Chronotherapeutic Considerations in Immunotherapy: A Case of Durable Response in Metastatic Non-small Cell Lung Cancer

Ivan Bivolarski

Oncology Centre in Burgas, Burgas, Bulgaria

Received July 9, 2025; Accepted June 2, 2026.

Key words: Pembrolizumab – Circadian rhythms – Tumour progression – Neutrophil-to-lymphocyte ratio – Treatment timing

Abstract: Chronotherapy, the alignment of treatments with biological rhythms, has been explored in oncology, particularly for chemotherapy and targeted therapies, but its impact on immune checkpoint inhibitor (ICI) efficacy remains underexplored. This research aimed to examine the potential chronotherapeutic effects of morning ICI administration on the long-term response to pembrolizumab in metastatic non-small cell lung cancer (NSCLC), emphasising immune function stability via the neutrophil-to-lymphocyte ratio (NLR), and to assess the clinical significance of circadian synchronisation in immunotherapy. This study utilised a retrospective case study methodology, examining the treatment records and serial hematologic data of a patient with metastatic NSCLC. A patient diagnosed with metastatic NSCLC in 2019 showed a lasting full response to pembrolizumab for more than 6 years. A retrospective analysis revealed consistent morning ICI administration (08:00–11:00 h) and a stable NLR (2.16–3.66) during the treatment period. In addition, serial haematologic analysis showed a stable NLR, ranging between 2.16 and 3.66 throughout the treatment course. This immunological stability may reflect enhanced immune function aligned with early-day innate immune activity, particularly neutrophil and antigen-presenting cell priming, which is known to follow circadian patterns. Although specific molecular circadian markers were not examined, this case highlights a significant issue in current medical practice: there is almost no consistency in the timing of immunotherapy treatments. Taken together with emerging retrospective data, these findings underscore the need for prospective studies evaluating the influence of treatment timing on immunotherapy efficacy and durable immune surveillance in solid tumours.

Mailing Address: Dr. Ivan Bivolarski, Oncology Centre in Burgas, 8001, 1041 Democracy Blvd., Burgas, Bulgaria; e-mail: bivolarskiivan274@gmail.com

https://doi.org/10.14712/23362936.2026.18

Prague Medical Report / Vol. 127 (2026) No. 2, p. 119–131

Introduction

Chronotherapy, the practice of aligning medical treatments with the body's circadian rhythms, has been extensively explored. This therapeutic approach represents a fundamental shift from the traditional "one-size-fits-all" dosing paradigm toward a more personalized, biologically-informed treatment strategy. That is, by timing drug administration to align with periods of optimal tolerance and efficacy, clinicians aim to enhance therapeutic benefit while simultaneously reducing toxicity – a dual objective that has become increasingly important as cancer treatments have grown more sophisticated and targeted (Fey et al., 2025). This chronotherapeutic method is especially pertinent for immunotherapy, as synchronising treatment with circadian rhythms may increase the immune system's capacity to prevent cancer. Immune checkpoint inhibitors (ICIs), including pembrolizumab, nivolumab, and atezolizumab, have profoundly altered cancer treatment paradigms, especially for conditions such as metastatic non-small cell lung cancer (NSCLC).

NSCLC is a predominant cause of cancer mortality worldwide, representing over 85% of all lung cancer cases (Xiao et al., 2023). The prevalence of NSCLC is elevated, with tobacco consumption being the primary risk factor. Recently, the growing acknowledgement of environmental contaminants, genetics, and occupational hazards as contributing factors has offered a more comprehensive epidemiological approach. The prognosis for NSCLC is frequently unfavourable due to a late-stage diagnosis, as the majority of patients come with metastatic illnesses. Although surgery, radiation, and chemotherapy have constituted conventional treatments, immunotherapy has transformed the therapeutic paradigm (Kaloshi et al., 2014; Latka et al., 2024). ICIs, including pembrolizumab, nivolumab, and atezolizumab, have demonstrated substantial survival advantages by strengthening the body's immunological response to tumour cells (Santry et al., 2024). Immunotherapy in NSCLC targets the programmed death-1 (PD-1) receptor or its ligand PD-L1, thereby blocking the tumour's capacity to avoid immune surveillance. The expanding significance of immunotherapy is particularly encouraging for patients with advanced or metastatic NSCLC, where survival prospects have historically been dismal with traditional therapies.

Circadian rhythms, governed by the body's internal clock, regulate numerous physiological functions, including sleep-wake cycles, hormone secretion, metabolism, and immune system function (Ding et al., 2024). These rhythms influence the immune system, causing immune cells like T-cells, macrophages, and dendritic cells to fluctuate in activity levels throughout the day. This temporal regulation boosts immune responses to infections and maintains homeostasis. Specifically, innate immune processes, including neutrophil migration and antigen-presenting cell priming, exhibit heightened efficacy during the early hours, which may elucidate the variations in immune responses correlated with the time of day (Chulenbayeva et al., 2018; Nurgaziyev et al., 2024). The circadian clock orchestrates the timing of cytokine production and immune cell trafficking, by optimising immune system activity during critical periods and facilitating rest during low-risk intervals. Comprehending these circadian fluctuations is essential for enhancing treatment options in immunotherapy, as misalignment between treatment and the body's circadian rhythms may result in inadequate responses or heightened side effects.

Clock genes, including BMAL1, PER2, and CRY1/2, serve as the molecular regulators of circadian rhythms. These genes form a transcriptional feedback loop that regulates the timing of biological activities. BMAL1 and CLOCK constitute a heterodimer that initiates the transcription of other clock genes, including PER and CRY proteins, which subsequently suppress the activity of BMAL1 and CLOCK to sustain rhythmic oscillations. These genes are expressed in diverse immune cells, and their expression affects immunological function. BMAL1 governs the metabolic reprogramming associated with T-cell activation, whereas PER2 influences the synthesis of inflammatory cytokines (Carbone et al., 2024). The CRY proteins facilitate the regulation of immune cell movement and the formation of immunological memory. Disruption of circadian rhythms, frequently caused by genetic abnormalities or environmental influences like shift work or jet lag, can compromise immune responses and facilitate the onset of illnesses, including cancer (Matiichuk et al., 2025). Moreover, understanding how these clock genes control the immune system may yield insights that optimise the timing of immunotherapy, potentially enhancing the effectiveness of treatments like ICIs.

The historical development of chronotherapy can be traced back to early observations in the 1970s and 1980s, when researchers first noted that the timing of chemotherapy administration could significantly impact both treatment outcomes and side effect profiles. Initial studies focused primarily on traditional cytotoxic agents, where the concept of therapeutic windows – periods during which normal tissues are least susceptible to damage while tumour cells remain vulnerable – became a cornerstone of treatment optimisation. Over time, this approach has evolved to encompass not only conventional chemotherapy but also oral agents and targeted therapies, reflecting

the growing understanding of how circadian biology influences drug metabolism, cellular repair mechanisms, and treatment response (Scheiermann et al., 2013; Mamontov et al., 2023).

The molecular machinery underlying these circadian immune rhythms involves several key clock genes, including Brain and Muscle ARNT-Like 1 (BMAL1), Period Circadian Regulator 2 (PER2), and the cryptochrome genes CRY1 and CRY2. These transcriptional regulators are known to modulate not only the amplitude and timing of immune responses but also the sensitivity of immune cells to various stimuli and their capacity for sustained activation. Research has demonstrated that BMAL1, for instance, plays a crucial role in regulating the metabolic reprogramming that occurs during T-cell activation, while PER2 influences the production of key inflammatory mediators. The CRY proteins, meanwhile, help coordinate the timing of immune cell migration and the establishment of immunological memory (Haspel et al., 2020; Karaboué et al., 2024). These biological mechanisms provide a strong theoretical foundation for the hypothesis that the timing of ICI administration could influence clinical outcomes.

