Chapter

Integration of Chronobiological Concepts for NSCLC Management

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Abstract

The authors reviewed pertinent experimental and clinical data allowing to consider the interest of taking into account the temporal dimension ('circadian') for prevention and management of the majority of cancers, i.e., non-small cell lung carcinoma (NSCLC). The universal importance of circadian rhythms has been acknowledged in animal or human situations regarding carcinogenesis and cancer promotion; cell kinetics, apoptosis, molecular genetics, as well as DNA repair mechanisms, platinum resistance...; molecular targets (i.e., epidermal growth factor reception-EGFR); and all lymphoid and immunology machinery components. Also chronotolerance to all chemotherapeutic agents useful for treating human lung cancer has also been evidenced. A few randomized clinical chronotherapy trials were performed in human NSCLC. One limited trial has shown apparent chronoefficiency, while in another one, chronotolerance to 5-fluorouracil and a platinum derivative were confirmed. The limited improvement of outcome in human NSCLC, even through the use of targeted and biological therapies (such as tyrosine-kinase (TKI) or vascular-endothelial-growth-factor (VEGFR) inhibitors; immunotherapy), allows to consider launching specific trials in human NSCLC aiming at either restoring a normal circadian structure of the host or taking into account circadian variations of specific targets. By now unfortunately, no targeted or immunotherapy trials have been launched considering temporal dimension.

Keywords: circadian rhythms NSCLC review

1. Introduction

1.1 Circadian timing system

Life is structured in space but also in time. Biological rhythms have been documented in all processes involved in the malignant transformation of cells as well as in the cellular proliferation of both healthy and tumor tissues [1–5]. All physiological functions expressed their metabolic or specific activities according to a circadian variation [1–5]. This is the case not only for actively dividing tissues but also for all other tissues, such as the myocardium, central nervous system, or organs involved in the metabolization, detoxification and excretion of drugs (kidney, liver) [3–5].

Recent advances identify critical molecular events that rhythmically control drug metabolism and detoxification, cell cycle, molecular targets, deoxyribonucleic acid (DNA) repair, apoptosis, and angiogenesis [3–5]. The coordination of these processes along the 24-h period is ensured by the circadian timing system (CTS) whose hierarchical organization determines chronotherapeutic effects [3–5]. The CTS coordinates physiology and cellular functions over a 24-h period (**Figures 1** and **2**). This circadian physiology is generated or controlled by a central pacemaker, the suprachiasmatic nuclei (SCN) in the hypothalamus. The SCN generate circadian physiology through diffusible signals, including transforming growth factor-alpha (TGF-alpha), epidermal growth factor (EGF), prokineticin-2, cardiotrophin-like cytokine, and neuroanatomic sympathetic and parasympathetic pathways [1–8].

A dozen specific clock genes constitute the core of the molecular clock in mammals [3–6]. These genes are involved in transcriptional and posttranscriptional activation and inhibition regulatory loops that result in the generation of the circadian oscillation in all physiological systems and individual mammalian cells [3–5]. In particular, the circadian locomotor output cycles kaput-brain and muscle ARNT-like protein-1 (CLOCK-BMAL1) or NPAS2-BMAL1 protein dimers play a key role in the molecular clock through the activation of transcriptional clock genes period's (Per's), cryptochrome (Cry's), and Reverb's [3–5, 9] (**Figures 1** and **2**).

Proper circadian regulation is essential for the well-being of the organism, and disruption of circadian rhythms is associated with pathological conditions including cancer [1, 5, 10, 11]. In mammals, the core clock genes, Per1 and Per2, are key regulators of circadian rhythms in central clock, in the hypothalamus, and in peripheral tissues [9–13]. Recent findings revealed molecular links between Per

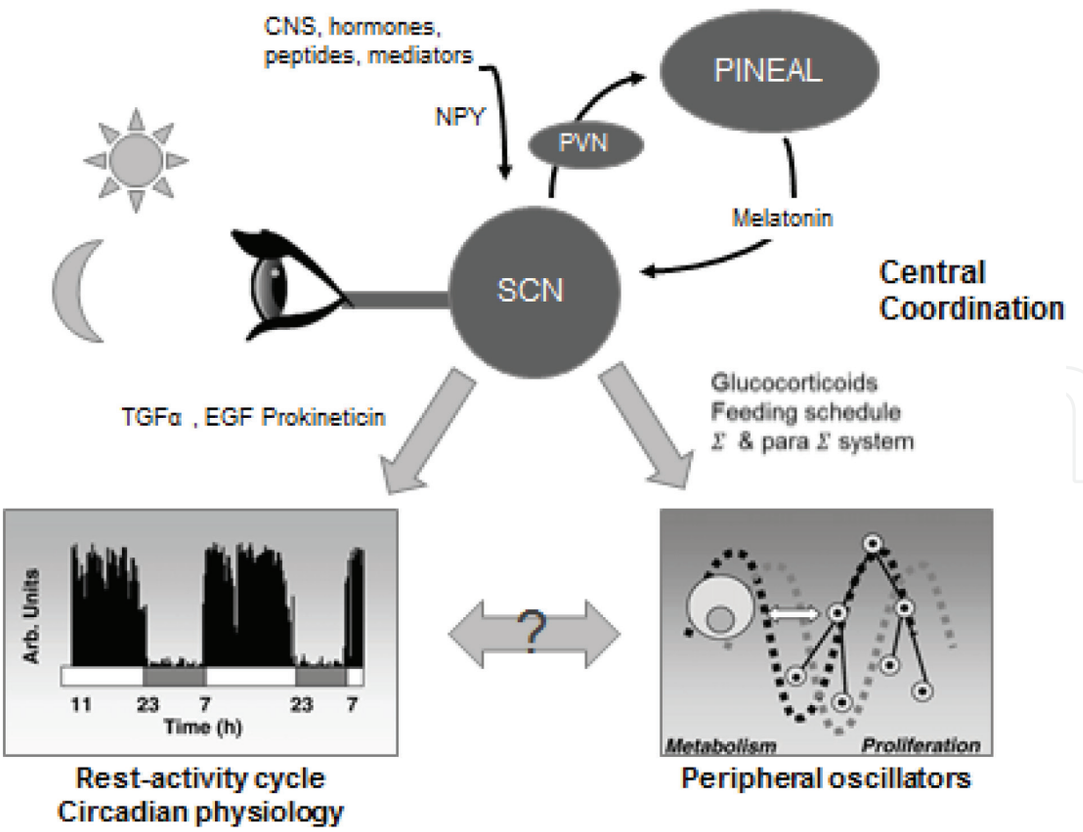

Figure 1.
Schematic view of the circadian timing system (CTS). The suprachiasmatic nucleus (SCN) is a biological clock located at the floor of the hypothalamus. Its period (cycle duration) is calibrated by the alternation of light (L) and darkness (D) through the rhythmic melatonin secretion by the pineal gland. The SCN controls or coordinates circadian rhythms in the body. Abbreviations: PVN, paraventricular nucleus; NPY, neuropeptide Y; TGF-alpha, transforming growth factor α; EGF, epidermal growth factor; Σ, sympathetic (after Levi et al. [3]; adapted).

