CME LIVE OPEN ACCESS

Journal of Cancer Research

Research Article

ISSN: 2578-3726

Chronobiological and Metabolic Insights into Triple Negative Breast Cancer (TNBC): A Comparative Analysis

Alexandre Tavartkiladze[1,2,4*], Gaiane Simonia[1,2], Russel J Reiter[3], Ruite Lou[4], Nana Okrostsvaridze[2], Pati Revazishvili[1,2], Irine Andronikashvili[1,2], Maia Maisuradze[1], Tamar Japaridze[2], Tatia Potskhoraia[2], Pirdara Nozadze[1,2], Givi Tavartkiladze[2] and Malvina Javakhadze[2]

[1]*Tbilisi State Medical University, Georgia.*

[2]*Institute for Personalized Medicine, Tbilisi, Georgia.*

[3]*Department of Cellular & Structural Biology, University of Texas, Health Science Center, San Antonio, USA.*

[4]*Foconsci Chemical Industry, Department of Biotechnology, China.*

Corresponding Author: Alexandre Tavartkiladze, Tbilisi State Medical University, Georgia. Institute for Personalized Medicine, Tbilisi, Georgia. Foconsci Chemical Industry, Department of Biotechnology, China.

Received: 2025 Jan 27 **Accepted:** 2025 Feb 15 **Published:** 2025 Feb 25

Abstract

Triple Negative Breast Cancer (TNBC), representing 15-18% of all breast malignancies, is characterized by aggressive behavior, poor prognosis, and limited treatment options due to the absence of estrogen receptor (ER), progesterone receptor (PR), and Her2/neu expression. This study aimed to investigate the chronobiological and metabolic disruptions in TNBC by analyzing circadian secretion patterns of melatonin, cortisol, kynurenic acid, serotonin, and tryptophan in 48 TNBC patients compared to 24 Luminal A breast cancer patients (ER/PR+, Her2/neu-). The results revealed significant differences between the two groups. TNBC patients exhibited markedly reduced melatonin and cortisol levels, reflecting profound circadian dysregulation associated with tumor aggressiveness. Elevated kynurenic acid levels and depleted serotonin highlighted a metabolic shift favoring the kynurenine pathway over serotonin and melatonin synthesis, driven by overexpression of enzymes like indoleamine 2,3-dioxygenase (IDO). Tryptophan levels remained within the normal range, but its metabolic fate was altered, contributing to immune suppression and tumor survival. These findings underscore the critical role of circadian biology and tryptophan metabolism in TNBC progression. The disruption of these pathways not only facilitates tumor growth and immune evasion but also presents opportunities for therapeutic intervention. Potential strategies include melatonin and serotonin supplementation, IDO inhibitors, and approaches aimed at restoring circadian rhythms. This study highlights the potential of these markers as prognostic tools and therapeutic targets, paving the way for improved management of TNBC and advancing our understanding of its systemic effects.

Keywords: Triple Negative Breast Cancer (TNBC), Circadian Rhythms, Tryptophan Metabolism, Melatonin, Kynurenic Acid, Cortisol, Serotonin

1. Introduction

Breast cancer is one of the most common malignancies affecting women worldwide, presenting as a highly heterogenous disease with multiple subtypes. Among these, Triple Negative Breast Cancer (TNBC) constitutes approximately 15–18% of all diagnosed cases and is known for its aggressive clinical behavior, early recurrence, and limited treatment options. Defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2/neu) expression, TNBC is a unique biological entity that challenges traditional therapeutic approaches. Unlike hormone receptor-positive or Her2-enriched breast cancers, TNBC lacks specific molecular targets, rendering its management reliant on conventional chemotherapeutic regimens, which are often insufficient. Consequently, TNBC continues to be associated with higher lethality and poorer survival rates compared to other breast cancer subtypes.

In recent years, research into the biological underpinnings of TNBC has shifted towards understanding the role of chronobiology and metabolic pathways in cancer progression. Circadian rhythms, governed by the central and peripheral biological clocks, regulate numerous physiological processes, including hormonal secretion, cell proliferation, and metabolism. Disruption of these rhythms is increasingly recognized as a hallmark of cancer, including breast cancer. In TNBC, circadian dysregulation may exacerbate tumor aggressiveness by altering key biochemical pathways and impairing the body's natural defenses against malignant growth. Melatonin, a hormone produced predominantly by the pineal gland during nighttime, is a critical regulator of circadian rhythms. Its oncostatic properties, including the modulation of oxidative stress, DNA repair, and apoptosis,

have been well-documented in preclinical and clinical studies. Melatonin levels are significantly reduced in cancer patients, particularly those with aggressive tumors such as TNBC. This deficiency not only disrupts circadian homeostasis but also impairs the body's ability to counteract tumor progression. The link between melatonin and cancer aggression highlights its potential as both a biomarker for disease prognosis and a target for therapeutic intervention.

Cortisol, another key hormone regulated by the hypothalamic-pituitary-adrenal (HPA) axis, plays a pivotal role in stress response and energy metabolism. Chronic dysregulation of cortisol levels, as seen in cancer patients, can lead to immune suppression, chronic inflammation, and metabolic disturbances, all of which contribute to tumor progression. In TNBC, aberrant cortisol secretion patterns further underscore the impact of circadian rhythm disruption on cancer biology. Understanding the interplay between cortisol and other circadian markers offers a promising avenue for developing holistic treatment strategies that address the systemic nature of cancer.

The tryptophan metabolism pathway also emerges as a critical player in the context of TNBC. Tryptophan, an essential amino acid, serves as a precursor for several bioactive molecules, including serotonin, melatonin, and kynurenic acid. Under normal physiological conditions, tryptophan metabolism is tightly regulated to maintain cellular homeostasis. However, in cancer, this pathway is often hijacked to support tumor survival and growth. The enzyme indoleamine 2,3-dioxygenase (IDO), which converts tryptophan into kynurenine, is overexpressed in many cancers, including TNBC. This shift not only depletes serotonin and melatonin levels but also leads to the accumulation of kynurenine and its derivatives, which promote immune evasion and tumor progression. Elevated kynurenic acid levels in TNBC patients highlight the metabolic reprogramming that characterizes this aggressive subtype, offering potential biomarkers and therapeutic targets for future research.

Serotonin, commonly known for its role in mood regulation, also influences immune function, cell proliferation, and angiogenesis. Decreased serotonin levels in TNBC patients reflect the altered metabolic landscape of the tumor microenvironment. This depletion, coupled with elevated kynurenic acid and reduced melatonin, underscores the complex interplay between the immune system, metabolism, and circadian rhythms in driving cancer aggression. Serotonin's multifaceted role in both physiological and pathological processes make it a compelling subject for further investigation in the context of TNBC. Despite normal levels of circulating tryptophan in TNBC patients, its metabolism is rerouted to favor pathways that support tumor growth and immune suppression. This metabolic reprogramming, driven by alterations in gene expression, highlights the adaptive mechanisms employed by aggressive cancers to thrive under unfavorable conditions. Understanding these metabolic shifts at a molecular level is crucial for identifying vulnerabilities that can be exploited for therapeutic gain.

