Chronotherapy for Cancer Treatment: A Review of Timing Strategies

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Abstract

Chronotherapy, the delivery of drugs in accordance with the body's circadian cycle, has become a promising strategy to increase the effectiveness and lower the toxicity of cancer treatment. Cell division, DNA repair, and metabolism are three physiological functions regulated by the circadian clock that are known to affect how well anti-cancer treatments work. This study reviews the present status of research on timing techniques in order to offer a thorough overview of chronotherapy in the treatment of cancer. The review starts off by going over the basic ideas of circadian rhythms and how they relate to cancer biology. The justification for adopting chronotherapy to improve treatment results is then discussed, including the possibility of increasing therapeutic efficacy while reducing side effects. A full analysis of preclinical research is then provided, emphasizing the influence of treatment scheduling on drug metabolism, host-tumor interactions, and tumor growth inhibition. The significance of taking circadian rhythms into account while developing cancer treatment plans is emphasized in this study. The complicated interactions between circadian biology, tumor dynamics, and treatment effects are highlighted, underscoring the need for more study. In the end, utilizing the power of chronotherapy has the potential to transform cancer treatment by customizing regimens based on each patient’s inherent rhythms.

Keywords: Chronotherapy, Circadian rhythms, Cancer treatment, Timing strategies, Circadian clock, Chemotherapy

INTRODUCTION

Current therapeutic approaches and recent innovations in cancer treatment:

In the past ten years, a lot of research has been done on cancer, one of the leading causes of mortality in the world, with the goal of developing new treatments that would lessen the adverse effects of current ones. Tumours develop into very heterogeneous structures as cancer progresses, resulting in a mixed population of cells with a variety of molecular characteristics and therapeutic response patterns [1]. This
heterogeneity can be seen both spatially and temporally, and it is essential to the creation of resistant phenotypes induced by a selective pressure following drug delivery. Cancers are typically thought of as a group of cells, and cancer is treated as a single, homogenous condition. Consequently, understanding these intricate processes is essential for developing precise and effective drugs [2].

**Current therapeutic approaches:**

**Nanomedicine:**

It provides a flexible platform of biocompatible and biodegradable technologies that may deliver traditional chemotherapeutic medications in vivo, enhancing their bioavailability and concentration near cancer tissues, as well as their release profile. Applications for nanoparticles range from therapeutic use to medical diagnostics. The unique physicochemical features of nanoparticles (sizes ranging from 100 to 1,000 nm) are attributed to their small size and high surface-to-volume ratio. Biocompatible nanoparticles are used in cancer treatment to circumvent some of the issues with conventional therapy, such as the inadequate bioavailability and specificity of drugs or contrast agents [3]. The encapsulating of the active medications in nanoparticles will boost their solubility, biocompatibility, stability in bodily fluids, and retention duration in the tumour vasculature. It is also possible to design nanoparticles that release the drug in a controlled way in response to a specific stimulus and that are extremely selective for a certain target (refer to the section on "Targeted therapy and immunotherapy") [4, 9–11]. This includes the liposomal formulation ThermoDox, which can release doxorubicin in response to a temperature increase [4].

**Extracellular vesicles in cancer treatment and diagnosis:**

EVs can be categorised into two types according to their biogenesis. To be more precise, shed microvesicles (sMVs), which typically range in size from 50 to 1,300 nm, are found in nearly all extracellular body fluids and are in charge of molecular material exchange between cells. Exosomes are tiny vesicles that are discharged from endosomes in both normal and pathological situations. They have an average diameter of 30-150 nm. Exosomes are involved in the building of the environment necessary for the creation of pre-metastatic niches and the advancement of metastatic processes, as well as in the growth and metastasis of cancer and the reciprocal information exchange between tumour cells and surrounding tissues. Circulating vesicles are therefore important for clinical purposes in the identification, assessment, and monitoring of malignancy. Exosomes are acknowledged as trustworthy diagnostic agents, but they can also be extracted and used as Nano scale medication carriers or anti-cancer vaccinations in cancer treatment [5].

**Natural antioxidants in the treatment of cancer:**

The human body is constantly exposed to many harmful substances such as air pollution, UV radiation, and cigarette smoke. These insults lead to the synthesis of reactive species, specifically oxidants and free radicals, which are the initial cause of numerous disorders, including cancer. These molecules can also result from the therapeutic administration of drugs, but they are also naturally created by peroxisomes, mitochondria, and macrophage metabolism during normal physiological aerobic activities in our cells and tissues. Oxidative stress and radical oxygen species can damage DNA and other biomacromolecules, such as lipids (membrane peroxidation and necrosis), proteins (significantly altering the regulation of transcription factors and, as a result, of essential metabolic pathways and chromosomal aberrations) [6].