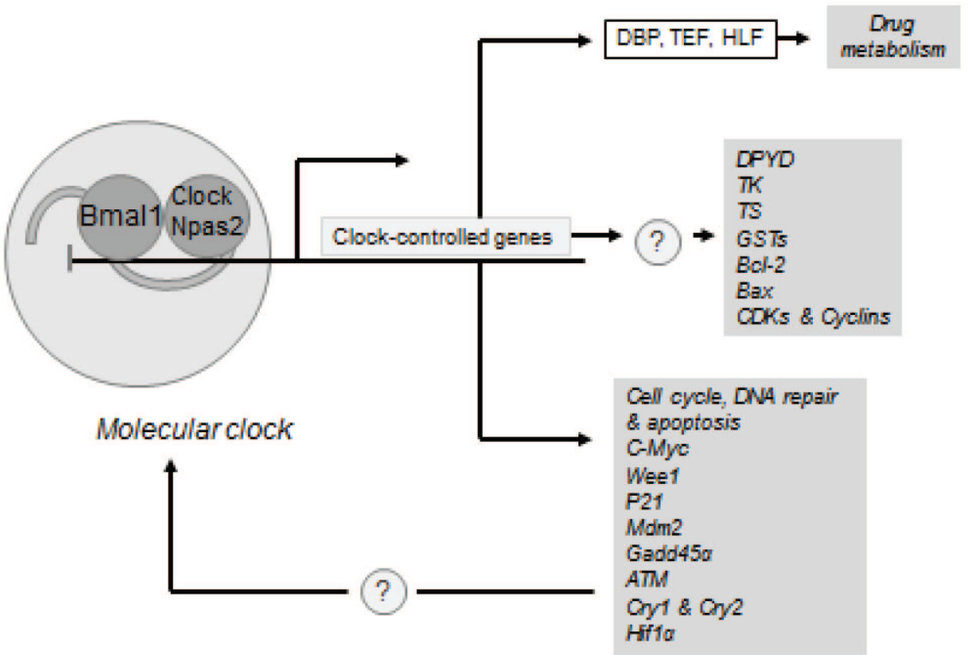

Figure 2.
Schematic representation of the molecular clock and the pathways involved into the control of relevant drug metabolism, cell cycle, DNA repair, and apoptosis in mammalian tissues. The protein dimer BMAL1-CLOCK or BMAL1-NPAS2 (a CLOCK homolog) plays an essential role in the rhythmic transcription of clock-controlled genes. After Levi et al. [3]; modified.

genes and cellular components that control fundamental cellular processes such as cell division and DNA damage [9–13]. New data also shed light on mechanisms by which circadian oscillators operate in peripheral organs to influence tissue-dependent metabolic and hormonal pathways [4, 5]. Circadian cycles are linked to basic cellular functions, as well as to tissue-specific processes through the control of gene expression and protein interactions. By controlling global networks such as chromatin remolding and protein families, which themselves regulate a broad range of cellular functions, circadian regulation impinges upon almost all major physiological pathways including immunological ones [4, 5].

1.2 Aim of this review

In 2002, we performed an overview of accessible experimental and clinical data allowing to believe in possible improvement in NSCLC management through chronobiological considerations. Here we will update our previous review with experimental and clinical recent contributions considering only circadian rhythmicity [14].

It is to be emphasized that we were unable to find any study on that subject using new biological alternatives such as targeted therapies or immunotherapy approaches.

2. Carcinogenesis

Studies performed by Hashimoto et al. on a murine model with circadian-varying lung tumor induction through timed single- or split-dose irradiation have already been reviewed [14, 15].

Cancer development associated to circadian disturbances both in damaged (target) and undamaged tissues and systems [1, 3–5] has been described some years

ago. Experimentally, the importance of deregulation of circadian rhythms before the development of liver cancer in rats exposed to diethyl-nitrosamine, a carcinogen that can also induce lung tumors, was reported by Filipsky et al. [16].

Molecular insights illustrate how dysregulation of circadian rhythms might influence the susceptibility to cancer development and provide further support for the emerging role of circadian genes in tumor suppression [12]. Silencing of tumor suppressor genes, such as the Per1—clock core gene, resulting from epigenetic alterations may occur early in lung cancer tumorigenesis [9, 17].

These observations allow considering Per1 gene as a potential target for chemoprevention.

Epidemiologic studies in human beings have evidenced a probable relationship between altered circadian rhythms and tumorigenesis. A high incidence of cancer has been observed in long-term shift workers such as flight attendants, nurses, or industrial workers [18–20]. Recent studies have also suggested that alterations of sleep quality were susceptible to enhance the risk of various cancers including lung cancers [21, 22]. Disruption of melatonin circadian rhythms (peak at night after dim-lighting) could partially explain such observations [23]. However, a large epidemiologic on thousands of Chinese female textile workers apparently failed to confirm an increased risk of lung carcinoma [24].

On the other hand, sport practice and regular physical activity, which are known to facilitate and maintain circadian general activities, may have an inverse effect, thus minimizing the risk of developing a lung cancer [25].

3. Cell kinetics and molecular biology

3.1 Cell kinetics

Hashimoto et al. recently reported that DNA synthesis activity in the normal lung was low but higher during the night [15]. Also a large number of experimental animal studies document circadian rhythm in cell proliferation in spontaneous or transplanted tumors, growing in ascitic fluid or solid phases [1, 2]. Precisely, Burns et al. studied alterations of DNA synthesis rhythmicity in selected organs of mice (i.e., bone marrow) bearing a transplanted Lewis lung carcinoma (LLC) [26].

Colombo et al. [27] reported day/night differences of spontaneous apoptosis in two different murine tumors, one of these being a lung one, in addition to circadian rhythms of division, peaks of apoptosis matching with mitoses valleys [27].

