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Circadian Regulation of Drug Responses: Toward Sex-Specific and Personalized Chronotherapy

Francis A. Lévi,[1,2,3] Alper Okyar,[4] Eva Hadadi,[5,6] Pasquale F. Innominato,[7,8] and Annabelle Ballesta[9]

[1]Chronotherapy, Cancers and Transplantation Research Unit, Faculty of Medicine, Paris-Saclay University, Villejuif, France; email: francis.levi@universite-paris-saclay.fr

[2]Gastrointestinal and General Oncology Service, Paul-Brousse Hospital, Assistance Publique-Hôpitaux de Paris, Villejuif, France

[3]Department of Statistics, University of Warwick, Coventry, United Kingdom

[4]Faculty of Pharmacy, Department of Pharmacology, Istanbul University, Beyazit-Istanbul, Turkey

[5]Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium

[6]Laboratory for Myeloid Cell Immunology, Center for Inflammation Research VIB, Zwijnaarde, Belgium

[7]Oncology Department, Ysbyty Gwynedd Hospital, Betsi Cadwaladr University Health Board, Bangor, United Kingdom

[8]Warwick Medical School and Cancer Research Centre, University of Warwick, Coventry, United Kingdom

[9]Inserm Unit 900, Cancer Systems Pharmacology, Institut Curie, MINES ParisTech CBIO—Centre for Computational Biology, PSL Research University, Saint-Cloud, France

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Abstract

Today's challenge for precision medicine involves the integration of the impact of molecular clocks on drug pharmacokinetics, toxicity, and efficacy toward personalized chronotherapy. Meaningful improvements of tolerability and/or efficacy of medications through proper administration timing have been confirmed over the past decade for immunotherapy and chemotherapy against cancer, as well as for commonly used pharmacological agents in cardiovascular, metabolic, inflammatory, and neurological

conditions. Experimental and human studies have recently revealed sexually dimorphic circadian drug responses. Dedicated randomized clinical trials should now aim to issue personalized circadian timing recommendations for daily medical practice, integrating innovative technologies for remote longitudinal monitoring of circadian metrics, statistical prediction of molecular clock function from single-timepoint biopsies, and multiscale biorhythmic mathematical modelling. Importantly, chronofit patients with a robust circadian function, who would benefit most from personalized chronotherapy, need to be identified. Conversely, nonchronofit patients could benefit from the emerging pharmacological class of chronobiotics targeting the circadian clock.

1. INTRODUCTION

Circadian rhythms are defined as endogenous biological rhythms with an approximate period of 24 h (admittedly within a range from 20 to 28 h) (1). In mammals, these rhythms are generated by genetic molecular clocks at the single-cell level. They are coordinated by the suprachiasmatic nuclei (SCN), the hypothalamic circadian pacemaker, which also adjusts them to the environmental 24-h cycle (2).

Prior reviews have examined complementary aspects of chronopharmacology (3–8). Here, we emphasize that the study of circadian drug responses has now become a continuum from cell cultures to organoids, animal models, and patients. Sexual dimorphism is currently emerging as an important factor for the circadian regulation of the main absorption, distribution, metabolism, elimination and toxicity (ADMET) mechanisms at molecular, tissue, and organ levels. We summarize recent advances in the chronopharmacology of those agents that target immunity, cancer, coagulation, metabolism, the cardiovascular system, and inflammatory and rheumatologic processes. We examine the implication of sexually dimorphic effects for chronotherapy, that is, the administration of treatments according to circadian rhythms to reduce adverse events and/or to enhance efficacy. We further analyze the current developmental status of new molecular clock–targeted agents and their promises for chronotherapy.

Predictable chronopharmacologic effects require well-coordinated and functional circadian clocks that need to be measured because reported between- and within-subject differences in circadian rhythms have large implications for optimal drug timing (4, 5). Information on the environmental time cues that synchronize the circadian timing system (CTS) needs to be complemented by molecular and physiological CTS measures for mapping the main mechanisms of circadian rhythms in drug responses in individual subjects.

Dedicated digital platforms and wearable technologies, coupled with machine learning methodologies, now enable the continuous remote measurement of circadian biomarkers and the automatic computation of their parameters in individual patients in real time (6, 9–11). The disruption of host circadian biomarkers has emerged as an independent predictor of poor survival in cancer patients (6, 12). Even the functionality of molecular circadian clocks in healthy or malignant human tissues has become predictable from a single-timepoint biopsy through new omics-based algorithms, further promoting chronotherapy into the era of clock-based precision medicine (13). We illustrate how the combination of biological, clinical, and mathematical frameworks can properly address the unmet need of clock-based precision medicine through the design of precise systems medicine approaches that recapitulate the molecular mechanisms of whole-body drug chronopharmacology in animal models and patients (14, 15).

2. THE CIRCADIAN TIMING SYSTEM

2.1. Main Components: Hierarchical Network of Connected Molecular Circadian Clocks

The hypothalamic SCN pacemaker contains neurons and glial cells whose functions spontaneously oscillate with endogenous periods of nearly 24 h while being synchronized to an exact 24-h period by external time cues such as light-dark cycles and social interactions (3) (**Figure 1*a***). The SCN further generates physiological rhythmic signals, including hormonal or temperature cycles, which can entrain the circadian clocks in peripheral organs. Each cell is endowed with a molecular clock composed of approximately 15 genes interconnected through positive and negative feedback loops that generate circadian oscillations in intracellular processes (16) (**Figure 1*b***). In the absence of external synchronizers or internal rhythmic signals, individual cells may oscillate with different circadian periods, amplitudes, and phases (17, 18). The human chrono-exposome can shift or desynchronize molecular clocks and circadian physiological signals, with implications for chronic disease development (19, 20) (**Figure 1*a***). As an example, meal timing can reset liver clocks through alterations in insulin or glucagon secretions, while rhythmic food intake itself can also drive most of the rhythmic gene expression independently of the cell-autonomous hepatic clock (21).

2.2. Clock-Controlled Pathways and Sex Specificities

Genome-wide studies have uncovered circadian regulation of general cellular mechanisms such as chromatin remodeling, transcription, translation, and posttranslational regulations (22, 23). More than 40% of the 20,000 protein-coding genes display circadian expression in at least 1 out of 12 male mouse organs (24, 25). In male baboons, approximately 80% of the detected protein-coding transcripts exhibited 24-h rhythms in at least one of the 64 studied tissues (26). Consequently, time-dependent activities characterize several intracellular pathways that drive cell metabolism, division, repair, and survival or death (**Figure 1*b***). For instance, a dynamic coupling between the molecular circadian clock and the cell cycle through gene transcriptional control and direct protein-protein interactions was demonstrated at the single-cell level (6).