**Immunotherapy and targeted treatment:**

Traditional cancer therapy suffers from low chemotherapeutic drug selectivity for cancer cells. Because they influence both healthy and unhealthy tissues, most drugs cause unfavourable side effects. One of the main study topics is figuring out how to only focus on the intended spot. The propensity of nanoparticles to accumulate more in cancer cells has garnered significant attention due to their enhanced permeability and retention effect (EPR). Passive targeting is dependent on the minuscule size of nanoparticles, the vascular leakiness, and the inadequate lymphatic outflow from malignant tissues. However, passive targeting is hard to control and might result in multidrug resistance
(MDR). However, by concentrating on a subset of overexpressed receptors, active targeting boosts absorption by tumour cells. To functionalize nanoparticles, for example, ligands that bind particular cells or subcellular regions might be employed. Many ligands, such as aptamers, proteins, peptides, tiny molecules, and antibodies, can be used [7].

**Gene therapy as a cancer treatment:**

In order to treat particular disorders, gene therapy involves inserting a healthy copy of a damaged gene into the genome. In 1990, a retroviral vector was used to transfer the adenosine deaminase (ADA) gene to T cells in patients with severe combined immunodeficiency (SCID). Subsequent research indicated that gene therapy may be utilised to treat cancer, but also a number of other unusual and chronic human disorders. There are now 2,900 active gene therapy clinical studies, 66.6% of which are focused on treating cancer. Various approaches are being investigated for cancer gene therapy: 1) Expression of proapoptotic and chemo-sensitizing genes; 2) Expression of wild-type tumour suppressor genes; 3) Expression of genes capable of triggering specific antitumor immune responses; and 4) Targeted oncogene inhibition [8].

**Magnetic hyperthermia and thermal ablation:**

The phrase “thermal ablation of tumours” refers to several techniques that employ heat (hyperthermia) or cold (hypothermia) to kill malignant cells. The temperature range between 40°C and 60°C is known to cause cell necrosis. It is also effective to destroy tumor cells over extended periods of time at temperatures between 41°C and 55°C. Furthermore, it has been established that cancer cells are more sensitive to high temperatures than healthy ones. Hypothermic ablation is caused by the formation of ice crystals during cooling, which tear apart cell membranes and destroy cells. Argon gas is the most effective cooling agent, lowering the temperature of the tissues around it to -160°C. Furthermore, because gases like nitrogen have a higher heat capacity than argon when at their critical point, they can be used in this manner. However, the technology required to control and manage them is still in their infancy [9]. Clinics most frequently use RF ablation since it is a safe and effective therapy. An insulated electrode tip delivers an RF wave alternative current to a target zone, and the circuit is completed by placing a second electrode on the skin’s surface. Heat is produced as a result of the ion oscillation in the extracellular fluid caused by the current's contact. The more conductive the substance, the more effectively the process functions. Because of this, the lungs are less affected by RF ablation than the liver and other tissues that have significant water and ion contents. Furthermore, the efficacy of the treatment decreases with lesion size, with portions no larger than 3 cm² showing the best effects [10].

**Radiomics and pathomics are recent innovations in cancer treatment:**

Effective cancer treatment today relies on surgery and, in around 50% of patients, radiotherapy, which can be administered via an external beam source or by locally inserting a radioactive source (this method is known as brachytherapy), resulting in targeted irradiation. Image-guided radiotherapy (IGRT), in which pictures of the patient are captured throughout the treatment to allow the optimal quantity of radiation to be set, now makes it easier to localize the beam. As a result of the development of intensity-modulated radiotherapy (IMRT), radiation fields of various intensities may be produced, assisting in lowering the doses absorbed by healthy tissues and reducing unfavorable side effects. Lastly, stereotactic ablative radiotherapy (SABR) has made it possible to provide an ablative dosage of radiation just to a tiny target volume, considerably lowering unfavorable effects [11].

Radioresistance, which might develop during therapy and reduce its effectiveness, is unfortunate. As a result, focusing on certain activities has shown to be effective in restoring the anti-cancer benefits that have been lost due to mitochondrial abnormalities. According to a recent study, radioresistance in an oesophageal adenocarcinoma model is associated with abnormal mitochondrial structure and size, and measuring patients’ energy metabolism has made it possible to distinguish between those who are resistant to treatment and those who are sensitive to it. Treatment for gastrointestinal cancer is being researched using tiny compounds that function as radiosensitizers and target mitochondria [12].

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The areas of radiomics and pathomics, which combine quantitative image characteristics from radiology and pathology screens as therapeutic and prognostic predictors of illness outcome, are both promising and cutting-edge. In order to handle and elaborate the enormous number of gathered datasets and to precisely anticipate the treatment efficacy, the clinical result, and the illness recurrence, several artificial intelligence technologies, such as machine learning applications, have been launched. Finding an ad hoc adaption for the best prognosis and outcome can be aided by prediction of the therapy response. Biomedical pictures are essential to enable real-time monitoring of disease development, being firmly associated to cancer molecular characterization. Personalized treatment nowadays necessitates an integrated interpretation of the data produced by numerous diagnostic techniques [13].