In human, circadian rhythmicity of cell proliferation has been reported for squamous cell carcinomas of the lung, as those of the skin and cervix [1, 2, 14, 28]. Various mechanisms responsible for the deregulation of the cell cycle and enhanced susceptibility to oncogenesis through activation of cell proliferation and cancer promotion have been identified. For example, in NSCLC, overexpression of cyclin D1, and mutation of p16 leading to a shortened and accelerated G1-phase and permanent phosphorylation (and inactivation) of pRb are known; in addition, mutations of p53 (with further impaired apoptosis) or pRb have been observed both in NSCLC and SCLC [29].

3.2 Molecular biology

3.2.1 Clock genes and circadian regulation

Tissues such as the liver, pituitary, and kidney but also the lung exhibit robust circadian rhythmicity in cultures [3–5]. Circadian timers are important for lung functions; for example, there is a well-documented link between diurnal variations

in lung physiology (i.e., airway narrowing and inflammation) and nocturnal asthma [30]. Gibbs et al. have studied the cellular localization of core clock genes in both mouse and human organotypic lung slices [30]; they also established the effects of glucocorticoids on pulmonary clock [30]. They were able to demonstrate a marked circadian rhythm in PER2 expression that is responsive to glucocorticoids. Immunohistochemical techniques were used to localize specific expression of core clock proteins and glucocorticoid receptor on the epithelial cells lining the bronchioles (Clara cells and type II pneumocyte cells) in both mouse and human lung tissues [30]. Selective ablation of Clara cells resulted in the loss of circadian rhythm in lung slices, demonstrating these cells to be critical for maintaining coherent circadian oscillations in the lung tissue. The coexpression of glucocorticoid receptor and core clock components establishes them as a likely interface between humoral suprachiasmatic nucleus output and circadian lung physiology [30].

Clock genes or proteins PER1 and PER2 have been linked to DNA damage response pathways in a series of studies, involving among others Lewis lung carcinoma (LLC) cells [3, 6, 9, 11–13]. Overexpression of either PER1 or PER2 in cancer cells inhibits their neoplastic growth both in vitro and in vivo and increases their apoptotic rate [13]. Also high expression of circadian gene mPer2 is able to diminish radiosensitivity of LLC and EMT6 cells with decreased expression of bax and p53 and increased expression of c-myc and bcl-2 [12, 13, 31]. This type of observations illustrates that the circadian system is involved in the protection and restoration of tumor cells, i.e., those of LC, against environmental detriments, such as gamma irradiation [13]. The gene, mPer2, might be considered as an inhibitor of tumor radiotherapy effects [32].

Downregulation of Per1 or Per2 enhanced tumor growth (i.e., of LLC cells) in vitro [12, 13, 31, 33]. Thus Per1 and Per2 exert their tumor suppressor functions in a circadian time-dependent manner [9, 31–33]. Also downregulation of Per1 or Per2 increases tumor growth only at given specific times of the day [12]. These optimal times may be shifted in tumors that have mutant period genes [12].

Overexpression of Per1 makes human cancer cells sensitive to DNA damage-included apoptosis; in contrast, inhibition of Per1 in similarly treated cells blunted apoptosis [9]. The apoptotic phenotype was associated with altered expression of key cell cycle regulators. In addition, Per1 interacted with the checkpoint proteins ATM and Chk2. Ectopic expression of Per1 in human NSCLC cell lines led to significant growth reduction [6, 9]. Per1 m-RNA expression was high in the normal lung and downregulated in a large panel of tumor samples from NSCLC patient samples as well as in lung cancer cell lines [3–6, 8, 11]. In addition, Gery et al. showed that ectopic or forced expression of Per1 in NSCLC cell lines led to growth inhibition, G2M cell cycle arrest, apoptosis, and reduced clonogenic potential [6, 17]. The influence of Per1 on cell cycle and apoptosis seems to be p53-status independent [5, 13].

Timeless (TIM) a homolog of a drosophila circadian rhythm gene has circadian properties in exploration in mammals [34]. Precisely its expression is enhanced in lung cancer cell lines where its knockdown was related to the induction of apoptosis, suppression of proliferation, and clonogenic growth [34]. In surgically resected specimens from 88 consecutive patients, high TIM protein levels as gauged by immunohistochemistry (IHC) correlated with poor overall survival [34, 35].

Taken together those results support clearly the hypothesis that circadian rhythm disruption plays an important role in lung tumorigenesis, as well as a link between circadian epigenetic regulation and cancer development.

3.2.2 Circadian regulation of tumor blood flow and angiogenesis

Hori et al. working on experimental Sato lung tumor were able to correlate biological time of greatest tumor growth and highest tissue blood flow (during

dark span) [36]. This finding strongly suggests that tumor tissue blood flow has a determining influence on tumor proliferative activity and that tumor growth is influenced by circadian variation in tumor tissue blood flow [36].

These results were confirmed by Blumenthal et al. who also showed that the blood flow rhythm may differ between tumor and normal tissues, thus creating a window of opportunity when tumors could be targeted with a therapeutic agent such as vascular endothelial growth factor (VEGF) inhibitors [37].

The molecular mechanism regulating circadian expression of VEGF in tumor cells (Lewis lung carcinoma cells among others) has been investigated by Koyanagi et al. [38]. They found that the expression of VEGF in hypoxic tumor cells was affected by the circadian organization of molecular clockwork. The core circadian oscillator is composed of an autoregulatory transcription-translation feedback loop in which clock and BMAL1 are positive regulators and period (Per) and cryptochrome (Cry) genes whose expression in the implanted tumor cells showed also a circadian oscillation act as negative ones. The levels of VEGF m-ribonucleic acid (RNA) in tumor cells implanted in mice rose substantially in response to hypoxia, but the levels fluctuated rhythmically in a circadian fashion. These findings support the notion that monitoring of circadian rhythm in VEGF production may be useful for choosing the most appropriate time of day (i.e., when VEGF production is increased) for administrating antiangiogenic agents [38].

In order to identify possible mechanisms underlying tumor progression related to circadian disrhythmicity, Yasumina et al. injected epidermoid HeLa cells in nude mice exposed to a 24-h light cycle (L/L) or to a "normal" 12-h light/dark cycle (L/D) [39]. A significant increase in tumor volume in the L/L group compared with the L/D group was observed. In addition, tumor microvessels and stroma were strongly increased in L/L mice but were not associated to an increase in the production of VEGF. DNA microarray analysis showed enhanced expression of WNT10A (wingless gene 10A). WNT10A could stimulate growth of both microvascular endothelial cells and fibroblasts in tumors from light-stressed mice, along with marked increases in angio-/stromagenesis [39]. Thus, WNT10A may be a novel angio-/stromagenic growth factor. These findings also suggest that circadian disruption induces the progression of malignant tumors via a WNT signaling pathway in models involving tumor cells similar to that encountered in human NSCLC [39].

