

Melatonin-Induced Oncostasis, Mechanisms and Clinical Relevance

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Abstract

Melatonin is a natural substance ubiquitously distributed and present in almost all living species, from unicellular organisms to humans. Melatonin is synthesized not only in the pineal gland but also in most tissues in the body where it may have a cytoprotective function via paracrine or autocrine effects. Melatonin is effective in suppressing neoplastic growth in a variety of tumors. The mechanisms involved include antiproliferative effects via modulation of cell cycle, ability to induce apoptosis in cancer cells, anti-angiogenic and antimetastatic effects, anti-estrogenic activity, the capacity to decrease telomerase activity, immune modulation, and direct and indirect antioxidant effects. Besides these oncostatic properties, melatonin deserves to be considered in the treatment of cancer for two other reasons. First, because its hypnotic-chronobiotic properties, melatonin use that can allow the clinician to effectively address sleep disturbances, a major co-morbidity in cancer. Second, because melatonin's anxiolytic and antidepressant effects, it has a possible application in two other major co-morbidities seen in cancer patients, i.e. depression and anxiety. This report summarizes the possible mechanisms involved in melatonin oncostasis and reviews what is known about the clinical application of melatonin as an adjuvant therapy in cancer patients.

Keywords: Melatonin; Cancer; Apoptosis; Antioxidant; Angiogenesis; Estrogen signaling pathway; Metastasis

Abbreviations: 13-HODE: 13-hydroxyoctadecadienoic Acid; AKT: Protein Kinase B; AIF: Apoptosis-Inducing Factor; Bax: B cell lymphoma Bcl-2 associated X protein; Bid: BH3 Interacting-Domain Death Agonist; Ca²⁺/CaM: Calcium/Calmodulin; cAMP: Cyclic Adenosine Monophosphate; Cdk: Cyclin/Cyclin-Dependent Kinase; COX: Cyclooxygenase; E2: Estradiol; EGF: Epidermal Growth Factor; EGFR: Epidermal Growth Factor Receptor; EMT: Epithelial-Mesenchymal Transition; ER: Estrogen Receptor; ERE: Estrogen Response Element; ERK: Extracellular-Signal-Regulated Kinase; ET-1: Endothelin-1; GSK3 β : Glycogen Synthase Kinase 3; Hif1 α : Hypoxia-Inducible Factor 1; IGF: Insulin-Like Growth Factor; IL: Interleukin; iNOS: Inducible Nitric Oxide Synthase; L1: Element 1 Retrotransposon; MAPK: Mitogen-Activated Protein Kinase; MET: Mesenchymal-To-Epithelial Transition; MT: Melatonin Receptor; mTOR: Mammalian Target Of Rapamycin; MyD88: Myeloid Differentiation Primary Response Gene 88; NF- κ B: Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells; NK: Natural Killer; NO: Nitric Oxide; PI3K: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase; PK: Protein Kinase; RNS: Reactive Nitrogen Species; ROS: Reactive Oxygen Species; SCN: Suprachiasmatic Nuclei; Smad: Mothers Against Decapentaplegic Homolog; Sirt: Sirtuin; TERT: Telomerase Catalytic Protein Component; TGF: Transforming Growth Factor; Th: T Helper; TNF: Tumor Necrosis Factor; uPA: Urokinase-Type Plasminogen Activator; VEGF: Vascular Endothelial Growth Factor

Introduction

Melatonin is a ubiquitous methoxyindole present in most living species, including unicellular microorganisms, plants, most invertebrates and vertebrates and humans. The first function of melatonin in phylogeny may have been cytoprotective [1]. As such, melatonin could be among the natural molecules that are effective in treating neoplastic malignancies. Despite a number of studies that have established the potentiality of melatonin as an adjuvant in the treatment of cancer melatonin's importance on cancer therapy

remains largely unappreciated. Several aspects of this subject have been reviewed elsewhere [2-8]. The aim of this report is to update the present knowledge on the possible mechanisms involved in melatonin oncostasis (Figure 1) and to assess what is known about the therapeutic application of melatonin in cancer patients.

Melatonin Oncostasis

Antiproliferative effects

Numerous studies have shown that melatonin has remarkable oncostatic properties and can reduce the promotion and/or progression of tumors. Its antiproliferative properties have been demonstrated in an extensive variety of tumors including breast, endometrial, prostate, colon, and ovarian cancers, choriocarcinoma, melanoma, neuroblastoma, osteosarcoma, and leukemia, with particular efficacy in lymphoproliferative tumors [9-15] (Figure 2).

Melatonin exerts direct anticancer actions by inhibiting the proliferation and growth of tumor cells. The potential signaling pathway responsible for inhibiting cell proliferation requires further investigation, but several explanations are possible, as follows.

Modulating the cell cycle: Melatonin increases the duration of the cell cycle in cancer cells by either expanding the G1 phase (thus

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Received December 29, 2015; **Accepted** February 02, 2016; **Published** February 19, 2016

Citation: Cardinali D, Escames G, Acuña-Castroviejo D, Ortiz F, Fernández-Gil B, et al. (2016) Melatonin-Induced Oncostasis, Mechanisms and Clinical Relevance. J Integr Oncol S1: 006. doi:10.4172/2329-6771.S1-006

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Figure 1: The several mechanisms through which melatonin can exert oncostatic effects. They include antiproliferative effects via modulation of cell cycle, ability to induce apoptosis in cancer cells, metabolic, anti-angiogenic and antimetastatic effects, anti-estrogenic activity, the capacity to decrease telomerase activity, the regulation of genomic instability and of the immune system, and direct and indirect antioxidant effects. See text for details.

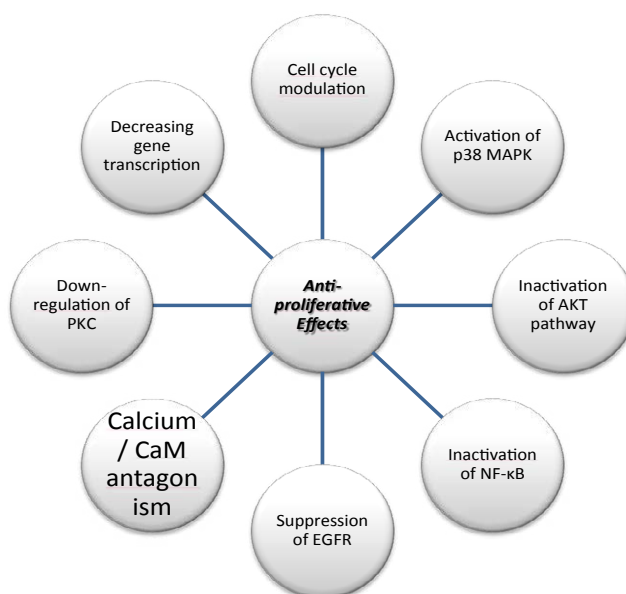


Figure 2: The antiproliferative effects involved in melatonin oncostasis comprise the modulation of cell cycle, activation of p38 MAPK and inactivation of the AKT pathways, inactivation of NF-κB, suppression of EGFR, calcium/CaM antagonism, down-regulation of PKC and the decrease of gene transcription associated with cell proliferation. See text for details.

delaying the entrance of cells into the S phase) or by arresting cells in the G2/M phase [15]. Prolonging G1 and delaying progression to S phase is one way that melatonin drives tumor cells to become more differentiated. These data are important because aggressive tumors are associated with poor differentiation [16,17], an effect that seems to be mediated by modulation of expression of genes related to the cell cycle [18,19].

In cancer, the cell cycle is commonly deregulated, contributing to tumorigenesis by amplification or overexpression of cyclins. Melatonin downregulates the activity of cyclin-dependent kinase (CDK) 4 and CDK2 [20]. The cyclin D/CDK4 complex initiates phosphorylation of retinoblastoma protein, which is then further phosphorylated by cyclin E/CDK2. Phosphorylation of phosphorylated retinoblastoma protein triggers the steps required for the cell to enter S phase [21]; thus, inhibition of these CDKs by melatonin may block cancer cell cycle progression. Cyclin B is associated with CDK1, and melatonin also inhibits the transcriptional activity of cyclin B and CDK1 which after association promotes entry into mitosis, thereby blocking cell progression at the G1 and G2/M phases [13,22].

Although melatonin induces alterations in cell cycle progression, these effects depend on the overall metabolic and differentiation state of the cancer cells. Loureiro et al. [23] demonstrated that forcing mitochondrial metabolism in embryonal carcinoma stem cells leads to reduced proliferation and pluripotency and to spontaneous differentiation. Therefore, it can be presumed that the melatonin-dependent antiproliferative effect requires an active mitochondrial metabolism.

Inducing activation of p38 mitogen activated protein kinase (MAPK): Melatonin induces phosphorylation (activation) of p38 MAPK, suggesting that this signaling kinase plays a key role in cell growth inhibition [24-26]. P38 MAPK activates kinases involved in phosphorylation of cyclin D1, enhancing its proteasome-dependent degradation and delaying progression through G1 [27]. In addition, evidence suggests that p38 MAPK can induce B cell lymphoma Bcl-2 associated X protein (Bax) activation, leading to its mitochondrial translocation prior to apoptosis. MAPK signaling pathways are responsible for melatonin antiproliferative effects in some cancer cells [18,24,26,28].

Inactivation of the AKT pathway: Activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/ protein kinase B (AKT)/ mammalian target of rapamycin (mTOR) signaling pathway is related to advanced tumor stage [29]. AKT is another important protein kinase (PK) that phosphorylates key signaling molecules controlling cell proliferation, size, differentiation, survival and apoptosis [30]. Evidence suggests that melatonin induces downregulation of the phosphorylation of mTOR and AKT, thus attenuating the expression of survival genes such as McL-1, Bcl-xL, cyclin D1, and cyclin E, both in breast cancer [30,31] and hepatoma cells [32].

Inactivation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)

Toll-like receptors are cell surface sensors that can initiate pathways to stimulate cell proliferation, as well as recruiting immune cells to provide support for cancer progression. Toll-like receptors activation, via a myeloid differentiation primary response gene 88 (MyD88)-dependent pathway, induces an inflammatory response and promotes activation of the transcription factor NF-κB. The antiproliferative effect of melatonin involves negative regulation of MyD88 [33] and NF-κB [34]. These factors have proliferative effects by direct action on

cyclin D1. NF-κB also induces interleukins, cytokines, chemokines and cyclooxygenase (COX)-2. COX-2 overexpression and prostaglandin E₂ production correlate significantly with invasiveness, prognosis, and survival in several types of cancer [35]. Therefore, COX-2 inhibitors can be important anticancer agents and, additionally, act additively with chemotherapy. Again, the action of melatonin in repressing the expression of COX-2 may account for its oncostatic effects [36].

Suppression of the epidermal growth factor receptor (EGFR) mechanism: Mitogenic regulators such as peptide growth factors, including epidermal growth factor (EGF) and related peptides such as transforming growth factor (TGF)-α, which share the same EGFR, are well recognized for playing pivotal roles in cancer progression via autocrine/paracrine stimulation of malignant epithelial cell growth [37]. Thus, an important antiproliferative action of melatonin may depend on the suppression of the EGFR mechanism [38].

Acting as a calcium/calmodulin (Ca²⁺/CaM) antagonist: Ca²⁺/CaM is another important molecule that plays a role in cell proliferation. It is involved in cell cycle progression and cytoskeletal integrity [39]. The antiproliferative effects of melatonin in certain cancer cells may depend on melatonin binding to Ca²⁺/CaM as well as on melatonin-induced changes in the intracellular distribution of CaM [39]. CaM binds to many enzymes and activates them, e.g., adenylate cyclase. Repressed adenylate cyclase activity is associated with reduced cAMP levels within cells, which can lead to altered PKA, cAMP binding protein, and p300 coregulator expression/activation, as well as the attenuation of phospho-activation and transactivation of various transcription factors [40]. Therefore, melatonin, acting as a CaM antagonist, may block reentry of cells into the cell cycle and mitosis [38].