Unambiguous data gathering criteria must be defined immediately. Quantitative imaging network and the German National Cohort Consortium are two examples of steps that have previously been made to standardize processes and simplify clinical translation. To establish reliable procedures for the construction of models in radiation treatment, precise description of the parameters essential for image capture, the design and application of computational and statistical approaches, is required. The US National Library of Medicine reports that some 50 radiomics clinical studies are presently enrolling patients, and a few have already were completed [14].

**Purpose and significance:**

The purpose of this review article on chronotherapy for cancer treatment aims to offer a thorough and critical assessment of the body of knowledge and research about the timing tactics applied in cancer treatment. Chronotherapy is the practice of delivering treatments or drugs at specified times of the day or night in order to maximize their efficacy and minimize the side effects. To consolidating and summarizing existing knowledge which means finding of several investigations, clinical trials and research articles on chronotherapy for cancer treatment. Clinicians can use this information to help them decide when to deliver particular cancer therapies to get greatest results for their patients. In the area of cancer treatment timing methods, chronotherapy is a useful resource that compiles and assesses the body of information, identifies best practices, points out gaps, and helps evidence-based decision-making. It significantly contributes to the development of medical knowledge and the enhancement of patient outcomes [15].

**Biological Basis of Chronotherapy**

**Circadian rhythms and their relevance to cancer biology:**

Currently, cancer is the illness that has the biggest global impact on both health and the economy. More than 10 million people died from cancer in 2020, and 19.3 million new cases were diagnosed. Since biological rhythms are involved in the beginning, progression, and treatment of the illness, it is not surprise that novel methods of treating cancer should do the same. This section examines many cancer-related processes where the circadian clock is active (Table 1).

**Cell Cycle Progression**

The circadian gating of the cell cycle, which is controlled by time windows set by the biological clock, regulates changes from one phase of the cell cycle to another. By planning DNA replication, for instance, during periods of low solar irradiation, this phenomena is highly conserved and helps to reduce DNA failures. Understanding this circadian gating is crucial for cancer therapy in order to determine when anti-proliferative medicines should be administered. As both are based on cycles controlled by transcriptional and translational feedback loops, protein modification, and degradation, circadian rhythm control is analogous to cell cycle regulation [16]. In order to enable normal cell division, the cell cycle is tightly regulated by a number of molecular processes, including mitosis, which occurs only at specific times of the day in mammals, and some genes whose expression is influenced by circadian rhythms, which govern various stages of the cell cycle. Wee1, whose expression changes during the day due to CLOCK/ BMAL1 activation and PER/CRY repression, controls the circadian activity of CDK1/cyclin B1, the complex that initiates mitosis. The expression of Wee1 is actually inhibited by PER1, another protein with circadian action. Additionally, the p16-INK4A
gene, which inhibits CDK/cyclin complexes, is controlled by PER1 through its interaction with the checkpoint kinase Chk1. Contrarily, CLOCK/BMAL1 regulates and inhibits c-Myc production, and PER1 stabilizes it, which prevents p21, another inhibitor of CDK/cyclin complexes, from being expressed and permits the cell cycle to proceed. (Figure-1).

Table 1 The key novel cancer therapy strategies benefits and drawbacks

<table>
<thead>
<tr>
<th>Strategy</th>
<th>benefits</th>
<th>drawbacks</th>
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</table>
| Nanoparticles                 | Superior stability and precision  
High levels of bioavailability and biocompatibility | Depending on the specific nanoparticle                                   |
| EVs                           | Secreted physiologically  
Molecular characterization is done well  
High degree of biocompatibility  
Loadable and modifiable in vitro | Absence of preclinical protocols for drug loading, measurement, isolation, and storage |
| Natural antioxidants          | Easily accessible in substantial amounts  
Taking advantage of their inherent qualities | Restricted bioavailability  
Potentially harmful                                                        |
| Targeted therapy              | Increased specificity  
decrease in unfavourable reactions | Insufficient details about enduring adverse consequences                 |
| Gene therapy                  | Gene expression that promotes apoptosis and chemosensitivity  
Expression of tumour suppressor genes of the wild-type  
Gene expression capable of eliciting certain anti-tumour immune responses  
targeted oncogene and safety (RNAi) silencing | Integration of genomes  
restricted effectiveness in some patient populations  
high likelihood of immune system neutralisation  
Interactions off-target and inflammation (RNAi)  
Ad hoc delivery systems (RNAi) are required.  
Configuring dosages and appropriate settings for RNAi controlled release |
| Thermal ablation Magnetic hyperthermia | Accurate handling of the relevant domain.  
Treatment with the option of MRI imaging (magnetic hyperthermia) coupled with the procedure. | High efficacy limited to specific regions  
low strength of penetration  
Requirement for a qualified operator to administer the treatment |
| Radiomics/pathomics           | Creation of the entire tridimensional volume of the cancer using non-invasive imaging methods  
Prognostic and therapeutic markers of illness outcome | Defining clear standards for acquiring data  
Standardisation of processes to enable clinical translation; characterization of parameters and employment of computational and statistical techniques to provide reliable protocols for the creation of therapeutic models |
Various biological clock elements are in charge of regulating the cell cycle's machinery at various stages.