The body's circadian cycles influence numerous fundamental biological systems, potentially enhancing the efficacy of morning drug delivery, particularly in immunotherapy (Al-Maqtari et al., 2024). Circadian rhythms regulate various immunological processes, determining the peak activity times of immune cells, including neutrophils, T-cells, and dendritic cells. Research shows that early in the day optimally prepares the immune system for increased activity, particularly for innate immune responses (Nagy et al., 2025). This includes the increased migration of neutrophils and the enhanced activity of antigen-presenting cells, which are crucial for beginning and maintaining immune responses against tumours. Administering drugs to align with peak immune responses may enhance the efficacy of ICIs such as pembrolizumab.

The circadian regulation of cytokine synthesis, with immune cell activity, plays a significant role. Cytokines, encompassing pro-inflammatory signals that activate immune cells, exhibit a predictable cycle, with production peaking at specific times of the day. Cytokine levels are elevated in the morning, rendering it an ideal period for treatment when immune cells exhibit heightened responsiveness. Administering ICIs at elevated cytokine levels may augment therapy efficacy, as it corresponds with the body's inherent immunological activation (Li et al., 2025).

Moreover, the study of how the time of day affects the body's metabolic processes, known as chronopharmacology, plays a significant role (Nagy et al., 2025). Drug metabolism, absorption, and elimination adhere to circadian rhythms. Enzymes responsible for medication metabolism typically exhibit heightened activity in the morning, perhaps leading to more effective digestion of immune checkpoint inhibitors and prolonged drug concentrations. This timing may also alleviate side effects by synchronising treatment with intervals when the immune system is better equipped to manage therapeutic medicines with fewer unexpected reactions.

However, to date, no prospective clinical trials have systematically evaluated the impact of circadian timing on ICI efficacy. Although retrospective studies and meta-analyses have begun to shed light on this relationship – particularly suggesting improved survival with morning administration in cancers such as melanoma and NSCLC – the data remain fragmented (Amiama-Roig et al., 2022; Wang et al., 2022a; Patel et al., 2024). Moreover, case-level evidence illustrating chronotherapeutic consistency is virtually absent from the literature.

The primary aim of this study was to document and analyse a real-world case of prolonged immunotherapy response in metastatic NSCLC with retrospective evaluation of treatment timing patterns and associated immunological parameters. This study's main hypothesis posits that timing the administration of ICIs with the body's circadian cycles may improve treatment efficacy and immunological responses in patients with metastatic NSCLC. The main objectives of the study were:

1) To assess the possible chronotherapeutic impact of morning ICI administration (between 08:00 and 11:00) on the long-term response to pembrolizumab in a real-world patient with metastatic NSCLC.
2) To examine the stability of immunological markers, specifically the neutrophil-to-lymphocyte ratio (NLR), as an indicator of systemic immune reactivity about treatment scheduling.
3) To delineate deficiencies in the existing chrono-immunotherapy literature and suggest avenues for additional investigation, particularly concerning circadian rhythms and the efficacy of immune checkpoint inhibitors in solid tumours.
4) To investigate the necessity for prospective trials assessing the impact of treatment scheduling on immunotherapy results and sustained immune surveillance.

Case report

A 58-year-old female initially presented in January 2019 with progressive dyspnoea, persistent dry cough,

Prague Medical Report / Vol. 127 (2026) No. 2, p. 119–131

Table 1: Longitudinal laboratory monitoring of lymphocyte and granulocyte percentages in a patient with stage IV lung adenocarcinoma (2019–2025).

Time	18.04.2019 10:59	10.05.2019 09:26	14.05.2019 09:42	31.05.2019 09:37	13.06.2019 08:49	21.06.2019 08:41	11.07.2019 08:55	02.08.2019 09:37	23.08.2019 08:53	05.09.2019 08:40
Lym	26.8	30.1		23.2		29.4		61.9	60.7	
Gran	62.6	58.0		67.1		61.7		29.6	29.3	
Time	06.12.2019 09:10	06.12.2019 09:11	27.12.2019 10:44	08.01.2020 09:10	17.01.2020 11:08	05.02.2020 09:02	07.02.2020 11:45	07.02.2020 12:29	28.02.2020 09:17	05.03.2020 09:09
Lym	72.1	72.1	73.8	67.7	77.0	71.8	57.9		73.1	66.0
Gran	14.0	13.8	15.6	18.6	15.6	15.3	27.5		17.9	22.2
Time	31.07.2020 09:11	05.08.2020 11:51	21.08.2020 09:20	03.09.2020 09:23	11.09.2020 09:26	02.10.2020 11:59	26.10.2020 12:14	02.11.2020 09:11	16.11.2020 12:05	30.11.2020 11:38
Lym		72.0		62.0	65.8			67.6		65.8
Gran		19.4		25.6	21.2			20.6		23.4
Time	05.05.2021 11:45	05.05.2021 12:04	26.05.2021 10:48	04.06.2021 09:12	15.06.2021 10:44	05.07.2021 09:03	06.07.2021 10:36	27.07.2021 09:28	02.08.2021 09:31	17.08.2021 09:07
Lym				67.5				66.8		51.7
Gran				22.1				22.3		31.9
Time	30.11.2021 09:33	22.12.2021 12:38	06.01.2022 09:27	12.01.2022 12:26	03.02.2022 09:52	24.02.2022 09:04	17.03.2022 09:16	17.03.2022 09:17	07.04.2022 09:38	14.04.2022 09:58
Lym					64.6	58.4	58.1	59.5		58.2
Gran					22.1	27.8	27.4	27.3		28.7
Time	12.08.2022 09:40	02.09.2022 14:50	08.09.2022 10:56	23.09.2022 09:43	14.10.2022 09:43	14.10.2022 09:50	17.10.2022 15:50	08.11.2022 12:38	14.11.2022 09:34	29.11.2022 09:15
Lym	68.2	57.5	56.3	69.4	60.0	58.4			59.3	58.4
Gran										
Time	18.05.2023 09:35	01.06.2023 09:55	07.06.2023 09:43	29.06.2023 09:22	11.07.2023 11:26	20.07.2023 11:00	09.08.2023 11:15	09.08.2023 12:18	09.08.2023 12:19	09.08.2023 12:39
Lym	65.2	60.8	58.5	60.4	66.0	67.9	66.2	66.8		
Gran										
Time	14.03.2024 10:57	04.04.2024 09:47	25.04.2024 11:09	16.05.2024 10:00	06.06.2024 11:29	27.06.2024 11:10	25.07.2024 09:04	19.08.2024 08:56	22.08.2024 10:38	09.09.2024 09:47
Lym	64.6	59.4	57.7	60.8	67.4	61.9	61.4	60.2	63.0	68.5
Gran										

Source: compiled by the author

and general fatigue. A chest X-ray on January 31, 2019, revealed a suspicious mass in the right lung, prompting further evaluation. On February 27, 2019, a right pneumonectomy with mediastinal lymph node dissection was performed. Histopathological analysis confirmed a grade 3 poorly differentiated adenocarcinoma with acinar, papillary, and lepidic components infiltrating the parietal pleura and pericardium. The final staging was pT4 pN1 cM1a (stage IV).