Down-regulation of PKC: PKC appears to promote tumor growth because cytoskeletal proteins phosphorylated by PKC are necessary for mitosis. Melatonin down-regulation of PKC can be thus another mechanism involved in its oncostatic effect [38].

Decreasing gene transcription associated with cell proliferation: The transcription of Nestin, Bmi-1, and Sox2 has been used as a marker of cell proliferation. These genes are involved in the development of the nervous system [41] and in cancer progression [42-44]. Melatonin at high concentrations decreases cell viability and decreases transcript levels of genes associated with cell proliferation [45]. In addition, melatonin significantly increases gene expression of endocan and downregulates the activity of alkaline phosphatase and lactate dehydrogenase, thereby promoting differentiation of cancer cells, which may concur to melatonin's anticancer properties [46].

Ability to induce apoptosis

Although melatonin protects normal cells from apoptosis [47,48] it promotes apoptotic cell death in several types of cancer cells [10,49-53]. The reason for this discrepancy is not known. Therefore, a mechanistic clarification is needed regarding the differential responses of normal and cancer cells to melatonin in terms of apoptosis regulation.

The effects of melatonin on apoptosis are mediated by different and interacting pathways as

Activation of p53-related pathways: Melatonin-based apoptosis is assumed to involve activation of p53-related pathways. The p53 protein acts in apoptosis, cell cycle arrest, and DNA repair [54]; it causes cell cycle arrest primarily by activating the transcription of a cyclin-dependent kinase inhibitor, p21/waf1, and induces apoptosis via the

transcriptional activation of the pro-apoptotic Bcl-2 family member Bax gene [55].

Melatonin enhances p53 protein expression and that of several pro-apoptotic proteins including Bax and p21 [56] and depresses phosphorylated mouse double minute 2 homolog (MDM2), a major physiological antagonist of p53. AKT activation is performed through PI3K-dependent phosphorylation, and phosphorylated AKT (AKT-P) is required to phosphorylate MDM2, thus allowing MDM2 to enter into the nucleus and interact with p53. Melatonin significantly reduces the AKT-P/total AKT ratio [28].

Inactivation of the p53 gene is commonly observed in human cancer and is associated with resistance to cell death [57]. More than 50% of human cancers exhibit loss of normal p53 function and/or defects in the p53 signaling pathway. Therefore, agents that might inhibit the development of resistance to chemotherapy could be useful in clinical practice.

Activation of intrinsic and extrinsic apoptotic pathways: Several studies using different tumor cell types have also reported that melatonin induces caspase 3 and 9 activation [24,58,59]. Other studies have shown the release of pro-apoptotic agents from mitochondria, triggered by melatonin in tumor cells [10,24]. The mechanisms of melatonin's induction of apoptosis include mitochondrial membrane depolarization and permeability transition pore induction, which strongly suggests involvement of the mitochondrial-mediated pathway of apoptosis. Melatonin treatment in cholangiocarcinoma increases intracellular reactive oxygen species (ROS), which increase caspase activation because of their toxicity. Therefore, activation of ROS production by melatonin is associated with cytotoxicity in cancer cells [60].

Furthermore, melatonin increases calcium uptake, and the rise in calcium levels may lead to activation of PKC α together with PKC δ and, in consequence, could trigger the extrinsic apoptotic cascade [6]. In the extrinsic apoptotic pathway, melatonin could induce a pronounced rise in caspase 8 associated with augmented expression of both Fas and its ligand FasL.

The intrinsic and extrinsic pathways are connected by the caspase-8-mediated cleavage of the pro-apoptotic Bcl-2 family member BH3 interacting-domain death agonist (Bid), which translocates to mitochondria to trigger the release of cytochrome C. Melatonin increases the activation and association of Bax and Bid and is associated with a detectable rise in the expression of both proteins [11,49]. For example, melatonin upregulates Bax and the conversion of caspase-3 to cleaved form in human colorectal cancer cells [15]. Melatonin also promotes Bcl-2 down-regulation [10], suggesting that it may be an important endogenous cell death modulator. Moreover, melatonin synergistically promotes chemotherapy-induced apoptosis, mainly through downregulation of Bcl2 and elevation of pro-apoptotic proteins.

On the other hand, melatonin induces Bim expression [61]. Bim interacts with other Bcl-2 proteins to antagonize their anti-apoptotic activities, leading to apoptosis. Moreover, Bim is modulated by several transcription factors such as forkhead box proteins O (FOXO). Melatonin upregulates FOXO3a-mediated activation of the pro-apoptotic protein Bim and enhances endoplasmic reticulum stress-induced apoptosis through inhibition of COX-2 expression and reduction of Bcl-2 levels, and by an elevation of the pro-apoptotic transcription factor C/EBP homologous protein (CHOP). Woo et al. demonstrated that melatonin has an antitumor function through

down-regulation of COX-2 expression by inhibition of NF- κ B and p38 MAPK activation [62]. Therefore, the stimulatory effects of melatonin on apoptosis in cancer cells involve both the intrinsic and the extrinsic apoptotic pathways [6].

Regulation of histone deacetylases (HDACs): HDACs are critical regulators of gene expression that enzymatically remove the acetyl group from histones. Recent work has shown evidence of a close relationship between transcriptional repression by blockade of acetylation of histones and apoptosis induction. HDAC4, one of the class IIa HDACs, is an important regulator of gene expression as a part of transcriptional corepressor complexes. HDAC4 nuclear import is necessary for melatonin-induced H3 deacetylation on the bcl-2 promoter and subsequent bcl-2 suppression [63].

HDAC1s are frequently overexpressed in various types of human cancer, melatonin treatment decreasing expression of HDAC1 [64]. Fan et al. reported that melatonin acts as a suppressor in colorectal cancer cells and osteosarcoma cells via HDAC signaling inhibition. On the other hand, HDAC1 inhibitors induce ROS production, so oxidative stress might be an important mechanism by which melatonin induces cancer cell death [64].

Melatonin also downregulates sirtuin (Sirt) 1, thus leading to increased p53 acetylation. Acetylated p53 is preserved from degradation and triggers the intrinsic apoptotic pathway. Moreover, proliferation and viability of cancer cells are impaired through Sirt1 melatonin-dependent inhibition [39,65].

Activation of the TGF β -1 pathway: Melatonin-dependent late apoptosis is associated with activation of the TGF β -1 pathway, leading to increased phosphorylated mothers against decapentaplegic homolog (Smad) 2 and Smad3 levels and enabling interaction with Smad4 [16,28,66]. Smad2/Smad4 or Smad3/Smad4 complexes can thus enter the nucleus where they lead to the transcriptional induction of TGF β -1-related genes.

These data suggest that different apoptotic pathways are triggered by melatonin because TGF β 1 is involved only in a late stage of apoptosis. It seems that an early programmed cell death is associated with a significant increase in the p53/MDM2 ratio and with apoptosis-inducing factor (AIF) release, and that a late apoptotic process is TGF β 1-dependent, in which activated caspase 7 is associated with both caspase 9 activation and a reduced Bcl2/Bax ratio [66].

Thus, although melatonin can reduce cell proliferation by mechanisms that involve cell death by apoptosis [17,67], the exact pathways by which melatonin influences apoptosis and why it has both pro- and anti-apoptotic actions remain to be defined [68,69]. Because melatonin increases the population of necrotic cells, it may play an important role as a tumor suppressor and/or chemotherapeutic agent against tumors.

Modulation of the immune response

Natural killer (NK) cells are potent effectors of cancer immunoediting and can destroy tumors directly via exocytosis of cytotoxic granules. Other apoptotic-inducing mechanisms of NK cells, such as antibody-dependent cellular cytotoxicity, Fas ligand and tumor necrosis factor (TNF)- α secretion, have been discovered [70,71]. In addition to direct cytotoxicity, NK cells play an important role in the regulation of the anti-tumor adaptive immune response because they produce cytokines such as interferon- γ , TNF- α , interleukin (IL)-10, and several chemokines and growth factors. Hence, NK cells influence

macrophages, neutrophils and dendritic cells during the immune response [72].

A reduction in circulating NK cells has been described in cancer patients [73]. Numerous reports have demonstrated that melatonin increases the number of NK cells under a variety of conditions. Melatonin administration to leukemic mice results in a quantitative and functional enhancement of NK cells [74]. Although the mechanism has not been defined directly, one possibility is that melatonin acts through increased IL-2 production via melatonin receptors in T-helper cells [75], which leads to an increase in NK cell number and function [76]. T-helper cells play a crucial role in protection against malignancy. In addition, studies performed in patients with cancer have documented that immunological treatment with IL-2 plus melatonin induces a significant increase in the number of NK cells [77]. Therefore, the oncostatic actions of melatonin can also include direct augmentation of NK cell activity [2].

Melatonin increases not only the number of NK cells, monocytes, and leukocytes but also their production of interleukins (IL-2, IL-6, IL-10, IL-12) [9,78]. Melatonin exerts immunomodulatory anticancer activity by (a) augmenting the antitumor immune response by promoting IL-2 release from Th-1 lymphocytes, which may express melatonin receptors; (b) enhancing IL-12 production by dendritic cells in response to IL-2; and (c) decreasing oncogene expression and therefore biological malignancy [79]. Indeed, IL-2 is fundamental as a growth factor for T lymphocytes, with these cells playing an essential role in the generation of an effective anticancer immunity. Also, melatonin decreases expression of CD4+ CD25+ regulatory T cells and Foxp3 in tumor tissue [80].

Because of its antioxidative effects, melatonin reduces chemotherapy-induced lymphocyte damage [81]. An important strategy in cancer therapy is the activation of the immune system to induce a potent anti-tumor response. Thus the role of melatonin as an immunoenhancer deserves consideration [82] (Figure 3).

Metabolic effects

Two metabolic effects of melatonin are relevant for its oncostatic activity (Figure 3C).

Inhibition of aerobic glycolysis: Cancer cells use elevated amounts of glucose to enhance lactate production via glycolysis, which is maintained in conditions of high oxygen tension. This type of glucose metabolism is termed aerobic glycolysis [83], a phenomenon inhibited by melatonin, thus reducing glucose metabolism. Melatonin decreases the uptake of glucose and modifies the expression of the GLUT1 transporter in prostate cancer cells, supporting a critical role in the uptake of glucose by cancer cells [84].

Inhibition of linoleic acid uptake: In addition to glucose metabolism, linoleic acid serves as an energy source for tumor growth and as a specific tumor growth–signaling molecule. Several investigations have shown that high intake of linoleic acid increases growth rates of human and murine tumors [40,85]. Linoleic acid is converted within the tumor to 13-hydroxyoctadecadienoic acid (13-HODE), which augments the mitogenic effects of EGF and insulin-like growth factor-1 (IGF-1) to enhance downstream phosphorylation of ERK1/2 and AKT, leading to amplified cell proliferation and survival responses [86]. In addition, 13-HODE induces p38 MAPK [86].

Blask et al. [87,88] demonstrated that melatonin directly inhibits tumor growth by suppressing the cAMP-dependent tumor uptake of linoleic acid and its metabolism to the mitogenic molecule 13-

HODE. Melatonin may exert its suppressive effects on tumor linoleic acid metabolism via a MT_1 melatonin receptor–mediated reduction in cAMP formation. Blask et al. [89] demonstrated that in human breast cancer xenografts, suppression of the circadian amplitude of nocturnal melatonin production causes a significant increase in linoleic acid uptake and its conversion to 13-HODE, stimulating tumor growth. Light at night suppresses the direct antiproliferative effects of endogenous melatonin on human cancer [90,91]. Therefore, some antitumor effects of melatonin correlate with melatonin receptor (MT_1/MT_2)-dependent inhibition of linoleic acid uptake [89]. In addition, melatonin could suppress the Warburg effect by reducing 13-HODE formation and thus activation of AKT, which is a major stimulatory pathway for aerobic glycolysis [92].