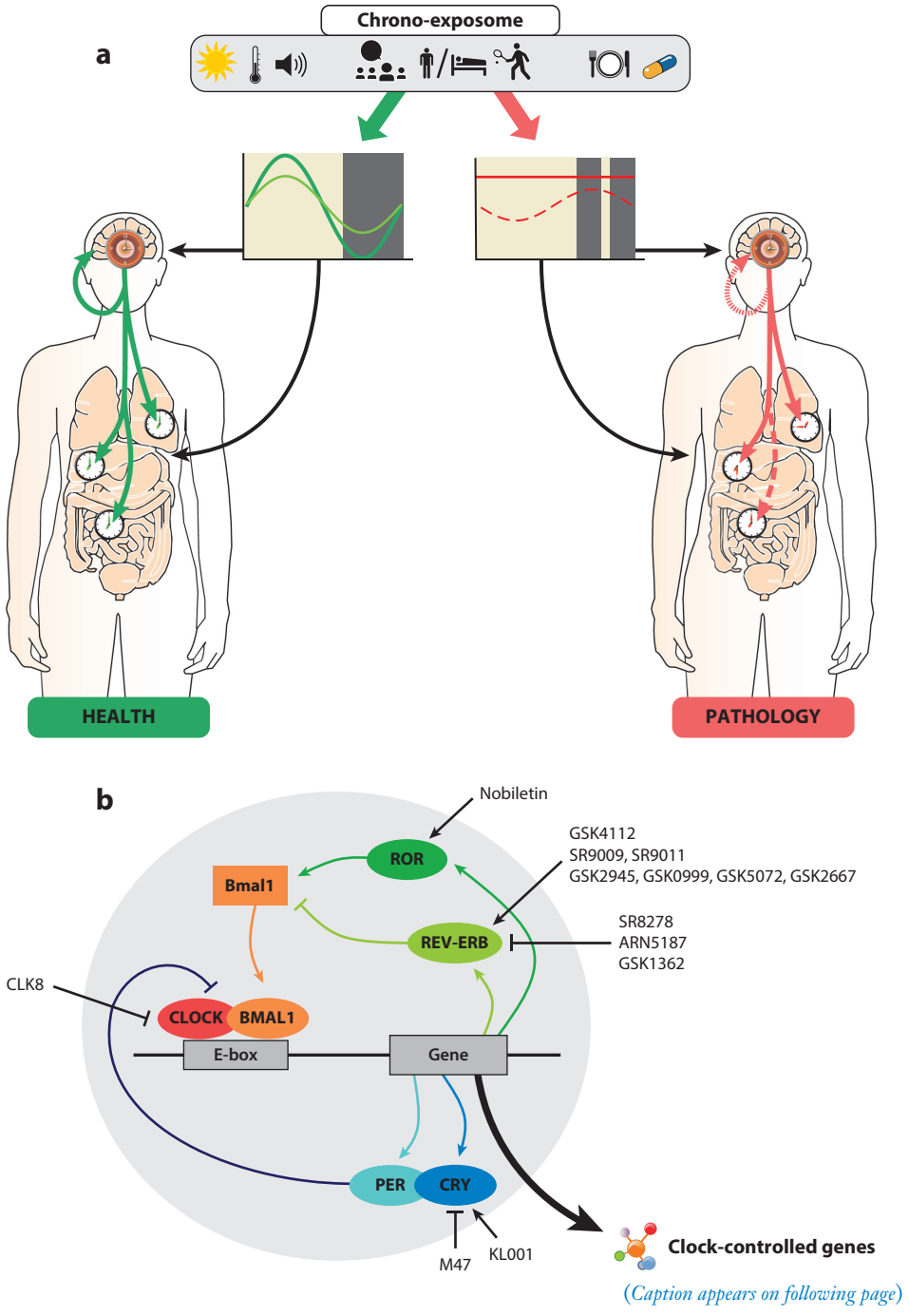
Sex hormone receptors show distinct expression patterning within the SCN that likely contributes to some CTS sexual dimorphism (27). Female rodents often show higher-amplitude rest-activity, body temperature, and corticosterone rhythms and earlier activity onset (27). In mice or humans, 24-h mean levels and circadian amplitudes and/or phase could display different patterns of messenger RNA (mRNA) expression for clock genes and downstream clock-controlled genes, as well as the activities they encode according to sex (28–31) (**Figure 2**; **Supplemental Table 1**).

Supplemental Material >

2.3. The Circadian Immune System

The discovery of autonomous circadian clocks within immune cells (32) and recent results in chrono-immunopharmacology (see Section 4) have established a major role for the circadian immune system at the crossroads of many chronopharmacologic effects.

Large-amplitude circadian rhythms in T and B lymphocyte proliferation, trafficking, and functions have long been known, both in rodents and in humans. For instance, the circulating counts of total lymphocytes—T ($CD3^+$); naive, effector, and memory T ($CD4^+$); and naive T ($CD8^+$) subsets—were nearly twice as high around 2:00 AM compared to 8:00 AM in healthy men. In contrast, lower-amplitude circadian changes were found for effector T ($CD8^+$) and natural killer (NK) cells, with the highest blood counts in the afternoon, and circulating B cells, with a maximum



(*Caption appears on following page*)

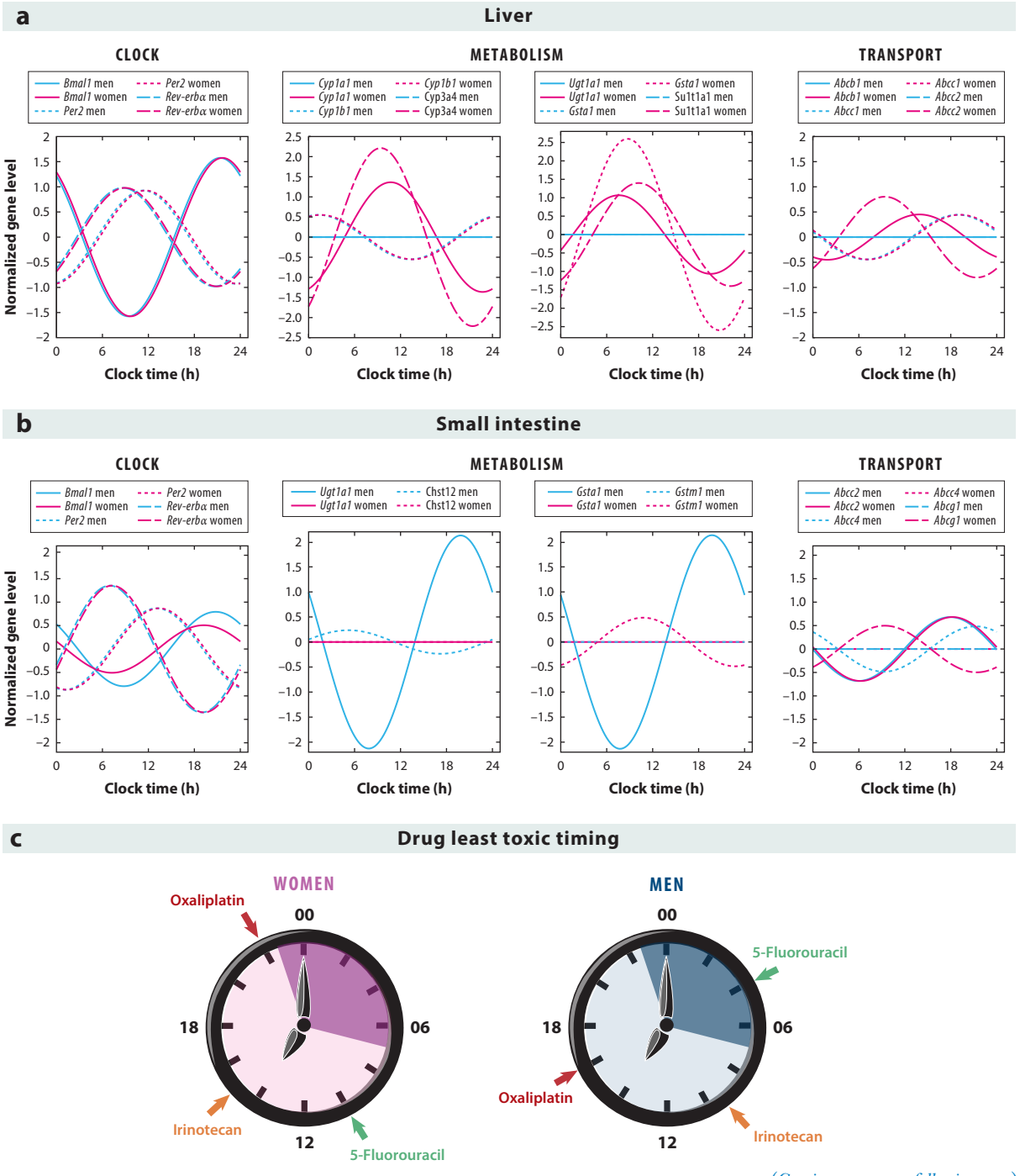
Figure 1 (*Figure appears on preceding page*)