Cell proliferation is elevated in cancer and circadian clock genes are frequently severely dampened, most likely as a result of gene alterations. Desynchronization between the cell cycle and the body clock is another effect of these changed rhythms. The influence of circadian rhythms of anticancer medication delivery as well as the cytotoxic effect of a drug during the various cell cycle phases may be studied and understood using computational models that account for this desynchrony. The optimal timing for drug delivery for 5-FU and oxaliplatin was determined via a model, and it was also shown that various cell populations can be toxicated differently by the same temporal pattern of administration, such as normal vs malignant cells. Utilizing computational models enables the discovery of novel elements that might enhance therapeutic effectiveness as well as tolerance. As a result, tracking circadian rhythms in cancer patients may provide a fresh treatment option. Although the circadian clock may affect the cell cycle, the latter may function without the former given that there are mice that lack the circadian clock, and these animals do not exhibit phenotypes that are linked to cell cycle problems or have a higher risk of developing cancer [17].

**Repair Mechanisms for DNA**

The circadian clock regulates several biological processes, including apoptosis, DNA damage checkpoints, and nucleotide excision repair (NER). A cell goes through apoptosis when DNA damage builds up and DNA repair processes fail. One of our body's defense mechanisms, programmed cell death removes cells that have sustained harm over time. One of the genome's protector genes, p53, triggers this apoptotic reaction in response to DNA damage. In order to avoid MDM2-induced p53 localization to the proteasome, the clock protein PER2 binds to both p53 and MDM2, the p53 inhibitor. This makes PER2 a unique downstream effector in the DNA-damage pathway. Therefore, p53 levels are impacted by PER2 downregulation, whereas p53 protein stability and the transcription of p53-targeted genes are impacted by PER2 overexpression. In actuality, p53 controls PER2, further enhancing control over the cell cycle, the biological rhythm, and the p53-mediated response [18].

Current data further demonstrates that only the NER mechanism is directly controlled by the clock via the repair factor XPA. It has been suggested that this component, which is involved in the rhythmic nature of the repair activity in mice, is activated by CLOCK-BMAL1 and repressed by CRY-PER. As a result, the highest peak of NER activity also serves as the maximum peak of XPA, which establishes the repair capacity. Since cisplatin is less hazardous to the remainder of the organism when administered in the downstream phase of NER, it may have a greater therapeutic effect at lower dosages. (Figure 2) [19].

**Figure 1: Cell cycle and the molecular circadian clock’s relationship.**

**Figure 2: DNA damage and the circadian rhythm of molecules are related.**

The circadian clock regulates all of these activities, which include DNA damage accumulation, malfunctions in DNA repair pathways, and cell death.
Dysfunction of the mitochondria
Mitochondrial malfunction is one of the key developmental stages that appear to be involved in the emergence and spread of cancer. Higher amounts of several reactive oxygen species (ROS), hypoxia, and apoptotic inhibitory signals are seen in tumor tissues' mitochondria. The dynamic mechanism through which mitochondria continually fuse and divide is evident. Circadian clocks appear to be in charge of controlling these alterations in mitochondrial morphology. Low amounts of several mitochondrial fusion proteins are seen in mice with BMAL1 gene knockouts. Similar to this, mitochondrial respiration is different in mice missing PER1/2. This would suggest that interference with mitochondrial dynamics jeopardizes homeostasis and organismal health. Due to this, type 2 diabetes, obesity, dyslipidemia, and cardiovascular disease are all made more likely to occur as a result of impaired mitochondrial activity. As a result, it is important to research and maybe employ as a therapeutic target the circadian dependency of mitochondrial morphology and its interaction with metabolic and energy cycles [20].

Metabolism Reprogramming
The reprogramming of tumor cells' metabolism to engage in glycolysis and lactic fermentation even in environments with enough oxygen is another feature of cancer. By increasing glycolysis, the microenvironment becomes more acidic, ROS accumulate, DNA is damaged, and the circadian rhythm of the niche containing the tumor cells is changed. Due to its function in the body's metabolism, the pancreas is an organ that is significantly impacted by the rhythm of the body. Pancreatic differentiation is controlled by the biological clock through the cell cycle, Wnt, and Notch pathways throughout embryonic development. Additionally, the pancreas' exocrine secretion rises in the dark period. Misaligned meals have been shown in studies on mice to decouple insulin and corticosterone cycles in the exocrine portion of the pancreas. Thus, unfavorable dietary practices, such as those typically linked to shift employment, may influence the pancreas' rhythm and lead to pancreatic-related diseases. Furthermore, in young persons with type 1 diabetes, changes in sleep patterns are linked to high levels of hemoglobin A1c (HbA1c) and higher insulin needs. As a result, the molecular circadian clock's components are related to metabolism and may be exploited to generate novel therapeutic treatments for diseases like pancreatic cancer and other pathologies as well as new prognostic indicators [21].