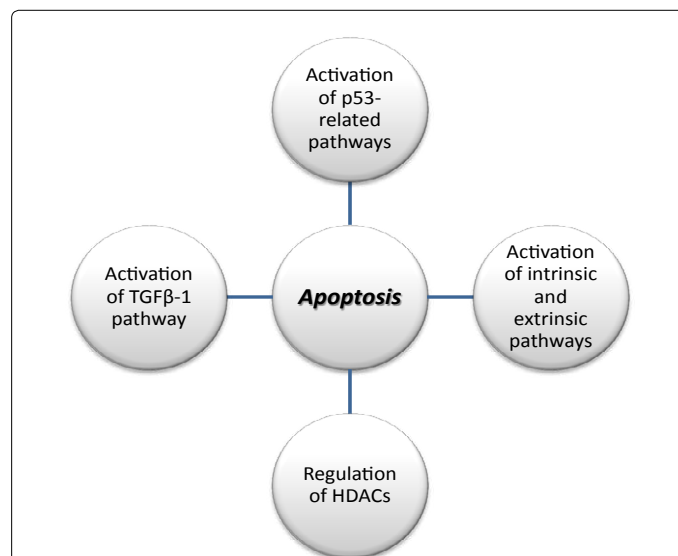


Figure 3A: The promotion of apoptosis in cancer cells produced by melatonin is given by the activation of p53-related pathways, activation of intrinsic and extrinsic apoptotic pathways, regulation of HDACs and activation of TGFβ-1 pathway.

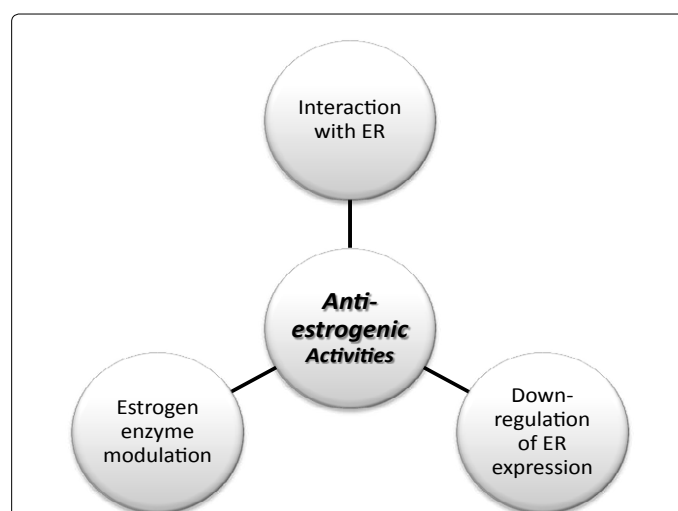


Figure 3B: Melatonin antiestrogenic activity includes the interaction with ER, down-regulation of ER expression and inhibition of testosterone aromatization. C. Melatonin metabolic effects related to oncostasis includes inhibition of aerobic glycolysis and of linoleic acid uptake. D. Melatonin effects on genomic instability comprise regulation of circadian gene disruption and normalization of L1 retrotransposon expression. See text for details.

Anti-angiogenic activity

Angiogenesis is an essential step in the development of primary tumors. Cancer cell growth relies on new vessel formation for nutrients and oxygen supply [93]. Tumor-induced angiogenesis is a complex process mediated and controlled by growth factors, cellular receptors and adhesion molecules. Hypoxia-inducible factor 1 (HIF1a) induces the expression of several genes such as vascular endothelial growth factor (VEGF), thus increasing new vessel formation and allowing metastatic spreading by connection to the preexisting vessels [94]. Moreover, VEGF appears to be frequently overexpressed in cancer cells, which consistently correlates with tumor size and histologic tumor grade [95].

The anti-angiogenic properties of melatonin have been reported in numerous studies. Melatonin decreases serum levels of VEGF in metastatic cancer patients [96]. Several studies have reported that melatonin-related anti-angiogenic activity in cancer cells is mediated, at least in part, by inhibition of HIF1a nuclear translocation, which is required for its transcriptional activation and subsequent VEGF expression [96,97]. Park et al. [98] demonstrated that melatonin suppresses tumor angiogenesis by inhibiting HIF-1 and VEGF via sphingosine kinase 1 in colon cancer cells. On the other hand, miRNAs play critical roles as modulators of angiogenesis [99]; e.g., overexpression of miRNA 3195 and miRNA 374b inhibits mRNA expression of HIF-1alpha, HIF-2alpha, and VEGF in hypoxia in prostate cancer cells, melatonin enhancing the expression of these miRNAs [100]. Therefore, the anti-angiogenic activity of melatonin can be the result of suppressing of HIF-1alpha by miRNA.

Endothelin-1 (ET-1) synthesis in blood vessels is considered to be one of the main stimulants of angiogenesis in primary tumors and contributes to angiogenesis and metastasis [101]. Melatonin suppresses the formation of ET-1 by inhibiting endothelin-converting enzyme 1 [102]. Additionally, this inhibition is associated with a reduction in edn-1 mRNA expression (the first step in ET-1 synthesis), which in turn results from the inactivation of FOXO1 and NF-B transcription factors [103].

Melatonin also inhibits other tumor growth factors, such as IGF and EGF, which are strong mitogens that stimulate tumor angiogenesis [102,104]. Therefore, melatonin seems to suppress cancer angiogenesis through several complementary mechanisms.

Antimetastatic effects

Metastasis formation involves changes in tumor cells to acquire greater migration and invasion capacity via mechanisms that are not yet fully understood [105]. Melatonin has antimetastatic effects mediated by the inhibition of p38 MAPK and matrix metalloproteinases 2 and 9 [106,107], which are involved in the degradation of the basement membrane and metastatic cell extravasation. Since PKC induces stress fiber thickening and decreases focal adhesion to promote tumor cell migration and invasion, melatonin may reduce stress fiber formation and thickening via PKC inhibition [108].

Epithelial-mesenchymal transition (EMT) has also been seen in cancer cells as they acquire invasive and metastatic phenotypes [109]. EMT is characterized by cellular changes, including the loss of cell adhesion proteins, cytoskeleton reorganization, and increased motility and invasiveness. Also linked to the progression of EMT is the Wnt/ β -catenin pathway, since β -catenin is a core component of the adherent junctions due to binding to E-cadherin [110]. Inhibition of glycogen synthase kinase (GSK) 3 β by AKT or Wnt signaling leads to metastasis.

However, when this pathway is activated, GSK3 β phosphorylates β -catenin, triggering its ubiquitination and subsequent proteasome-mediated degradation. Therefore, GSK3 β regulates EMT and metastasis via its phosphorylation by AKT [111]. Melatonin, by inhibition of AKT, leads to inhibition of EMT and the development of a metastatic phenotype [112]. Melatonin's activation of GSK3 β and inhibition of β -catenin may thus promote mesenchymal-to-epithelial transition (MET) to suppress the metastatic potential of tumors [113]. Melatonin could suppress metastasis by the blockade of AKT-mediated phosphorylation of GSK3 β , leading to the ubiquitination of β -catenin, as well as by the suppression of p-p38 MAPK [40].

An increase in inducible nitric oxide synthase (iNOS) expression is associated with early recurrence [114] and metastatic processes [115,116]. Belgorosky et al. [117] showed that human colorectal adenocarcinoma cells that express iNOS are more invasive than the non-iNOS-expressing cells. In addition, NO inhibition reduces vascularization, and the inhibition of angiogenesis is accompanied by tumor growth reduction [117]. It is well known that melatonin inhibits both the expression and activity of iNOS and NO levels [118-121].

Anti-estrogenic activities

The extensively studied anti-estrogenic properties of melatonin are the basis for its oncostatic actions in hormone-dependent mammary cancer [16,41] (Figure 3B).

Interaction with estrogen receptors and down-regulation of their expression, binding to DNA, and transactivation: Melatonin interferes with the activation of the estrogen receptor (ER), behaving as a selective ER modulator [122-124]. ER is a member of a superfamily of ligand-inducible transcription factors that bind to specific recognition sequences in the DNA of responsive genes. These genes become transcriptionally activated to produce mRNAs and proteins involved in numerous cell processes such as proliferation and differentiation. In the absence of estradiol (E2), the inactive receptor is complexed with a variety of proteins that block its ability to interact with DNA whereas in the presence of E2, the receptor undergoes a conformational change that allows its binding to coactivators, initiating the transcription of target genes. Melatonin downregulates ER expression by suppressing ER gene transcription, resulting in a reduction in ER mRNA and

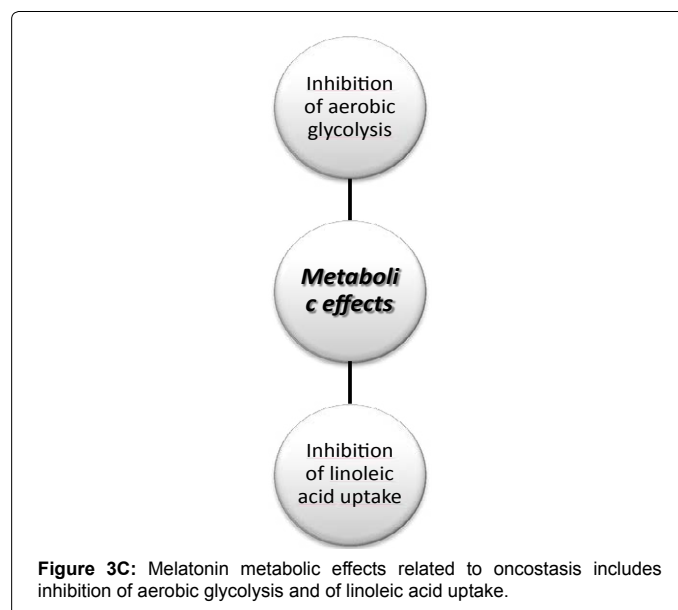


Figure 3C: Melatonin metabolic effects related to oncostasis includes inhibition of aerobic glycolysis and of linoleic acid uptake.

protein levels [125,126]. Melatonin also inhibits the mitogenic effects of E2 in cancer cells by blocking its ability to stimulate binding of ER to DNA: melatonin inhibits binding of the E2-ER complex to the estrogen response element (ERE) on DNA [38]. Melatonin cannot by itself affect the transcriptional activity of ER in the absence of E2.

The inhibitory effect of melatonin on ER is augmented in the presence of EGF. ER transactivation by melatonin plus EGF renders the receptor less sensitive to E2 and thus less efficient in regulating the transcription of E2-responsive genes that are critical for breast cancer cell proliferation [38]. Furthermore, melatonin not only inhibits the action of growth stimulatory factors but also stimulates the production or release of growth inhibitory factors [127].

The anti-estrogenic effects of melatonin seem to be mediated through MT₁ receptors, which are coupled to Gi proteins and inhibit adenylate cyclase activity, thus decreasing the activity of the cAMP/PKA signaling pathway [16]. ER α is activated by elevated intracellular cAMP levels. E2 increases cAMP, thus enhancing ER-mediated transcription [16]. The reduction of cAMP could be the mechanism underlying the decreased E2-induced ER α transcriptional activity brought about by melatonin [124].

In addition, the association of CaM with the E2-ER α complex facilitates its binding to DNA. Therefore, the inactivation of CaM by melatonin could also explain the anti-estrogenic actions of the methoxyindole [128]. Melatonin can be considered a specific inhibitor of E2-induced ER α -mediated transcriptional activation, whereas it does not inhibit ER β -mediated transactivation [16]. These data suggest that melatonin has an important influence on gene expression in human breast cancer cells.