Immunohistochemistry on March 29, 2019, demonstrated high PD-L1 expression (>50%) and negative status for EGFR and ALK mutations. A positron emission tomography/computed tomography (PET/CT) scan on April 15, 2019, revealed multiple bone metastases and abdominal lymph node involvement, confirming advanced disease. At treatment initiation, her ECOG performance status was 1. By the end of the first six months, clinical improvement was observed, and the patient was reassessed as ECOG 0, a status maintained throughout follow-up.

Following multidisciplinary tumour board discussion, first-line pembrolizumab monotherapy was initiated on April 22, 2019. A retrospective review of the hospital's pharmaceutical software revealed that all recorded infusions were consistently prepared and administered in the morning hours (08:00–11:00), with minimal deviations. This pattern was not based on a predefined chronotherapeutic strategy but rather emerged from routine hospital logistics. Even after checking for diseases and giving radiotherapy, the timing stayed the same, indicating an unintentional but ongoing match with the body's natural immune rhythms.

16.09.2019 08:23	17.09.2019 09:01	04.10.2019 09:13	04.10.2019 09:41	10.10.2019 12:32	15.10.2019 10:39	25.10.2019 09:00	07.11.2019 08:55	15.11.2019 08:50	26.11.2019 10:57
63.8			78.5			62.3		66.3	
26.7			15.5			27.0		22.8	
23.03.2020 08:52	02.04.2020 08:50	10.04.2020 10:40	07.05.2020 11:05	29.05.2020 10:49	04.06.2020 11:21	19.06.2020 09:42	19.06.2020 09:43	07.07.2020 11:08	10.07.2020 09:32
67.2		70.5	73.7			73.3		71.5	65.4
20.7		17.9	18.2			16.6		19.1	21.6
07.12.2020 13:11	29.12.2020 12:53	06.01.2021 09:50	15.01.2021 09:30	03.02.2021 09:50	26.02.2021 11:13	04.03.2021 10:58	19.03.2021 09:24	02.04.2021 09:33	14.04.2021 10:44
		63.6	59.6	56.0	69.4	63.0	63.4		
		23.3	25.7	28.2	19.2	21.5	26.1		
01.09.2021 09:37	03.09.2021 09:57	03.09.2021 09:58	03.09.2021 11:24	27.09.2021 09:41	27.09.2021 09:42	04.10.2021 11:54	19.10.2021 12:17	04.11.2021 09:52	09.11.2021 11:07
62.8									
23.6									
28.04.2022 09:49	12.05.2022 09:38	17.05.2022 10:57	09.06.2022 09:18	09.06.2022 09:19	27.06.2022 10:37	01.07.2022 10:58	07.07.2022 09:34	25.07.2022 09:14	03.08.2022 09:41
		55.9	56.8	57.3	70.2	65.1	64.4	60.2	64.3
		28.7	27.8	27.1					
20.12.2022 09:06	10.01.2023 09:22	31.01.2023 09:38	21.02.2023 09:33	28.02.2023 09:57	14.03.2023 09:24	28.03.2023 11:27	06.04.2023 08:33	27.04.2023 09:15	27.04.2023 09:16
55.0	63.1	66.7	50.7	56.9	58.8	67.0	60.0	60.4	61.0
31.08.2023 10:49	15.09.2023 09:40	21.09.2023 10:56	13.10.2023 09:24	06.11.2023 09:59	24.11.2023 10:55	15.12.2023 09:45	11.01.2024 09:53	01.02.2024 10:56	22.02.2024 08:25
66.4	62.1	66.6	58.3	52.0	66.5	62.7	63.1	66.0	62.9
27.09.2024 10:44	18.10.2024 09:35	08.11.2024 09:06	29.11.2024 09:43	20.12.2024 09:49	10.01.2025 11:37	31.01.2025 11:36	06.02.2025 10:53	21.02.2025 11:20	14.03.2025 10:59
66.5	66.5	64.7	60.5	67.5	70.7	65.9	63.8	72.3	69.1

In the context of treating patients with locally advanced or metastatic NSCLC, monitoring immunological status is of great importance as a prognostic and predictive factor (Labrecque and Cermakian, 2015). The NLR in peripheral blood has established itself as a reliable biomarker of systemic inflammatory response and overall immune reactivity, correlating with patient survival, especially in the context of immunotherapy.

Considering the chronobiological patterns of the immune system's functioning, the timing of immunotherapy administration may potentially affect the clinical efficacy of treatment through synchronisation with the circadian rhythms of immune cells. In the presented clinical case, the patient received the immune checkpoint inhibitor pembrolizumab at stable morning hours, providing a unique opportunity to study long-term changes in the cellular components of the blood (Catozzi et al., 2024).

To illustrate and analyse these dynamics, a retrospective compilation of data on the percentage of lymphocytes and granulocytes in the patient's blood serum was conducted over more than 6 years of treatment. Table 1 illustrates the time sequence of laboratory indicators, allowing visualisation of the stability of the immune profile and assessment of the potential impact of the regularity and timing of drug administration on the patient's immunological status. This approach opens perspectives for further research on chronotherapy in the field of immunotherapy for oncological diseases.

This heatmap illustrates the temporal dynamics of lymphocyte and granulocyte percentages in

Prague Medical Report / Vol. 127 (2026) No. 2, p. 119–131

peripheral blood samples collected from a 58-year-old female patient with stage IV poorly differentiated lung adenocarcinoma (pT4 pN1 cM1a), who received continuous first-line immunotherapy with pembrolizumab. Data span from April 2019 to May 2025, covering 60 laboratory assessments. Notably, immune cell proportions remained within a stable physiological range throughout the observation period. The predominance of morning blood draws, coinciding with consistently timed ICI administration (08:00–11:00), allowed for an exploratory assessment of chronotherapeutic alignment.

High lymphocyte percentages (≥70%) and corresponding reductions in granulocytes were transiently observed during late 2019 and early 2020, suggesting possible reactive haematological shifts. However, the NLR calculations derived from these data remained consistently below the prognostically adverse threshold (>5), reinforcing the hypothesis of a sustained favourable immunological milieu under stable circadian treatment conditions. To explore the potential implications of this pattern, a retrospective analysis was conducted on serial haematological parameters, with a focus on the NLR, a surrogate marker of immune status (Mazzoccoli et al., 2020; Nelson et al., 2022; Mok et al., 2024). Sixty paired measurements were extracted from blood tests. The NLR values ranged from 2.16 to 3.66, remaining consistently below the prognostically unfavourable threshold (>5) and showing minimal variability. This immunological stability may reflect a favourable biological environment, possibly influenced by consistent ICI doses in the morning.

In October 2019, the patient received palliative radiotherapy to the Th1 vertebra. Pembrolizumab continued without interruption. Localised progression in July 2022 prompted radiotherapy at a mediastinal lymph node (25 Gy), while immunotherapy remained unchanged. In August 2024, a PET/CT scan showed that the lymph nodes in the chest area were still affected, which resulted in another round of radiotherapy (24 Gy) for that area. In the context of studying the immune status of patients or evaluating the function of the immune system, analysing long-term changes in the ratio of lymphocyte and granulocyte cell populations is of great importance. The percentage content of these cells in peripheral blood is an important biomarker of systemic immune reactivity, reflecting the balance between adaptive and innate immunity (Hergenhan et al., 2020; Ding et al., 2024; Quist et al., 2024).

In particular, the dynamics of these indicators allow assessment of the overall state of the immune response at different times, which can be useful in monitoring chronic processes or long-term treatment. Considering the chronobiological rhythms of the immune system's functioning, analysing changes in the cellular composition of blood over a long period opens up opportunities for studying the influence of circadian rhythms on immune indicators and their stability (Keller et al., 2009; Balachandran et al., 2023). Figure 1 illustrates a retrospective analysis of the percentage ratio of lymphocytes and granulocytes in the blood over a period of more than 6 years, enabling visualisation of trends and fluctuations in these cell populations.