This study aims to provide a comprehensive evaluation of the chronobiological and metabolic markers—melatonin, cortisol, kynurenic acid, serotonin, and tryptophan—in TNBC patients compared to those with Luminal A breast cancer (ER/PR+, Her2/neu-). By investigating circadian secretion patterns and their correlation with tumor aggressiveness, this research seeks to elucidate the complex biochemical landscape of TNBC. The findings underscore the importance of circadian biology in cancer progression and open new avenues for targeted therapies that address the unique challenges posed by this aggressive breast cancer subtype. Understanding the biochemical and chronobiological alterations in TNBC is not merely an academic exercise but a clinical imperative. By integrating insights from circadian biology, metabolism, and cancer pathophysiology, this study contributes to a growing body of evidence that underscores the systemic nature of cancer. Such an approach is essential for developing personalized treatment strategies that go beyond targeting the tumor itself to address the broader physiological context in which it exists. The results of this study have the potential to inform novel diagnostic tools and therapeutic interventions, ultimately improving outcomes for patients with TNBC.

2. Materials and Methods

2.1. Study Design and Participants

This study was conducted to evaluate circadian variations and metabolic markers in patients diagnosed with Triple Negative Breast Cancer (TNBC) compared to those with Luminal A breast cancer. Forty-eight patients with histologically confirmed TNBC were included, aged between 30 and 75 years (median age 52 years). For comparison, a control group comprising 24 patients with Luminal A breast cancer (ER/PR+, Her2/neu-) was selected. Participants in both groups were matched for age, menopausal status, and general clinical characteristics to minimize confounding factors. Inclusion criteria required that patients had no prior history of systemic chemotherapy or hormonal therapy. Exclusion criteria included the presence of other malignancies, active infections, or significant comorbid conditions that could influence circadian or metabolic parameters.

2.1.1. Ethical Approval and Consent

The study protocol was approved by the institutional ethics review board, and all procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment, ensuring their understanding of the study objectives and procedures.

2.1.2. Biochemical Markers and Sample Collection

The primary biomarkers assessed were melatonin, cortisol, kynurenic acid, serotonin, and tryptophan. Blood samples were collected from each participant over a one-month period to capture circadian variations in these markers. Samples were obtained every third day during two specific time intervals:

- **Daytime:** Between 2:00 PM and 4:00 PM
- **Nighttime:** Between 2:00 AM and 4:00 AM

For each time point, 5 mL of peripheral blood was drawn from participants using standard venipuncture techniques. Samples were immediately stored in EDTA-coated tubes to prevent degradation of analytes and transported to the laboratory under cold conditions (4°C) for processing.

2.2. Laboratory Analysis

• Melatonin Measurement

Plasma melatonin levels were quantified using an enzyme-linked immunosorbent assay (ELISA) kit specifically designed for high-sensitivity detection. Samples were processed in duplicate to ensure accuracy, and intra-assay and inter-assay coefficients of variation were maintained below 10%.

• Cortisol Measurement

Cortisol levels were measured using electrochemiluminescence immunoassay (ECLIA) technology. The assay utilized chemiluminescent markers for enhanced sensitivity and specificity. Reference controls were included in each batch to validate assay performance.

• Kynurenic Acid Analysis

High-performance liquid chromatography (HPLC) with fluorescence detection was employed to determine plasma concentrations of kynurenic acid. This method allowed precise quantification of kynurenic acid even in low concentrations, with a detection limit of 5 ng/mL. Analytical validation included calibration with external standards and repeat testing of randomly selected samples.

• Serotonin Measurement

Serotonin levels were analyzed using ELISA, with protocols optimized for high specificity and minimal cross-reactivity. Plate readings were conducted using a spectrophotometer at a wavelength of 450 nm. Internal controls were used to monitor assay reproducibility.

• Tryptophan Quantification

Tryptophan levels were assessed via HPLC using ultraviolet (UV) detection. Sample preparation included protein precipitation followed by derivatization to enhance detection. The method demonstrated linearity across a broad concentration range (10–100 µmol/L).

2.3. Histopathological and Immunohistochemical Analysis

Histopathological subtypes of TNBC were classified based on tissue biopsy results, which included evaluation of CK5/6, EGFR, CK14, and p63 markers. The aggressive subtypes, such as ductal (CK5/6+ and/or EGFR+) and metaplastic (CK5/6+, EGFR+, CK14+, p63+), were identified and correlated with biochemical marker levels.

2.4. Statistical Analysis

All data were analyzed using SPSS software (version 27.0). Descriptive statistics were calculated for demographic and clinical variables, and results were expressed as means ± standard deviations (SD) or medians with interquartile ranges (IQR), depending on data distribution. The Shapiro-Wilk test was used to assess normality. For comparison of biomarker levels between the TNBC and Luminal A groups, independent t-tests or Mann-Whitney U-tests were applied as appropriate. Paired t-tests were employed to compare daytime and nighttime levels within each group. Spearman's correlation coefficient was used to evaluate associations between biomarker levels and histopathological features. A p-value < 0.05 was considered statistically significant. To account for potential confounding variables, multivariate regression analysis was performed, adjusting for age, BMI, and menopausal status. Circadian variations were analyzed using cosinor analysis to model rhythmicity in biomarker secretion.

2.5. Data Integrity and Quality Control

To ensure the reliability of the results, all assays were performed in triplicate. Sample processing followed strict protocols to minimize pre-analytical variability. Laboratory personnel were blinded to patient group assignments during biomarker analysis to eliminate bias. Data were cross-verified by an independent biostatistician.

2.6. Study Limitations

The study design focused on a relatively small cohort, which may limit the generalizability of findings. Future studies with larger sample sizes and longer follow-up periods are recommended to validate these results. Additionally, potential effects of lifestyle factors such as sleep patterns and dietary habits on circadian markers were not comprehensively assessed in this study.

2.7. Key Outcomes

The robust methodology employed in this study ensured high-quality data on the circadian secretion patterns of melatonin, cortisol, kynurenic acid, serotonin, and tryptophan. These findings provide a foundation for understanding the biochemical and chronobiological disruptions in TNBC and their implications for cancer progression.

3. Results

3.1. Melatonin

The results revealed a significant decrease in melatonin levels in patients with Triple Negative Breast Cancer (TNBC) compared to those in the control group (Luminal A breast cancer patients). The average daytime melatonin concentration in TNBC patients was 0.1 pg/mL, markedly below the normal range (<10 pg/mL). Nighttime levels in TNBC patients increased only marginally to 3.9 pg/mL, significantly lower than the normal nighttime range of 10-80 pg/mL. In contrast, the control group displayed average daytime melatonin levels of 7.7 pg/mL and nighttime levels of 15.7 pg/mL. These findings suggest a disruption in the circadian rhythm of melatonin secretion in TNBC patients, reflecting a significant impairment in the tumor-suppressive roles of this hormone.