Estrogen enzyme modulator action: In breast cancer occurring in postmenopausal women, estrogens are synthesized in the mammary tissue from androgenic precursors of adrenal origin. Estrogens are the product of androgen metabolism catalyzed by the aromatase enzyme complex [124]. Aromatase activity in breast cancer tissue is higher than in non-malignant breast tissue, resulting in an increased production of estrogen within breast tumors [129]. Melatonin inhibits the expression and activity of enzymes, such as P₄₅₀ aromatase, estrogen sulfatase, and 17 β -hydroxysteroid dehydrogenase, involved in the synthesis and transformation of biologically active estrogens, thus behaving as a selective enzyme modulator [130,131]. Melatonin also inhibits the increased proliferation of MCF-7 breast cancer cells induced by testosterone [132]. Because testosterone ultimately leads to proliferation via its transformation into estrogens, the inhibitory effects of melatonin could be due to the blockade of the formation of estrogens from androgens [132]. In addition, melatonin potentiates the effects of other anti-aromatases such as aminoglutethimide [16].

The ability of melatonin to modulate aromatase activity and expression has been explained by the binding of melatonin to MT₁ receptors [133]. Thus, cAMP can be the link between melatonin and aromatase activity in breast cancer cells. In addition, melatonin stimulates the expression and activity of estrogen sulfotransferase, the enzyme responsible for the transformation of E2 into the biologically inactive estrogen sulfates [16].

Capacity to decrease telomerase activity

Telomerase is a specialized ribonucleoprotein DNA polymerase that extends the telomeres of eukaryotic chromosomes. Activation of telomerase plays an important role in carcinogenesis, providing a mechanism for an unlimited neoplastic cell division capacity [90].

Telomerase is activated in most human cancers, and the death of tumor cells is associated with a decline in detectable telomerase activity [134]. In normal cells, melatonin increases telomerase activity [135] but in cancer cells, it attenuates telomerase activity both in vivo and in vitro [136]. All telomerases contain a telomerase catalytic protein component (TERT) and a RNA subunit, which constitute the minimum structure for telomerase activity. In MCF-7 cancer cells, melatonin inhibits telomerase activity and the expression of the TERT mRNA subunit [136]. Agonism of the nuclear receptor of melatonin decreases TERT RNA levels, whereas agonism of the MT₁ membrane receptor increases it [137]. This finding suggests an interaction between the membrane and nuclear melatonin signaling pathways to modulate telomerase activity.

Telomerase is considered an important therapeutic target because telomerase inhibition leads to cancer cell death. The reduction of telomerase expression by melatonin can be an important event in the ability of this molecule to limit tumor growth.

Function as a free radical scavenger

Melatonin has a marked dose-dependent antioxidative effect, providing protection against damage from carcinogenic substances and acting as a free radical scavenger [138]. ROS generation is a major factor involved in carcinogenesis [139]. Several transcription factors with roles in cell growth and death can be activated by ROS through distinct intracellular pathways. Moreover, free radicals damage all cellular components such as lipids, proteins, and DNA, and damage of DNA by oxidative stress has been implicated as a main contributing factor in the development of cancerous growth [139].

The oxidative damage of mitochondria is also strongly involved in carcinogenesis [140]; e.g., it is hypothesized that mitochondrial changes can be associated with cholangiocarcinoma development [141]. Mitochondrial impairment produces ROS and reactive nitrogen species (RNS) that in turn reduce mitochondrial bioenergetics, favoring cell damage and death [142,143]. Effectively, melatonin treatment significantly inhibits cholangiocarcinoma development [141]. Melatonin is a special class of antioxidant because when scavenging free radicals, it is processed in a series of metabolites that are also free radical scavengers [144]. In addition to direct scavenger activity, melatonin has a genomic effect, inducing expression of antioxidant enzymes such as glutathione peroxidase, glutathione reductase and superoxide dismutase [145] as well as reducing the expression of pro-oxidative enzymes, e.g., iNOS [118,121]. Therefore, melatonin may inhibit cancer growth through its ability to directly or indirectly neutralize ROS and RNS production.

Another interesting aspect of melatonin is that it is taken up by mitochondria, providing in situ protection against oxidative damage [146,147]. Several reports have shown that melatonin improves mitochondrial function, reduces mitochondrial oxidative status, and increases the activity of the respiratory chain [146,148]. Melatonin, but not other antioxidants (e.g., vitamins C and E, N-acetylcysteine) is highly efficient in maintaining mitochondrial glutathione homeostasis in extremely oxidative conditions, closing the mitochondrial permeability transition pore [149] and promoting mitochondrial survival [150,151].

Free radicals and their derivative products can activate nuclear factors such as NF- κ B, leading to the production of proinflammatory cytokines, which in turn enhance inflammation and further ROS generation [152]. Therefore, antioxidants capable of decreasing intracellular free radicals also can reduce NF- κ B activation and

proliferation of cancer cells [18]. As already mentioned, melatonin has potent anti-inflammatory properties [118,119,153-156] and prevents NF- κ B activation by oxidative stress [157]. It inhibits growth of glioma cells, and this inhibition is associated with a decrease in basal levels of intracellular free radicals [19,158] and inhibition of NF- κ B transcription [18]. Antioxidant agents can enhance the cytotoxic action of chemotherapeutic drugs [159].

Regulation of genomic instability

Two aspects of genomic stability are relevant for melatonin-induced oncostasis. They include regulation of circadian gene expression and that of the long interspersed element 1 (L1) retrotransposon (Figure 3D).

Circadian disruption: The production of melatonin at night by the pineal gland represents a highly reliable output signal of the circadian clock, and the suppression of pineal melatonin production in response to light at night might explain the rise in cancer that has accompanied industrialization [160,161]. The repression of the nocturnal circadian melatonin signal promotes tumor aerobic glycolysis and the expression and activation of the signaling pathways involved in tumor proliferation and survival that drive resistance in cancer cells to endocrine therapies and chemotherapies [40]. Therefore, the melatonin signal regulates metabolic and cell signaling activities to inhibit cancer initiation, promotion, and progression [162], and these effects can be mediated by melatonin MT₁/MT₂ receptors [163].

Circadian synchronization is coordinated by the suprachiasmatic nuclei (SCN) of the hypothalamus, as the master clock located in SCN neurons controls peripheral circadian clock genes. At the same time, these genes can subsequently regulate the clock-controlled genes that are involved in the cell cycle [164]. Light may directly affect tumor growth through PER1 and PER2, which in turn regulate cell cycle and apoptosis-regulated genes. Effectively, the deregulation of per and *Cry* clock genes is related to cancer development. Melatonin is involved in modulating clock genes and therefore could restore abnormal apoptotic processes [6].

Melatonin can also alter DNA methylation patterns, thus decreasing

the expression of oncogenic genes while simultaneously up-regulating the expression of tumor suppressor genes. Light exposure at night may affect overall DNA methylation and clock gene expression, including PER2, to promote tumor progression [40].

Regulation of L1 expression: One well-known cause of cancer is genomic instability, and one of the intrinsic DNA-damaging agents that can cause different types of genomic instability in human cancers is the L1 retrotransposon. It has been reported that melatonin suppresses the expression of L1 in human cancer in a receptor-mediated manner [165].

On the other hand, it seems that light exposure at night promotes genomic instability. Therefore, upregulation of L1 expression and light exposure at night could be contributing factors driving genomic instability relevant to cancer risk. De Haro et al. reported that expression of endogenous L1 elements in prostate cancer is suppressed by circulating melatonin and that this regulation is disrupted by exposure to light at night, which suppresses nocturnal melatonin production.

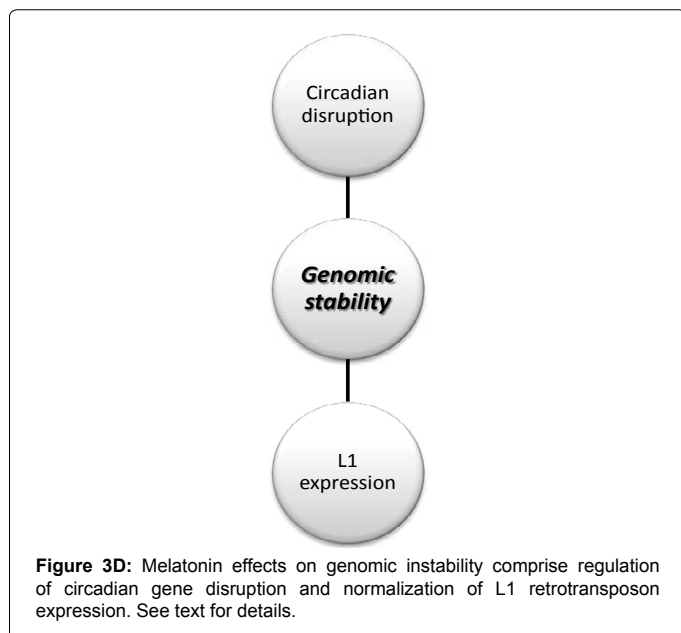
The general conclusion is that melatonin exerts anticancer effects by multiple pathways and in a number of experimental models in vivo and in vitro. Table 1 summarizes relevant data on this subject. Melatonin decreases growth in most tumor cell lines by increasing necrosis, decreasing proliferation, and increasing apoptosis [14,141,166]. Taken together, the different pathways involved in carcinogenesis, including cell proliferation and metastasis, are important targets of melatonin, which represents a unique molecule able to interact with these different pathways, reducing oncogenesis. Further studies are needed to determine the multiple oncostatic mechanisms of action of melatonin.

In addition, melatonin increases the efficacy of anticancer drugs and has multiple protective effects against drug toxicity [167,168]. The data support the clinical use of melatonin in the co-treatment of cancer.

Clinical Application of Melatonin in Cancer

Three are the major reasons why melatonin deserves to be considered in the treatment of cancer. First, melatonin is a hypnotic-chronobiotic agent that can allow the clinician to effectively address sleep disturbances, a major co-morbidity in cancer. Second, the anxiolytic and antidepressant effects of melatonin underline its possible application in two other major co-morbidities (i.e. depression and anxiety) in cancer patients. Third, melatonin has a number of oncostatic properties (as reviewed in the previous sections of this article) that could make it an effective adjuvant of chemotherapy and radiotherapy. We will briefly review these three possible applications of melatonin from a clinical standpoint.

Sleep disorders are very common among cancer patients [169]. However, they generally remained underdiagnosed and poorly treated [170-173]. In a cross-sectional survey study on nearly 1,000 cancer patients to examine the prevalence of sleep problems, sleep disturbance was most prevalent among the lung and breast cancer patients. Sleep complaints included excessive fatigue (44% of patients), restlessness leg syndrome (41%), insomnia (31%) and excessive diurnal somnolence (28%) [174]. It must be noted however that an imprecise conceptualization of sleep has led to narrowly focused interventions being diffusely targeted to symptoms, rather than focused and specific to one or more sleep disorders underlying those symptoms [175]. This is important because although many interventions for sleep in cancer have shown efficacy, the majority of these studies are too targeted to undefined subtypes of insomnia.