Figure 1 shows the change in the percentage of lymphocytes (indicated in blue) and granulocytes (indicated in orange) in the blood over the period from 2019 to 2024. The X-axis represents the time scale in years, divided by observation dates, and the Y-axis represents the percentage ratio of the respective cells. Lymphocytes demonstrate an overall trend of fluctuations within approximately 50 to 80%,

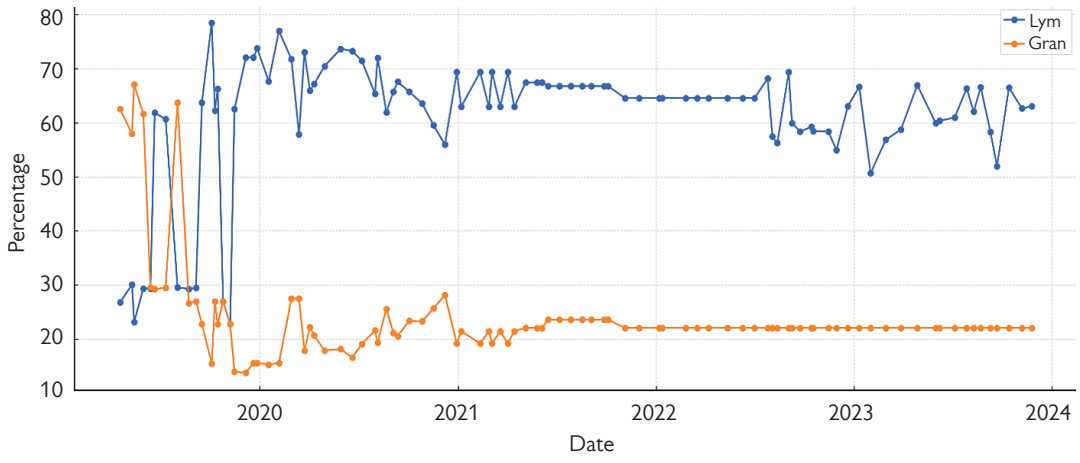

Figure 1: Dynamic changes in the percentage ratio of lymphocytes and granulocytes in the blood from 2019 to 2024. Source: compiled by the author.

stabilising at around 65–70% in the second half of the period. Granulocytes, on the other hand, initially have a high percentage (over 60%) but quickly decrease to around 20–30% and remain stable in subsequent years. The dynamics show an inverse relationship between the percentage of lymphocytes and granulocytes, which is typical for maintaining a balance in the number of different types of leukocytes in the blood.

An increase in lymphocytes is accompanied by a decrease in granulocytes, which may indicate adaptive changes in the immune system in response to various factors such as infections, inflammatory processes, or other changes in the body (Łątka et al., 2024). The stabilised indicators in the period after 2021 indicate a certain equilibrium in the cellular composition of the blood, which may be characteristic of the normal functioning of the immune system in the absence of acute pathological processes. Thus, this Figure 1 clearly demonstrates important features of the cellular immune response, which can be used for further analysis in medical and biological research.

Throughout her 6-year treatment journey, the patient received pembrolizumab infusions exclusively in the morning. Despite several tumour board evaluations, treatment adjustments, and progression events, the timing remained unchanged. Treatment compliance was excellent, with only two missed cycles (Cycle 74 and Cycle 84), both due to logistical reasons. As of March 2025, a total of 101 cycles of pembrolizumab have been completed, resulting in long-term disease control limited to localized progression, with no significant immune-related adverse events. In the context of studying the immune status of patients or evaluating the functioning of the immune system, analysing long-term changes in the ratio of lymphocytes and granulocytes is of great importance. The percentage content of these cells in peripheral blood is a significant biomarker of systemic immune reactivity, reflecting the balance between adaptive and innate immunity (Labrecque and Cermakian, 2015; Du and Holme, 2020).

In particular, the dynamics of these indicators allow assessment of the overall state of the immune response at different time intervals, which is useful for monitoring chronic processes or long-term treatment. Considering the chronobiological rhythms of the immune system's functioning, analysing changes in the cellular composition of blood over a long period opens up opportunities to study the influence of circadian rhythms on immune indicators and their stability. Figure 2 presents a retrospective analysis of the percentage ratio of lymphocytes (blue line) and granulocytes (orange line) in peripheral blood over a period exceeding 6 years, enabling visualisation of trends and fluctuations in these cell populations.

Figure 2 illustrates the changes in the percentage of lymphocytes (shown in blue) and granulocytes (shown in orange) in peripheral blood over a time span exceeding 6 years, from April 2019 to March 2025. The X-axis represents the chronological timeline divided by specific observation dates, while the Y-axis shows the percentage ratio of these two immune cell populations. Lymphocytes exhibit fluctuations generally ranging between approximately 50 and 80%, with a tendency to stabilise around 65–70% in the later stages of the observation period. In contrast, granulocytes start with a higher percentage, exceeding 60%, but rapidly decline to a range of about 15–30%, maintaining this relatively stable level throughout the subsequent years.

The graph clearly demonstrates an inverse relationship between the relative proportions

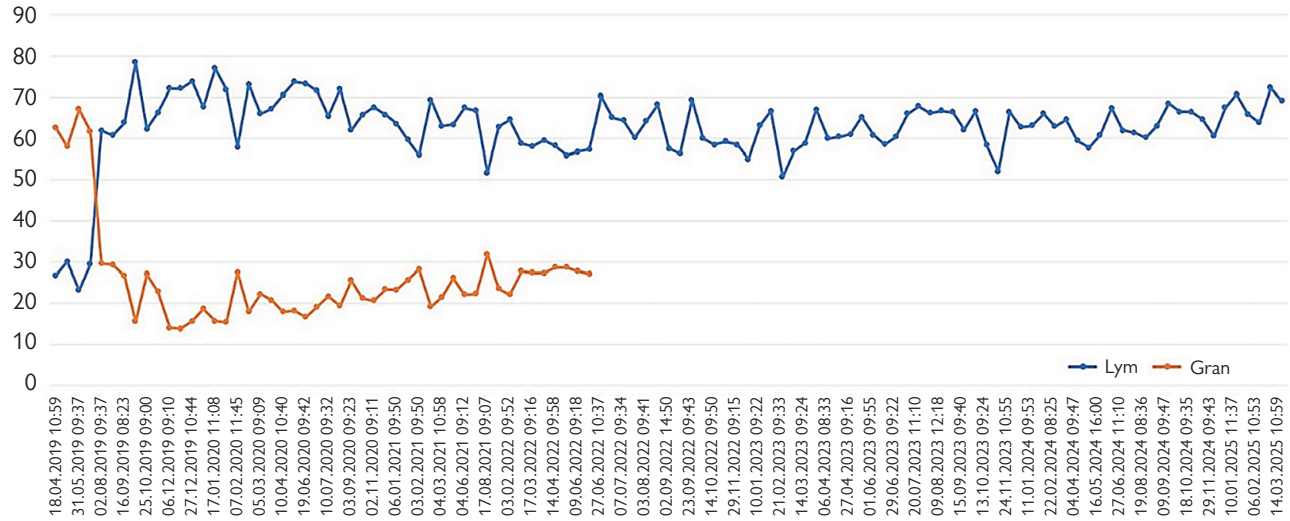

Figure 2: Dynamics of the percentage ratio of lymphocytes and granulocytes in peripheral blood over a long period.
Source: compiled by the author.

