival and immunologic correlates in patients with metastatic renal cell carcinoma: a multicenter cohort analysis. J Immunother Cancer 2024, 12(3): e008011.
88. Rousseau A, Tagliamento M, Auclin E, Aldea M, Frelaut M, Levy A, Benitez JC, Naltet C, Lavaud P, Botticella A et al: Clinical outcomes by infusion timing of immune checkpoint inhibitors in patients with advanced non-small cell lung cancer. Eur J Cancer 2023, 182: 107-114.
89. Centanni M, Moes D, Trocóniz IF, Ciccolini J, van Hasselt JGC: Clinical Pharmacokinetics and Pharmacodynamics of Immune Checkpoint Inhibitors. Clin Pharmacokinet 2019, 58(7): 835-857.
90. Conforti F, Pala L, Pagan E, Corti C, Bagnardi V, Queirolo P, Catania C, De Pas T, Giaccone G: Sex-based differences in response to anti-PD-1 or PD-L1 treatment in patients with non-small-cell lung cancer expressing high PD-L1 levels. A systematic review and meta-analysis of randomized clinical trials. ESMO Open 2021, 6(5): 100251.
91. Ozturk N, Ozturk D, Pala-Kara Z, Kaptan E, Sancar-Bas S, Ozsoy N, Cinar S, Deniz G, Li XM, Giacchetti S et al: The immune system as a chronotoxicity target of the anticancer mTOR inhibitor everolimus. Chronobiol Int 2018, 35(5): 705-718.
92. Otasowie CO, Tanner R, Ray DW, Austyn JM, Coventry BJ: Chronovaccination: Harnessing circadian rhythms to optimize immunisation strategies. Front Immunol 2022, 13: 977525.
93. Zhang H, Liu Y, Liu D, Zeng Q, Li L, Zhou Q, Li M, Mei J, Yang N: Time of day influences immune response to an inactivated vaccine against SARS-CoV-2. Cell Res 2021, 31(11): 1215-1217.
94. Fontova P, Colom H, Rigo-Bonnin R, van Merendonk LN, Vidal-Alabró A, Montero N, Melilli E, Meneghini M, Manonelles A, Cruzado JM et al: Influence of the Circadian Timing System on Tacrolimus Pharmacokinetics and Pharmacodynamics After Kidney Transplantation. Front Pharmacol 2021, 12: 636048.
95. Satoh S, Tada H, Murakami M, Tsuchiya N, Li Z, Numakura K, Saito M, Inoue T, Miura M, Hayase Y et al: Circadian pharmacokinetics of mycophenolic Acid and implication of genetic polymorphisms for early clinical events in renal transplant recipients. Transplantation 2006, 82(4): 486-493.
96. Levi F, Le Louarn C, Reinberg A: Timing optimizes sustained-release indomethacin treatment of osteoarthritis. Clin Pharmacol Ther 1985, 37(1): 77-84.
97. Arvidson NG, Gudbjörnsson B, Larsson A, Hällgren R: The timing of glucocorticoid administration in rheumatoid arthritis. Ann Rheum Dis 1997, 56(1): 27-31.
98. Buttgereit F, Doering G, Schaeffler A, Witte S, Sierakowski S, Gromnica-Ihle E, Jeka S, Krueger K, Szechinski J, Alten R: Efficacy of modified-release versus standard prednisone to reduce duration of morning stiffness of the joints in rheumatoid arthritis (CAPRA-1): a double-blind, randomised controlled trial. Lancet 2008, 371(9608): 205-214.
99. Buttgereit F, Mehta D, Kirwan J, Szechinski J, Boers M, Alten RE, Supronik J, Szombati I, Romer U, Witte S et al: Low-dose prednisone chronotherapy for rheumatoid arthritis: a randomised clinical trial (CAPRA-2). Ann Rheum Dis 2013, 72(2): 204-210.
100. To H, Yoshimatsu H, Tomonari M, Ida H, Tsurumoto T, Tsuji Y, Sonemoto E, Shimasaki N, Koyanagi S, Sasaki H et al: Methotrexate chronotherapy is effective against rheumatoid arthritis. Chronobiol Int 2011, 28(3): 267-274.
101. Sion B, Bégou M: Can chronopharmacology improve the therapeutic management of neurological diseases? Fundam Clin Pharmacol 2021, 35(3): 564-581.
102. Klerman EB, Brager A: Keeping an eye on circadian time in clinical research and medicine. Clin Transl Med 2022, 12(12): e1131.