Subjects	Measured	Results	Ref
20 women with clinical stage I or II breast cancer	Plasma melatonin levels over 24 h	In 10 patients, whose tumors were estrogen receptor positive, the nocturnal increase in plasma melatonin was much lower than that observed in 8 control subjects. Women with the lowest peak concentration of melatonin had tumors with the highest concentrations of estrogen receptors. A significant correlation was found between the peak plasma melatonin concentration and the tumor estrogen receptor concentration in 19 of the patients	[218]
Normal individuals, women with breast cancer, and women at high risk for breast cancer	Plasma melatonin levels over 24 h	The mean daytime and nighttime plasma levels, and the range of melatonin day to night differences for women with breast cancer and women at high risk for breast cancer were comparable to each other and to the normal subjects. Women with estrogen or progesterone receptor-positive tumors had a significantly lower mean plasma melatonin day to night difference. A strong inverse correlation was observed between the plasma melatonin concentration and the quantities of estrogen or progesterone receptor in the primary tumor. Plasma melatonin did not correlate with tumor glucocorticoid receptor content or stage of breast cancer among these patients, or with menopausal status, age, parity, or the plasma levels of estrone, estradiol, progesterone or gonadotropins among all individuals studied.	[219]
42 cancer patients of both sexes (breast cancer, 10; lung cancer, 13; colon cancer, 11; soft tissue sarcoma, 4; testicular cancer, 1; Hodgkin's disease, 1; peritoneal mesothelioma, 2).	Melatonin serum levels before and 28 days after each cycle of chemotherapy	Regardless of the type of tumor and chemotherapeutic regimen, 12/16 patients (75%) whose melatonin enhanced after chemotherapy had an objective regression. In contrast, 2/26 patients only (8%) whose melatonin did not enhance after chemotherapy had a clinical response. The percentage of objective responses was statistically significantly higher in patients with a chemotherapy-induced melatonin increase than in those with no melatonin increase	[220]
35 patients with breast cancer (23 primary tumors); 28 patients with untreated benign breast disease	Serum melatonin levels over 24 h	A 50 % depression of peak and amplitude found in the group of patients with primary breast cancer. The peak significantly declined with increasing tumor size: 27 % at Stage T1, 53 % at T2 and 73 % at T3. Patients with secondary breast cancer had a melatonin profile similar to controls	[221]
86 patients with breast cancer	Daytime plasma melatonin values	Patients in the advanced disease group had significantly higher levels than those in the adjuvant treatment group, and patients with progressive disease had significantly higher values than those in remission or with stable disease. No significant differences were found between different dominant metastatic disease sites. Multiple-regression tests showed a significant inverse correlation between survival and melatonin values	[197]
Patients with benign (14) or malignant (10) breast cancer vs. 160 controls	Daily pattern of urinary aMT6s	Women with malignant tumors had significantly lower 24 h concentrations of urinary aMT6s with a decrease in the amplitude of the rhythm compared to women with benign tumors. The amount of urinary aMT6s was dependent upon the age of the subject but was not affected by either menopausal status or body mass index. However, when the women with malignant tumors were compared with a large group of normal women of the same age their urinary aMT6s levels were not outside the normal range	[222]
17 patients with breast cancer (9 primary tumors); 4 patients with untreated benign breast disease	Serum melatonin and aMT6s levels over 24 h	Nocturnal melatonin and aMT6s levels and their circadian amplitudes were significantly depressed in the group of patients with primary breast cancer. In contrast, patients with secondary breast cancer showed nocturnal melatonin and aMT6s concentrations and amplitudes similar to benign breast disease	[223]
8 young men, 7 elderly patients with benign prostatic hyperplasia and 9 patients of similar age with primary prostate cancer	Serum and urine melatonin and aMT6s levels over 24 h	The circadian patterns of melatonin and aMT6s in serum were very similar in the different groups. Mean value and amplitude were significantly depressed by 40-60% in with primary prostate cancer (40-60%) as compared to the other groups. Circadian rhythms similar to those of serum were found in urine	[224]
138 women (68 were diagnosed with endometrial cancer, 70 had abnormal bleeding)	Plasma melatonin levels	A significant correlation was found between melatonin plasma levels and the presence of endometrial cancer. The mean plasma melatonin value was 6.1 pg/ml in the cancer-positive group and 33.2 pg/ml in the cancer-negative control group.	[225]
10 patients with untreated non small cell lung cancer vs. 10 healthy subjects	Diurnal plasma rhythm of melatonin, cortisol and other hormones	Melatonin levels and melatonin/cortisol ratio were significantly lower in patients. Disruption of circadian rhythmicity of melatonin levels were found in cancer patients.	[226]
127 patients diagnosed with breast cancer and 353 matched control subjects	Levels of aMT6s in 24-h urine samples	No statistically significant differences in urinary aMT6s concentrations were observed between women who developed breast cancer and control premenopausal or postmenopausal women	[198]
147 women with invasive breast cancer and 291 matched control subjects	Levels of aMT6s in the first morning urine	In logistic regression models, the relative risk of invasive breast cancer for women in the highest quartile of urinary aMT6s compared with those in the lowest was 0.59. This association was essentially unchanged after adjustment for breast cancer risk factors or plasma sex hormone levels but was slightly weakened when the analysis included 43 case patients with in situ breast cancer and their 85 matched control subjects. The exclusion of women who had a history of night-shift work left our findings largely unchanged.	[227]
3,966 postmenopausal women	Levels of aMT6s in 12-h overnight urine samples	Increased melatonin levels were associated with a statistically significantly lower risk of invasive breast cancer in postmenopausal women. Among the 3966 women in the cohort, 40 of the 992 women in the highest quartile of aMT6s developed breast cancer during follow-up, compared with 56 of the 992 women in the lowest quartile	[228]
33,528 women (follow-up 11 years). 525 incident cases of breast cancer	Self-reported sleep duration. Levels of aMT6s in 24-h urine samples	Among women postmenopausal at baseline, breast cancer risk decreased with increasing sleep duration. Irrespective of gender, urinary aMT6s levels increased with increasing self-reported hours of sleep.	[229]
180 premenopausal women with incident breast cancer and 683 matched controls	Levels of aMT6s in 12-h overnight urine samples	In logistic regression models, the relative risk of invasive breast cancer for women in the highest quartile of total overnight aMT6s output compared with the lowest was 1.43. A relative risk (OR= 0.68) was found between overnight aMT6s level and breast cancer risk in women with invasive breast cancer diagnosed >2 years after urine collection and a significant inverse association in women with a breast cancer diagnosis >8 years after urine collection (OR, 0.17).	[230]

Subjects	Measured	Results	Ref
640 nurses and midwives, 321 working on rotating night shifts vs 319 working on only day basis	Morning levels of aMT6s in urine samples. Computer-assisted measure of mammo-graphic density	The adjusted means of percentage of mammographic density and absolute density were slightly, non significantly, higher among women working rotating night shifts. There was no significant association between morning aMT6s and mammographic density	[231]
Prospective cohort study (1977-2009) of 251 breast cancer cases and 727 matched controls	Morning levels of aMT6s in urine samples.	No significant association was found between aMT6s and breast cancer risk, either overall (for highest third vs. lowest, multivariable-adjusted odds ratio = 0.90, 95% confidence interval: 0.61, 1.33) or by menopausal status.	[232]

aMT6s: 6-sulfoxy-melatonin

Table 1: Melatonin levels in cancer patients.

The possible causes of sleep disorders in patients with cancer are diverse; when pre-existing sleep problems occur they often seem to be aggravated by cancer. Cancer itself (e.g. tumor invasion symptoms, pain), chemotherapy, corticosteroid treatment, environmental factors or psychological distress are among the factors to contribute to disruption of sleep. Sleep loss can be followed by immunosuppression thus impacting on the course of the disease [169,170,176,177].

The type and frequency of hypnotic medication were recorded in a sample of 1984 patients who had previously underwent cancer treatment [178]. Hypnotics were taken by 22.6% of patients. Among the factors associated with increased use of hypnotic medication, to be older, to have more stressful life events experienced during the past 6 months, to suffer higher levels of anxiety or past or current chemotherapy treatment, were quoted. Almost 80% of participants who were taking drugs were prescribed benzodiazepines (mostly lorazepam and oxazepam), followed by zopiclone (9%). It must be noted that regardless that many drugs are currently approved for the treatment of insomnia, very few have been tested for safety or efficacy in patients with cancer [179].

Melatonin can have a place in treating sleep disorders in cancer patients. Its potentiality in treating sleep disturbances is relevant because the sleep-promoting compounds that are usually prescribed in cancer patients, like benzodiazepines and related Z drugs, have many adverse effects, such as next-day hangover, dependence and impairment of cognition. Indeed, a number of studies point out to a beneficial effect of melatonin in a wide variety of sleep disorders [180] and melatonin is increasingly recognized as an effective medication to stop benzodiazepine/Z drug abuse in patients [181]. Melatonin has been used for improving sleep in patients with insomnia mainly because it does not cause hangover or show any addictive potential. Melatonin's efficacy has been demonstrated in most [182,183] but not all meta-analysis [184]. Brain imaging studies in awake subjects have revealed that melatonin modulates brain activity pattern to one resembling that of actual sleep [185]. A consensus of the British Association for Psychopharmacology on evidence-based treatment of insomnia, parasomnia and circadian rhythm sleep disorders concluded that melatonin is the first choice treatment when a hypnotic is indicated in patients over 55 years [186].

As melatonin exhibits hypnotic and chronobiotic properties of a short duration, the need for the development of prolonged release preparations of melatonin or of melatonin agonists with a longer duration of action on sleep regulatory structures in the brain arose. Slow release forms of melatonin (e.g., Circadin®, a 2 mg- preparation developed by Neurim, Tel Aviv, Israel, and approved by the European Medicines Agency, EMEA, in 2007) and the melatonin analogs ramelteon (approved by the Food and Drug Administration, FDA, in 2005), agomelatine (approved by EMEA in 2009) and tasimelteon

(approved by FDA in 2013) are examples of this strategy. It must be noted that as shown by the binding affinities, half-life and relative potencies of the different melatonin agonists it is clear that studies using 2-5 mg melatonin/day are unsuitable to give appropriate comparison with the effect of the above mentioned compounds, which in addition to being generally more potent than the native molecule are employed in considerably higher amounts [180].

Concerning the second reason why melatonin can be useful in cancer patients, depression is a frequent and serious comorbid condition affecting the quality of life. Such comorbidity reduces the compliance with treatment and aggravates the physical consequences of the disease. Although there are studies showing that about 40% of tumor patients need professional psycho-oncological support [187] only less than 10% of patients are referred for psychosocial intervention in daily clinical practice [188]. Studies of effective pharmacotherapy are relatively scarce in cancer patients with depression and they are biased by a high number of dropouts due to side effects relating to the use of antidepressants compared to placebo [179]. It is therefore difficult to determine with clarity as to what is the best pharmacological treatment for major depression in cancer patients.

Circadian rhythm abnormalities, as shown by the sleep/wake cycle disturbances, constitute one of the most prevalent signs of depression [189]. The disturbances in the amplitude and rhythm of melatonin secretion that occur in patients with depression resemble those seen in subjects with chronobiological disorders, thus suggesting a link between melatonin secretion disturbance and depressed mood. Since melatonin is involved in the regulation of both circadian rhythms and sleep, any antidepressant drug with effects on melatonin receptors could be an advantage in treatment. Melatonin treatment has been found effective to treat circadian rhythm disorders [180,190]. As far as its antidepressant activity, melatonin (10 mg/day) was inactive to affect bipolar affective disorder [191] and improved sleep with no effect on symptoms of depression in major depressive disorder [192,193]. Among the analogs developed to improve the efficacy of melatonin's effects, agomelatine (Valdoxan®, Servier, France) has been licensed by the EMEA for the treatment of major depression disorder in adults. Agomelatine has a unique pharmacological profile as it is both a MT₁/MT₂ melatonin receptor agonist and an antagonist of 5-HT_{2C} receptors. As the first melatonergic antidepressant, agomelatine displays a non-monoaminergic mechanism of action [180]. MT₁ and MT₂ receptors also appear to be involved in sedating and anxiolytic effects of melatonergic drugs which have been linked to a facilitatory role of melatonin on γ -aminobutyric acid transmission [194]. This antiexcitatory action of melatonin may underlie the anxiolytic, antihyperalgesic and antinociceptive effects of melatonergic agents, all them of potential application in cancer patients [4,181]. In a double-blind, placebo-controlled study of 54 women undergoing surgery for breast cancer and receiving 6 mg of melatonin or placebo for 3 months,

the risk of developing depressive symptoms was significantly lower than placebo [195]. Likewise, health-related quality of life assessment in patients with advanced, non-small cell lung cancer and receiving 10–20 mg melatonin daily was better than placebo, particularly in social well-being [196]. A higher extent of DNA damage was observed in the placebo group, and this was associated with a lower survival, implying the protective effect of melatonin in healthy cells [196]. In another study, 95 postmenopausal women with a prior history of stage 0-III breast cancer and who had completed active cancer treatment, 3 mg of melatonin or placebo was used for 4 months. Subjects receiving melatonin experienced significantly greater improvements in subjective sleep quality but there were no significant differences in measures of depression or hot flashes, presumably because of the low amounts of melatonin used [171] (Table 2).