The Immune System
The immune system has been linked to circadian clocks, demonstrating circadian rhythmicity. For instance, undifferentiated T lymphocytes and NK cells are more abundant in human blood during the sleep phase than they are during the activity phase. Circadian clocks also regulate the synthesis of certain hormones. Proinflammatory cytokines (like IL-1 and TNF-) and anti-inflammatory cytokines (like IL-4 and IL-10) both exhibit their acrophase during the active phase of the cell cycle, whereas anti-inflammatory cytokines (like IL-4 and IL-10) do not. A powerful immunosuppressant whose release peaks in the morning, glucocorticoids are another excellent example. Cortisol levels, for instance, are greater in the morning and decrease throughout the second portion of the night. Because of this, hormones may affect the effectiveness of immunotherapy in the treatment of cancer [22]. The circadian clock acts as a gate that regulates several immune system functions, such as lymphocyte trafficking and antigen presentation; although more research is required to fully understand their influence. In fact, cancer patients have altered versions of certain of these biological cycles. It may be possible to create cancer immunotherapies that are more successful by knowing the rhythmicity of the immune system and the part that immune cells play in the growth, encouragement, or demise of tumor cells.

The Molecular Mechanisms of the Circadian Timing System (CTS) and Circadian Clock
Mammals' CTS are arranged hierarchically, with "master" pacemaker clocks that respond to light being situated in the suprachiasmatic nucleus (SCN) of the hypothalamus and controlling rhythmic cycles in peripheral tissues and extra-SCN neurons. By using both direct autonomic nervous system efferents and neuroendocrine inputs, the SCN syncs peripheral tissue clocks with the ambient light cycle (Figure 3A). A number of
Proteins that take part in both positive and negative transcriptional feedback loops are essential to the molecular clockwork of the cell (Figure 3B). Because they bind E-box elements (CACGTG) in the Period (Per) and Cryptochrome (Cry) genes, the transcription factors Brain and muscle aryl hydrocarbon receptor nuclear translocator 1 (BMAL1) and CLOCK have a positive impact on circadian transcription. BMAL1/CLOCK heterodimer-driven transcription is negatively regulated by the mammalian PER and CRY proteins. After interacting with casein kinase I (CKI), PER and CRY create heterodimers that go into the nucleus where CRY functions as a negative regulator of BMAL1/CLOCK-driven transcription. Circadian gene expression is mediated by transcription at the ROR/REV-ERB and the DBP-E4BP4 (D-box) binding elements NR1Ds and RORs (subfamilies of nuclear hormone receptors), which either activate or repress gene transcription and form the ROR components in a number of clock genes. This is in addition to the primary biochemical feedback loop that controls cycling at the E-box. Microarray research has

### Table 2: An overview of the relationships between cancer and circadian rhythms

<table>
<thead>
<tr>
<th>Cell Cycle Progression</th>
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<tr>
<td>CDK/cyclin complexes</td>
<td>both take place during replication, hence the clock has an indirect affect. (62,66)</td>
</tr>
<tr>
<td>Mismatch repair (MMR) Double-strand breaks (DSBs)</td>
<td>Directly controlled by the clock through the XPA repair factor. (65–67)</td>
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<tr>
<td>Nucleotide excision repair (NER)</td>
<td>Minimal concentrations of several mitochondrial fusion proteins. (70)</td>
</tr>
<tr>
<td>BMAL1 knockout mice</td>
<td>Altered mitochondrial respiration. (71)</td>
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<td>PER1/2 knockout mice</td>
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examined the role of specific clock genes in peripheral oscillators. When rat hepatocytes' circadian clocks were specifically disrupted, for instance, oscillations were lost in 90% of the transcripts, indicating that the remaining 10% of rhythms were triggered by systemic stimuli that originated outside the liver yet had an impact on its function. According to genetic and bioinformatics research, mammals have more clock genes that either directly control essential clock processes or post-translationally control the activity of essential clock proteins [23].

**A. Timing-based drug administration:**

**Morning Administration:** Some drugs are more effective when administered in the morning due to the body's natural rhythms. For example, glucocorticoids, which are used to manage inflammation and immune-related conditions, are often given in the morning to mimic the body's natural cortisol peak.

**Evening or Nighttime Administration:** Some drugs may have improved effectiveness or reduced toxicity when administered in the evening or at night. For instance, certain chemotherapy agents have been shown to be less toxic to healthy cells and more toxic to cancer cells when given during nighttime [24].

**Chronomodulated Drug Delivery:** This approach involves developing drug delivery systems that release medications at specific times of the day, synchronized with the body's circadian rhythms. These systems may include time-release formulations, programmable pumps, or smart drug delivery technologies.

**Chronotherapy for Cancer:** In cancer treatment, researchers have explored the concept of chronochemotherapy, where chemotherapy drugs are administered at specific times to coincide with the circadian rhythm of tumor growth and cell division. This approach aims to increase the drug's effectiveness against cancer cells while reducing its impact on healthy tissues [25].