Prague Medical Report / Vol. 127 (2026) No. 2, p. 119–131

of lymphocytes and granulocytes, reflecting the homeostatic balance typical of the immune system's cellular composition. This inverse dynamic suggests adaptive modulation of the immune response, possibly influenced by various internal and external factors such as infections, inflammation, or other physiological changes. The stabilisation of both lymphocyte and granulocyte percentages after approximately 2021 indicates a maintained equilibrium in the cellular makeup of the blood, which may correspond to normal immune function in the absence of acute pathological conditions. This Figure provides valuable insight into the long-term dynamics of key leukocyte populations, offering a basis for further immunological and clinical investigations.

A 58-year-old female with metastatic NSCLC undergoing pembrolizumab monotherapy offers important information regarding the possible influence of circadian rhythms on enhancing immunotherapy efficacy. Although there was no deliberate chronotherapeutic approach, the patient's treatment was regularly provided in the morning, aligning with natural peaks in immune system activity. Over 6 years of treatment, her haematological data exhibited stable immunological profiles, notably a consistently advantageous NLR, indicating a balanced immune response. Variations in lymphocyte and granulocyte populations suggested adaptive immune mechanisms, potentially enhanced by morning delivery. This case emphasises the advantages of synchronising immunotherapy with circadian rhythms, as the patient exhibited a beneficial immunological milieu with minimal side effects, underscoring the necessity for additional research into the impact of treatment timing on immune responses and long-term efficacy in cancer therapy.

Gaps in the current literature on chrono-immunotherapy

Even though more and more past studies and combined analyses indicate that when ICIs are given, they might affect results, there are still important gaps in the research that make it hard to apply these findings in practice. First, most published studies lack precise control over administration timing consistency. While several analyses stratify patients into "morning" and "afternoon" groups using fixed cut-offs (typically 11:30, 13:00, or 16:30), few account for whether this timing was maintained across treatment cycles. The possibility that inconsistent scheduling dilutes potential circadian effects has not been adequately addressed.

Second, the majority of available data comes from retrospective cohorts. Although the sample size is large, these studies are often affected by confounding variables – including performance status (PS), corticosteroid use, treatment line, or centre-specific logistics – that may influence both the timing of administration and outcomes. For instance, the scheduling of afternoon appointments may favour sicker patients or those receiving more aggressive supportive care, leading to bias. Although several studies attempt propensity score matching, the absence of random prospective trials continues to hamper definitive conclusions. Third, studies vary considerably in their biological depth. The mechanistic rationale behind chrono-immunotherapy often remains speculative. Only a few publications discuss the role of core circadian genes (such as BMAL1, PER2, or CRY1/2), immune cell trafficking rhythms, or cytokine dynamics. Also, there haven't been any human studies so far that have used direct signs of circadian function, like measuring gene activity or the cycles of melatonin and cortisol, to verify overall rhythmic alignment.

Fourth, in many studies, immune-related biomarkers such as NLR, PD-L1 expression levels, and tumour mutational burden are not uniformly reported or integrated into timing analyses. These parameters may interact with circadian factors and could help stratify patients more meaningfully. Fifth, only a minority of publications provide long-term follow-up data, particularly beyond 24 months. This makes it hard to understand how regular chrono-aligned dosing might affect ongoing immune monitoring, which is important for lasting responses, especially in NSCLC and melanoma.

Lastly, there is an evident gap in the literature regarding single-patient, high-resolution clinical observations – cases where timing was deliberately maintained, responses were thoroughly documented, and clinical confounders were minimised. Such cases may serve as valuable "probes" about the biological plausibility of chrono-immunotherapy, especially when randomised trials are still lacking. Given these limitations, there is a clear need for studies that carefully control the timing of treatments, utilize biomarkers for patient stratification, and monitor both clinical and biological factors longitudinally. Until such research is available, well-documented real-world cases may provide crucial insights into the clinical significance of circadian alignment in cancer immunotherapy.

Clinical observation and timing consistency

This case presents a compelling example of prolonged immunotherapy efficacy potentially influenced by treatment timing. A patient with metastatic NSCLC

has maintained a complete metabolic response for over 6 years under pembrolizumab monotherapy. A retrospective review revealed a consistently morning-aligned administration schedule (08:00–11:00), which, although unintentional, overlapped with known circadian peaks in innate immune activity, particularly neutrophil and antigen-presenting cell function, suggesting a possible chrono-immunological synergy.

To further investigate this observation, haematological parameters were analysed with particular attention to the NLR as a surrogate marker of systemic immune balance. Sixty NLR measurements demonstrated minimal fluctuation, with all values remaining below the prognostically unfavourable threshold (>5). This immunologic stability, coupled with sustained disease control and minimal toxicity, raises the hypothesis that consistent early-day dosing may enhance ICI efficacy by aligning with circadian immune rhythms.

While causality cannot be inferred from a single case, these findings resonate with the broader theoretical framework of chronobiology. Immune system processes – including T-cell activation, cytokine production, and antigen presentation – are governed by core circadian clock genes such as BMAL1, PER2, and CRY1/2 and follow well-established diurnal oscillations (Walker et al., 2021). Despite this, the integration of chronotherapy into immuno-oncology remains largely uncharted.

Retrospective studies and meta-analyses have recently begun to explore this link, suggesting improved survival with morning ICI administration in melanoma and NSCLC. However, substantial limitations persist in the current literature that restrict its translational potential.

Limitations in current evidence

First, most studies lack control over timing consistency – patients are typically categorised into "morning" and "afternoon" groups based on initial treatment times without documentation of whether this timing was maintained across treatment cycles. This procedure introduces variability that may obscure potential chrono-therapeutic effects. Second, the available evidence is predominantly retrospective in nature and susceptible to confounding factors such as performance status, corticosteroid use, treatment setting, and institutional scheduling preferences. These uncontrolled variables can significantly influence both treatment timing and outcomes.

Third, the mechanistic rationale behind chrono-immunotherapy remains largely speculative. Few studies incorporate direct molecular circadian markers, such as clock gene expression, melatonin or cortisol rhythms – to verify systemic rhythmic alignment. Moreover, immune-related biomarkers, like NLR, PD-L1 expression, or tumour mutational burden, are not uniformly analysed in relation to timing, further limiting their interpretability.

Fourth, long-term follow-up data are scarce. Most available studies report outcomes within 12–24 months, making it unclear how consistent, circadian-aligned dosing might contribute to sustained immune surveillance – an essential component of durable immunotherapy responses. Finally, the literature lacks high-resolution, single-patient reports with detailed timing data, immune marker tracking, and prolonged follow-up. These individual cases, although based on personal experiences, can offer helpful details about the potential of chrono-immunotherapy, especially since there are no planned studies.

Impact of chronotherapeutic strategies on immune checkpoint inhibitor efficacy and immune system responsiveness

The analysis of clinical data regarding the timing of ICI administration highlights not only the importance of considering dosing time but also expands the understanding of potential mechanisms underlying enhanced therapeutic efficacy with chronologically optimised administration.

Yeung et al. (2023) demonstrated that morning administration of ICIs in melanoma patients is associated with improved survival outcomes. This finding aligns with clinical observations where pembrolizumab infusions were consistently performed during morning hours, approximately between 08:00 and 11:00. Such temporal consistency in dosing may enhance immune responsiveness and contribute to sustained disease control without the emergence of significant immune-related adverse events. These results emphasise the role of circadian rhythms in the pharmacokinetics and pharmacodynamics of ICIs, potentially resulting in improved efficacy and safety profiles.