With few exceptions [197-199], melatonin levels were found to be decreased in cancer patients (see Supplementary Table 1). One active issue in scientific research is the possibility that working non-day hours is associated with an increased risk of cancer, most notably breast and prostate cancer. The major idea behind this is that the reduced melatonin secretion plays a crucial role in the occurrence of cancer. A number of studies have addressed this question by employing both case-control and cohort designs, thus supporting [200,201] or not supporting [202] such an association. The inconsistencies may depend on the definition of “shift work” [203]. A major support derives from meta-analysis studies, a procedure that combines data from several studies and treats those data as one large study. Although meta-analyses

can be informative, they are questionable because of the dissimilarity among studies.

This argument is also relevant for results on the third aspect of clinical application of melatonin in cancer, i.e. the therapeutic effect of melatonin in cancer patients. Literature data on this point are summarized in the Supplementary Table 2. Two meta-analyses have been published to assess melatonin efficacy in treating cancer patients. The first meta-analysis was a systematic review of randomized controlled trials of melatonin in solid tumor cancer patients and its effect on survival at 1 year [204]. It included 10 studies published between 1992 and 2003 and comprised 643 patients. Melatonin reduced the risk of death at 1 year with effects consisting across melatonin dose, and type of solid cancer. No relevant adverse events were reported.

In the second meta-analysis 8 eligible randomized controlled trials of solid tumor cancers (n=761) were selected [205]. The dosage of melatonin used was 20 mg orally, once a day. Melatonin significantly improved the complete and partial remission and 1-year survival rate and decreased radiochemotherapy-related side effects. It must be noted that all trials examined in both meta-analyses included solid tumor cancers and were unblinded and all except one were conducted by the same group of researchers at the same hospital network.

In addition, relevant negative results concerning melatonin efficacy in cancer patients have been published. In a trial designed to compare whole brain radiation therapy alone to radiation therapy and 20 mg/day melatonin for patients with brain metastases from solid tumors, neither

Subjects	Design	Study's duration	Treatment	Measured	Results	Ref.
33 patients with metastatic renal cancer	Randomized open trial	5 days	IL-2 alone or IL-2 plus melatonin (10 mg/day p.o. at 2000 h)	Clinical outcome	The frequency of episodes of severe hypotension and depressive symptomatology were significantly greater during IL-2 alone than during IL-2 + melatonin. No other toxicity, including capillary leak syndrome, vomiting and fever, were significantly influenced by the concomitant treatment with melatonin	[233]
54 patients with metastatic solid tumors	Open trial	2 months	Melatonin was given i.m. at a daily dose of 20 mg at 1500 h for 2 months	Clinical outcome	The clinical response was as follows: 1 partial response (cancer of pancreas), 2 minor responses (colon cancer and hepatocarcinoma) and 21 with stable disease. The remaining 30 patients rapidly progressed within the first 2 months of therapy	[234]
42 patients with advanced melanoma	Open label study	Median follow-up of 33 weeks	Melatonin from 5 to 700 mg/m ² /day in four divided doses p.o.	Clinical outcome. Serum FSH, LH and TSH levels	6 patients had partial responses and 6 additional patients had stable disease. The median response duration was 33 weeks for the partial responders. The toxicity encountered was minimal and consisted primarily of fatigue in 17 of 40 patients. Decreased levels of FSH were found	[235]
63 consecutive metastatic non small cell lung cancer patients	Randomized open trial	1 year	Patients were randomized to receive melatonin (10 mg p.o. at 1900 h, n = 31) or supportive care alone (n = 32)	Clinical outcome	The percentage of both stabilizations of disease and survival at 1 year was significantly higher in patients treated with melatonin. No drug-related toxicity was seen in patients treated with melatonin	[236]
20 metastatic non small cell lung cancer patients	Open trial	4 weeks	Melatonin was given p.o. at a daily dose of 10 mg at 2000 h starting 7 days before the onset of IL-2 administration. IL-2 was given s.c. at a dose of 3 x 10 ⁶ IU.m ² every 12 h for 5 days/week for 4 weeks. In responder patients a second cycle was given after a rest-period of 21 days.	Clinical outcome	A partial response was achieved in 4/20 (20%) patients. Ten other patients had a stable disease (50%), whereas 6 patients progressed. Toxicity was low in all cases	[237]

Subjects	Design	Study's duration	Treatment	Measured	Results	Ref.
30 advanced cancer patients	Randomized open trial	4 weeks	Patients were randomized to receive IL-2 (3×10^6 IU.m ² twice/daily for 6 days/week for 4 weeks, with or without melatonin 10 or 50 mg given daily p.o. at 2000 h	Immune parameters	IL-2 together with melatonin, but not IL-2 alone, induced a significant increase in mean number of lymphocytes, T lymphocytes, NK cells, CD25-positive cells and eosinophils. Soluble IL-2 receptor and neopterin increase was significantly higher during IL-2 given twice/daily than during IL-2 plus melatonin, while no difference was seen in TNF rise	[238]
35 patients with advanced tumors of the digestive tract (colorectal cancer: 14; gastric cancer: 8; hepatocarcinoma: 6; pancreas adenocarcinoma: 7)	Open trial	4 weeks	Melatonin was given p.o. at a daily dose of 50 mg at 2000 h starting 7 days before the onset of IL-2 administration. IL-2 was given s.c. at a dose of 3×10^6 IU.m ² every 12 h for 6 days/week for 4 weeks)	Clinical outcome	A complete response was achieved in two patients (gastric cancer: 1; hepatocarcinoma: 1). Six other patients obtained a partial response: (gastric cancer: 2; hepatocarcinoma: 2; colon cancer: 1; pancreas cancer: 1). Stable disease was obtained in 11/35 (31%) patients, whereas the remaining 16 patients (46%) progressed. The response rate was significantly higher in untreated patients than in those previously treated with chemotherapy	[239]
82 patients, 72 of whom showed distant organ metastases (non-small cell lung cancer: 19; hepatocarcinoma: 16; colon cancer: 15; gastric cancer: 11; cancer of pancreas: 11; breast cancer: 6; other: 4)	Open trial	4 weeks	Melatonin was given p.o. at a daily dose of 40 mg at 2000 h starting 7 days before the onset of IL-2 administration. IL-2 was given s.c. at a dose of 3×10^6 IU/m ² every 12 h for 6 days/week for 4 weeks)	Clinical outcome, immune parameters	Objective tumor regression were achieved in 17/82 (21%) patients. The median duration of response was 8+ months. A stabilization of disease was obtained in 30 patients, while the other 35 patients progressed. The lack of progression was associated with a significantly higher increase in lymphocyte and eosinophil mean number and with a significantly lower increase in neopterin mean levels. The treatment was well tolerated in all patients	[240]
14 patients with metastatic gastric cancer	Open trial	Variable	Melatonin was given p.o. at a daily dose of 50 mg at 2000 h starting 7 days before the onset of IL-2 administration. IL-2 was given s.c. at a dose of 3×10^6 IU/m ² every 12 h for 6 days/week for 4 weeks). In patients in whom the disease did not progress, a second cycle was given after a rest period of 21 days	Clinical outcome	A tumor regression was obtained in 3/14 (21%) patients, complete response in 1 and partial in 2, with a median duration of 13 + months. The disease stabilized in 6/14 (43%) patients and progressed in the remaining 5 (36%). Survival was significantly longer in patients with response or stable disease than in those with progression. Toxicity was low in all cases	[239]
14 patients with un-resectable hepato-cellular carcinoma	Open trial	Variable	Melatonin was given p.o. at a daily dose of 50 mg at 2000 h starting 7 days before the onset of IL-2 administration. IL-2 was given s.c. at a dose of 3×10^6 IU/m ² every 12 h for 6 days/week for 4 weeks). In patients in whom the disease did not progress, a second cycle was given after a rest period of 21 days	Clinical outcome	Objective tumor regressions were obtained in 5/14 (36%) patients (one complete response, four partial responses), with a median duration of 7 months. 6 patients had stable disease, while the other 3 progressed. Toxicity was low in all cases	[241]
50 patients with brain metastases due to solid tumors	Randomized open trial	1-2 years	Supportive care alone (steroids plus anticonvulsant agents) or supportive care plus melatonin (20 mg/day p.o. at 2000 h)	Clinical outcome	The survival at 1 year, free-from-brain-progression period, and mean survival time were significantly higher in patients treated with melatonin than in those who received the supportive care alone	[242]
22 patients with metastatic renal cell carcinoma	Open trial	12 months	Human IFN, 3 mega- units i.m.3 times per week, plus melatonin, 10 mg p.o. every day	Clinical outcome	There were seven remissions (33%): three complete, involving lung and soft tissue and four partial. Nine patients achieved stable disease, and five progressed. General toxicity was mild. Fever, chills, arthralgias, and myalgias occurred rarely. Leukopenia and hepatic enzyme elevation were modest and always reversible	[243]
14 metastatic solid tumor patients	Randomized open trial	12 days	Recombinant human TNF was given at a daily dose of 0.75 mg i.v. for 5 consecutive days. Melatonin was given p.o. at a daily dose of 40 mg, starting 7 days before TNF	Clinical outcome	Lymphocyte mean number observed at the end of TNF infusion was significantly higher in patients treated with TNF plus melatonin than in those receiving TNF alone. Asthenia and hypotension were significantly less frequent in patients treated with TNF plus melatonin, whereas no difference occurred in the frequency of fever and chills	[244]

Subjects	Design	Study's duration	Treatment	Measured	Results	Ref.
60 patients with locally advanced or metastatic non-small cell lung cancer who were randomized to receive immune-therapy or chemo-therapy	Randomized open trial	6 months	Immunotherapy consisted of IL-2 (3 million IU/day s.c. for 6 days/week for 4 weeks) and melatonin (40 mg/day orally every day, starting 7 days before IL-2); in nonprogressing patients, a second cycle was repeated after a 21-day rest period, then they underwent a maintenance period consisting of one week of therapy every month until progression. Chemotherapy consisted of cisplatin and etoposide; cycles of chemotherapy were repeated every 21 days until progression	Clinical outcome	No complete response was obtained. Mean progression-free period and the percentage survival at 1 year were significantly higher in patients treated with immunotherapy than in those treated with chemotherapy. Toxicity was substantially lower in patients receiving immunotherapy than in those given chemotherapy	[245]
30 patients with gastro-intestinal tract tumors	Randomized open trial	1 week	A high-dose IL-2 (18 million IU/day s.c. for 3 days) or low-dose IL-2 (6 million IU/day s.c. for 5 days) plus melatonin (40 mg/day orally). Patients underwent surgery within 36 hours from IL-2 interruption	Clinical outcome Immune parameters	IL-2 plus melatonin were able to prevent surgery-induced lymphocytopenia but rather an increased mean number of lymphocytes, T lymphocytes and T helper lymphocytes were found. Toxicity was less in patients treated with IL-2 plus melatonin	[246]
14 patients with untreatable endocrine tumors (mostly thyroid cancer, carcinoid and endocrine pancreatic tumors)	Phase II pilot study	2 months	Melatonin was given p.o. at a daily dose of 50 mg at 2000 h starting 7 days before the onset of IL-2 administration. IL-2 was given s.c. at a dose of 3×10^6 IU/m ² every 12 h for 6 days/week for 4 weeks). In patients in whom the disease did not progress, a second cycle was given after a rest period of 21 days	Clinical outcome	A partial response was achieved in 3/12 (25%) patients (carcinoid tumor: 1; neuroendocrine lung tumor: 1; pancreatic islet cell tumor: 1). Another patient with gastrinoma had a more than 50% reduction of tumor markers. Toxicity was low in all patients	[247]
14 advanced solid tumor patients, affected by thrombocytopenia	Open trial		Melatonin was given p.o. at a daily dose of 40 mg at 2000 h starting 7 days before the onset of IL-2 administration. IL-2 was given s.c. at a dose of 3×10^6 IU.m ² every 12 h for 6 days/week for 4 weeks)	Clinical outcome. Platelet number	A normalization of platelet number occurred in 10/14 (71%) patients, and platelet mean number significantly increased on treatment. No important therapy-related toxicity was observed	[248]
40 patients with estrogen receptor-negative post-menopausal metastatic breast cancer patients	Randomized open trial	1 year	Patients were randomized to receive tamoxifen alone (20 mg/day orally) or tamoxifen plus melatonin (20 mg/day p.o. in the evening)	Clinical outcome	No complete response was seen. Partial response rate was significantly higher in patients treated with tamoxifen and melatonin than in those receiving tamoxifen alone (7/19 vs 2/21). Percent of survival at 1 year was significantly higher in patients treated with tamoxifen plus melatonin than in those treated with tamoxifen alone (12/19 vs 5/21)	[249]
50 patients with metastatic colorectal cancer	Randomized open trial	1 year	Patients were randomized to receive supportive care alone or s.c IL-2 (3 million IU/day for 6 days/week for 4 weeks) plus melatonin (40 mg/day orally)	Clinical outcome	No spontaneous tumor regression occurred in patients receiving supportive care alone. A partial response was achieved in 3/25 patients treated with immunotherapy. Percent survival at 1 year was significantly higher in patients treated with immunotherapy than in those treated with supportive care alone (9/25 vs. 3/25)	[250]
30 patients with glioblastoma	Randomized open trial	1 year	Patients were randomized to receive radiotherapy alone or radiotherapy plus melatonin (20 mg/daily orally) until disease progression	Clinical outcome	Both the survival curve and the percent of survival at 1 year were significantly higher in patients treated with radiotherapy plus melatonin than in those receiving radiotherapy alone (6/14 vs. 1/16). Radiotherapy or steroid therapy-related toxicities were lower in patients concomitantly treated with melatonin	[251]
116 patients with advanced solid tumors	Randomized open trial	1-5 weeks	Patients treated with IL-2 (3×10^6 IU/day s.c. every day, 6 days/week for 4 weeks) or with TNF (0.75 mg/day i.v. for 5 days) were randomized to receive or not a concomitant melatonin administration (40 mg/day orally in the evening, starting 7 days prior to cytokine injection)	Clinical outcome	The occurrence of hypotension was significantly less frequent in patients concomitantly treated by melatonin than in those who received the cytokine alone, during either IL-2: or TNF immunotherapy	[252]