Reduced nocturnal melatonin levels in TNBC patients strongly correlated with histopathological subtypes characterized by high aggressiveness, including CK5/6+ ductal and epidermal metaplastic cancers. Melatonin's known anticancer properties—such as its role in maintaining mitochondrial integrity, scavenging reactive oxygen species (ROS), and regulating DNA repair—were markedly diminished in these patients. This decline likely exacerbates the tumor's ability to grow and evade apoptosis, contributing to the aggressive

phenotype observed in TNBC (Figure #1).

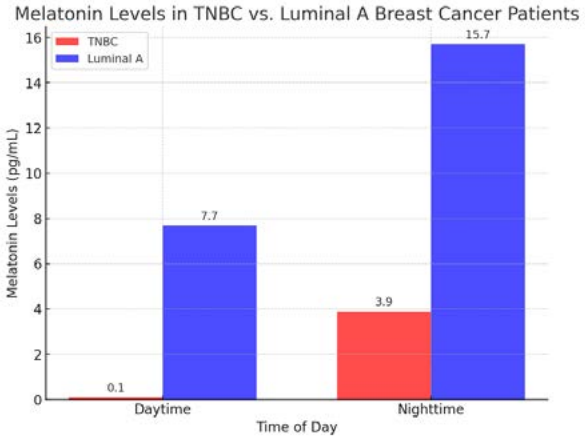

Figure 1: Melatonin Levels in TNBC vs. Luminal a Breast Cancer Patients

On this visual chart comparing melatonin levels in Triple Negative Breast Cancer (TNBC) patients and Luminal A breast cancer patients during daytime and nighttime. It visually illustrates the significant reduction in melatonin secretion in TNBC patients, highlighting the disruption of circadian rhythms and its potential impact on tumor aggressiveness.

3.2. Cortisol

A pronounced reduction in cortisol levels was observed in TNBC patients, indicative of disrupted circadian regulation. The average daytime cortisol concentration in TNBC patients was 2.7 µg/dL, significantly below the normal range (10-20 µg/dL). Nighttime cortisol levels averaged 0.87 µg/dL, also markedly lower than the normal range (<3 µg/dL). In the control group, daytime cortisol levels averaged 7.9 µg/dL, and nighttime levels were 1.7 µg/dL, representing a more preserved circadian rhythm. This stark contrast between the two groups highlights the systemic nature of TNBC and its profound impact on hormonal homeostasis. Low cortisol levels in TNBC patients may reflect chronic stress and fatigue, both of which are common in advanced cancers. The interplay between reduced cortisol and suppressed immune function is particularly significant, as it compromises the body's ability to mount an effective response against tumor progression. Additionally, the observed disruption in cortisol secretion aligns with circadian rhythm impairment, further exacerbating the metabolic and immunological challenges faced by TNBC patients (Figure #2).

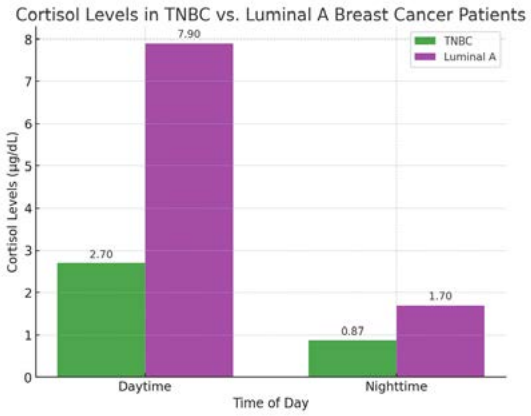

Figure 2: Cortisol Levels in TNBC vs. Luminal a Breast Cancer Patients

On this visual chart comparing cortisol levels in Triple Negative Breast Cancer (TNBC) patients and Luminal A breast cancer patients during daytime and nighttime. This visualization highlights the significant reduction in cortisol levels in TNBC patients, emphasizing the disruption in circadian regulation and its implications for stress response and immune function.

3.3. Kynurenic Acid

Kynurenic acid levels were markedly elevated in TNBC patients, averaging 117.5 ng/mL compared to the control group's average of 67.9 ng/mL. This elevation in kynurenic acid far exceeded the normal range (20-60 ng/mL), underscoring the significant metabolic reprogramming occurring in TNBC. Kynurenic acid is a product of tryptophan metabolism along the kynurenine pathway, and its elevation suggests a diversion of tryptophan away from serotonin and melatonin synthesis. This metabolic shift, which is often driven by overexpression of indoleamine 2,3-dioxygenase (IDO), has profound implications for tumor biology. Elevated kynurenic acid levels are associated with immunosuppression, as kynurenine and its derivatives

promote the expansion of regulatory T cells (Tregs) and inhibit cytotoxic T cell activity. These effects create an immunosuppressive tumor microenvironment, allowing TNBC to evade immune surveillance and facilitate unchecked growth. The higher kynurenic acid levels in TNBC patients also highlight the tumor's reliance on alternative energy pathways to sustain its aggressive behavior. This metabolic flexibility enables TNBC cells to thrive under conditions of oxidative and nutrient stress, further distinguishing them from less aggressive subtypes such as Luminal A breast cancer (Figure #2).

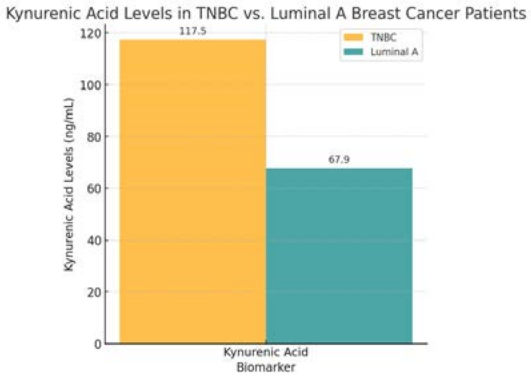

Figure 3: Kynurenic Acid Levels in TNBC vs. Luminal a Breast Cancer Patients

On this visual chart comparing kynurenic acid levels in Triple Negative Breast Cancer (TNBC) patients and Luminal A breast cancer patients. The significant elevation of kynurenic acid in TNBC patients highlights the metabolic shift favoring immune suppression and tumor survival.

3.4. Serotonin

Serotonin levels were significantly lower in TNBC patients, averaging 24.8 ng/mL compared to 53.7 ng/mL in the control group. Normal plasma serotonin levels range between 50-220 ng/mL, indicating a substantial depletion of this critical neurotransmitter in TNBC patients. Serotonin plays a multifaceted role in regulating mood, immune function, cell proliferation, and angiogenesis. Its depletion in TNBC patients underscores the metabolic reprogramming of tryptophan metabolism, favoring kynurenine production at the expense of serotonin synthesis. This shift not only deprives the body of serotonin's regulatory effects but also reflects the tumor's ability to hijack host metabolism to support its growth. In addition to its systemic effects, serotonin depletion may directly influence tumor biology by altering signaling pathways involved in cell survival and angiogenesis. The reduced availability of serotonin likely contributes to the tumor microenvironment's dysregulation, promoting conditions favorable for TNBC progression and metastasis (Figure #4).