Lévi et al. (2023) highlighted significant differences between morning and evening dosing of ICIs, linking these differences to diurnal variations in immune system activity. Although deliberate chronotherapeutic strategies were not employed in the referenced clinical cases, the observed stability in administration time could have supported the maintenance of a favourable immunological milieu, thus potentiating the therapeutic effect. This observation corresponds with the concept that circadian rhythms regulate the expression of key immune molecules, including cytokines, chemokines,

Prague Medical Report / Vol. 127 (2026) No. 2, p. 119–131

and receptors, suggesting that synchronising treatment timing with endogenous biological cycles may optimise treatment response (Kucherenko et al., 2019; Dyba and Berezenko, 2023).

Furthermore, Qian et al. (2021) provided additional evidence supporting the association between morning ICI administration and improved overall survival in melanoma patients. Their findings suggest the existence of critical temporal windows in which immune system activity peaks, and ICI administration during these intervals may more effectively stimulate antitumor immune responses. This accumulation of evidence strengthens the rationale for integrating temporal considerations into immunotherapy planning.

The foundational work by Cheng et al. (2022) offers valuable insights into the physiology of circadian synchronisation and its impact on pharmacological strategies, including immunotherapy. Morning administration of pembrolizumab may facilitate optimal interaction between the circadian system and immune mechanisms, enhancing T-cell activity, upregulating key cytokines, and reducing immunosuppressive factors. Such biological effects are crucial for achieving durable clinical responses in melanoma treatment (Montayeva et al., 2015, 2016). Additionally, contemporary chronopharmacological studies indicate that time of day influences not only drug pharmacokinetics but also the functional state of immune cells, such as lymphocytes and dendritic cells, which are essential for initiating and sustaining antitumor immunity (Dyba et al., 2024; Tutchenko et al., 2024). Circadian rhythms regulate immune cell trafficking, activation, and effector molecule production, directly impacting ICI efficacy. Maintaining consistent dosing times may thus help align therapy with peak immune responsiveness.

Johnson et al. (2022) discussed the long-term consequences of immunotherapy-related toxicity. The absence of significant immune-mediated adverse effects in this clinical context may be associated with the optimisation of drug administration timing. This observation aligns with Johnson and colleagues' emphasis on minimising toxicity during prolonged immunotherapy to improve patient outcomes. Boesch et al. (2023) investigated non-pharmacological interventions aimed at optimising cancer immunotherapy. Consistent morning administration of pembrolizumab can be considered such an intervention, potentially contributing to improved clinical outcomes. This finding corresponds with the conclusions of authors, who highlighted the importance of considering dosing time to enhance therapeutic efficacy.

Ortega-Campos et al. (2023) examined the interaction between circadian rhythm genes and cancer characteristics. Stable morning administration of pembrolizumab may support maintenance of a favourable immunological status, consistent with Ortega-Campos et al.'s (2023) conclusions regarding the critical role of circadian rhythm considerations in immunotherapy planning. Wang et al. (2022b) explored the role of dendritic cells in circadian antitumor immune responses. The observed stable morning dosing regimen likely contributes to sustaining an advantageous immune environment, in line with findings by Wang et al. (2022b) emphasising circadian rhythm alignment in immunotherapeutic strategies.

Thomas et al. (2023) discussed the gut microbiome as a potential biomarker for cancer immunotherapy. Maintaining a consistent morning dosing schedule of pembrolizumab may promote a beneficial immunological profile, which is in agreement with Thomas et al. (2023) recognition of circadian rhythm importance in immunotherapy design. Guillot et al. (2023) investigated manipulations of the gut and tumour microbiome to improve immunotherapy outcomes. Stable morning administration of pembrolizumab appears to support a favourable immune status, aligning with Guillot et al. (2023) findings on the significance of circadian rhythm considerations in the context of immunotherapy.

Chronotherapy may also reduce the incidence of immune-related adverse effects by modulating immune tolerance and inflammatory processes, both of which are under circadian control. Morning administration likely provides a more favourable balance between immune activation and immunopathology, consistent with observed reductions in severe adverse events (Missori et al., 2016). Collectively, these findings underscore the significance of incorporating dosing time into immunotherapy protocols and suggest promising avenues for further investigation in chrono-immunotherapy.

This study's findings indicate that synchronising ICI doses with the body's circadian cycles may enhance therapeutic success, especially in metastatic NSCLC. This might be clinically implemented by delivering ICIs throughout the early hours (between 08:00 and 11:00), a timeframe linked to enhanced innate immune activity, including elevated neutrophil and antigen-presenting cell functionality. Clinicians may consider using standardised morning dose regimens for patients undergoing immunotherapy to optimise therapeutic results. Moreover, the regular morning delivery could be integrated into clinical procedures, encouraging additional research into the ideal time of ICIs for various cancer types. Moreover, regular assessment of immune-related biomarkers, including the NLR, may facilitate the evaluation of the chronotherapeutic strategy's efficacy and inform subsequent treatment choices.

The stable NLR identified in this study indicates a balanced and advantageous immunological milieu, essential for successful cancer treatment. A continuously low NLR, as evidenced here (range from 2.16 to 3.66), correlates with enhanced immune reactivity and presumably decreased systemic inflammation, both of which facilitate superior treatment outcomes. The stability in NLR may indicate the alignment between circadian immunological rhythms and ICI treatment, thereby enhancing a more vigorous and enduring immune response. Nonetheless, it is crucial to acknowledge that additional factors, like the patient's overall health, concomitant therapies (e.g., radiation), and unique immunological attributes, may also affect NLR and treatment effectiveness.

Other studies have also suggested that the time of immunotherapy delivery, especially morning administration, may enhance clinical outcomes. Retrospective studies in melanoma and NSCLC indicate enhanced survival with morning ICI treatment, aligning with the results of this trial. Nevertheless, although our study underscores the relationship between timeliness and persistent immunological profiles, additional factors such as tumour burden, the specific medication administered, and patient characteristics may also influence the outcomes. Comparisons with research examining various circadian-based chronotherapeutic techniques might enhance the validation of the broader application of these findings. Future prospective trials that account for these characteristics will be crucial to validate the clinical significance of morning administration in immunotherapy. In light of these insights, it is proposed that future research prioritise prospective studies with stringent control of treatment timing, biomarker-based patient stratification, and incorporation of circadian molecular assessments. Until such trials are conducted, well-documented real-world cases may continue to offer critical insight into the therapeutic potential of chrono-immunotherapy.

Conclusion

This case highlights a potential association between the timing of immunotherapy administration and prolonged treatment response, raising a hypothesis that merits further investigation. The observed effect was not the result of a predefined chronotherapeutic strategy but rather an incidental finding, retrospectively derived from pharmacy records within routine clinical care. This observation underscores the current absence of formal frameworks guiding time-of-day considerations in immunotherapy protocols.

In addition to the consistent morning-aligned administration of pembrolizumab over more than 6 years, serial haematological analysis demonstrated a remarkably stable NLR, ranging from 2.16 to 3.66, without significant fluctuations or inflammatory peaks. This prolonged immunological equilibrium may reflect a chronobiologically favourable alignment between treatment timing and innate immune system activation.

At present, no large-scale retrospective or prospective studies have systematically examined the influence of circadian timing on immune checkpoint inhibitor efficacy. Case reports documenting such observations remain rare, and the field of chrono-immunotherapy is still in its infancy. Nevertheless, the established role of circadian biology in regulating immune function suggests that this relationship deserves formal exploration. Until robust evidence becomes available, this report serves as a hypothesis-generating observation – an early signal that biological timing may play a clinically relevant role in optimising long-term outcomes in cancer immunotherapy.