Subjects	Design	Study's duration	Treatment	Measured	Results	Ref.
100 untreatable metastatic solid tumor patients	Randomized open trial	Variable	Patients were randomized to receive supportive care alone or supportive care plus melatonin (20 mg/daily p.o. in the evening) until disease progression	Clinical outcome. TNF levels	There were 86 evaluable patients, the other 14 patients having died from rapid progression of disease. The per cent of weight loss greater than 10% was significantly higher in patients treated by supportive care alone than in those concomitantly treated by melatonin, with no difference in food intake. Mean serum levels of TNF significantly decreased in patients concomitantly treated with melatonin	[253]
25 metastatic solid tumor patients other than breast cancer and prostate cancer	Phase II pilot study	Variable	Tamoxifen (20 mg/day) and melatonin (20 mg/day) were given p.o. until disease progression	Clinical outcome	Three patients had a partial response (12%, one cervix carcinoma; one melanoma; one unknown primary tumor). A stable disease was achieved in 13 other patients, whereas the remaining 9 patients progressed. Performance status improved in 9/25 patients and a survival longer than 1 year was observed in 7/25 patients	[254]
30 node-relapsed melanoma patients	Randomized open trial	31 months	Patients were randomized to receive no treatment or melatonin (20 mg/ daily p.o. in the evening) until disease progression		The percent of disease-free survival was significantly higher in melatonin-treated individuals than in controls. No melatonin-related toxicity was observed	[255]
14 metastatic prostate cancer patients refractory to a previous therapy with LHRH analogue	Open trial	1-2 years	The LHRH analogue triptorelin was injected i.m. at 3.75 mg every 28 days. Melatonin was given p.o at 20 mg/ day in the evening every day until progression, starting 7 days prior to triptorelin	Clinical outcome. Serum levels of PSA, prolactin and IGF-1	A survival longer than 1 year was achieved in 9/14 (64%) patients. PSA mean concentrations significantly decreased on therapy of triptorelin plus melatonin. In addition, a normalization of platelet number was obtained in 3/5 patients with persistent thrombocytopenia prior to study	[256]
70 advanced non-small cell lung cancer patients	Randomized open trial	1 year	Chemotherapy alone with cisplatin (20 mg/m ² /day i.v. for 3 days) and etoposide (100 mg/m ² /day i.v. for 3 days) (Cycles were repeated at 21-day intervals) or chemotherapy plus melatonin (20 mg/day orally in the evening)	Clinical outcome	A complete response was achieved in 1/34 patients concomitantly treated with melatonin and in none of the patients receiving chemotherapy alone. Partial response occurred in 10/34 and in 6/36 patients treated with or without melatonin, respectively. The percent of 1-year survival was significantly higher in patients treated with melatonin plus chemotherapy than in those who received chemotherapy alone (15/34 vs. 7/36). Chemotherapy was well tolerated in patients receiving melatonin, and the frequency of myelosuppression, neuropathy, and cachexia was significantly lower in the melatonin group	[257]
80 patients with metastatic solid tumors who were in poor clinical condition (lung cancer: 35; breast cancer: 31; gastro-intestinal tract tumors: 14)	Randomized open trial	Variable	Lung cancer patients were treated with cisplatin and etoposide, breast cancer patients with mitoxantrone, and gastrointestinal tract tumor patients with 5-FU plus folates. Patients were randomized to receive chemotherapy alone or chemotherapy plus melatonin (20 mg/day p.o. in the evening)	Clinical outcome	Thrombocytopenia was significantly less frequent in patients concomitantly treated with melatonin. Malaise, asthenia, stomatitis and neuropathy were less frequent in the melatonin group	[258]
31 patients with advanced solid tumors		3 months	Melatonin (10 mg/day p.o. in the evening)	Clinical outcome. Serum levels of TNF- α , IL-1, IL-2, IL-6 and IFN- γ	After 3 months of therapy, 19 patients (61%) showed disease progression. The other 12 (39%) achieved disease stabilization. A significant decrease of IL-6 circulating levels was found	[259]
50 patients suffering from lung cancer, gastro-intestinal tract tumors, breast cancer or brain glioblastoma			Melatonin alone (20 mg/day orally in the dark period) or melatonin plus aloe vera tincture (1 ml twice/day)	Clinical outcome	A partial response was achieved in 2/24 patients treated with melatonin plus aloe and in none of the patients treated with melatonin alone. Stable disease was achieved in 12/24 and in 7/26 patients treated with melatonin plus aloe vera or melatonin alone, respectively. The percent 1-year survival was significantly higher in patients treated with melatonin plus aloe vera	[260]

Subjects	Design	Study's duration	Treatment	Measured	Results	Ref.
20 previously untreated patients with inoperable lung cancer	Double blind placebo controlled study	2 months	2 cycles of carboplatin on day 1 and etoposide on days 1-3 every 4 weeks. Melatonin 40 mg or placebo (double-blind) was given orally in the evening for 21 consecutive days, starting 2 days before chemotherapy. Patients were randomized to receive melatonin either with the first or the second cycle	Hematologic parameters	The hematologic parameters--depth and duration of toxicity for hemoglobin, platelets and neutrophils were not significantly different between cycles with/without melatonin	[261]
14 metastatic breast cancer women	Open trial	Variable	Epirubicin. at weekly intervals. Melatonin was given orally at 20 mg/day in the evening every day, starting 7 days prior to chemotherapy	Clinical outcome. Hematologic parameters	Evaluable patients were 12/14. The induction phase with melatonin induced a normalization of platelet number in 9/12 evaluable patients, and no further platelet decline occurred in chemotherapy. Objective tumor regression was achieved in 5/12 (41%) patients	[262]
250 metastatic solid tumor patients (lung cancer, 104; breast cancer, 77; gastro-intestinal tract neoplasms, 42; head and neck cancers, 27)	Randomized open trial	1 year	Chemotherapy consisted of cisplatin plus etoposide or gemcitabine alone for lung cancer, doxorubicin alone, mitoxantrone alone or paclitaxel alone for breast cancer, 5-FU plus folinic acid for gastro-intestinal tumors and 5-FU plus cisplatin for head and neck cancers. Patients were randomized to receive melatonin (20 mg/day p.o. every day) plus chemotherapy, or chemotherapy alone	Clinical outcome	The 1-year survival rate and the objective tumor regression rate were significantly higher in patients concomitantly treated with melatonin than in those who received chemotherapy alone (tumor response rate: 42/124 chemotherapy + melatonin versus 19/126 chemotherapy only; 1-year survival: 63/124 chemotherapy + melatonin versus 29/126 chemotherapy only). Melatonin significantly reduced the frequency of thrombocytopenia, neurotoxicity, cardiotoxicity, stomatitis and asthenia	[263]
30 metastatic renal cell cancer patients under chronic therapy with morphine	Randomized open trial	3 years	Oral doses of morphine ranged from 60 to 120 mg/day. Patients were randomized to receive morphine alone or morphine plus melatonin (20 mg/day p.o. in the evening). IL-2 was s.c. administered at a dose of 6 million IU/day for 6 days/week for 4 consecutive weeks. In non progressing patients, a second cycle was planned after a 21-day rest period	Clinical outcome	The percent of partial responses achieved in patients treated with morphine alone was significantly lower than that observed in patients concomitantly treated with melatonin (1/16 vs. 4/14). The 3-year percent of survival was significantly higher in patients concomitantly treated with melatonin	[264]
12 advanced hematologic malignancies which did not respond to previous standard therapies (non-Hodgkin's lymphoma 6; Hodgkin's disease, 2; multiple myeloma, 2; acute myelogenous leukemia, 1 and chronic myelo-monocytic leukemia, 1)	Open trial	30 months	IL-2 was s.c. administered at a dose of 3 million IU/day for 6 days/week for 4 consecutive weeks. Melatonin was given orally at 20 mg/day in the evening, without interruption. In non-progressing patients, a second IL-2 cycle was planned after a 3 week-rest period	Clinical outcome	A partial response was achieved in one patient with multiple myeloma. Stable disease occurred in 7 other patients, whereas the other 4 patients progressed. The lack of progression was obtained in 8 out of 12 (67%) patients, with a median duration of 21+ months (14-30+ months). The treatment was well tolerated in all patients	[9]
30 patients with cancer-related thrombocytopenia who did not respond to melatonin alone	Randomized open trial	Variable	Patients were randomized to receive melatonin alone (20 mg/day orally in the evening) or melatonin plus 5-methoxytryptamine (1 mg/day orally in the early afternoon)	Hematologic parameters	A normalization of platelet count was achieved in 5/14 (36%) patients treated with melatonin plus 5-methoxytryptamine and in none of the patients treated with melatonin alone. Mean platelet number significantly increased only in the patients treated with melatonin plus 5-methoxytryptamine	[265]
20 metastatic patients, who progressed on previous antitumor therapies	Open trial	2 months	Melatonin was given orally at 20 mg/day in the evening	Clinical outcome. VEGF levels in plasma	The clinical response consisted of minor response in 2, stable disease in 6 and progressive disease in the remaining 12 patients. Non-progressing patients showed a significant decline in VEGF mean concentrations, whereas no effect was achieved in progressing patients	[266]