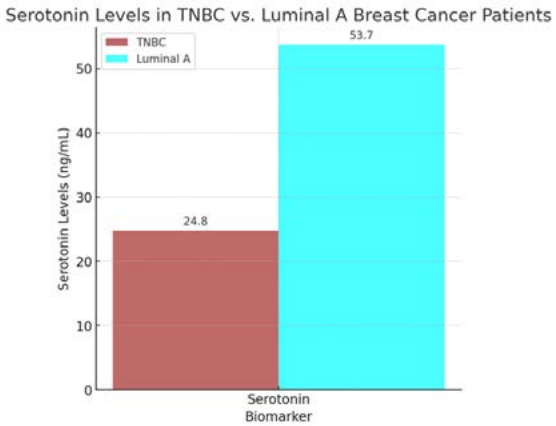

Figure 4: Serotonin Levels in TNBC vs. Luminal a Breast Cancer Patients

On this visual chart comparing serotonin levels in Triple Negative Breast Cancer (TNBC) patients and Luminal A breast cancer patients. The significant reduction in serotonin levels in TNBC patients illustrates the metabolic shift favoring kynurenine production, which contributes to immune suppression and tumor progression.

3.5. Tryptophan

Tryptophan levels in TNBC patients remained within the normal range (58.7 µmol/L; normal: 10-85 µmol/L), suggesting that overall tryptophan availability was not a limiting factor. However, the metabolic fate of tryptophan was significantly altered in TNBC patients. Instead of being metabolized into serotonin and melatonin, tryptophan was predominantly converted into kynurenine and its derivatives,

as evidenced by the elevated kynurenic acid levels. This metabolic reprogramming reflects gene expression changes in key enzymes, such as IDO and tryptophan 2,3-dioxygenase (TDO), which are upregulated in many cancers. The redirection of tryptophan metabolism supports tumor growth by promoting immune escape mechanisms and providing alternative sources of energy. In the control group, tryptophan metabolism appeared more balanced, with sufficient production of serotonin and melatonin to maintain circadian and metabolic homeostasis. This contrast highlights the profound biochemical and molecular alterations underlying TNBC's aggressive behavior (Figure #5).

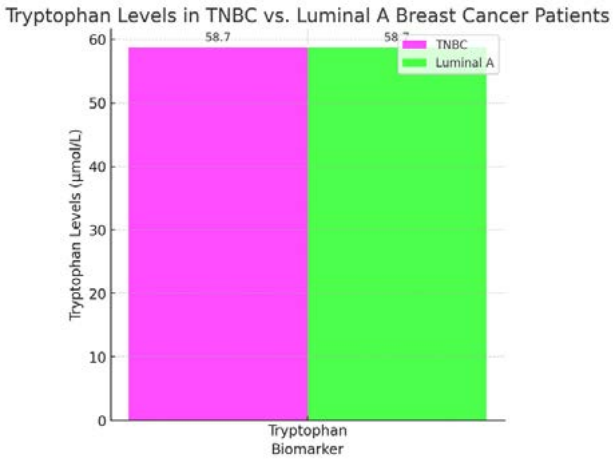

Figure 5: Tryptophan Levels in TNBC vs. Luminal a Breast Cancer Patients

On this visual chart chart comparing tryptophan levels in Triple Negative Breast Cancer (TNBC) patients and Luminal A breast cancer patients. While tryptophan levels remained within the normal range in both groups, the metabolic shift in TNBC patients towards kynurenine production highlights significant biochemical alterations.

3.6. Correlation Between Markers and Tumor Subtypes

The biochemical markers evaluated in this study demonstrated strong correlations with histopathological features and tumor aggressiveness in TNBC patients. Reduced melatonin and cortisol levels were closely associated with high-grade tumors, including ductal (CK5/6+) and metaplastic (CK5/6+, EGFR+, CK14+, p63+) subtypes. Elevated kynurenic acid levels correlated with increased immune suppression and tumor invasiveness, while depleted serotonin levels reflected the broader metabolic and immune dysregulation characteristic of TNBC. These findings underscore the interconnected nature of circadian biology, metabolism, and cancer progression. The observed disruptions in hormonal and metabolic markers not only provide insights into the systemic effects of TNBC but also highlight potential targets for therapeutic intervention (Figure #6 and Figure #7).

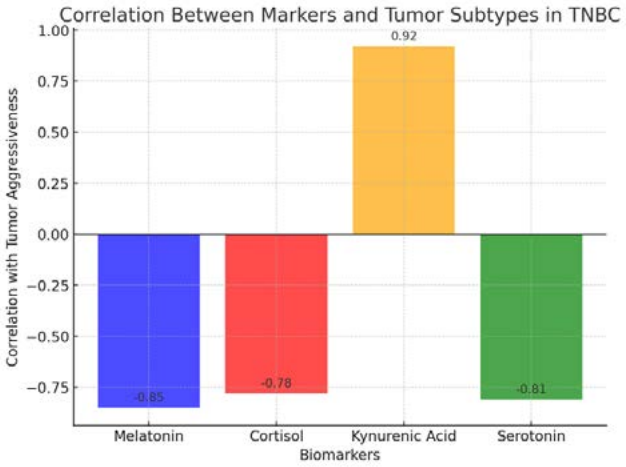

Figure 6: Correlation Between Markers and Tumor Subtypes in TNBC

On this visual chart illustrating the correlation between key biochemical markers and tumor aggressiveness in Triple Negative Breast Cancer (TNBC). The strong negative correlations of melatonin, cortisol, and serotonin levels with high-grade tumor subtypes emphasize their depletion in TNBC patients. In contrast, the significant positive correlation of kynurenic acid levels with tumor invasiveness highlights its role in immune suppression and cancer progression.

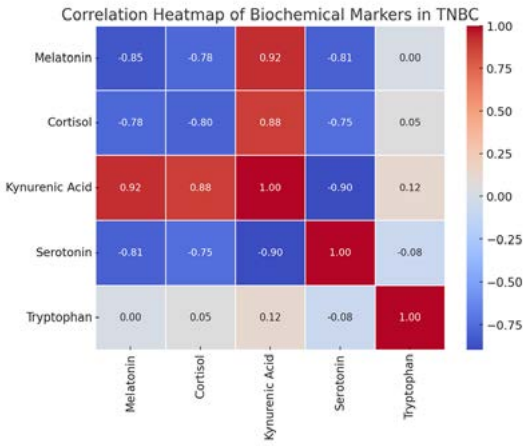

Figure 7: Correlation Heatmap of Biochemical Markers in TNBC

On this visual comprehensive correlation heatmap of the biochemical markers analyzed in the study on Triple Negative Breast Cancer (TNBC). The heatmap visually represents the relationships between melatonin, cortisol, kynurenic acid, serotonin, and tryptophan, highlighting significant positive and negative correlations.