The circadian timing system. (*a*) The suprachiasmatic nuclei (SCN) display autonomous circadian rhythms, which are synchronized to an exact 24-h period by external cues. This central pacemaker sends physiological signals to molecular clocks located in each nucleated cell of the organism. Elements of the human exposome, including light, temperature, noise, socioprofessional interactions, food intake, and drug administration, can either reinforce or alter the functions of the central and peripheral clocks, depending on their 24-h exposure patterns, with implications for the development of pathology. (*b*) Simplified view of the molecular clock. The core of the cellular clock is the protein complex involving circadian locomotor output cycles kaput/basic helix-loop-helix ARNT like 1 (CLOCK/BMAL1) that promotes the transcription of Period homologs 1, 2, and 3 (PER1, PER2, PER3); cryptochromes (CRY1, CRY2); retinoic acid–related orphan receptors (RORAαβγ, RORB, RORC); and nuclear receptor subfamily 1 group D, i.e., REV-ERB (REV-ERBα, REV-ERBβ). PER and CRY proteins undergo posttranslational modifications in the cytoplasm followed by nuclear translocation, probably under the form of a PER/CRY complex, and inhibit CLOCK/BMAL1 positive action on transcription, including on their own. Consequently, cytoplasmic PER and CRY protein levels decrease, allowing intranuclear CLOCK/BMAL1 complexes to trigger transcription of their target genes again, thus starting a new cycle. As a second feedback loop, ROR and REV-ERB proteins activate and inhibit BMAL1 transcription, respectively. Selected small molecules targeting the core clock genes are shown (see Section 5).

near 8:00 PM (33–36). In mice and humans, both innate and adaptive immune cells [monocytes (37); macrophages (32); dendritic cells (38); neutrophils (39); and $CD4^+$ T (40), $CD8^+$ T (41), NK (42), and B cells (38)] are further endowed with a functional circadian machinery. The SCN induces neuronal and hormonal rhythmic signaling especially through cortisol and catecholamines, which directly act on the immune cells to fine-tune their rhythmic functional activity (43–47) (**Figure 3**). Current efforts aim to understand how sex, microbiome, and circadian rhythms align to affect the immune system, including drug responses. Indeed, a sex bias characterizes the prevalence of autoimmune diseases (48) and lethal infections (49). Sexual dimorphism was reportedly attenuated in germ-free mice, and consequently, the prevalence of sex-biased diseases (e.g., type 1 diabetes or liver cancer) was altered (50–52). A recent murine study illustrated a more pronounced circadian pattern in the rhythms of intestinal microbiota-host interactions in females as compared to males. Recent evidence suggests the importance of the microbiome in sex-specific circadian gene expression rhythms and metabolism (50–54). These data reveal an important potential role for the CTS in the lifelong fundamental impact of the microbiota on the development and function of the mammalian immune system (55–57) and, consequently, on the modulation of drug responses.

2.4. Diagnostic Tools for Circadian Timing System Metrics in Individual People

The large between-subject differences in CTS function and phase according to sex, lifestyle, genetics, and treatments support a paradigm shift of chronotherapy from one-size-fits-all approach toward personalized treatment delivery over the 24-h cycle. Such a shift requires the identification of circadian rhythm metrics in individual patients using novel diagnostic tools, analyses, and care logistics designed to inform between- and within-subject variations in the CTS. Chronotype questionnaires assessing diurnal orientation preferences have unraveled large sex- and age-related differences in the daily timing of activity and sleep (58, 59). The time of dim light melatonin onset (DLMO), assessed from repeated saliva or blood sampling in subjects under dim light conditions (approximately 10 lux) from 6:00 PM to 12:00 AM, has been proposed as a metric of CTS phase (59). Large between-subjects variations were identified in salivary or plasma DLMO from a total of 152 published studies in 4,397 healthy subjects (60). In this report, no difference in DLMO was found between men and women. Because of its related technical, environmental, dietary, and



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Sex-related differences in clock and pharmacological gene expression and in drug chronotoxicity in humans. (*a*,*b*) Sex-specific expression of selected clock genes, drug metabolism enzymes, and transporters in the liver (*a*) or the small intestine (*b*) of deceased donors from the Genotype-Tissue Expression project (29). For each donor, the tissue internal phases were estimated using the CHIRAL statistical algorithm. (*c*) Least-toxic administration timing of irinotecan, oxaliplatin, and 5-fluorouracil in female and male metastatic colorectal cancer patients (see Section 4).

pharmacological constraints, the use of DLMO as a CTS phase marker may appear limited in medical practice (61, 62). Alternative methods of CTS phase determinations have been proposed. Recent machine learning algorithms have identified CTS phase predictors based on mRNA expression of Period homolog 2 (PER2), nuclear receptor subfamily 1 group D member 2 (REV-ERBβ), and approximately 10 other genes in circulating monocytes from a single blood sample (63). Further artificial intelligence models provide metrics that estimate molecular clock functionality and phase from a single transcriptome or RNA sequencing in a given tissue biopsy (13, 64). The identification of constitutive or somatic clock gene polymorphisms or mutations can also affect patients' CTS phase, sleep disorders, and disease risk (65, 66). Lately, the development of wearable sensors and dedicated telemonitoring platforms has allowed for remote continuous and longitudinal monitoring of circadian biomarkers from individuals in their daily routines (10). Rest-activity, position, and chest surface temperature recordings and telemonitoring have revealed up to 12-h between-subject differences in times of maximum physical activity or temperature, both in healthy people and in cancer patients (6, 67).

3. KEY ADMET CIRCADIAN REGULATORS AND DRUG RESPONSES

The circadian regulation of ADMET has been established, with its implications for tolerability and efficacy, over the past decades (3–7, 68–70). A comprehensive literature search, however, reveals that most circadian investigations are performed in male animals or male volunteers, while sexual dimorphism is seldom addressed for circadian rhythms or ADMET, despite long-known differences in drug pharmacology and treatment effects between men and women (59, 71).

3.1. Sexual Dimorphism in Circadian Patterns in Absorption, Distribution, and Elimination

Sex-related differences have been documented for processes of oral drug absorption, including gastric emptying time, intestinal motility, intestinal and liver blood flows, and bile secretion and excretion. For instance, the higher gastric alcohol dehydrogenase activity in women accounted for a lower alcoholic toxic threshold, thus accounting for faster alcoholic liver damage compared to men (72). The higher activity of the P450 enzyme CYP3A4 in women compared to men (73) could account for the larger oral clearance of midazolam in women compared to men (74). In addition, the higher average fat mass in females accounts for larger distribution volumes (V_d) of lipid-soluble drugs such as benzodiazepines as compared to males (75). In contrast, the V_d of water-soluble drugs such as salbutamol or ofloxacin is larger in males compared to females due to sex differences in lean body mass (76).

Circadian rhythms have been found for CYPs and drug transporters, including ATP-binding cassette (ABC) transporter P-glycoprotein (P-gp) and solute-carrier transporters (SLCs), in the gastrointestinal tract. The molecular clock was probed to induce large-amplitude circadian transcription rhythms of intestinal P-gp component genes as well as peptide transporters (77–80).

