The Sleep-Cancer Mystery: An Investigation of How Surgery Affects the Association Between Sleep Deprivation and Glioblastoma Through Neuroinflammation and Immunosuppressive Modulation.

[Redacted by Managing Editor]

Abstract

Glioblastoma is the most common and aggressive primary malignant brain tumor in adults. GBM progression is facilitated by cytokines, chemokines, tumor-associated macrophages, and other immune molecules that can lead to an immunosuppressive tumor microenvironment and infiltrate the blood-brain barrier. Sleep deprivation is a pervasive and dangerous condition that can lead to immunosuppression and neuroinflammation. Numerous studies have found links between sleep deprivation and elevated markers of systemic inflammation and impaired anti-tumor immunity, which facilitate cancer progression. Chronic sleep deprivation will lead to increased neuroinflammation and immunosuppression, facilitating the progression of glioblastoma. While prior studies have explored individual therapeutic strategies, including surgery and immunotherapy, results have been limited. Thus, surgical interventions combined with various therapeutic approaches can modulate neuroinflammatory and immunosuppressive pathways linking chronic sleep deprivation to the progression of glioblastoma. Given the challenging nature of directly addressing sleep deprivation and surgery's possible linkage to increased inflammation, combinative therapy could be more effective. The direct connection between sleep deprivation and glioblastoma proliferation and progression has not been sufficiently researched. Thus, the purpose of this review is to understand how surgery and a potential combined approach affect the association between sleep deprivation and glioblastoma progression through neuroinflammation and immunosuppressive modulation. The implications of this review can offer a greater understanding of the association between sleep deprivation and the proliferation and progression of glioblastoma, and whether a combination therapy or surgery alone is the most feasible and effective approach in addressing this prevalent and dangerous disease.

Introduction

Sleep deprivation is a widespread and severe condition that can lead to impaired immune function and neuroinflammation, creating a pro-tumor microenvironment [1] [2] [3]. Cytokines and other immune molecules facilitate this response and have made the treatment of glioblastoma, a common form of brain cancer, very challenging [2] [3].

Sleep is essential for maintaining homeostasis, supporting immune, neural, hormonal, and metabolic function [1] [2]. Sleep deprivation, the reduced sleep below physiological needs,

affects an estimated 50–70 million Americans across all ages and socioeconomic groups, and has become a growing issue [2] [3] [4].

Chronic sleep deprivation is associated with impaired immune responses, including lower cytotoxic activity (reduced CD8+ T cells and NK cells) and increased pro-tumor inflammation [3]. More specifically, sleep deprivation can contribute to neuroinflammation, which is marked by proinflammatory cytokines [2] [5], promoting tumor-favorable conditions [1] [5] [6]. Furthermore, sleep deprivation weakens antitumor immunity and decreases T-cell activity [2], suggesting a correlation between sleep deprivation and accelerated GBM progression. Lastly, sleep loss impairs the blood-brain barrier, leading to an influx of inflammatory agents [3].

Patients with glioblastoma (GBM), the most common and aggressive primary malignant brain tumor in adults [3] [6] [7] [8], have a survival time of fewer than 15 months [9] [10] [11] [12]. The severity of GBM can be attributed to its anatomical location and the disruption of the blood-brain barrier [11]. GBM proliferation is complex and driven by epigenetic alterations and mutations in oncogenes, tumor suppressor genes, and DNA repair pathways [11] [12] [13] [14] [15]. That being said, sleep deprivation induced neuroinflammation amplifies signaling pathways involved in GBM progression

GBM is marked by neuroinflammation that promotes tumor growth, invasion, and therapy resistance by altering the tumor microenvironment [13]. The Tumor Microenvironment (TME) is composed of tumor cells, immune cells (microglia, macrophages, T cells, NK cells), stromal cells, and the extracellular matrix [2] [6] [8] [9] [11]. Glioma-associated microglia/macrophages (GAMs) release TGF-β, IL-6, IL-10, and other molecules to inhibit T cells [11]. Furthermore, CD8+ cells and NK cells, key to the anti-tumor response, are suppressed or exhausted in the GBM TME [6].

Multiple therapeutic strategies exist to combat GBM, including immunotherapy, pharmacological means, lifestyle and sleep management, and surgical interventions. [2] [6] [8] [13] Surgery is especially vital in GBM treatment and can relieve symptoms by reducing tumor mass [13]. Surgery, however, can disrupt the BBB by increasing the infiltration of inflammatory macrophages and decreasing the integrity of tight junctions [16].

Despite advances in neuroscience and oncology, treatment options for GBM remain uncertain and are only marginally effective. Recent studies have shown that sleep deprivation plays a major role in increasing neuroinflammation and immunosuppression facilitated through proinflammatory cytokines, macrophages, microglia, and T-cells [3] [5] [6]. While previous studies have associated sleep deprivation with neuroinflammation and immune suppression, and others have found a correlation between neuroinflammation/immune suppression and glioblastoma progression, a direct association has yet to be identified.

Chronic sleep deprivation heightens neuroinflammatory and immunosuppressive responses, accelerating GBM progression. Furthermore, surgical interventions' modulation of neuroinflammatory and immunosuppressive pathways linking chronic sleep deprivation and the progression of glioblastoma must be researched to improve clinical outcomes. However, due to inflammatory responses caused by surgery, a combined approach involving multiple therapeutic

methods could lead to the most effective and long-lasting outcomes. Thus, the purpose of this review is to understand how surgery and a potential combined approach affect the association between sleep deprivation and glioblastoma progression through neuroinflammation and immunosuppressive modulation.