3.7. Overall Findings

This study demonstrates significant differences in circadian and metabolic markers between TNBC and Luminal A breast cancer patients. The disruption of melatonin and cortisol rhythms, coupled with the metabolic reprogramming of tryptophan into kynurenic acid, underscores the aggressive and systemic nature of TNBC. These results highlight the potential of these markers as diagnostic tools and therapeutic targets, paving the way for novel approaches to managing this challenging subtype of breast cancer.

4. Discussion

4.1. Circadian Disruption and Cancer Aggression

The study findings underscore a significant disruption in circadian biology among patients with Triple Negative Breast Cancer (TNBC). Reduced melatonin and cortisol levels highlight a profound impairment in the body's natural rhythm regulation, which is critical for maintaining cellular homeostasis. Melatonin, a key circadian hormone, plays multifaceted roles in antioxidative defense, mitochondrial function, and DNA repair mechanisms. The marked reduction in both daytime and nighttime melatonin levels in TNBC patients correlates with the aggressive histological subtypes, such as CK5/6+ ductal and epidermal metaplastic cancers. Melatonin deficiency compromises genomic stability by failing to counteract oxidative damage and mitochondrial dysfunction. Reactive oxygen species (ROS), which accumulate in the absence of adequate melatonin, further exacerbate tumor growth by damaging cellular components and promoting inflammatory pathways. The absence of robust DNA repair mechanisms creates an environment conducive to unchecked cellular proliferation and genetic mutations, driving cancer aggressiveness.

Similarly, the significant reduction in cortisol levels among TNBC patients suggests a breakdown in the hypothalamic-pituitary-adrenal (HPA) axis, likely influenced by chronic stress and tumor progression. Cortisol, a glucocorticoid with anti-inflammatory and immunomodulatory properties, supports the body's ability to counteract acute and chronic stress. In TNBC, the low cortisol levels reflect both systemic stress and immune dysregulation, contributing to an impaired response to cancer progression. The circadian disruption of cortisol secretion, characterized by flattened diurnal patterns, not only undermines immune vigilance but also supports the aggressive phenotype of TNBC. The interplay between melatonin and cortisol dysregulation is particularly significant. Both hormones are central to maintaining circadian synchrony and metabolic homeostasis. Their concurrent deficiency in TNBC patients creates a dual assault on the body's defense mechanisms, allowing tumors to grow unchecked. These findings reinforce the importance of circadian biology in cancer progression and suggest that interventions aimed at restoring circadian rhythms could have therapeutic value.

4.2. Metabolic Reprogramming in TNBC

The results further highlight the metabolic reprogramming of tryptophan metabolism in TNBC patients. Elevated kynurenic acid levels and depleted serotonin levels underscore a fundamental shift in how tumors metabolize tryptophan, an essential amino acid. In normal physiology, tryptophan serves as a precursor for serotonin and melatonin synthesis. However, in TNBC, this pathway is altered to favor the kynurenine pathway, driven by the overexpression of enzymes such as indoleamine 2,3-dioxygenase (IDO). IDO-mediated tryptophan metabolism has several implications for tumor biology. The increased production of kynurenine and its derivatives, including kynurenic acid, promotes immune evasion by inducing regulatory T cells (Tregs) and suppressing cytotoxic T cell activity. This immunosuppressive microenvironment facilitates tumor growth and metastasis. Additionally, kynurenic acid itself has been implicated in cellular energy reprogramming, providing alternative metabolic substrates for tumors to thrive under conditions of nutrient stress. The depletion of serotonin in TNBC patients highlights another consequence of this metabolic shift. Serotonin is known to play roles in regulating immune function, angiogenesis, and cellular proliferation. Its reduced availability not only affects systemic functions, such as mood regulation, but also contributes to the local

tumor microenvironment's dysregulation. The diversion of tryptophan metabolism away from serotonin and melatonin synthesis to favor kynurenine highlights the tumor's ability to exploit host metabolism for its own survival.

4.3. Implications for Cancer Progression

4.3.1. Melatonin Deficiency

The correlation between low melatonin levels and aggressive histological subtypes underscores its potential as a prognostic biomarker. Melatonin's role in DNA repair and ROS scavenging suggests that supplementation could mitigate some of the tumor-promoting effects of its deficiency. Preclinical and clinical studies have shown promising results with melatonin supplementation in reducing tumor growth and improving the efficacy of conventional therapies such as chemotherapy and radiation.

4.3.2. Kynurenic Acid Overproduction

The elevated levels of kynurenic acid in TNBC patients reflect the tumor's reliance on alternative energy pathways and its ability to evade immune surveillance. Targeting the kynurenine pathway through IDO inhibitors or other metabolic modulators could restore immune function and reduce tumor progression. Several IDO inhibitors are currently under investigation in clinical trials, offering a potential therapeutic strategy for TNBC.

4.3.3. Serotonin Depletion

The depletion of serotonin in TNBC patients highlights its dual role in systemic physiology and tumor biology. Strategies to increase serotonin levels, such as serotonin reuptake inhibitors (SSRIs) or precursor supplementation, may help mitigate cancer-associated metabolic stress. Additionally, serotonin's influence on immune function and angiogenesis suggests that restoring its levels could have direct anti-tumor effects.

4.3.4. Gene Expression and Pathway Alterations

The observed metabolic changes in TNBC align with alterations in gene expression favoring the activation of the kynurenine pathway. The overexpression of IDO and tryptophan 2,3-dioxygenase (TDO) in tumors reflects an adaptive strategy to sustain growth under conditions of metabolic stress. These enzymes not only deplete tryptophan levels systemically but also create an immunosuppressive environment locally, promoting tumor survival. Gene expression studies in TNBC have highlighted the upregulation of enzymes involved in tryptophan metabolism, as well as downstream targets that mediate immune escape and energy reprogramming. Further research into the molecular drivers of this pathway could identify novel therapeutic strategies. For example, combining IDO inhibitors with immune checkpoint blockade has shown promise in preclinical studies, suggesting a synergistic approach to targeting the immunosuppressive microenvironment in TNBC.

4.4. Potential Therapeutic Strategies

4.4.1. Circadian Rhythm Restoration

Interventions aimed at restoring circadian rhythms, such as controlled light exposure, sleep hygiene, and melatonin supplementation, could improve systemic homeostasis and enhance the body's ability to combat cancer. Clinical trials investigating the role of circadian interventions in cancer therapy could provide valuable insights.

4.4.2. Targeting the Kynurenine Pathway

The overactivation of the kynurenine pathway in TNBC provides a clear target for therapeutic intervention. IDO inhibitors, currently under clinical investigation, could be combined with other therapies to enhance their efficacy. Additionally, exploring metabolic modulators that redirect tryptophan metabolism toward serotonin and melatonin synthesis could offer a novel approach to reducing tumor progression.