3.2.3 Circadian regulation of epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) pathways

Binding parameters and constant dissociation of EGFR circadian variations, peaking late in dark span, were related to DNA synthesis activity variations in actively dividing mouse tissues [40]. EGFR by itself was found to be capable of phase shifting the prominent circadian rhythm of DNA synthesis, i.e., in the esophagus [40, 41]. Phosphorylation of the cyclic monophosphate (cAMP) response element-binding protein (CREB) and SER-133-phospho-CREB (PCREB) is a transcriptional factor that may regulate circadian cell rhythmicity [41]. Concentrations of EGF (and nerve growth factor (NGF)) were monitored in mouse saliva [42]; both growth factors exhibited identical diurnal variations, peaking between 12:00 and 20:00 h. Otherwise, the effect of EGF injections on cell kinetics of mouse tongue epithelium appeared to be time-dependent [41, 42].

Of interest, some studies were also performed in humans. Salivary (on the contrary to urinary or plasmatic) EGF level followed an apparent diurnal rhythm related to feed [43]. It was markedly reduced in the case of oral inflammation or head and neck cancer; in those situations, the capacity of oral mucosal defense could therefore be impaired [43].

EGFR is present in the majority of tumor cells in head and neck cancer [43]. Though circadian rhythmicities in cell proliferation and clock genes have been demonstrated in oral mucosal [45] and in related cancers [1, 2], so far, no study has dealt with the search for circadian variability in EGFR expression in squamous cell carcinomas. However, a circadian rhythm in plasmatic EGF level (with an acrophase around 14:20, a peak-to-trough interval of 26% and a superimposed 12-h frequency) has been reported in metastatic breast cancer patients [45].

As stated earlier, the circadian axis comprises a central clock (SCN; paraventricular areas) and a downstream network of hypothalamic relay station that modulates arousal, feeding, and sleeping behavior among others [4, 46] (**Figure 2**).

Communication between the clock and these hypothalamic signaling centers is mediated in part by diffusible substances that include ligands of the EGFR [4–8, 44, 46] (**Figures 1** and **2**). A significant functional role for EGFR in the suprachiasmatic nucleus is suggested by recent findings showing that epidermal growth factor receptor and its ligand TGF-α are highly expressed in the suprachiasmatic nucleus. Also EGFR activation induces behavioral and physiological effects, strengthening the notion that EGFR can modulate suprachiasmatic nucleus neural function and behavior [4–8, 44, 46]. Furthermore, Vadigepalli et al. confirmed that gene expression response to EGFR is circadian time dependent [8]; this response includes several genes encoding different neuropeptide receptors, ion channels, and kinases. In order to hypothesize the transcription factors underlying the EGFR response, different circadian time-dependent gene expression groups were analyzed for enriched transcriptional regulatory elements in the promoters. Results indicate that several transcription factors such as Elk 1 and cAMP-responsive element-binding protein/activating transcription factor family, known to be "input points" to the core clock network, are playing a role. Taken together, these results indicate that EGFR has a circadian time-dependent neuromodulatory function in the suprachiasmatic nucleus [7, 8].

3.2.4 Circadian regulation of immune pathways

Diurnal variation in immune and inflammatory function is evident in the physiology and pathology of animals and humans [47, 48].

Studies highlight the extent to which the molecular clock, most notably the core clock proteins BMAL1, CLOCK, and REV-ERBα, controls fundamental aspects of the immune response [47–49]. Examples include the BMAL1-CLOCK heterodimer-regulating Toll-like receptor 9 (TLR9) expression and repressing expression of the inflammatory monocyte chemokine ligand (CCL2) as well as REV-ERBα suppressing the induction of interleukin-6 (IL-6) [49].

Disruption of the circadian clockwork in macrophages (primary effector cells of the innate immune system) by conditional targeting of a key clock gene (bmal1) removed all temporal gating of endotoxin-induced cytokine response in cultured cells and in vivo. The loss of circadian gating was coincidental with suppressed REV-ERBα expression. This work demonstrates that the macrophage clockwork provides temporal gating of systemic responses to endotoxin and identifies REV-ERBα as the key link between the clock and immune function. REV-ERBα may therefore represent a unique therapeutic target in human inflammatory disease [49].

Also mechanistically, Bmal1 deficiency in macrophages increases pyruvate kinase M2 (PKM2) expression and lactate production, which is required for expression of the immune checkpoint protein PD-L1 (programmed cell death-ligand 1) in a STAT1-dependent manner (signal transducer and activator of transcription 1). Consequently, targeted ablation of PKM2 in myeloid cells or administration of anti-PD-L1-neutralizing

antibody or supplementation with recombinant interleukin-7 (IL-7) facilitates microbial clearance, inhibits T cell apoptosis, reduces multiple organ dysfunction, and reduces septic death in Bmal1-deficient mice [47–49].

4. Chronopharmacology of anticancer drugs active against human NSCLC

4.1 Animal data

Circadian variation in pharmacokinetics (PK) has been observed in rodents for all the drugs routinely administered to LC patients, i.e., pyrimidine derivatives, anthracyclines, vinca-alkaloïds (vinorelbine), topoisomerase inhibitors, taxanes, platinum derivatives, gemcitabine, other antimetabolites, etc. [1, 3–5]. These chrono-PK were expressed though circadian-varying metabolization, detoxification, excretion, and also maximal concentration (CMax) or area under the curve (AUC) [1, 3–5].

Chronotolerance has been observed in rodent studies long time ago for any chemotherapy agents routinely used for NSCLC patients [1–5]. As a recent example, best tolerance and chronoefficacy of gemcitabine alone or in combination with cisplatin were observed with best antitumor efficacy when both drugs were given around their least toxic time schedule, respectively, 11 and 15 hours after light onset (HALO) in animal facility [50]. In an older study, Flentje et al. had also documented the circadian chronoefficacy of cyclophosphamide (CPA) in LLC [51].

Chronotolerance to an experimental radioimmunotherapy with 131 I-anti-carcinoembryonic antigen (CEA) IgG was reported [52]. A 30% increase in maximum tolerated dose was possible when the drug was given at the trough of the bone marrow division activity (around 9 HALO) [52].