**Personalized Chronotherapy:** Individual variations in circadian rhythms and drug metabolism are taken into account to customize the timing of drug administration for each patient. Personalized chronotherapy may involve monitoring a patient's circadian rhythm through various methods, such as continuous glucose monitoring, wrist actigraphy, or temperature monitoring.

**Circadian Rhythm Optimization:** In some cases, efforts are made to optimize a patient's circadian rhythm before drug administration. This could involve using bright light therapy or other approaches to align a patient's circadian rhythms with the desired drug administration schedule. It's crucial to remember that research on chronotherapeutic tactics is still ongoing and that not all treatments or disorders may considerably benefit from timing-based methods. Depending on

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**Figure 3:** Mammalian CTS and calibrators are organised hierarchically. (A) The CTS comprises of three parts: an input component, a clock work, and an output component. (B) The circadian oscillator interlocked positive and negative transcriptional feedback loops.
the specific medication, the patient's unique illness, and other considerations, these tactics may or may not be helpful. Chronotherapeutics shows potential for boosting patient quality of life and bettering treatment results as the area develops [26].

**Light-based therapies and phototherapy:**

**PHOTODYNAMIC THERAPY:**

In photodynamic therapy (PDT), cancer cells are killed using specialized medications, often known as photosensitizing agents, and light. Only when specific types of light have activated or "turned on" the medications will they begin to function. PDT is sometimes referred to as phototherapy, photo radiation therapy, and photochemotherapy. The photosensitizing chemical is either injected into a vein or applied topically, depending on the part of the body being treated. The drug is absorbed by cancer cells gradually. The treated area is then lighted. The drug interacts with light and produces a special type of oxygen molecule that kills cells. PDT may also be advantageous since it triggers the immune system to attack the tumour and cuts off the blood supply to the cancer cells. The drug-to-light interval is the period between giving the drug and turning on the lights. Depending on the treatment used, it could take anything from a few hours to a few days [27].

PDT light is produced using specific types of lasers or light-emitting diodes (LEDs). The type of light used depends on the type of cancer and its location in the body. PDT is frequently conducted as an outpatient operation, so you will not need to remain in the hospital, but it may also be combined with surgery, chemotherapy, or other anti-cancer treatments, as well as radiation therapy [28].

**Photodynamic therapy has limits too [29]:**

1. PDT can only treat regions that receive light. This implies that it is mostly employed to treat conditions that are present on, immediately under, or in the lining of organs that may be illuminated. Because light cannot penetrate deeply into bodily structures,
2. Large tumors and malignancies that have encroached deeply into the skin or other organs cannot be treated with PDT.
3. PDT does not treat cancers that have spread to many places.
4. When injecting or using PDT drugs, it's important to take precautions as the chemicals might cause excessive sensitivity to light for a period of time.
5. Individuals with specific blood conditions cannot use PDT.

**Significance of Photodynamic therapy:** PDT can be used to prolong life and enhance quality of life in persons with certain forms of cancer. It is becoming acknowledged as a beneficial therapeutic option for some forms of localized malignancies (cancers that have not spread very far from their initial site of growth).

**US-approved PDT cancer treatment drugs [30]:**

The US Food and Drug Administration (FDA) has currently licensed a number of photosensitizing medications for the treatment of certain malignancies and pre-cancers. Two of the most popular are listed below:

1. **Porfimer sodium** (Photofrin) is extensively used and researched. It is turned on by a laser’s red light. The FDA has authorized it for the treatment of individuals with certain esophageal and lung malignancies, and research are ongoing with regard to other cancer types.
2. **Aminolevulinic acid** (ALA, often known as Levulan) is a medication applied directly to the skin. It is exclusively applied to the face or scalp and is used to treat actinic keratosis (AK), a skin disease that can progress to cancer. Instead of laser light, a specific blue light is employed to activate this medication.

**Cancer therapy using chronomodulating drugs:**

One appealing concept is the use of chronomodulating drugs to reset or hold the circadian cycle at a specific time point in order to treat patients when the therapy would be most effective or to help restore the dampened clock caused by the disease or the therapy. Circadian oscillation in cultured cells can be caused by a variety of substances, including high serum concentration, dexamethasone (GR agonist), forskolin (adenylate cyclase activator), phorbol-12-myristate-13-acetate (PMA, protein
kinase C activator), fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin, calcium ionophore calcimycin (induces apoptosis by intracellular Ca2+), endothelin, glucose, prostaglandin E2, NAD, heme, and cAMP. These substances produce rhythm in various ways. As a result, a wide variety of substances might be employed to alter the circadian clock at various periods during the circadian cycle. One of the experimental methods involves using stably transfected Luc reporter cell lines to screen various compounds and examine their impact on the circadian cycle. One of these screenings led to the discovery of a medication that blocks GSK-3, which shortens the time of oscillations in U2OS cells. GSK-3 has previously been discovered as a kinase in mammals that either promotes nuclear translocation (PER 2), increases stabilization (REV-ERBα), or directly phosphorylates a number of essential clock proteins and mediates their degradation (CRY2) CLOCK, and BMAL1) [31].