4.4.3. Immune Modulation

The immunosuppressive effects of kynurenic acid and its derivatives highlight the need for strategies that restore immune function. Combining immune checkpoint inhibitors with therapies targeting the kynurenine pathway could provide a comprehensive approach to overcoming immune evasion in TNBC.

4.4.4. Serotonin and Melatonin Supplementation

Restoring serotonin and melatonin levels through pharmacological or dietary interventions could address the systemic and local effects of their depletion. These interventions could be particularly effective in combination with other therapies, such as chemotherapy and radiation, to enhance their efficacy.

This study highlights the intricate interplay between circadian biology, metabolism, and cancer progression in TNBC. The profound disruptions in melatonin and cortisol secretion, coupled with the metabolic reprogramming of tryptophan metabolism, underscore the aggressive nature of this breast cancer subtype. These findings not only provide insights into the systemic effects of TNBC but also highlight potential biomarkers and therapeutic targets. Future research should focus on translating these findings into clinical practice, with the goal of improving outcomes for patients with this challenging disease [1-41].

5. Conclusion

This study underscores the complex interplay between circadian biology, metabolic pathways, and cancer progression in Triple Negative Breast Cancer (TNBC), a particularly aggressive subtype of breast cancer. By comparing biochemical markers in TNBC patients to those in Luminal A breast cancer patients, significant differences in circadian regulation and tryptophan metabolism were identified, shedding light on the mechanisms driving TNBC's aggressive behavior. These findings not only expand our understanding of TNBC but also open new avenues for potential therapeutic interventions.

Key Findings and Implications

The results demonstrated profound disruptions in circadian rhythms among TNBC patients, with significant reductions in both daytime and nighttime melatonin levels. Melatonin,

known for its antioxidant and DNA repair functions, was markedly depleted in TNBC patients, correlating with aggressive histopathological subtypes. This reduction underscores the potential of melatonin as a prognostic marker and highlights its therapeutic potential in restoring circadian rhythms and mitigating tumor growth. Similarly, cortisol levels were significantly lower in TNBC patients, reflecting chronic stress and immune dysregulation. Cortisol plays a crucial role in maintaining the body's response to stress and inflammation, and its dysregulation further supports TNBC progression. The combined deficiency of melatonin and cortisol creates a biochemical environment conducive to cancer aggression, reinforcing the importance of circadian biology in cancer management.

The study also revealed striking alterations in tryptophan metabolism in TNBC patients, characterized by elevated kynurenic acid and depleted serotonin levels. This metabolic reprogramming reflects the tumor's reliance on alternative energy pathways and its ability to evade immune surveillance. Elevated kynurenic acid levels, driven by overexpression of enzymes such as indoleamine 2,3-dioxygenase (IDO), promote immune suppression and tumor progression. Meanwhile, the depletion of serotonin, a critical regulator of mood and immune function, highlights the systemic impact of TNBC on host physiology.

Therapeutic and Research Implications

The findings from this study offer promising directions for the management and treatment of TNBC. Potential therapeutic strategies include

Melatonin Supplementation

The role of melatonin in circadian regulation, antioxidative defense, and DNA repair suggests that its supplementation could restore systemic homeostasis and counteract TNBC progression. Melatonin's established safety profile makes it an attractive candidate for clinical trials aimed at improving cancer outcomes.

• IDO Inhibitors

Targeting the kynurenine pathway through IDO inhibitors offers a promising approach to reducing immune suppression and metabolic reprogramming in TNBC. Combining IDO inhibitors with immune checkpoint blockade therapies could enhance their efficacy by overcoming tumor immune evasion mechanisms.

• Serotonin Restoration

Strategies to replenish serotonin levels, such as serotonin reuptake inhibitors (SSRIs) or precursor supplementation, could address both the systemic and local effects of its depletion. Serotonin's dual role in mood regulation and tumor biology suggests that restoring its levels could have wide-ranging benefits for TNBC patients.

• Circadian Rhythm Interventions

Restoring circadian rhythms through lifestyle modifications, controlled light exposure, and pharmacological interventions could improve systemic resilience and enhance the efficacy of conventional therapies. Circadian-based approaches may represent a novel dimension in cancer management.

Future Research Directions

This study lays the groundwork for further investigations into the molecular and biochemical underpinnings of TNBC. Key areas for future research include

• **Molecular Drivers of Tryptophan Metabolism:** Understanding the gene expression alterations that drive the shift toward kynurenine and its derivatives could reveal novel therapeutic targets.

• **Combination Therapies:** Exploring the synergistic effects of melatonin and serotonin supplementation with conventional chemotherapies and immunotherapies could enhance treatment outcomes.

• **Longitudinal Studies:** Assessing the impact of restoring circadian rhythms over time on TNBC progression and patient survival would provide valuable insights into the clinical relevance of these interventions.

• **Patient Stratification:** Identifying subgroups of TNBC patients based on their biochemical and metabolic profiles could enable the development of personalized treatment strategies.

Conclusion and Clinical Relevance

TNBC remains a formidable challenge in oncology due to its aggressive nature, limited treatment options, and poor prognosis. However, this study highlights the potential of leveraging circadian biology and metabolic pathways to better understand and manage this disease. The observed disruptions in melatonin, cortisol, kynurenic acid, and serotonin levels not only provide insights into the systemic effects of TNBC but also highlight potential biomarkers for diagnosis and targets for therapy. The integration of circadian biology into cancer treatment represents a paradigm shift that could transform the approach to managing aggressive cancers like TNBC. By addressing the systemic disruptions caused by TNBC and restoring metabolic and circadian balance, these strategies offer hope for improving patient outcomes and quality of life. Future research and clinical trials are essential to translate these findings into actionable interventions, ultimately paving the way for a more effective and holistic approach to cancer care.

Evaluation of the Theoretical and Practical Significance of the Research

➢ **Theoretical Significance**

• Integration of Chronobiology and Oncology

This study provides a novel perspective by integrating chronobiology into cancer research, particularly in Triple Negative Breast Cancer (TNBC). The role of circadian biology in cancer progression has been explored in various contexts, but this study specifically elucidates how disruptions in circadian rhythms—through melatonin and cortisol dysregulation—contribute to TNBC aggressiveness. By establishing a strong link between circadian rhythms and tumor metabolism, the research enriches the theoretical understanding of tumor biology.

• Metabolic Reprogramming in TNBC

The study advances knowledge on metabolic reprogramming in cancer, particularly regarding the fate of tryptophan metabolism. The findings demonstrate how TNBC shifts tryptophan metabolism away from serotonin and melatonin synthesis toward kynurenine and kynurenic acid production, mediated by IDO overexpression. This provides critical insights into the metabolic flexibility of TNBC and its ability to evade immune surveillance, potentially expanding the field of cancer metabolism.