Clock, as a member of histone acetyltransferases, controls acetylation of histone 4 required for repair of DNA double-strand breaks thanks to several repair genes such as excision repair cross-complementing group 1 (ERCC1) or activator protein 1 (AP1) [6]. Expression of histone acetyltransferase genes is associated with cisplatin resistance [6, 53]. Histone acetyltransferase inhibition (i.e., by vorinostat) may increase carboplatin and paclitaxel activity in NSCLC cells [54]. The acetyl-CoA-binding motive is found in clock and shows sequence similarity with MYST members, i.e., Tip 60. Tip 60 which is overexpressed in human epidermoid cisplatin-resistant cancer cells [53] exerts a control regulation on several genes implicated in DNA repair (i.e., ERCC1 and AP1) [53].

Furthermore, the promoter region of the Tip 60 gene contains several E-boxes, and its expression is regulated by the E-box-binding circadian transcription factor clock! Thus, clock and Tip 60 regulate not only transcription but also DNA repair, through periodic (diurnal) histone acetylation in cell populations that can be found in human NSCLC [53].

Finally, of interest, diurnal-varying pharmacokinetics of erlotinib (a largely used tyrosine kinase inhibitor (TKI) for treating human NSCLC) has been reported both in xenograft-bearing nude mice [55] and in Lewis tumor-bearing mice. [56]. Circadian rhythm plays a critical role in the pharmacokinetics of erlotinib in mice, and the mechanisms may be attributed to gene expression rhythms of drug-metabolizing enzymes in liver tissues [56]. The inhibitory effect of erlotinib on phosphorylation of EGFR, AKT (type of serine/threonine protein kinase, also called protein kinase B), and mitogen-activated protein kinase (MAPK) varies with its administration time. The results indicate that the antitumor effect of erlotinib is more potent when the drug is administered when the activities of EGFR and its downstream factors increase [55, 56].

5. Human beings

In human as well, pharmacokinetics of some major drugs used in NSCLC have been reported to be circadian varying [1–5, 28, 44]. Circadian variation of plasma 5-fluorouracil (5 FU) concentration has been repeatedly observed when the drug is infused for a few days at a constant rate [57, 58]. This was reported when the drug was given either as a single agent or with a platinum derivative [58]. Similarly, plasmatic concentration of vindesine, a semisynthetic vinca-alkaloïd derived from vinblastine, exhibit circadian variation with peak between 9 am and 3 pm, when infused at a constant rate for 48 h [59]. Also the fixation of platinum ion to plasma proteins was shown to be circadian varying with an acrophase during late afternoon [60]. More recent assessment of circadian variability of cisplatin pharmacokinetics confirmed that cisplatin clearance was 1.38- and 1. 22-fold higher for total and unbound drug with administration at 06:00 pm vs. 06:00 am [61].

Host chronotolerance to anticancer drugs used in NSCLC patients has also been observed in clinical practice. Pyrimidine derivatives such as 5 FU are less toxic when infused during nighttime sleep [1, 3–5, 57, 58]. Also platinum derivatives such as cisplatin, carboplatin, and oxaliplatin are better tolerated between 3 and 6 pm while anthracyclines are less toxic in the morning [1, 3–5, 57, 58]. The first reported chronotherapy randomized trial, based on diurnal cell kinetics, treating mostly NSCLC patients, compared a 40-h sequential chemotherapy beginning either at 10 am or at 10 pm [28]. In this study, patients who received the sequential chronotherapy from 10 am experienced significantly greater granulocyte toxicity [14, 28].

Focan et al. also reported on host chronotolerance of 124 chemotherapy-naïve advanced NSCLC patients, receiving randomly etoposide for 3 days either at 6 am

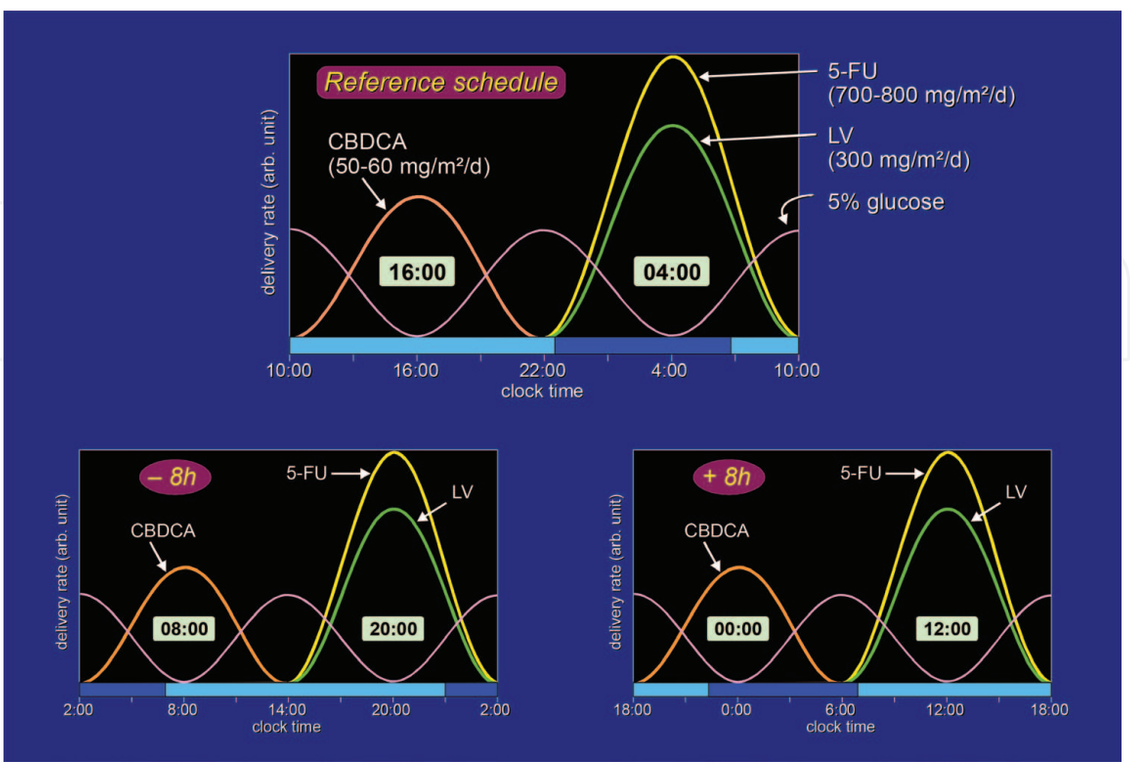

Figure 3.
Programs of ambulatory chronotherapy with 5 FU, folinic acid (leucovorin-LV), and carboplatin (CBDCA). The reference schedule is compared to two others with varying peaks (−8 h; +8 h). Daily delivery is automatically repeated by a chrono-programmable pump (Melodie) five times every 21 days (FFC5_16).