The magnitude and timing of the 24-h patterns in ATP binding cassette subfamily C member 2 (*Abcc2*) mRNA and protein expressions in ileum mucosa also differed between male and female

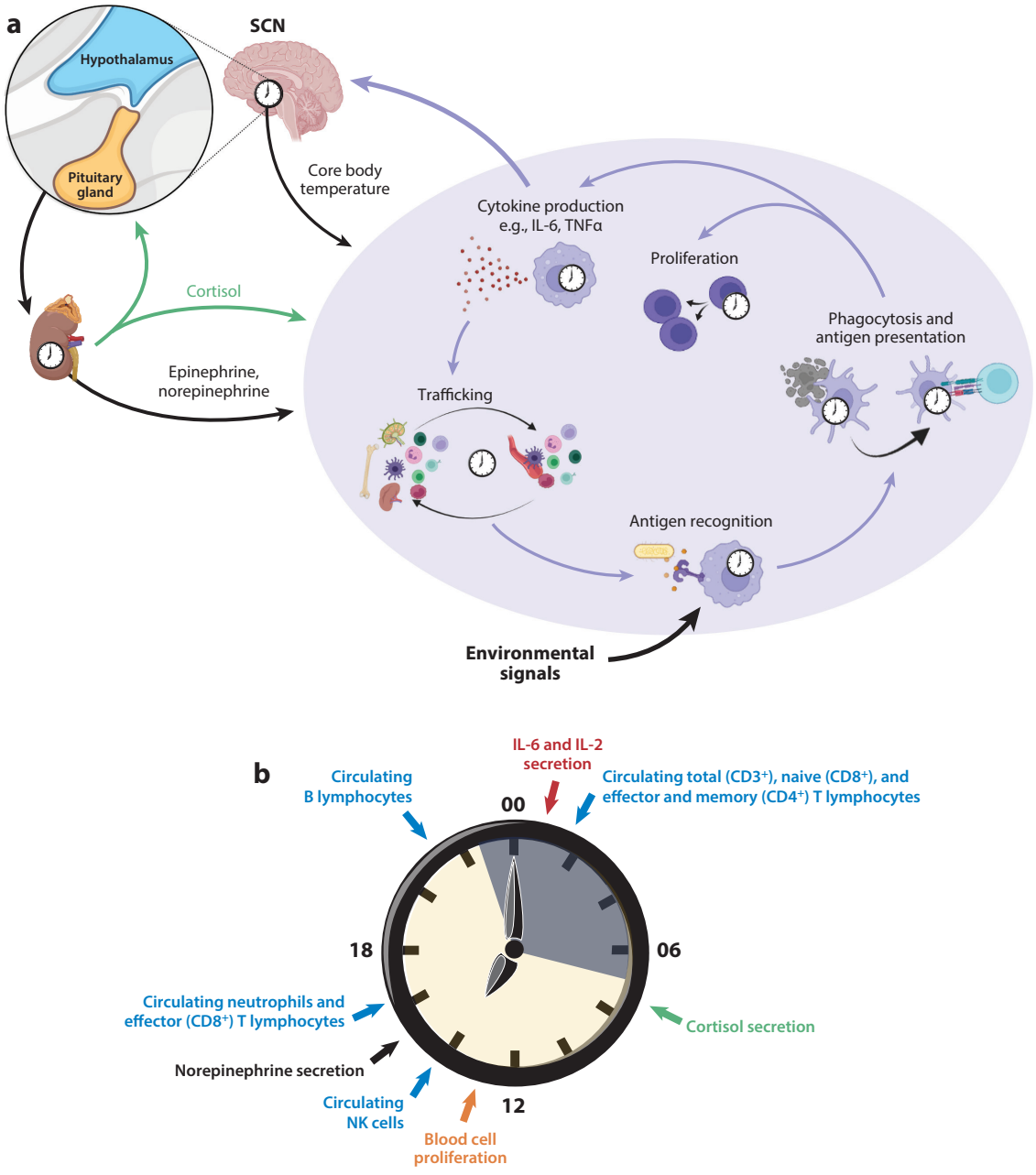


Figure 3

Interactions between the circadian timing system and the immune system. (*a*) The suprachiasmatic nuclei initiate hormonal and neuronal signaling to fine-tune the behavior and fitness of immune cells to the time of day. Cell-intrinsic molecular clocks regulate key immune functions, including migratory and defense mechanisms. (*b*) Peaks of circulating blood cell populations and key events in the 24-h span. Distinct immune cell types harbor different migratory behavior that is in line with their functional characteristics. In general, first-line defender and effector cells [neutrophils, natural killer (NK) cells, and $CD8^+$ T cells] show higher abundance in the blood during daytime with a peak in the afternoon, while naive or nonactivated cell populations are present in the blood circulation at higher abundance at night.

mice and were correlated with the circadian pattern in the intestinal tolerability of the topoisomerase I (TOP1) inhibitor irinotecan (81). Sex further moderated the circadian control of P-gp encoding *Abcb1a/Abcb1b* in mouse ileum, with circadian amplitudes being significantly larger in females (82). As a result of sex-specific rhythmic patterns, plasma, ileum, and liver concentrations of talinolol, a pure P-gp substrate, significantly differed according to its time of administration, sex, and fasting status (82).

A meta-analysis of publicly available microarray data further confirmed prominent rhythmic mRNA expression of ATP binding cassette subfamily G member 2 (*Abcg2*), *Abcc1*, and *Abcc5* in 14 mouse organs (83). The most prominent circadian patterns in solute carrier family (SLC) drug carriers were found for *Slc16a1* (monocarboxylate transporter MCT1), *Slc15a4* [proton-coupled oligopeptide transporter (PHT1)], and *Slc29a1* [equilibrative nucleoside transporter (ENT1)] (83).

Xenobiotic liver uptake depends in part upon SLC transporters, whose circadian regulation has been shown for the mRNA expression of organic anion-transporting polypeptides (*Oatp1a1*, *Slco1a1*), organic anion/dicarboxylate exchanger 2 (*Oat2*, *Slc22a7*), and *Oct1* (Slc22a1) in male mice (84).

Xenobiotic brain uptake displays circadian variations in male mice as a result of daily rhythms in blood-brain barrier ABC transporter activity, peaking around Zeitgeber time (ZT)12–16 (defined as the time of onset of an environmental entraining cue, most often light onset), that is, in the first half of the activity span. The circadian blood–cerebrospinal fluid barrier displayed sexual dimorphism in rat choroid plexus as (*a*) the peak in *Abcc4* mRNA occurred 6 h earlier in males as compared to females, (*b*) *Abcc1* was rhythmic in male rats only, and (*c*) *Abcg2* was rhythmic in female rats only (85).

Non-time-specified renal expression of *Abcb1a/1b* mRNA was larger in female compared to male mice (86), consistent with the reported lack of any *Abcb1a/1b* mRNA and P-gp protein rhythm in the kidney of male mice (87). In mouse liver, however, a circadian rhythm characterized P-gp protein expression in females, but not in males, thus supporting a sexually dimorphic biliary excretion of xenobiotics (82).

3.2. Sex Dimorphism in Circadian Patterns of Cellular Drug Metabolism

Circadian variations have long been known for phase I oxidation, reduction, and hydrolysis reactions through CYP's enzymatic activities (88, 89) and for phase II detoxification enzymes in rodent liver or intestine, mediated by key clock-controlled transcription factors (68, 90). Sex-related differences were found for the circadian mRNA expressions of D-binding protein (*DBP*), which peaked at ZT9 in mouse liver and at ZT12 in colon in both sexes, yet with larger amplitudes in females (28).

Most liver *Cyp* genes and nuclear receptors were more expressed in female mice as compared to males, and circadian rhythms were present in both sexes, yet with a larger amplitude in females as compared to males (91).