References

Al-Maqtari, H. M., Hasan, A. H., Suleiman, M., Ahmad Zahidi, M. A., Noamaan, M. A., Alexyuk, P., Alexyuk, M., Bogoyavlenskiy, A., Jamalis, J. (2024) Benzyloxychalcone hybrids as prospective acetylcholinesterase inhibitors against Alzheimer's disease: Rational design, synthesis, in silico ADMET prediction, QSAR, molecular docking, DFT, and molecular dynamic simulation studies. *ACS Omega* **9(30)**, 32901–32919.

Amiama-Roig, A., Verdugo-Sivianes, E. M., Carnero, A., Blanco, J. (2022) Chronotherapy: Circadian rhythms and their influence in cancer therapy. *Cancers* **14(20)**, 5071.

Balachandran, D. D., Bashoura, L., Sheshadri, A., Manzullo, E., Faiz, S. A. (2023) The impact of immunotherapy on sleep and circadian rhythms in patients with cancer. *Front. Oncol.* **13**, 1295267.

Boesch, M., Baty, F., Rassouli, F., Kowatsch, T., Joerger, M., Früh, M., Brutsche, M. H. (2023) Non-pharmaceutical interventions to optimize cancer immunotherapy. *Oncoimmunology* **12(1)**, 2255459.

Carbone, A., Vitullo, P., Di Gioia, S., Castellani, S., Conese, M. (2024) A new frontier in cystic fibrosis pathophysiology: How and when clock genes can affect the inflammatory/immune response in a genetic disease model. *Curr. Issues Mol. Biol.* **46(9)**, 10396–10410.

Catozzi, S., Assaad, S., Delrieu, L., Favier, B., Dumas, E., Hamy, A., Latouche, A., Crochet, H., Blay, J., Mullaert, J., Ballesta, A., Heudel, P. (2024) Early morning immune checkpoint blockade and overall survival of patients with metastatic cancer: An in-depth chronotherapeutic study. *Eur. J. Cancer* **199**, 113571.

Cheng, Y., Yao, J., Fang, Q., Chen, B., Zang, G. (2022) A circadian rhythm-related biomarker for predicting prognosis and immunotherapy efficacy in lung adenocarcinoma. *Aging* **14(23)**, 9617–9631.

Chulenbayeva, L., Ilderbayev, O., Taldykbayev, Z., Ilderbayeva, G., Argynbekova, A. (2018) Phytocorrection of immunological and biochemical changes in the combined impact of coal dust and high dose of radiation. *Georgian Med. News* **Issue**, 141–150. (in Russian)

Ding, J., Chen, P., Qi, C. (2024) Circadian rhythm regulation in the immune system. *Immunology* **171(4)**, 525–533.

Du, Z., Holme, P. (2020) Coupling the circadian rhythms of population movement and the immune system in infectious disease modeling. *PLoS One* **15(6)**, e0234619.

Dyba, M. B., Berezenko, V. S. (2023) Possibilities of shear wave elastography in the diagnosis of liver fibrosis and monitoring of autoimmune liver diseases in children. *Modern Pediatr. Ukraine* **8**, 34–41.

Dyba, M., Berezenko, V., Zabara, D., Bezpala, A., Donskoi, B. (2024) Monocyte subpopulations in children with autoimmune liver disease. *Pathol. Res. Pract.* **263**, 155622.

Fey, R. M., Billo, A., Clister, T., Doan, K. L., Berry, E. G., Tibbitts, D. C., Kulkarni, R. P. (2025) Personalization of cancer treatment: Exploring the role of chronotherapy in immune checkpoint inhibitor efficacy. *Cancers* **17(5)**, 732.

Guillot, N., Roméo, B., Manesh, S. S., Milano, G., Brest, P., Zitvogel, L., Hofman, P., Mograbi, B. (2023) Manipulating the gut and tumor microbiota for immune checkpoint inhibitor therapy: From dream to reality. *Trends Mol. Med.* **29(11)**, 897–911.

Haspel, J. A., Anafi, R., Brown, M. K., Cermakian, N., Depner, C., Desplats, P., Gelman, A. E., Haack, M., Jelic, S., Kim, B. S., Laposky, A. D., Lee, Y. C., Mongodin, E., Prather, A. A., Prendergast, B., Reardon, C., Shaw, A. C., Sengupta, S., Szentirmai, É., Thakkar, M., Walker, W. E., Solt, L. A. (2020) Perfect timing: Circadian rhythms, sleep, and immunity – An NIH workshop summary. *JCI Insight* **5(1)**, e131487.

Hergenhan, S., Holtkamp, S., Scheiermann, C. (2020) Molecular interactions between components of the circadian clock and the immune system. *J. Mol. Biol.* **432(12)**, 3700–3713.

Johnson, D. B., Nebhan, C. A., Moslehi, J. J., Balko, J. M. (2022) Immune-checkpoint inhibitors: Long-term implications of toxicity. *Nat. Rev. Clin. Oncol.* **19(4)**, 254–267.

Kaloshi, G., Roji, A., Seferi, A., Cakani, B., Bushati, T., Roci, E., Petrela, M. (2014) Spinal dissemination of intracranial glioblastoma in bevacizumab era: A potential bevacizumab-induced mechanism. *Acta Inform. Med.* **22(2)**, 142–144.

Karaboué, A., Innominato, P. F., Wreglesworth, N. I., Duchemann, B., Adam, R., Lévi, F. A. (2024) Why does circadian timing of administration matter for immune checkpoint inhibitors' efficacy? *Br. J. Cancer* **131(5)**, 783–796.

Keller, M., Mazuch, J., Abraham, U., Eom, G. D., Herzog, E. D., Volk, H., Kramer, A., Maier, B. (2009) A circadian clock in macrophages controls inflammatory immune responses. *Proc. Natl. Acad. Sci. U. S. A.* **106(50)**, 21407–21412.

Kucherenko, A. M., Moroz, L. V., Bevz, T. I., Bulavenko, V. I., Antypkin, Y. G., Berezenko, V. S., Dyba, M. B., Pampukha, V. M., Gorodna, O. V., Livshits, L. A. (2019) Investigation of rs11536889 + 3725G/C polymorphism of the TLR4 gene in patients with autoimmune and chronic viral hepatitis C. *Cytol. Genet.* **53(4)**, 300–306.

Labrecque, N., Cermakian, N. (2015) Circadian clocks in the immune system. *J. Biol. Rhythms* **30(4)**, 277–290.

Latka, K., Kolodziej, W., Pawus, D., Bielecki, M., Latka, D. (2024) Performance of successful ambulatory cervical spine surgery: Safety, efficacy, and early experiences of first 100 cases in Poland. *Br. J. Neurosurg.* **39(6)**, 807–812.

Łątka, K., Kołodziej, W., Pawuś, D., Waligóra, M., Trompeta, J., Klepinowski, T., Lasowy, P., Tanaka, M., Łabuz-Roszak, B., Łątka, D. (2024) Extremely rare complications in uniportal spinal endoscopy: A systematic review with unique case analyses. *J. Clin. Med.* **13(6)**, 1765.

Lévi, F. A., Okyar, A., Hadadi, E., Innominato, P. F., Ballesta, A. (2023) Circadian regulation of drug responses: Toward sex-specific and personalized chronotherapy. *Annu. Rev. Pharmacol. Toxicol.* **64(1)**, 89–114.

Li, X., Rothämel, P., Nussbaum, C., Sperandio, M., Scheiermann, C. (2025) Development of a circadian immune system. *Trends Immunol.* **46(9)**, 614–623.