(group A) or 6 pm (group B) and cisplatin at day 4 at 6 pm [62]. A lesser degree of hematological toxicity was documented in group A, while cisplatin was better tolerated in group B [62]. Similar results were reported by Krakowski et al. [63].

Focan et al. also performed a randomized phase I–II trial comparing as first-line treatment, a complex sequential chronotherapy with 5 FU, folinic acid (FOL), and carboplatin with 24-h sinusoidal variation of drug delivery [64] (**Figure 3**). The reference schedule (peaks of 5 FU and FOL at 4 am, peak of carboplatin at 4 pm vs. two other schedules peaking, respectively, at + or – 8 hours, repetition for 5 days every 3 weeks) appeared to be the least toxic one with an overall excellent clinical tolerance [14, 64] (**Figure 4**). Toxicity data were reviewed in order to detect a possible gender effect as had been observed in metastatic colorectal cancer [3–5, 65]. Despite of no significant difference in treatment adaptation or dose intensity between men and women, overall increased serious toxicities were recorded in women versus men. Severe leukopenia and mucositis occurred more than twice as frequently in women than in men (grade 3–4 leukopenia per course, 7.7 vs. 3.2%; grade 3–4 mucositis, 6.6 vs. 1.2%) [14].

According to the results of the phase I–II trial described above [64], a phase II study was further performed on 68 advanced NSCLC previously untreated patients

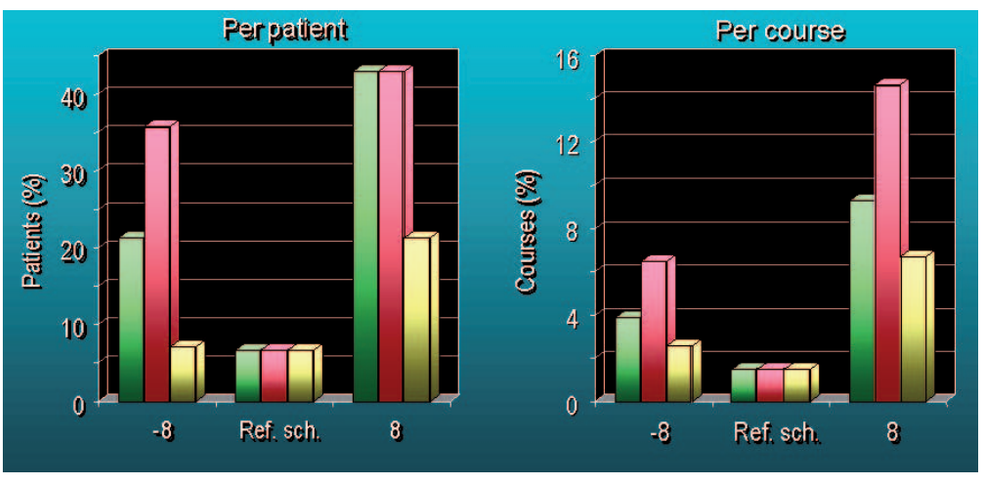

Figure 4.
Tolerability of chronomodulated 5 FU-LV-CBDCA infusion (grades 3–4 toxicities; in green, leucocytes; in red, granulocytes; in yellow, mucositis). Reference schedule was clearly the least toxic one ($p < 0.007–0.025$).

VARIABLE	MALES (275)	FEMALES (84)	p
Hb	10.5	26.2	0.001
WBC	9.8	16.6	0.023
Mucitis	8.3	21.4	0.016

Phase II trial chrono-FFC5_16. Grades 2–4 toxicities according to gender (% courses-p from chi-square)

Table 1.
Gender effect in NSCLC.

with the best circadian schedule [3, 14]. An excellent therapeutic index with a maximum of 17%, grades II–IV toxicities were observed and a gender effect was confirmed (**Table 1**) [3, 14].

Further analyses in advanced colorectal cancers have shown that the pattern of chronotolerance in men was rather sinusoidal with an optimal time corresponding to the reference modality; conversely in women the pattern was damped with optimal peaks of delivery possibly located 6 h later than in men [66]. The male subgroup showed a mean clearance (CL) value twice larger than the value observed in the female subgroup [66]. On the other hand, one has also to remind that the distribution of genes with circadian-varying expression was quite different in men and women in oral mucosa [44].

6. Circadian biological and behavioral determinants in LC patients

6.1 Hormones, immune functions, and tumor markers

A number of groups studied tumor-marker (CEA-carcinoembryonic antigen, alpha-fetoprotein, and others) rhythms but with similar disappointing results [1, 2, 67]. If in controls, a clear group circadian rhythmicity with an afternoon peak around 03:00 pm was evident, in cancer patients, individual variability or absence of rhythm were evidenced [1, 67].

Hormonal, hematological, and rest-activity cycles perhaps might constitute more promising markers of the host's internal circadian time structure. The most prominent hormonal circadian rhythms are cortisol (peak time occurs early in the morning in diurnally active persons) and melatonin (peak time occurs during the first half of the dark period in diurnally active people) [3–5, 67]. Important alterations of the normal circadian profile of these rhythms have been described in lung and other cancer patients with low performance status and high tumor burden [67].

Bartsch and colleagues [68] reported peculiarities in the cortisol and melatonin circadian rhythms in LC patients. Experimental data suggest interactions between interleukin-2 (IL-2; antitumor immune response is an IL-2-dependent phenomenon) and the pineal gland, which also may play a role in the control of immunity and cancer growth [69, 70]. The melatonin rhythm was evaluated in a group of LC patients receiving subcutaneous IL-2 treatment [70]. Prior to IL-2 therapy, none of the patients showed a normal 24-h rhythm of melatonin; IL-2 administration induced a normalization of the melatonin circadian rhythm with a nighttime peak in the majority of cases [70]. This observation suggests that abnormal pineal function in some lung cancer patients might arise in part from altered endogenous IL-2 production [70]. On the other hand, IL-2 administration induced a rise in cortisol with maintenance of 24-h rhythmicity [70].

Melatonin, tryptophan, and 6-sulfatoxymelatonin circadian profiles in blood and urine were compared in 30 advanced NSCLC cancer versus 63 healthy volunteers [66, 71]. All three molecule concentrations were significantly lower in cancer patients with a significant inverse correlation between melatonin and tryptophan levels [71].