Subjects	Design	Study's duration	Treatment	Measured	Results	Ref.
100 patients with inoperable advanced primary hepato-cellular carcinoma	Open trial	2 years	Patients were treated separately by transcatheter arterial chemoembolization (n=50) and by transcatheter arterial chemoembolization + melatonin (20 mg/day at 2000 h orally, starting 7 days before (n=50)	Clinical outcome: IL-2 and sIL-2R levels.	The effectivity rates of transcatheter arterial chemoembolization were significantly lower than that of transcatheter arterial chemoembolization + melatonin. Melatonin protected liver function from the damage caused by transcatheter arterial chemoembolization. IL-2 levels of all patients significantly increased, whereas sIL-2R expressions decreased in the melatonin group	[267]
13 patients with metastatic melanoma patients progressing on dacarbazine plus IFN- α .	Open trial	1 year	Cisplatin was injected i.v. for 3 days every 21 days. IL-2 was administered s.c. at 3 million IU/day from days 4 to 9 and from days 11 to 16 of the cycle. Melatonin was given orally at 20 mg/day in the evening, every day without interruption	Clinical outcome	One patient obtained a complete response, while a partial response was achieved in 3 other patients. A stable disease occurred in 5 patients, whereas the remaining 4 patients had a progressive disease. The treatment was well-tolerated and no cisplatin-related neurotoxicity was observed	[268]
14 consecutive untreatable metastatic solid tumor patients	Cross-over randomized study	Variable	Two consecutive immunotherapeutic cycles at 21-day intervals with IL-2 plus melatonin alone or with IL-2 plus melatonin (20 mg /day) and naltrexone (100 mg in the morning every next day)	Hematologic parameters	The association of naltrexone further amplifies the lymphocytosis obtained after IL-2 plus melatonin	[269]
30 patients with metastatic colorectal cancer	Randomized open trial	1 year	Patients were randomized to be treated with irinotecan alone for 9 consecutive weeks or irinotecan plus melatonin (20 mg/day)	Clinical outcome	No complete response was observed. A partial response (was achieved in 2 out of 16 patients treated with irinotecan alone and in 5 out of 14 patients concomitantly treated with melatonin. Percent of disease-control achieved in patients concomitantly treated with melatonin was significantly higher than that observed in those treated with chemotherapy alone	[270]
100 consecutive metastatic non-small cell lung cancer patients	Randomized open trial	5 years	Patients were randomized to receive chemotherapy (cisplatin and etoposide) alone or chemotherapy and melatonin (20 mg/day).	Clinical outcome	Both the overall tumor regression rate and the 5-year survival results were significantly higher in patients concomitantly treated with melatonin. In particular, no patient treated with chemotherapy alone was alive after 2 years, whereas a 5-year survival was achieved in three of 49 (6%) patients treated with chemotherapy and melatonin. Chemotherapy was better tolerated in patients treated with melatonin	[271]
24 patients not amenable to standard anticancer treatment and with weight loss and/or decreased serum albumin	Open trial	8 weeks	4.9 g of eicosapentaenoic acid and 3.2 g of docosahexanoic acid, or 18 mg/day of melatonin for 4 weeks	Serum or plasma TNF- α , IL1 β , sIL2R, IL-6, IL-8 and specific fatty acid levels	Serum levels of fatty acids increased with fish oil. No major changes in biochemical variables and cytokines were observed with any intervention. In the fish oil group, 5 of 13 patients (38%) showed weight stabilization or gain compared with 3 of 11 patients (27%) in the melatonin group. After combining interventions, approximately 63% of patients showed such responses	[272]
370 metastatic solid tumor patients, suffering from non-small cell lung cancer or gastro-intestinal tumors	Randomized open trial	2 years	Patients were randomized to receive chemotherapy alone or chemotherapy plus melatonin (20 mg/day orally in the evening every day)	Clinical outcome	The overall tumor regression rate achieved in patients concomitantly treated with melatonin was significantly higher than that found in those treated with chemotherapy alone. The 2-year survival rate was significantly higher in patients concomitantly treated with melatonin	[273]
126 Radiation Therapy Oncology Group recursive partitioning analysis Class 2 patients with brain metastases	Phase II randomized trial	Variable	Class 2 patients with brain metastases were randomized to 20 mg of melatonin, given either in the morning or in the evening. All patients received radiation therapy in the afternoon. Melatonin was continued until neurologic deterioration or death	Clinical outcome	Neither of the randomized groups had survival distributions that differed significantly from the historic controls of patients treated with whole-brain radiotherapy	[206]
846 patients with metastatic solid tumor (non-small cell lung cancer or gastro-intestinal tract tumors)	Randomized open trial	3 years	Patients were randomized to receive the best supportive care only, supportive care plus melatonin (20 mg/day, orally in the evening), or melatonin plus supportive care plus a low-dose of IL-2 for 5 days/week, for 4 consecutive weeks	Clinical outcome	Melatonin alone was able to induce a significant increase of disease stabilization and survival time with respect to supportive care alone. The association of IL-2 with melatonin provided a further improvement in the percentage of tumor regressions and of 3-year survival with respect to melatonin alone	[274]

Subjects	Design	Study's duration	Treatment	Measured	Results	Ref.
20 consecutive patients, who underwent pelvic irradiation	Randomized open trial		The patients were randomized to be concomitantly treated with melatonin alone, with melatonin plus 5-mthoxytryptamine or with s.c. low-dose IL-2	Hematologic parameters	Radiotherapy induced a significant decline in the mean number of lymphocytes while neither melatonin alone, nor melatonin plus 5-methoxytryptamine were able to significantly reduce this decline.	[275]
14 untreatable metastatic cancer patients showing alterations of cortisol rhythm	Open trial	3 months	Melatonin at 20 mg/day orally in the evening	Clinical outcome. Plasma cortisol rhythm.	Normalization of cortisol rhythm was achieved in 4/14 (29%) patients. Moreover, stable disease was obtained in 6/14 (43%) patients under melatonin therapy, whereas the other 8 patients had progressive disease. The percentage of cortisol rhythm normalization achieved in patients with stable disease was significantly higher than that observed in patients with progressive disease	[276]
20 patients with untreatable metastatic solid tumor	Open trial	1 year	Melatonin at 20 mg/day orally in the evening	Immune parameters	Melatonin induced a statistically significant decline in mean T-reg cell numbers in patients who achieved disease control, whereas no effect was seen in those who had progressed. No in vitro effect of melatonin incubation was apparent	[277]
95 post-menopausal women with a prior history of stages 0-III breast cancer who had completed active cancer treatment	Double-blind, placebo-controlled study	4 months	Patients were randomly assigned to either 3 mg oral melatonin (n = 48) or placebo daily for 4 months	Compliance and change in breast cancer biomarkers (estradiol, IGF-1, IGF-binding protein 3 and the IGF-1/IGFBP-3 ratio)	Short-term melatonin treatment did not influence the estradiol and IGF-1/IGBBP-3 levels	[207]
Patients with advanced lung or gastro-intestinal cancer and cachexia	Double-blind, placebo-controlled study	28 days	Melatonin 20 mg p.o. versus placebo	Clinical outcome	After interim analysis of 48 patients, the study was closed for futility. There were no significant differences between groups for appetite or other symptoms, weight, toxicity, or survival from baseline to day 28	[208]
Advanced, non-small cell lung cancer	Double-blind, placebo-controlled study	7 months	Patients were randomized to receive 10 mg or 20 mg of melatonin p.o. or placebo.	Assessment of health-related quality of life (HRQoL) was completed at baseline, and at 2, 3 and 7 months. DNA damage was measured during the first three months of chemo-therapy	Patients in the melatonin-treated group had significantly better HRQoL scores, particularly in social well-being. Median survival was 7.3 months in the two groups. A greater amount of DNA damage marker was observed in the placebo-treated group, and this was associated with lower survival.	[196]
54 women undergoing surgery for breast cancer and without signs of depression on Major Depression Inventory (MDI)	Double-blind, placebo-controlled study	3 months	6 mg of melatonin p.o. or placebo	The primary outcome was the incidence of depressive symptoms measured by MDI.	The risk of developing depressive symptoms was significantly lower with melatonin than with placebo	[195]
95 post-menopausal women with a prior history of stage 0-III breast cancer	Placebo controlled study	4 months	3 mg of melatonin or placebo	Pittsburgh Sleep Quality Index (PSQI). Depression and hot flash assessment.	Compared to subjects on placebo, subjects randomized to melatonin experienced significantly greater improvements in subjective sleep quality as measured by the PSQI, including domains on sleep quality, daytime dysfunction and total score. There were no significant differences in measures of depression or hot flashes	[171]

5-FU: 5-fluorouracil; i.m. intramuscularly; IFN: interferon; IGF: insulin-like growth factor; IL: Interleukin; NK : natural killer; p.o. per os; PSA: prostatic specific antigen; s.c.: subcutaneous; sIL-2R: soluble IL-2 receptor; TNF : tumor necrosis factor; VEGF: Vascular endothelial growth factor.

Table 2: Effect of Melatonin in Cancer Patients.

of the randomized groups had survival distributions that differed significantly from the historic controls of patients treated with whole-brain radiotherapy [206]. In 95 post-menopausal women with a prior history of stages 0-III breast cancer who had completed active cancer treatment, a double-blind, placebo-controlled study with either 3 mg oral melatonin or placebo daily for 4 months, melatonin treatment did not affect breast cancer biomarkers [207]. In a double-blind, placebo-

controlled study in patients with advanced lung or gastro-intestinal cancer and cachexia, melatonin (20 mg/day) was ineffective to modify appetite, weight, toxicity, or survival from baseline to day 28 [208].

Conclusion

Melatonin can provide an innovative adjuvant strategy in cancer by combining their effects on the circadian rhythm with their oncostatic

and cytoprotective properties. As discussed in the present review article melatonin is effective in suppressing neoplastic growth in a variety of tumors. The mechanisms involved include antiproliferative effects via modulation of cell cycle, ability to induce apoptosis in cancer cells, anti-angiogenic and antimetastatic effects, anti-estrogenic activity, the capacity to decrease telomerase activity, immune modulation, and direct and indirect antioxidant effects. Besides these oncostatic properties, melatonin deserves to be considered in the treatment of cancer for two other reasons. First, because its hypnotic-chronobiotic properties, melatonin use that can allow the clinician to effectively address sleep disturbances, a major co-morbidity in cancer. Indeed as with many other diseases, evidence supports the hypothesis that metabolic rhythms attenuation and / or disruption contribute to the etiology of cancer. Second, because melatonin's anxiolytic and antidepressant effects, it has a possible application in two other major co-morbidities seen in cancer patients, i.e. depression and anxiety.

An important remaining question to be considered is the melatonin dose employed. From the basis aspects of melatonin activity discussed in previous sections of this article, it emerges the necessity to employ melatonin doses in the 100 – 500 mg/day range to produce full expression of cytoprotection in experimental cancer models. Indeed, melatonin has a high safety profile, it is usually remarkably well tolerated and, in some studies, it has been administered to patients at very large doses. Escalating doses of melatonin up to 100 mg were devoid of undesirable activity in humans [209,210]. Melatonin (300 mg/day for up to 3 years) decreased oxidative stress in patients with amyotrophic lateral sclerosis [211] with very few undesirable side effects. In children with muscular dystrophy, 70 mg/day of melatonin reduced cytokines and lipid peroxidation [212]. Doses of 80 mg melatonin hourly for 4 h were given to healthy men with no undesirable effects other than drowsiness [213]. In healthy women given 300 mg melatonin/day for 4 months there were no side effects [214]. In a randomized controlled double-blind clinical trial on 50 patients referred for liver surgery a single preoperative enteral dose of 50 mg/kg melatonin was safe and well tolerated [215]. This underlines the urgent need for large clinical trials in the field of melatonin and cancer [216,217].

Financial Support and Grants

PICT 2007 01045 and 2012 0984 from the Agencia Nacional de Promoción Científica y Tecnológica, Argentina; SAF2009-14037 and SAF2013-49019 from the Spanish Ministry of Economy and Competitiveness (MINECO); GREIB PT_2010_04 from the CEI Biotic Program of the University of Granada, Spain; RD12/0043/0005 (RETICEF) from the Instituto de Salud Carlos III, Spain, and CTS-101 from the Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía, Spain. The authors declare having no financial, consulting, or personal relationships with people or organizations that could be perceived as influencing their work.

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This article was originally published in a special issue, **Oncology and Biophysics: A Need for Integration** handled by Editor(s). Dr. Sarah S Knox, West Virginia University