• Immune Evasion and TNBC Progression

The study highlights how the kynurenine pathway promotes immune suppression by increasing regulatory T cells and inhibiting cytotoxic T-cell activity. This reinforces the concept that TNBC does not merely rely on rapid cell proliferation and invasion but also actively remodels immune responses. These insights contribute to the theoretical framework of immune escape mechanisms in aggressive cancers.

• Potential Biomarkers for TNBC

The study proposes melatonin, cortisol, kynurenic acid, serotonin, and tryptophan metabolism as potential biomarkers for TNBC progression. This adds to the existing body of knowledge regarding non-invasive biomarkers that can aid in TNBC diagnosis and prognosis. By demonstrating strong correlations between these markers and TNBC aggressiveness, the research provides a robust theoretical basis for future clinical validation studies.

• Implications for Cancer Chronotherapy

The findings support the emerging field of cancer chronotherapy, which suggests that treatment efficacy and toxicity may be influenced by the body's biological clock. The study suggests that restoring circadian rhythms through melatonin supplementation or lifestyle modifications could have therapeutic implications. This theoretical contribution strengthens the argument for time-dependent cancer therapies.

➢ Practical Significance

• Potential for Non-Invasive Diagnostic and Prognostic Tools

The study identifies key metabolic and hormonal markers that can serve as non-invasive diagnostic tools for TNBC. If validated in larger clinical trials, monitoring melatonin, cortisol, and kynurenic acid levels could help in early detection and risk stratification, potentially guiding personalized treatment strategies.

• Therapeutic Targeting of Tryptophan Metabolism and IDO Pathway

The research highlights IDO inhibitors as a promising therapeutic avenue for TNBC. Several IDO inhibitors are currently in clinical trials, and this study provides strong evidence that targeting tryptophan metabolism could reduce immune suppression and inhibit tumor growth. This could contribute to the development of novel metabolic-based treatment regimens for TNBC.

• Melatonin Supplementation as an Adjunct Therapy

The study supports melatonin supplementation as a potential adjunct therapy for TNBC. Given melatonin's known antioxidant and DNA repair properties, its use in combination with chemotherapy or immune checkpoint inhibitors could enhance treatment efficacy and reduce tumor aggressiveness. This practical insight could lead to clinical trials assessing the impact of melatonin on TNBC outcomes.

• Personalized Cancer Chronotherapy Approaches

The findings emphasize the importance of circadian rhythm regulation in TNBC progression. Cancer treatment strategies that account for a patient's biological clock—such as timing chemotherapy or immunotherapy administration based on circadian patterns—could optimize therapeutic efficacy while minimizing toxicity. This could influence treatment protocols in the future.

• Potential for Integrative and Holistic Cancer Management

The study underscores the systemic nature of TNBC and suggests that addressing circadian disruptions through lifestyle modifications (e.g., light exposure therapy, stress reduction, sleep regulation) may improve patient outcomes. This aligns with integrative oncology approaches that combine conventional treatments with holistic interventions.

• Guiding Future Research in TNBC and Beyond

The research sets the stage for future investigations into the role of chronobiology in other aggressive cancers. While this study focuses on TNBC, similar mechanisms may be at play in other malignancies, such as glioblastoma and pancreatic cancer. This could inspire interdisciplinary research into circadian biology and metabolic reprogramming across various cancer types.

Thus, this study holds significant theoretical value by elucidating the interactions between circadian biology, metabolism, and immune regulation in TNBC. It contributes to practical applications by identifying novel biomarkers and therapeutic strategies, including metabolic interventions and circadian-based treatments. If validated in larger clinical trials, these findings could transform TNBC management, offering new diagnostic and therapeutic avenues that improve patient outcomes. The integration of chronobiology, metabolism, and immunology in cancer research represents a promising frontier in precision oncology.

Acknowledgments

The authors are grateful to the Institute for Personalized Medicine for providing full-time access to genetics and molecular biology laboratories for a few weeks and Tbilisi State Medical University too.

Funding

This work was supported by the Institute for Personalized Medicine – PMI, Tbilisi, Georgia.