Phase II detoxification by glutathione *S*-transferase, which catalyzes the conjugation of reduced glutathione (GSH) to xenobiotics for their excretion processes, also displays circadian variations. GSH concentrations in liver or jejunum were nearly threefold higher in the second half of the nocturnal activity span as compared to the middle of the daily rest span in male mice (92, 93).

Sexually dimorphic toxicities were found for both acetaminophen and oxaliplatin toxicities in mice (94, 95). Acetaminophen was less toxic in female mice because of a faster recovery of mitochondrial GSH after its hepatic depletion (94, 96).

4. RECENT RESULTS ON CIRCADIAN RHYTHMS IN DRUG RESPONSES

4.1. Chronoimmunotherapy and Chronoimmunosuppression

The existence of a robust circadian organization in the immune system seems to strongly impact the efficacy and tolerability of immunotherapeutics. Large and consistent dosing time dependencies have been found for the anticancer efficacy of the recently developed immune checkpoint inhibitors, in line with earlier findings with interferons and interleukins. Circadian timing also influences the efficacy of vaccines, as well as that of different immunosuppressors used in transplant patients. Preliminary evidence also suggests sexual dimorphism could characterize both circadian amplitude and optimal timing of these agents.

4.1.1. Immune checkpoint inhibitors. Immune checkpoint inhibitors (ICIs) have become a major treatment for cancers since their initial regulatory approval in 2014 (97). Seven independent retrospective reports have revealed significant and consistent improvements in progression-free and/or overall survival through the administration of a larger proportion of ICI infusions in the morning or in the early part of the day in a total of 1,019 patients with metastatic melanoma (98), non-small-cell lung cancer (99–102), or renal cell or urothelial cancers (103, 104). The observed differences in median overall survival duration were up to fourfold higher according to the timing of administration (99). Moreover, the larger the proportion of late timing for ICI administrations, the poorer the outcome (102). The results call for the precise identification of optimal circadian timing and treatment schedules in patient strata, according to chronotype and CTS features, as well as ICI type and combinations, sex, molecular and phenotypic determinants, and cancer type and stage.

Such drastic dosing time-dependent efficacy of ICI antibodies with plasma half-lives of two to three weeks in humans (105) suggests a major mechanistic role for circadian pharmacodynamics in the tumor and its draining lymph nodes, which is now supported by experimental studies. For instance, no homing of T lymphocytes from blood to lymph nodes was demonstrated in the second half of the nocturnal activity span of mice, which would correspond to afternoon and evening in humans and might account for the impairment of circulating immune cells to reach their efficacy targets if initially exposed to ICIs at these times.

4.1.2. Vaccines. Circadian variation in multiple immune system functions also accounts for the observed differences in the strength of human immune response as a function of the time of vaccination (106). For instance, morning vaccination has been shown to be associated with stronger responses at the level of trained and adaptive immunity and of antibody production for Bacillus Calmette-Guérin and influenza virus, respectively, compared with evening vaccine administration. Consistent findings have been reported for SARS-CoV-2 as well (107).

4.1.3. Immunosuppressors. The pharmacology of immunosuppressors displays clinically meaningful circadian variations. Thus, chronopharmacokinetic differences according to the time of lipophilic tacrolimus or mycophenolate mofetil intake have been described in solid organ–transplanted patients, with greater peak plasma concentration (C_{max}) and area under the curve of the plasma concentration between 0 and 12 h after drug administration ($AUC_{0–12h}$) values reported with morning administrations, with potential implications for immunosuppressive treatment optimization (108–110).

4.1.4. Sex specificities in chronoimmunopharmacologic effects. Although some evidence suggests an impact of sex on ICI outcomes (111), its interaction with the timing of administration remains to be investigated, in view of recently reported sexually dimorphic circadian responses

of mouse immune system exposed to everolimus, a mammalian target of rapamycin inhibitor. Male mice were nearly threefold more susceptible to everolimus-induced immunosuppression compared to females, with the least toxic timing at ZT13, that is, near the onset of their daily activity span, in both sexes (112, 113). These experimental chronotoxicity findings support dedicated sex-specific preclinical research with a strong mechanistic component.

4.2. Cytotoxic Anticancer Agents

Cytotoxic agents embrace various classes of drugs that ultimately target proliferating cells and constitute the current backbone of systemic anticancer treatments. Circadian rhythms in response to arabinofuranosylcytosine, cyclophosphamide, doxorubicin, and cisplatin first revealed the striking relevance of dosing time for tolerability and efficacy of anticancer medications in mice or rats (4). Large circadian changes in the tolerability and/or efficacy of over 50 anticancer medications have been shown in experimental models (4, 5). While blood pharmacokinetics could vary according to circadian dosing time, it does not seem to constitute the main mechanism at work for chronotolerance and chronoefficacy (4, 5). In contrast, the circadian control of cellular pharmacokinetics and pharmacodynamics seems critical (14).

Clinical trials have evaluated the relevance of circadian timing for nearly 20 anticancer agents in colorectal, lung, breast, stomach, pancreatic, kidney, bladder, endometrial, ovarian, head and neck, skin, brain, and hematologic cancers (114). A systematic review of chronomodulated chemotherapy identified 18 randomized trials involving at least one group with chronomodulated chemotherapy and a total of 2,547 cancer patients (115). The authors emphasized that (*a*) chronomodulated chemotherapy improved the efficacy measure in three trials (17%), without any detrimental effect on efficacy in the other 15, and (*b*) significantly reduced all main toxicity end points in 11 trials (61%) or only some of them in 2 trials (11%) and increased toxicity in only 1 trial (6%). More numerous and larger clinical trials have been conducted in colorectal cancer, the third leading cause of cancer deaths worldwide (116). Fourteen chronotherapy trials involving nearly 1,700 cancer patients have led to (*a*) improved tolerability and efficacy of 5-fluorouracil (5-FU)-leucovorin and oxaliplatin through their maximum delivery near 4:00 AM at night and 4:00 PM in daytime, respectively (117–119); (*b*) the initial discovery of oxaliplatin efficacy and safety in patients with colorectal cancer (117, 118); (*c*) the initial medico-surgical strategy that allowed cures or prolonged survival in patients with metastatic colorectal cancer (120); (*d*) the need for development of sex-specific chronotherapeutic schedules for improving tolerability and efficacy (121–123); (*d*) the negative impact of treatment-induced circadian disruption, a novel adverse event; and (*e*) the negative impact of hematologic and clinical toxicities on efficacy, a finding divergent from the current paradigm where toxicity is considered as a surrogate for appropriate dosing, hence predicting for improved efficacy (124–127).

Anticancer agents are often administered as combination regimens to try to reduce the impact of resistance mechanisms. Both experimental evidence with several drug combinations (128) and clinical observations (121) support the delivery of each cytostatic at its most effective time, both as monotherapy and in combination (129).

The experimental, epidemiological, and clinical evidence linking the circadian clock, cancer processes, and chronotherapeutics has been extensively reviewed (3, 130–133). We focus here on novel translational approaches supporting the emergence of chronotherapy stratification factors, including sex and relevant circadian biomarkers, and systems medicine concepts.

4.2.1. Examples of molecular clock–controlled chronopharmacology mechanisms. Our team first demonstrated the cellular and molecular chronopharmacology mechanisms for the TOP1 inhibitor irinotecan, a main drug that prevents gastrointestinal malignancies, using

synchronized Caco-2 cell culture (78). Bmal1 silencing suppressed the several-fold increase in circadian changes in irinotecan bioactivation into SN38, as well as TOP1-DNA binding and apoptosis. The silencing, knock down, or mutation of Bmal1 or its molecular protein partner Clock also maintained toxicity at its worst circadian level for cyclophosphamide in male mice (134). These results have called for a novel artificial intelligence approach, based on physiologically based quantitative models, to recapitulate experimental and clinical data toward personalized chronotherapy (6).