Another team discovered a tiny chemical called longdaysin that extends the circadian period of mouse SCN explants as well as other cultured cells.[119] Longdaysin specifically targets the protein kinases ERK2, CK1α, and CKIδ. Although CK6 and ERK2 were previously recognized to play a part in the control of the circadian clock, CK1α was a novel player that appeared to phosphorylate PER1 directly and encourage its destruction. If additional factors like oscillation amplitude or rhythmicity are taken into account, a comprehensive screen discovered a number of tiny compounds that significantly increased the amplitude. The expression of clock-output genes like Dpb and Rev-Erbα is correlated with that. As demonstrated in the action of forskolin on SCN slices, several of the recently discovered small molecules mediated acute induction of Per2, followed by the phase delay. A tiny chemical that prolongs the circadian period and suppresses CRY breakdown was discovered through another screen. The fact that CRY negatively affects the transcription of the rate-limiting enzymes phosphoenolpyruvatecarboxykinase 1 (Pck1) and glucose-6-phosphatase (G6pc) makes it possible to study gluconeogenesis. Treatment with this substance inhibited the stimulation of Pck1 and G6pc by glucagon and the generation of glucose, making it a possible clock-based medication for the management of diabetes.

Many core clock proteins have additional clock-independent physiological functions, so tiny compounds that would affect particular clock proteins may be considered as more targeted therapeutic medication. The functioning of CLOCK/BMAL1 was altered by genotoxic treatment, therefore a search for modulators of its functionality was made. Although all of the mice in these investigations had behavioral arrhythmia, they all had a distinct reaction to the damage brought on by the chemotherapy drug cyclophosphamide. Cyclophosphamide was severely toxic to animals without clock activators (Clock mutant mice, Bmal1 KO mice), but it was not toxic to animals lacking clock repressors (Cry double KO mice). These findings imply that pharmacological regulation of circadian transcriptional activators may be a useful strategy for preventing genotoxic therapies from damaging normal tissues. A search for CLOCK/BMAL1 activity modulators turned up some well-known circadian function regulators in addition to some novel substances such as the organic selenium complex L-methyl selenocysteine. Selenium enhances BMAL1 transcription, increases BMAL1 protein, and likely activates the CLOCK/BMAL1 complex by preventing the transcription repressor Tieg1 from interacting to the SP-1 binding site in the Bmal1 promoter. Both in vitro and in vivo studies using a diet supplemented with selenium or selenium injections demonstrated this effect. Since selenium-induced BMAL1 effects were only observed in the liver and not in the SCN, the in vivo impact was tissue-specific. This indicates that the behavioral characteristics did not change. Given that it doesn’t interfere with the internal clock, this has enormous therapeutic potential. Selenium was unable to replicate the effects in Bmal1 KO rats, indicating that this pathway is solely mediated by BMAL1. Selenium already has two significant therapeutic effects: preventing tumors and guarding against DNA damage brought on by radiation therapy, an anticancer treatment. In patients with cervical and uterine cancer who are receiving treatment, selenium supplementation reduces the amount of diarrhea and mucositis that are brought on by fractionated ionizing radiation [32].

The Cry-deficient mouse model was used to study CLOCK/BMAL1 functioning, and there, an intriguing mechanism of pharmacological...
relevance was revealed. Cry double KO mice with a p53-null background have been reported to be more susceptible to UV-light-induced apoptosis. CLOCK/BMAL1 upregulation of p73-dependent apoptosis was the cause of this increase. Early growth response 1 (Egr1) gene expression is raised when Cry is downregulated in the absence of p53, which also increases p73 expression. Egr1 is a direct target of CLOCK/BMAL1 and positively activates p73. Due to BMAL1’s downregulation, Egr1 is continuously increased in Cry-deficient cells. EGR1 is a positive regulator that binds to the p73 promoter. At the p73 promoter, the negative regulator C-EBP is also present, but when exposed to UV radiation, only EGR1 remains linked to it. When oxaliplatin was used to treat tumor xenografts, a similar result was seen. These results point to an intriguing therapeutic approach for sensitizing tumor cells lacking in p53 activity, which involves activating the p73-dependent apoptotic pathway.

Comparison between Chronomodulated Chemotherapy and Conventional Chemotherapy:

Chemotherapy is a common kind of cancer treatment that includes giving patients cytotoxic medications that either kill or stop the growth of rapidly proliferating cells. Increasing the chemotherapy dose until maximal cytotoxicity occurs and a maximum tolerable dose is obtained is the fundamental tenet of conventional chemotherapy. However, because both cancerous and normal cells are impacted, significant toxicities frequently appear, which causes chemotherapy treatments to be discontinued and lowers survival rates. Therefore, discovering techniques to boost effectiveness while lowering side effects would significantly raise the potential of cancer therapy.