Mamontov, I. M., Tamm, T. I., Kramarenko, K. O., Ryabushchenko, D. D., Sytnik, D. A., Nepomniashchyi, V. V., Bardiuk, O. Y. (2023) Endoscopic retrograde cholangiopancreatography and endoscopic decompression in the malignant obstruction of the extrahepatic biliary tracts – A retrospective analysis. *Ukr. J. Radiol. Oncol.* **31(1)**, 28–37. (in Ukrainian)

Matiichuk, Y., Chemerys, O., Zimenkovsky, B., Drapak, I., Matiychuk, V. (2025) Synthesis and anticancer activity of aminothiazole-terminal phenoxycompounds hybrids and their analogs: A short review. *Chem. Chem. Technol.* **19(1)**, 61–78.

Mazzoccoli, G., Vinciguerra, M., Carbone, A., Relógio, A. (2020) The circadian clock, the immune system, and viral infections: The intricate relationship between biological time and host-virus interaction. *Pathogens* **9(2)**, 83.

Missori, P., Domenicucci, M., Paolini, S., Mancarella, C., Tola, S., D'Elia, A., Marotta, N., Seferi, A., Esposito, V. (2016) Emergency decompressive craniectomy after removal of convexity meningiomas. *Surg. Neurol. Int.* **7(1)**, 193098.

Mok, H., Ostendorf, E., Ganninger, A., Adler, A. J., Hazan, G., Haspel, J. A. (2024) Circadian immunity from bench to bedside: A practical guide. *J. Clin. Invest.* **134(3)**, e175706.

Montayeva, N. S., Kushaliyev, K. Z., Shalmenov, M., Gusmanov, M. G., Sadenov, M. M. (2015) Haematological, biochemical and immunomorphologic changes in dogs with melanoma and melanocytoma. *Biosci. Biotechnol. Res. Asia* **12(1)**, 321–326.

Montayeva, N. S., Kushaliyev, K. Z., Grabarević, Z. (2016) Early symptoms of malignancy in the appearance of melanoma in dogs. *Biol. Med. (Aligarh)* **8(1)**, BM-163-16.

Nagy, S., Hussein, A., Kesselman, M. M. (2025) The importance of timing in immunotherapy: A systematic review. *Cureus* **17(4)**, e82994.

Nelson, B. E., Andersen, C., Yuan, Y., Hong, D. S., Naing, A., Karp, D. D., Yap, T. A., Ahnert, J. R., Campbell, E., Tsimberidou, A. M., Dumbrava, E. E., Fu, S., Piha-Paul, S. A., Pant, S., Blumenschein, G. R., Altan, M., Tawbi, H. A., Tannir, N. M., Meric-Bernstam, F., Subbiah, V. (2022) Effect of immunotherapy and time-of-day infusion chronomodulation on survival in advanced cancers. *J. Clin. Oncol.* **40(16_Suppl.)**, 1588.

Nurgaziyev, M., Issilbayeva, A., Bersimbaev, R., Ilderbayev, O., Vinogradova, E., Jarmukhanov, Z., Nurgozhina, A., Sergazy, S., Kozhabergen, N., Akhmetova, Z., Meiramova, A., Chulenbayeva, L., Ibrayeva, A., Mukhanbetzhanov, N., Mukhanbetzhanova, Z., Kozhakhmetov, S., Ainabekova, B., Kushugulova, A. (2024) Gut microbiome-immune interactions and their role in rheumatoid arthritis development. *PeerJ* **12(7)**, e17477.

Ortega-Campos, S. M., Verdugo-Sivianes, E. M., Amiama-Roig, A., Blanco, J. R., Carnero, A. (2023) Interactions of circadian clock genes with the hallmarks of cancer. *Biochim. Biophys. Acta Rev. Cancer* **1878(3)**, 188900.

Patel, J. S., Woo, Y., Draper, A., Jansen, C. S., Carlisle, J. W., Innominato, P. F., Lévi, F. A., Dhabaan, L., Master, V. A., Bilen, M.

A., Khan, M. K., Lowe, M. C., Kissick, H., Buchwald, Z. S., Qian, D. C. (2024) Impact of immunotherapy time-of-day infusion on survival and immunologic correlates in patients with metastatic renal cell carcinoma: A multicenter cohort analysis. *J. Immunother. Cancer* **12(3)**, e008011.

Qian, D. C., Kleber, T., Brammer, B., Xu, K. M., Switchenko, J. M., Janopaul-Naylor, J. R., Zhong, J., Yushak, M. L., Harvey, R. D., Paulos, C. M., Lawson, D. H., Khan, M. K., Kudchadkar, R. R., Buchwald, Z. S. (2021) Effect of immunotherapy time-of-day infusion on overall survival among patients with advanced melanoma in the USA (MEMOIR): A propensity score-matched analysis of a single-centre, longitudinal study. *Lancet Oncol.* **22(12)**, 1777–1786.

Quist, M., Van Os, M., Van Laake, L. W., Bovenschen, N., Crnko, S. (2024) Integration of circadian rhythms and immunotherapy for enhanced precision in brain cancer treatment. *EBioMedicine* **109**, 105395.

Santry, L. A., van Vloten, J. P., AuYeung, A. W. K., Mould, R. C., Yates, J. G. E., McAusland, T. M., Petrik, J. J., Major, P. P., Bridle, B. W., Wootton, S. K. (2024) Recombinant Newcastle disease viruses expressing immunological checkpoint inhibitors induce a pro-inflammatory state and enhance tumor-specific immune responses in two murine models of cancer. *Front. Microbiol.* **15**, 1325558.

Scheiermann, C., Kunisaki, Y., Frenette, P. S. (2013) Circadian control of the immune system. *Nat. Rev. Immunol.* **13(3)**, 190–198.

Thomas, A. M., Fidelle, M., Routy, B., Kroemer, G., Wargo, J. A., Segata, N., Zitvogel, L. (2023) Gut oncomicrobiome signatures (GOMS) as next-generation biomarkers for cancer immunotherapy. *Nat. Rev. Clin. Oncol.* **20(9)**, 583–603.

Tutchenko, M., Rudyk, D., Aslanian, S., Chub, S., Besedinskyi, M. (2024) Recurrent variceal bleeding in alcoholic liver cirrhosis (a case report). *Gastroenterology* **58(3)**, 222–225.

Walker, W. H., Bumgarner, J. R., Becker-Krail, D. D., May, L. E., Liu, J. A., Nelson, R. J. (2021) Light at night disrupts biological clocks, calendars, and immune function. *Semin. Immunopathol.* **44(2)**, 165–173.

Wang, C., Barnoud, C., Cenerenti, M., Sun, M., Caffa, I., Kizil, B., Bill, R., Liu, Y., Pick, R., Garnier, L., Gkountidi, O. A., Ince, L. M., Holtkamp, S., Fournier, N., Michielin, O., Speiser, D. E., Hugues, S., Nencioni, A., Pittet, M. J., Jandus, C., Scheiermann, C. (2022a) Dendritic cells direct circadian anti-tumour immune responses. *Nature* **614(7946)**, 136–143.

Wang, C., Lutes, L. K., Barnoud, C., Scheiermann, C. (2022b) The circadian immune system. *Sci. Immunol.* **7(72)**, eabm2465.

Xiao, Y., Liu, P., Wei, J., Zhang, X., Guo, J., Lin, Y. (2023) Recent progress in targeted therapy for non-small cell lung cancer. *Front. Pharmacol.* **14**, 1125547.

Yeung, C., Kartolo, A., Tong, J., Hopman, W., Baetz, T. (2023) Association of circadian timing of initial infusions of immune checkpoint inhibitors with survival in advanced melanoma. *Immunotherapy* **15(11)**, 819–826.