4.2.2. Metallodrugs: past and future. The toxicity of platinum compounds, including cisplatin, carboplatin, and oxaliplatin, was reduced by up to threefold in female rats and male mice through drug dosing near ZT15–19, that is, near the middle of their nocturnal activity span (4). In agreement with these rodent data, clinical trials have found better tolerability of cisplatin, carboplatin, and oxaliplatin following their administration in the afternoon or early evening in ovarian, endometrial, bladder, lung, head and neck, and colon cancers, with improved or similar antitumor efficacies compared to those of early-morning or constant-rate administrations (135–138).

With regard to forthcoming drugs, the organo-osmium complex [OsII(η6-*p*-cym)(PhAzPy-NMe2)I]+(FY26) is a metabolic inhibitor that induces apoptotic cell death via reactive oxygen species generation (139). This promising anticancer metallodrug inhibited hepatocarcinoma (Hepa1–6) growth by 13% or 67% following dosing at ZT6 or ZT18, respectively, in male mice (139).

4.2.3. Sex dependencies in circadian responses to anticancer drugs. Despite mounting evidence that sex could profoundly impact the tolerability of chemotherapy, no recommendations have yet been made regarding any sex-specific dosing or scheduling of anticancer treatments (140). However, compared to male patients, female patients have been found to be largely and significantly more susceptible to time-unspecified administrations of 5-FU and irinotecan (141–144). Sex-related differences are also emerging in cancer chronotherapy, with such research being mostly clinically driven (145). For instance, the intravenous delivery of 5-FU at a constant rate resulted in circadian changes in the plasma concentration of the drug, with reduced mesor and circadian amplitude of 5-FU body clearance in female as compared to male cancer patients (146). The overall survival of metastatic colorectal cancer patients receiving a chronomodulated oxaliplatin and 5-FU-leucovorin combination was significantly prolonged in male patients and significantly reduced in female patients compared to conventional delivery in three international randomized trials involving 842 colorectal cancer patients (122). Moreover, retrospective analysis of prospectively collected data suggests that a 4–6-h delay in the least toxic timing of administration exists in women as compared to men (**Figure 2**). This has been found for both the chronomodulated oxaliplatin-5-FU-leucovorin combination in 113 metastatic colorectal cancer patients (135) and for irinotecan in combination with chronomodulated oxaliplatin-5-FU-leucovorin in 193 patients with metastatic colorectal cancer (121). Moreover, in a study involving 210 patients, the survival of women but not that of men was significantly improved through afternoon compared to morning administrations of rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone for diffuse large B cell lymphoma (147).

Regarding experimental models, sex-related differences were demonstrated for irinotecan-induced systemic and organ toxicities and plasma pharmacokinetics in mice. *Rev-Erbα* and *Bmal1* reciprocal transcription dynamics were identified as a main determinant of sex (and strain) differences in irinotecan chronopharmacology using a statistical approach (28, 148). Additionally, sex-related differences were found in the circadian organization of mouse bone marrow progenitors, which resulted in a 5–6-h delay in the least-hematotoxic time of oxaliplatin in females as compared to males (95). Albeit still limited, these rodent data support clinical observations,

therefore calling for prospective evaluations encompassing interactions between sex and timing in optimal circadian scheduling.

4.3. Cardiovascular Medications

Cardiovascular diseases have a high morbidity and mortality toll and usually require lifelong pharmacological treatments. Cardiovascular pathologies such as myocardial infarction, thrombosis, stroke, and hypertension have indeed been shown to be influenced by those circadian rhythms that orchestrate cardiovascular system physiology along the 24-h timescale. Furthermore, circadian time dependencies in the pharmacologic effects of cardiovascular medications, whether anticoagulant, antihypertensive or cholesterol-lowering, as summarized below, represent clinically-relevant findings towards the chronotherapeutic optimization of these commonly-used drugs.

4.3.1. Chronopharmacology of anticoagulants. Circadian fluctuations in thrombogenicity and hemostasis play a role in acute cardiovascular thrombotic events that occur most frequently in the early morning (149, 150). Early clinical studies revealed strikingly large-amplitude circadian changes in anticoagulation levels in patients with thrombosis receiving constant-rate unfractionated heparin, with values supporting a higher risk of rethrombosis in the morning and a higher risk of bleeding at night (151). More recent trials involving direct oral anticoagulants inhibiting factor X, such as rivaroxaban, demonstrated circadian-related changes in pharmacokinetic and pharmacodynamic end points. Thus, 8:00 AM dosing of rivaroxaban was associated with both nearly halved plasma concentrations at 12:00 PM (152) and reduced platelet aggregation in response to the ex vivo platelet activator ristocetin (153) in comparison to its administration at 8:00 PM. These clinical observations are supported by experimental evidence, with factor X activity displaying a significant circadian rhythm in male rats, with a peak at ZT4, in the early rest span (154). ZT4 also corresponded to the peaks of mRNA expression of the clock gene *Rev-Erbα* in circulating platelets, agonist-induced platelet aggregation, and in vitro thrombus formation in mice (155).

4.3.2. Antihypertensives and diuretics. Antihypertensives encompass a broad array of drugs that ultimately lower arterial blood pressure, which displays robust circadian oscillations in physiological conditions (14) so much so that the disruption of its circadian rhythm has been associated with a negative health impact (156). Intrinsically, most if not all targets of antihypertensives are involved in the complex regulation of the physiological mechanisms ultimately determining the 24-h pattern in arterial blood pressure (157). These drugs are generally administered as once-daily chronic doses, aiming at a sustained control of arterial blood pressure below an acceptable threshold (158). Guidelines do not recommend any specific time for antihypertensives; however, they tend to be taken in the morning (159). Notwithstanding, a systematic and comprehensive review of 153 randomized trials comparing morning versus evening intake of antihypertensives of various classes consistently favored evening intake for efficacy and safety end points (157). Interestingly, as for anticancer agents, when dual a combination of antihypertensives is required to obtain a satisfactory control of arterial blood pressure, evening/bedtime dosing is still consistently more effective and better tolerated than morning/awakening intake (157). Furthermore, even better results are expected with evening antihypertensive administration in patients with elevated asleep arterial blood pressure, which is physiologically expected to drop at nighttime and is referred to as a nondipper/riser phenotype (160). Diuretics, medications increasing urine output through different mechanisms of action, constitute an exception as bedtime intake, in association with their pharmacokinetics, would increase nocturnal polyuria, thus disrupting sleep, which has associated health implications (161). The prevalence of arterial hypertension, with its morbidity and mortality toll, as well as the complex, multilayered biological determinants of its circadian regulation,

including lifestyle choices, frequent comorbid conditions, and target organ pathophysiology, justifies a personalized, precise, and integrative therapeutic approach, as recently endorsed by the National Institutes of Health (156, 162).

4.3.3. Statins. Statins are a class of medications inhibiting hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase implicated in cholesterol biosynthesis, thus reducing low-density lipoprotein blood levels and ultimately attenuating the risk of cardiovascular events (163). HMG-CoA mRNA is expressed in a circadian fashion in the liver of rats (164) but is also influenced by other physiological rhythms, including feeding/fasting and hormones (165). Thus, in humans, intersubject differences, related to individual variability in lifestyle and CTS function, could impact the activity of the statin's target over 24 h. Indeed, although statins are usually recommended for evening intake (158), a systematic analysis of randomized trials failed to demonstrate a significant benefit conferred by the time of administration, at least with regard to intermediate end points (166). Given the complex multiple circadian and diurnal oscillations involved in cholesterol metabolism, it can be argued that statin chronopharmacology could be improved through chrononutritional interventions such as time-restricted eating (167), further supporting the concept of an integrative circadian-based pharmacological and behavioral interventional approach to improve disease outcomes (6).