The circadian clock regulates the molecular systems in charge of controlling pharmacological activities such drug absorption, distribution, metabolism, and excretion. Because the clock regulates the medication’s detoxification and excretion, which impact the drug’s effectiveness and toxicity on tumor cells and healthy cells, respectively, the best time to provide an anticancer drug therapy is determined. Rodents kept in alternating exposure to 12 hours of light and 12 hours of darkness (LD12:12) have demonstrated that anticancer medicines had increased effectiveness and tolerability as well as a 24-hour variability in medication toxicity. By giving anticancer drugs at certain times of the day, chronomodulated chemotherapy tries to take advantage of the circadian variation in drug response by striking cancer cells when they are most sensitive or normal cells when they are least vulnerable. Chronomodulated and circadian-based are words that have been used to denote comparable parenteral or oral delivery methods. We shall refer to all therapy avenues as chronomodulation for the purpose of clarity [33].

Chronomodulated chemotherapy aims to improve the quality of life and survival time of cancer patients by minimizing toxic side effects while maximizing the regimen’s effectiveness, This is based on the distinct circadian rhythms of DNA synthesis and cell proliferation found in tumour and normal cells. Chronomodulated chemotherapy is administered as a variable-rate infusion, with peak drug delivery times set to vary in accordance with circadian time or delivery limited to specific time periods.

Despite the fact that chronomodulated chemotherapy is a promising area of study that could significantly advance current and future cancer treatments, there is currently no medical consensus on the implementation of chronomodulated chemotherapy regimens, and conventional chemotherapy is typically given in accordance with hospital schedules and staff work schedules. Benefits of this strategy must be sufficiently validated by data from well-designed randomized clinical studies before it is implemented in clinical practice.

Challenges and Prospects for the Future of Chronotherapy

Chronotherapy is a permanent component of the scientific community’s attempts to enhance customized treatment. The 24-h periodicity in the circadian rhythms of the majority of tissues and metabolic processes has been confirmed by several researches concentrating on the metabolites of the circadian clock. Additionally, the molecular clock’s potential therapeutic targets have been discovered. But for now, timing the administration of medications to increase efficacy and decrease toxicity presents some challenges. The difficulties of chronotherapy are outlined by
Kuo and Ladurner from three angles: (a) to design clinical trials to gain a better understanding of the impact of biological clocks on the action of anticancer drugs and vice versa; (b) to investigate the influence of some factors, such as age, gender, comorbidities, and so on, on the response to chronotherapy; and (c) to encourage pharmaceutical companies to assess the effectiveness and toxicity of their drugs using chronotoxin. Furthermore, more effective delivery methods (such as nanoparticle systems) should be created and refined in order to ensure that medications are delivered at the proper moment [34].

Limitations of current chronotherapy:

The existence of chronotypes is one of the restrictions placed on the use of chronotherapy. As a result, the rhythmicity of the various stages varies depending on the individual. DLMO (dim light melatonin onset) is a technique for measuring the circadian phase objectively. The patient provides saliva samples for this non-invasive test, which involves measuring the amounts of melatonin in those samples. Melatonin production is inhibited by light, thus measurements are conducted every 30 to 60 minutes for 6 to 8 hours in extremely low light. However, this test is currently exceedingly difficult to administer due to both the cost and the length of the exam. Other theories include using blood tests to infer rhythms from the blood’s sequencing. Furthermore, created a straightforward yet accurate test (called Body Time) that can calculate the internal Cancers 2022, 14, 5071 15 of 21 circadian time in humans by measuring some clock biomarkers such as NR1D1, NR1D2, CRY1, PER1, PER2, PER3, CRISPLD2, KLF9, LGALS3, ELMO2, FKBP4, and HSPH1, from a single blood test.

Recently, a lot of emphasis has been placed on mathematical models of tumor growth known as control theory that take into account the potential impact of various environmental variables on the response to therapy. Particularly, the use of control theory in pharmacokinetic and pharmacodynamic models for anticancer treatments is becoming more and more popular. The creation of mathematical models that forecast the ideal therapy administration settings to manage the treatment response and the rate of progression is made possible by the growing availability of data from tumors. Before it can be effectively implemented, however, there is still a lot of study in this area to be done [35].

CONCLUSION

Finally, the investigation of chronotherapy as a unique approach to improving cancer treatment efficacy has shown a world of exciting possibilities. The examined literature emphasizes the complicated connection between circadian rhythms and anticancer drug pharmacodynamics, showing the possibility for improved therapeutic results by deliberate treatment scheduling.

The evidence presented in this review suggests that aligning cancer treatment with the body’s circadian rhythms can yield significant benefits, including enhanced drug absorption, reduced side effects, and improved treatment response. This underscores the importance of individualized approaches that consider not only the specific cancer type but also the patient’s inherent biological rhythm. However, it is crucial to recognize the difficulties and complexity of incorporating chronotherapy into conventional cancer treatment procedures. The variety of patient responses, the impact of outside stimuli on circadian rhythms, and practical issues all provide challenges that call for careful thought and more research.

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