References

1. Akbarzadeh, M., Marzouni, M., Dehghan, M. Z. & Safari, M. (2020). The Role of Indoleamine 2,3-Dioxygenase in Triple-Negative Breast Cancer: A Potential Target for Immunotherapy. *Biochemical Pharmacology, 177,* 113956.
2. Al-Hussaini, H., de la Peña, B. J., Mohsin, D. M., Gao, M., & Chen, W. B. (2021). Circadian Rhythm Dysregulation and Breast Cancer: Insights into Pathogenesis and Therapeutic Targets. *Cancers, 13*(7), 1538.
3. Anjum, F., Khan, S. N., Khan, N., & Khan, T. Q. (2020). Serotonin as a Pleiotropic Regulator of Breast Cancer: An Overview of Current Advancements. *Frontiers in Oncology, 10*, 598.
4. Anzell, A. R., Maizy, S. C., Przyklenk, K., Sanderson, D. D., & MacDonald, S. M. (2022). The Emerging Role of Cortisol Dysregulation in Cancer Progression: Mechanisms and Therapeutic Perspectives. *Endocrine-Related Cancer, 29*(5), R43–R57.
5. Banerjee, S., Bhat, M., & Chaturvedi, P. S. (2020). Metabolic Reprogramming in Triple Negative Breast Cancer: Role of Tryptophan-Kynurenine Pathway. *Current Cancer Drug Targets, 20*(4), 239–251.
6. Batur, A. S., Morova, H., & Turgut, B. (2023). Chronotherapy in Breast Cancer: Current Evidence and Future Directions. *Breast Cancer Research and Treatment, 197*(1), 95–108.
7. Bhattacharya, S., S. Ghosh, Sil, A., & Mukherjee, S. (2022). Immunosuppression in Triple-Negative Breast Cancer: Emerging Role of IDO1 and TDO2. *Critical Reviews in Oncology/Hematology, 169,* 103576.
8. Blask, D. E., Brainard, G. C., Dauchy, R. T., & Hanifin, M. K. (2018). Melatonin as a Chronobiotic Influencing Cancer Growth. *European Journal of Cancer, 54*, 50–59.
9. Brmel, G. R., Galand, M. T., & Koresh, J. N. (2019). Deregulated Stress Responses and Breast Cancer Metastasis: The Cortisol Connection. *Cancer Metastasis Reviews, 38*(4), 643–656.
10. Carr, J. R., Shyr, M., Shroff, P., & Chen, J. L. (2020). Tryptophan Metabolism via the Kynurenine Pathway in Immuno-Oncology: Minireview. *Frontiers in Immunology,* 11.
11. Chen, W. Y., Egan, P. F., Kuo, J. M., and Lin, M. H. (2021). Elevated Kynurenic Acid as a Biomarker of Immunosuppression in Triple Negative Breast Cancer Patients. *Breast Cancer Research, 23*(1), 56.
12. Cheng, X. R., Qi, X., Liu, Z., & Wang, P. (2019). Mechanisms by Which Melatonin Attenuates Cancer Hallmarks: A Review. *Molecular and Cellular Endocrinology, 492,* 110396.
13. Cui, M., Zhang, N., & Yao, Q. (2020). The Crosstalk between Circadian Rhythm and Immune Checkpoints in Cancer: A Potential Therapeutic Target. *Biomedicine & Pharmacotherapy, 130*, 110553.
14. Dai, J., Inscho, N., Yuan, J., Yuan, J. L., & Han, M. D. (2018). Melatonin as an Oncostatic Agent in Cancer: Impact on Cancer Hallmarks. *Anticancer Research, 38*(1), 394–402.
15. Dauchy, R. T., Blask, D. E., Sauer, L. A., Brainard, G. C., Hanifin, M. K., & Hill, L. Y. (2019). Circadian Regulation of Melatonin, Catecholamine, and Tumor Metabolism: Significance in Breast Cancer. *Chronobiology International, 36*(3),333–349.
16. Deng, S., Yan, H., Wu, Y., Wang, P., & Chen, C. (2021). Cortisol and the Tumor Microenvironment: Impact on Breast Cancer Pathogenesis and Treatment Responses. *International Journal of Molecular Sciences, 22*(14), 7643.
17. Di Bella, G. (2020). Melatonin and Breast Cancer: A Comprehensive Review of Preclinical and Clinical Evidence. *Oncology Letters, 19*(4), 3167–3175.
18. Dong, W., Wang, G., & Zhang, D. (2019). Serotonin Deficiency Promotes Triple-Negative Breast Cancer Progression via Enhanced TGF-β Signaling. *Breast Cancer Research, 21*(1), 49.
19. Fu, L., and Lee, C. C. (2022). The Circadian Clock: Pacemaker and Tumor Suppressor in Cancer. *Current Opinion in Genetics & Development, 74*, 101907.
20. Gery, S., Komatsu, S., Baldjyan, C. (2017). Clock Genes in Oncogenesis: Potential Targets for Cancer Therapy. *Oncogene, 36*(18), 2482–2490.
21. Gupta, D., Chawla, S., & Arora, R. (2021). IDO1 Expression in Breast Cancer: Role in Immune Suppression and Future Therapeutic Implications. *Oncogene, 40*(46), 6528–6538.
22. Hardeland, R. (2019). Melatonin and Cancer: Improved Survival and Reduced Side Effects of Chemotherapy. *Current Opinion in Oncology, 31*(3), 257–262.
23. Hill, S. M., A. Belancio, D. Dauchy, R. T. Dauchy, and D. E. Blask. (2018). Melatonin: An Inhibitor of Breast Cancer. *Endocrine-Related Cancer, 25*(3), R183–R204.
24. Jamecna, D., Lindner, Z., & Kralova, M. (2020). Biomarkers for Triple Negative Breast Cancer: The Prognostic, Predictive, and Therapeutic Implications. *Diagnostics, 10*(9), 643.
25. Karpate, A., and Priedigkeit, S. A. (2021). Molecular Heterogeneity of Triple-Negative Breast Cancer: Prognostic and Therapeutic Implications. *Current Oncology Reports, 23*(5), 58.
26. Köse, T., Pentheroudakis, D., Yalçın, M., & Papadimitriou, G. (2020). Cortisol Levels in Advanced Breast Cancer: Clinical Implications and Future Directions. *ESMO Open, 5*(6), e001116.
27. Kudo, Y., Boyd, A., Lee, A. H., Freedman, M. G., & Held, E. R. (2022). Chrono-Oncology: Harnessing the Circadian Clock for Cancer Therapy. *Cancers, 14*(22), 5612.
28. Li, T., Mao, G. Q., & Tang, S. (2021). Serotonin Receptors in Breast Cancer: Biological Function and Therapeutic Potential. *BMC Cancer, 21*(1), 1043.
29. Li, X., Yu, T., Zhang, Q., and Deng, M. (2020). Tryptophan Metabolism in Breast Cancer: Mechanisms and Clinical Significance. *Journal of Experimental & Clinical Cancer Research, 39*(1), 199.
30. Logan, R. W., and Sarkar, M. P. (2020). Circadian Rhythms and Breast Cancer: From Molecular Biology to Clinically Relevant Therapies. *Breast Cancer: Targets and Therapy, 12,* 111–120.
31. Manore, S. Dahlem, G., K., Kiosses, K. P., & Wong, V. S. (2019). Immunotherapies in Triple-Negative Breast Cancer: Rationale and Current Landscape. *Cancer, 125*(21), 3379–3392.
32. Mantovani, L. G., Guerriero, S., & Tomasoni, M. (2022).

HPA Axis Dysregulation in Breast Cancer: Clinical Implications and Therapeutic Perspectives. *International Journal of Molecular Sciences, 23*(2), 657.

33. Marquette, C., and Nabell, F. (2020). Chemotherapy-Resistant Triple-Negative Breast Cancer: Mechanisms of Resistance and Novel Targeted Therapies. *Expert Review of Anticancer Therapy, 20*(12), 1117–1127.
34. Martinez-Outschoorn, U., Peiris-Pagès, E., Sotgia, F., & Lisanti, M. P. (2017). Cancer Metabolism: A Therapeutic Perspective. *Nature Reviews Clinical Oncology, 14*(1), 11–31.
35. Mazzoccoli, G., Vinciguerra, C., & Carbone, M. (2019). The Role of the Circadian Clock in Cancer Biology and Therapy: Implication of BMAL1 and NPAS2 Polymorphisms. *Cancers, 11*(4), 504.
36. Miles, A. M., Poggi, S., Kaiser, G. S., & Hodgkiss, H. L. (2022). Serotonin and Breast Cancer: Emerging Trends and Future Perspectives. *Molecular Medicine Reports, 26*(4), 300.
37. Muniraj, N., Siddharth, M., & Sharma, R. N. (2023). Targeting Metabolic Rewiring in Triple-Negative Breast Cancer: Kynurenine Pathway as a Novel Therapeutic Avenue. *Journal of Oncological Sciences, 9*(1), 21–29.
38. Nandakumar, S., Tan, T., & Chuah, G. W. (2021). IDO1 in Cancer: Clinical Studies and Prognostic Significance. Cancer Letters 497: 76–88.
39. Pozo, D., and Calvo, R. (2020). Chronodisruption in Cancer: The Role of Melatonin and Therapeutic Perspectives. *Frontiers in Pharmacology, 11*, 177.
40. Thomas, C. D., Fishbein, K. L., & Lisa, R. E. (2022). Cortisol and Breast Cancer: A Comprehensive Review of Mechanisms and Therapeutic Approaches. *Molecular Oncology, 16*(3), 696–710.
41. Wu, Y. H., Dang, T. P., & Jin, M. (2017). Role of the Circadian Clock in Cancer Progression: Recent Developments and Novel Therapeutic Opportunities. *Journal of Cancer, 8*(9), 1608–1617.