4.4. Chronopharmacotherapy of Rheumatology and Analgesic Medications

The relevance of the time of administration for adverse events and efficacy has long been known for anti-inflammatory drugs in laboratory rodents and in patients and has been recently reviewed (168). Chronopharmacological evidence exists for both nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids. For instance, the sustained-release formulation of indomethacin was almost fourfold less toxic and twice as effective following evening rather than morning intake in patients with osteoarthritis (169). Short-acting prednisolone proved most effective for the reduction of morning stiffness after dosing near 2:00 AM in patients with rheumatoid arthritis (170). A modified-release prednisone formulation was designed for optimizing therapy timing without waking up patients for drug intake at night. The oral intake of such modified-release prednisone at 10:00 PM resulted in drug delivery starting 4 h later, that is, coincident with the high levels of circulating proinflammatory cytokines that determine joint stiffness. In large, randomized trials, this chronomodulated formulation significantly improved efficacy alone or in combination with standard disease-modifying antirheumatic drugs (DMARDs) (171, 172). Chronopharmacology also affected DMARDs, which mostly prevent or reduce the inflammation that damages joints and often target the immune system. Evening dosing of methotrexate, a dihydrofolate reductase inhibitor with anti-inflammatory properties, was associated with increased efficacy in comparison to standard thrice-daily dosing in patients with rheumatoid arthritis (173).

It is worth mentioning that circadian-based administration of central nervous system–acting drugs has also proven its clinical relevance in neurological and psychiatric conditions (6, 174–176). This review does not delve into these aspects, though.

5. CLOCK-TARGETED AGENTS AS NEW POTENTIAL THERAPEUTIC STRATEGIES

The development of new pharmacologic agents targeting molecular clock proteins represents an emerging domain of chronopharmacology (177, 178) (**Figure 1*b***). Such agents could shift the optimal timing of drugs to a desirable time window or restore molecular clock functions to reduce risk of disease development and further optimize chronotherapy. Small molecules regulating core clock proteins may be identified either through unbiased chemical library screening to identify

molecules affecting the clock function in cell culture or by virtual screening, a target-based method that proceeds with the crystal structure of the core clock proteins to select appropriate small molecules through molecular docking and dynamic simulations.

KL001, the first identified clock-targeted molecule, prevents ubiquitin-dependent degradation of CRY, resulting in a longer clock period (179). KL001 reduces glioblastoma growth and demonstrates antitumor efficacy through the inhibition of the CLOCK-BMAL1 complex in culture (180). Furthermore, KL001-mediated CRY stabilization inhibits glucagon-induced gluconeogenesis in primary hepatocytes, opening possibilities for diabetes treatments (177). Additional CRY modulators with improved affinity and pharmacokinetic properties have been subsequently identified (177), a few of them demonstrating hypoglycemic efficacy in diabetic mice (181–183). A recent study discovered a new selective CRY1 destabilizer, M47, which has an advantageous pharmacokinetics profile and crosses the blood-brain barrier in mice (184). M47 treatment of cancer-prone $p53^{-/-}$ mice enhanced mouse life span by 25% as compared to controls, thus suggesting its potential for treatment of p53-mutated cancers.

Regarding CLOCK and BMAL1 targeting, Clock inhibitor CLK8, the unique molecule that regulates the interactions between both proteins, was recently discovered (185). CLK8 interferes with the translocation of CLOCK into the nucleus, which enhances the clock amplitude without affecting period length. Since circadian rhythms are shown to be dampened in aging and in various diseases, amplitude-enhancer molecules such as CLK8 may hold great therapeutic promise.

GSK4112 is the first known synthetic agonist of REV-ERBs. It reduces *Bmal1* expression in cultured liver cells, inhibits gluconeogenesis in primary mouse hepatocytes, and decreases cytokine IL-6 protein secretion in macrophages. SR9009 and SR9011, although being initially used in humans as stenabolics, have been shown to be major REV-ERB agonists. Administration of either compound in mice at ZT6 resulted in loss of locomotor activity during the following dark phase, whereas normal circadian behavior was restored at the next cycle, consistent with a drug clearance of less than 24 h (186). REV-ERB agonists could be useful for treating jet lag and sleep disorders, as well as metabolic diseases, as they increased energy expenditure by altering the circadian expression of metabolic genes in the liver, skeletal muscle, and adipose tissue in healthy mice. Both SR9009 and SR9011 were also lethal to cancer cells or oncogene-induced senescent cells while demonstrating no toxicity in normal cells in culture. They further slowed down glioblastoma growth and prolonged survival in tumor-bearing mice, with acceptable tolerability (180, 187). However, GSK4112, SR9009, and SR9011 have important off-target effects as they also bind to the liver X receptor α (LXRα), which is involved in many physiological functions, including inflammation and energy metabolism. Thus, the compounds GSK2945, GSK0999, GSK5072, and GSK2667 have been synthesized and show a 1,000-fold increase in selectivity for REV-ERBα over LXRα as compared to former compounds.

SR8278, the first identified REV-ERB antagonist, reduced the expression of REV-ERB target genes in cells. In contrast to GSK4112, SR8278 inhibited glucagon secretion and cellular calcium signaling and triggered mania-like behavior in mice by altering dopaminergic neuron activity. However, SR8278's poor pharmacokinetics properties have hampered its in vivo use. ARN5187, which inhibits REV-ERB-mediated transcription and autophagy, displayed some activity against cancer cells. More recently, the REV-ERB inhibitor GSK1362 displayed efficacy against pulmonary inflammation by repressing cytokine production from alveolar macrophages and blocking SUMOylation (posttranslational modification involving the covalent binding of small ubiquitin-like modifier), thus inhibiting proteasomal degradation in mice. However, GSK1362's poor pharmacokinetics properties are also likely to limit its use in in vivo experiments. Finally, nobiletin, a retinoic acid–related orphan receptor (ROR) agonist, possesses amplitude-enhancing and period-lengthening effects and displays a positive effect on metabolism and longevity in mice

(188). In brief, numerous clock-targeted molecules have been recently developed in vitro but could not translate into mice because of poor pharmacokinetics properties.

6. DISCUSSION

The relevance of circadian rhythms for drug responses has been established in laboratory rodents for hundreds of medications of all pharmacologic classes over the last five decades. There is also growing evidence that the dosing time substantially matters for the tolerability and/or efficacy of many medications in patients, as shown for (*a*) chronomodulated chemotherapy in a systematic review of 18 randomized clinical trials in 2,547 cancer patients (115), (*b*) immunotherapy in a meta-analysis of seven retrospective studies in a total of 1,019 cancer patients (98, 99), (*c*) antihypertensive therapy in two randomized clinical trials involving more than 20,000 patients (189, 190), and (*d*) anti-inflammatory therapy in six randomized trials involving 517 osteoarthritic patients on NSAIDs (191) and 638 patients with rheumatoid arthritis on prednisone (171). However, many more randomized clinical trials appear to be needed to issue consensus timing recommendations for daily practice. Entering the term circadian on ClinicalTrials.gov on March 16, 2023, yielded only 301 of 64,020 recruiting registered clinical studies worldwide (0.05%), with only 7 of these 301 (2.3%) evaluating circadian drug timing. In particular, the recent class of agents targeting cyclin-dependent kinase 4/6 has not undergone any circadian-based development, despite the demonstrated coupling between the molecular clock and the cell cycle in nonmalignant cells (192, 193). This is clearly insufficient to advance the field of chronopharmacology and chronotherapy, thus carrying over the promises of the discovery of molecular clock mechanisms by Hall, Rosbash, and Young, the 2017 Nobel laureates for precision medicine and therapeutics. The data reviewed here stress the need for an urgent commitment by regulatory authorities, health and research institutions, charities, foundations, and pharmaceutical and biomedical industries toward careful consideration and support of developmental and clinical chronopharmacology and chronotherapeutics, including large-scale randomized trials. What is at stake is to complement dose and schedule recommendations with circadian timing ones to improve the safety and efficacy of medications, thus reducing both patient loss of chance and undue economic expenses for the society.