103. Roenneberg T, Pilz LK, Zerbini G: Chronotype and Social Jetlag: A (Self-) Critical Review. Biology (Basel) 2019, 8(3): 54.
104. Bailey M, Silver R: Sex differences in circadian timing systems: implications for disease. Front Neuroendocrinol 2014, 35(1): 111-139.
105. Kennaway DJ: The dim light melatonin onset across ages, methodologies, and sex and its relationship with morningness/eveningness. Sleep 2023, 46(5): zsad033.
106. Lévi F, Komarzynski S: Tele-Monitoring of Cancer Patients' Rhythms during Daily Life Identifies Actionable Determinants of Circadian and Sleep Disruption. Cancers (Basel) 2020, 12(7): 1938.
107. Wittenbrink N, Ananthasubramaniam B, Münch M, Koller B, Maier B, Weschke C, Bes F, de Zeeuw J, Nowozin C, Wahnschaffe A et al: High-accuracy determination of internal circadian time from a single blood sample. J Clin Invest 2018, 128(9): 3826-3839.
108. Vlachou D, Veretennikova M, Usselmann L, Vasilyev V, Ott S, Bjarnason GA, Dallmann R: TimeTeller: A tool to probe the circadian clock as a multigene dynamical system. PLoS Comput Biol 2024, 20(2): e1011779.
109. Komarzynski S, Huang Q: Relevance of a Mobile Internet Platform for Capturing Inter- and Intrasubject Variabilities in Circadian Coordination During Daily Routine: Pilot Study. J Med Internet Res 2018, 20(6): e204.
110. Ribeiro RFN, Cavadas C, Silva MMC: Small-molecule modulators of the circadian clock: Pharmacological potentials in circadian-related diseases. Drug Discov Today 2021, 26(7): 1620-1641.
111. Hirota T, Lee JW, St John PC, Sawa M, Iwaisako K, Noguchi T, Pongsawakul PY, Sonntag T, Welsh DK, Brenner DA et al: Identification of small molecule activators of cryptochrome. Science 2012, 337(6098): 1094-1097.
112. Dong Z, Zhang G, Qu M, Gimple RC, Wu Q, Qiu Z, Prager BC, Wang X, Kim LJY, Morton AR et al: Targeting Glioblastoma Stem Cells through Disruption of the Circadian Clock. Cancer Discov 2019, 9(11): 1556-1573.
113. Humphries PS, Bersot R, Kincaid J, Mabery E, McCluskie K, Park T, Renner T, Riegler E, Steinfeld T, Turtle ED et al: Carbazole-

containing amides and ureas: Discovery of cryptochrome modulators as antihyperglycemic agents. Bioorg Med Chem Lett 2018, 28(3): 293-297.
114. Gul S, Rahim F, Isin S, Yilmaz F, Ozturk N, Turkay M, Kavakli IH: Structure-based design and classifications of small molecules regulating the circadian rhythm period. Sci Rep 2021, 11(1): 18510.
115. Gul S, Akyel YK: Discovery of a small molecule that selectively destabilizes Cryptochrome 1 and enhances life span in p53 knockout mice. Nat Commun 2022, 13(1): 6742.
116. Doruk YU, Yarparvar D, Akyel YK, Gul S, Taskin AC, Yilmaz F, Baris I, Ozturk N, Türkay M, Ozturk N et al: A CLOCK-binding small molecule disrupts the interaction between CLOCK and BMAL1 and enhances circadian rhythm amplitude. J Biol Chem 2020, 295(11): 3518-3531.
117. He B, Nohara K, Park N, Park YS, Guillory B, Zhao Z, Garcia JM, Koike N, Lee CC, Takahashi JS et al: The Small Molecule Nobiletin Targets the Molecular Oscillator to Enhance Circadian Rhythms and Protect against Metabolic Syndrome. Cell Metab 2016, 23(4): 610-621.
118. Solt LA, Wang Y, Banerjee S, Hughes T, Kojetin DJ, Lundasen T, Shin Y, Liu J, Cameron MD, Noel R et al: Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature 2012, 485(7396): 62-68.
119. Sulli G, Rommel A, Wang X, Kolar MJ, Puca F, Saghatelian A, Plikus MV, Verma IM, Panda S: Pharmacological activation of REV-ERBs is lethal in cancer and oncogene-induced senescence. Nature 2018, 553(7688): 351-355.
120. Lee Y, Lahens NF: G1/S cell cycle regulators mediate effects of circadian dysregulation on tumor growth and provide targets for timed anticancer treatment. PLoS Biol 2019, 17(4): e3000228.