Circadian rhythmicity in growth hormone (GH) and insulin-like growth factor-1 (IGF-1) was studied in control subjects and LC patients [72]. GH stimulates IGF-1 production in the liver and other tissues, while IGF-1 can promote cell cycle progression and apoptosis inhibition. GH and IGF-1 also stimulate lymphopoiesis and immune function [72, 73].

In LC patients, a progressive increase in GH and a steady decline of IGF-1 serum levels with loss of circadian rhythmicity were observed. This loss of diurnal rhythms could play a role in carcinogenesis and tumor growth regulation [71–75]. All profiles of time-related neuroendocrine-immune system components seemed to be the advancing stage of disease [71–76].

Mazzocoli et al. assessed altered time neuroendocrine-immune system function in lung cancer patients [75]. Circadian rhythmicity with night acrophases was validated in the control group for hormone serum level (melatonin, TRH, TSH, GH,) and for lymphocyte subset variation (CD3-, CD4-, HLA DR-, CD20-, and CD25-expressing cells). Cortisol, CD6, CD8 bright, CD8 dim, CD16, TcR-delta-1, and delta-TcS1 presented circadian rhythmicity with acrophase in the morning/at noon. In LC patients cortisol, TRH, TSH, and GH serum level and all lymphocyte subsets (except for CD4) showed some altered circadian rhythmicity. Mesor of cortisol, TRH, GH, IL-2, and CD16 was increased, whereas that of TSH, IGF-1, CD8, CD8 bright, TcR-delta-1, and delta-TcS1 was decreased [75]. Peak times however are related similar to those of control subjects [75]. The melatonin/cortisol mean nocturnal level ratio was also decreased in LC patients [75, 76]. Taken together, these results suggested that lung cancer is associated with alteration in the proportions and 24-h profiles of various lymphocyte subsets; this may be related to disease stage and probably altered immune function [73–75, 76].

Lissoni et al. also observed in NSCLC treated by chemotherapy (+/− melatonin) that lymphopenia and altered cortisol rhythmicity were associated with worsened quality of life (QOL), loss of psychosexual identity, and lower spiritual and faith scores [77, 78].

6.2 Host marker rhythms

The persistence of a circadian time structure like that of control normal subjects seems to be an independent prognostic factor, at least in advanced breast or colon cancers [10, 18, 79, 80]. As stated earlier, the strongest circadian rhythms are those of cortisol (with the clinical interest being the morning-afternoon gradient) and melatonin (with a nighttime peak) [2, 4]. Noninvasive easy-to-repeat assessment techniques have been validated for the determination of the 24-h time structure: titrations of cortisol and melatonin in the saliva and 6-hydroxymelatonin sulfate in the urine [81]. Nocturnal urinary 6-sulfatoxymelatonin is also correlated with the proliferation of cell nuclear antigen (PCNA) in lung tumors [68]. Thus, its determination might constitute a noninvasive tool to estimate circadian tumor cell proliferation in lung or other tissues.

Cortisol diurnal rhythm and slope have been related to survival in metastatic breast cancer patients by Sephton et al. [18]. Similarly, the same authors together with Chinese counterparts could also link the quality of persistent diurnal cortisol rhythm to the prognosis (survival) in LC patients [19, 20].

Assessment of the rest-activity circadian cycle by actometry measurements also seems an easy way to estimate the general circadian profile of individuals [1, 4, 5, 82–84]. In advanced colorectal cancer, it was demonstrated in two studies that patients retaining a prominent rest-activity circadian rhythm will enjoy better quality of life and sleep, less fatigue, less depression, and improved survival [82–84]. Such evaluations were proposed to lung cancer patients and validated [82–84]. Focan et al. evaluated rest-activity rhythms in 28 advanced NSCLC before chronotherapy [83]. Better general physical activity and circadian rhythmicity were recorded in those patients receiving also corticosteroids [83]. Hrushesky and his group also studied circadian function in NSCLC patients by actigraphic recordings [82, 84]. They tried to correlate the rest-activity rhythms with sleep disturbances, quality of life (European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ-C30)) and mood (anxiety; depression—HADS—hospital anxiety and depression scale) [82, 84]. All patients suffered from severe disturbances of daily sleep-activity cycles, but each patient also maintained some degree of circadian organization. QOL measurements were correlated with circadian destructors, fatigue prominent during daytime, and altered moods [82, 84].

Before initiation of their radiotherapy, a high percentage (30–50%) of 185 patients experienced significant disturbances in sleep-wake circadian rhythmicity; these perturbations occurred in both sleep initiation and maintenance [85].

Recent clinical observations have shown that elevated levels of TGF-alpha are associated with fatigue, flattened circadian rhythms, loss of appetite, and depression in patients with metastatic cancer [46]. These data support the hypothesis that a symptom cluster of fatigue, appetite loss, and sleep disruption commonly seen in cancer patients may be related to EGFR ligands released either by the cancer itself or by the host in response to the stress of cancer and suggest that further examination of their role in the production of symptom clustering is warranted [46]. Those observations also suggest to consider the central clock as a possible target for restoration of normal circadian rhythmicity in cancer patients!

7. Clinical trials

7.1 General considerations

During the last decade, the management of NSCLC has evolved [86–97]. Platinum-based chemotherapy remains the standard front-line in treatment of advanced unresectable NSCLC in which cisplatin or carboplatin are combined with another chemotherapeutic agent such as taxanes, pemetrexed, or gemcitabine [87]. However the results in terms of response rate, progression-free survival, and median overall survival remained stable over time [86–97].

Thus, progresses confirmed by phase III trials came from targeted and immunotherapeutic biological approaches. Targeted therapies against EGFR mutations and anaplastic lymphoma kinase (ALK) gene rearrangement have improved the survival in a small proportion of patients whose tumors were expressing these molecular abnormalities [88–91].

Also the recent development and success of immune checkpoint inhibition of programmed cell death 1 (PD-1)/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) for treating metastatic cancers seemed to open a new pavement for fruitful research [86, 92–97].

Unfortunately to the best of our knowledge, despite precise theoretical observations depicted related to circadian expression, on targets of TKI inhibitors, EGFR blockers, antiangiogenic agents, and immune active-agents (lymphoid system; PD1; PDL1), by now no study has been launched to take into account any chronobiological considerations!

8. Chemotherapy

As a matter of fact, in NSCLC, only a few studies deal with considerations on temporal dimension for drug delivery.