Current trends in chronopharmacology are highlighting its great potential for precision circadian medicine and personalized chronotherapy. The recent discovery of sexual dimorphism in hormonal receptor expression in the SCN and for the circadian expression of clock and clock-controlled genes in peripheral organs has defeated the assumption of a similar CTS function in male and female mammals (59). Regarding ADMET, the mRNA and/or protein expressions and/or functions of several CYPs and ABC transporters could display large-amplitude rhythms in one sex and be arrhythmic in the other sex both in mice and in humans (**Figure 2**). Ad hoc designed studies in mice have further revealed sexually dimorphic circadian responses for irinotecan and oxaliplatin chronopharmacology (28, 96). The clinical relevance of sexually dimorphic circadian drug responses is supported by retrospective analyses from randomized clinical trials and a prospective meta-anaylsis in cancer patients. Strikingly, the respective dosing times associated with the least toxicity of 5-FU, oxaliplatin, and irinotecan were located 6 h later for women compared to men with colorectal cancer (121, 135) (**Figure 2**). The results call for experimental and clinical chronopharmacology designs to systematically address the possibility of sexually dimorphic circadian drug responses and to investigate their main mechanisms.

Large human studies have revealed between-subject differences in morningness/eveningness or chronotype (194) that translated into differences in the circadian phase of clock gene expressions in human oral mucosa (64). Physiologic, hormonal, or molecular CTS biomarkers could indeed help improve drug safety and efficacy through providing dynamic CTS metrics for

personalized drug administrations in chronofit patients, that is, those whose CTS is functional and rather robust. In the nonchronofit patients, who usually have a worse prognosis, there might be an indication for treating the defective CTS through integrative behavioral, environmental, and pharmacological interventions to maximize the potential benefit of chronotherapy (6).

A systems medicine approach appears mandatory for the identification of chronofitness and circadian phase through patient education and engagement, technological advances in telemonitoring, and real-time analyses of multiple circadian rhythms (6, 9). Indeed, artificial intelligence should help reach optimal actionable insight for personalized chronotherapy, based on critical host and disease CTS metrics derived from both the patient's multidimensional time series and across-species chronopharmacology models (**Figure 4**).

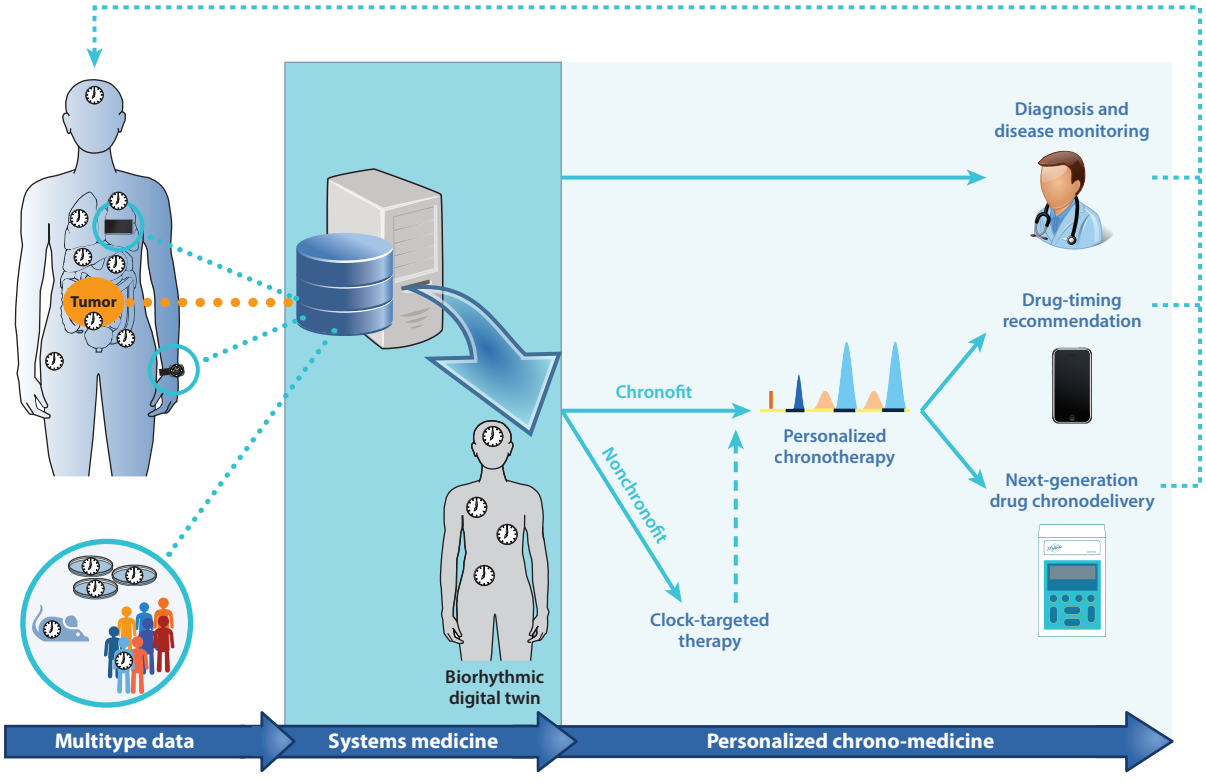


Figure 4

Pipeline to integrate digital technologies and artificial intelligence into daily medical practice toward personalized chronomedicine. Innovative wearable technologies now allow for the remote longitudinal monitoring of multiple circadian metrics in individual patients. In parallel, new statistical algorithms have been developed to predict the molecular clock function and phase from single-timepoint biopsies of diseased or healthy human tissues. Such multitype data sets may be combined with information available from cell cultures, laboratory animals, and patient populations to design biorhythmic digital twins of the patient. This systems medicine approach uses mathematical and machine learning methods to integrate multiscale data sets into complex mechanistic models recapitulating the numerous physiological components of the problem and translating them into personalized treatments (6, 9). Patient circadian data sets and their digital twin dynamic representations can uniquely inform on disease diagnosis and monitoring. They further provide educated information on the patient's circadian timing system functionality—or chronofitness—thus allowing patients who would benefit the most from treatment chronomodulation to be stratified. In the case of a nonchronofit patient, clock-targeted behavioral or pharmacological interventions can be considered. Innovative digital technologies and biorhythmic digital twins are critical tools to design sex-specific and personalized chronotherapies, to produce recommendations for daily medical practice, and to further initiate technological development for next-generation drug formulations and programmable administration devices.

While circadian drug responses are being identified for almost all drug classes in experimental models, clinical drug timing studies are needed for a thorough investigation of sex and between- and within-patient differences in the CTS to take full therapeutic advantage of circadian drug response mechanisms. We expect that future advances will stem from coordinated developmental chronotherapeutic strategies derived from studies in cells and rodents and from clinical trials, including randomized Phase III trials, with patient stratification according to sex and CTS metrics. It is now time for chronopharmacology to aim at issuing personalized drug timing recommendations that will yield a high benefit for patients.

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