Studies from Focan et al. have already been mentioned and reviewed elsewhere [14]. In the first pioneered study [14, 28], a significant better tumor outcome was observed in the group treated at the better dosing time, thus from 10 am for a 40-h sequential schedule [14]. In the second phase III trial using etoposide and cisplatin, despite varying toxicities, there were no differences in overall drug dose intensities nor in tumor outcome gauged by the frequency of tumor responses as well as survival [62]. On the contrary, Krakowski et al. observed an increased dose intensity of drugs when given at their best circadian schedule [63].

According to the results of the phase I-II trial described [59] that had confirmed diurnal tolerance of a complex infusion regimen including 5FU, Fol, and carboplatin for 5 days every 3 weeks, a phase II study was further performed on 68 advanced NSCLC previously untreated patients with the best circadian schedule [3, 14]. Tumor evolution remained within the frame of the literature, but patients enjoyed an improved therapeutic index [3, 14].

Lissoni et al. successively performed small randomized trials (with 20–40 patients per group) in advanced NSCLC patients [67, 68, 77, 78]. They observed progressive improvement of tumor outcome (response rate; 1-year survival, etc.) and quality of life through the association with standard cisplatin-based chemotherapy, melatonin, and sometimes interleukin-2 (IL2) [67, 68, 77, 78]. They have reviewed their results on 370 cases suffering from NSCL and gastrointestinal tract cancers [78]. They confirmed a higher rate of tumor responses and better long-term survival in patients who had received melatonin [78]. These observations were linked to positive circadian effects in cancer-relevant psycho-neuroendocrine and immune pathways [77, 78]. According to these authors, a high degree of faith may positively influence the efficacy of chemotherapy and the clinical evolution of NSCLC by improving the lymphocyte-mediated antitumor immune response and probably general host circadian rhythmicity [78].

Hrushesky's group performed also a randomized blinded trial on 84 advanced NSCLC patients receiving chemotherapy with etoposide and cisplatin together with melatonin or placebo (personal communication). Those patients receiving melatonin during the evening enjoyed higher response rates (29 vs. 8–11%) and longer survival in multivariate analysis (personal communication).

Two meta-analyses appeared to confirm survival benefits associated with melatonin therapy in cancer patients [98, 99]. One analysis has focused upon 8 trials that used a dosage of 20 mg of melatonin, while the second one reviewed 21 clinical trials on solid tumors using various doses of melatonin [98, 99]. Both analyses report that the administration of melatonin reduces the relative risk of death at 1 year by an average of 37%, doubles the frequency of complete response, and reduces the prevalence and/or severity of chemotherapy-induced nausea/vomiting, hypotension, and hematological toxicity. Thus some authors consider melatonin as a probable effective treatment for human NSCLC [100]!

9. Targeted treatments

Despite advances in research and a better understanding of the molecular pathways of NSCLC, few effective therapeutic options are available for most patients with NSCLC without druggable targets, especially for patients with squamous cell NSCLC [86–91].

Chrono-PKs of erlotinib have been investigated in rodents [55, 56]. On the other hand, Iurisci et al. observed an improvement of circadian rest-activity rhythms together with a relief of symptoms in a limited trial on advanced NSCLC patients [101].

Nevertheless, no trial taking into account temporal dimension has been launched at present time.

10. Immunotherapy

Immune checkpoint inhibitors such as anti-cytotoxic T-lymphocyte antigen-4 or anti-programmed death 1 (PD-1) or programmed death-ligand 1 (PD-L1) have induced durable response rates across a broad range of solid tumors. In NSCLC, pembrolizumab and similar antibodies have replaced chemotherapy as first-line

treatment for patients with PD-L1 tumor proportion score (TPS) of at least 50% [92–97], while pembrolizumab plus platinum and pemetrexed for those with nonsquamous histology irrespective of PD-L1 expression [92–97].

As discussed in previous chapters, it is conceivable to consider the temporal (diurnal) dimension when administering either targeted or immunologic treatments to lung cancer patients, but, to the best of our knowledge, no study on that subject has been launched to date. We could regret this lack of interest, while dissection of molecular pathways of the circadian clock system has been awarded in 2017 with a Nobel Prize!

11. Radiotherapy

In a single non-randomized limited study, gamma knife radiosurgery for brain metastases of NSCLC was delivered either in the morning (10.00 am to 12:30 pm, 58 cases) or in the afternoon (12:30 pm to 3:00 pm, 39 cases) [102]. Patients treated in the morning enjoyed better tumor local control at 3 months (97 vs. 67%), longer survival (9.5 months vs. 5 months), and a lower rate of central nervous system (CNS)-related cause of death. Those results, which may be related to circadian susceptibility of target cancer cells (? more cells in G2-M phase; more apoptosis in the morning?), prompted the activation of a prospective randomized RTOG study whose results have not yet been published.

With another methodology, Badiyan et al. [103] reevaluated the impact of daytime on tumor outcomes after stereotactic radiosurgery (SRS), in 437 patients NSCLC treated for CNS metastases. They confirmed a cut point of 11:41 am for providing the highest predictive value for overall survival [103].

12. Conclusions

In this review, we have tried to gather pertinent animal and human data supporting the need to take into account temporal dimensions (i.e., circadian) for prevention and treatment of human NSCLC. The importance of biological rhythmicity was evidenced regarding carcinogenesis, molecular biology and genetics, cells kinetics, apoptosis, DNA repair mechanisms, platinum resistance, etc. These observations are fully applicable to human NSCLC.

Some randomized clinical trials for human LC have confirmed chronotolerance and probably chronoefficacy of combined chemotherapy. Furthermore, theoretical considerations allow to propose the application of chronobiological concepts to improve the management of human NSCLC either by working on the host circadian rhythmicity and on circadian variation of targets expression, such EGFR and VEGF receptors, or on the new molecular targets or pathways also circadian varying in their expression (e.g., ERCC1, DNA repair, Tip 60, etc.).

Similarly circadian variations in multiple immunological pathways warrant further interest. These considerations would bring hope to improve overall tumor outcome by optimizing "classical" therapeutic index of chemotherapies but also circadian host rhythmicity by acting on the central clock (i.e., by TKI administration) and/or molecular machinery (receptors, various enzymatic pathways, DNA metabolism and repair, immunology pathways, etc.).

Also the potential role of melatonin as resynchronizing agent and as a potentially active agent warrants further evaluations.

Eventually the increased toxicity of chemotherapy in women is intriguing, peculiarly when host circadian rhythmicity seemed to be implicated.

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Conflict of interest

The authors have no conflict of interest to declare.

In homage

To Jeffrey C. Hall, Michael Rosbash, and Michael W. Young who received jointly the Nobel Prize in Physiology or Medicine 2017 "for their discoveries of molecular mechanisms controlling the circadian rhythm."

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