Sleep Deprivation, Inflammation, and Immune Suppression: Investigating Sleep's Crucial Role in Immune Function

Sleep is a vital physiological process essential for maintaining immune, metabolic, and neural homeostasis. Adequate sleep ensures that the immune system is sufficiently regulated, controlling the spread of diseases. New studies highlight how insufficient sleep triggers neuroinflammation, characterized by elevated pro-inflammatory cytokine levels, and the impairment of key immune cells like T-cells and natural killer cells [1] [2] [3]. Given the rising prevalence of sleep deprivation due to modern lifestyle changes, its immunological consequences must be addressed.

Sleep and Sleep Deprivation

Sleep is a fundamental physiological and neurological process that is crucial for maintaining immune, metabolic, and neural homeostasis [2] [3]. This process is shared among many organisms and is vital for healthy neural and brain functions [3]. Given the importance of sleep in immune function, sleep deprivation should be viewed as a serious health concern.

In contrast, sleep deprivation, the reduction in sleep time from the required amount [3], has become increasingly common in modern society, driven by environmental and lifestyle changes (socioeconomic environment and lifestyle) [2] [3]. Chronic sleep deprivation is excessive daytime sleepiness that occurs nearly every day for at least three months [4]. Sleep deprivation affects an estimated 50 to 70 million Americans of all ages and socioeconomic classes [4]. Alarmingly, the number of people getting sufficient sleep, at least 6 hours of sleep a night, has continued to decrease over the last 25 years [1].

Beyond fatigue, sleep deprivation is associated with an increased risk of cardio/cerebrovascular diseases, accidents, hypertension, stroke, cancer, and neurodegenerative diseases [4] [17]. These associations underscore the necessity of researching sleep deprivation's interactions with the immune system, specifically, inflammatory and anti-tumor functions.

Sleep Deprivation and Immune Regulation

Sleep deprivation disrupts immune regulation, increasing susceptibility to infections and tumor proliferation [4]. Individuals who are consistently sleep deprived exhibit a higher susceptibility to common infections and are more vulnerable to diseases [2] [5]. Past studies have

shown that sufficient sleep leads to lower cortisol levels, allowing the immune system to function optimally, reducing the risk of inflammation [2].

Sleep deprivation triggers an inflammatory response characterized by increased levels of proinflammatory cytokines, supporting protumor conditions. Neuroinflammation is an inflammatory response in the brain marked by the release of cytokines, chemokines, and growth factors [1] [6].

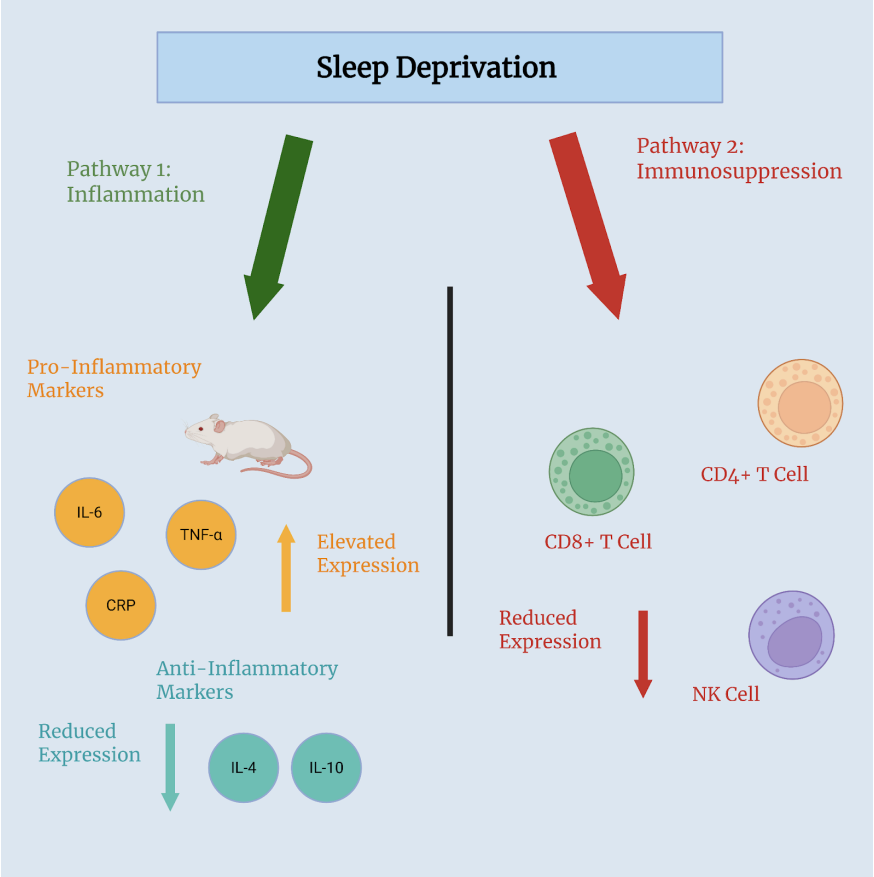

Figure 1: Sleep deprivation has been associated with increased levels of pro-inflammatory markers, including interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), and C-reactive proteins (CRP) [2] [3] [4] and suppressed levels of anti-inflammatory cytokines IL-10 and IL-4 [5]. Sleep deprivation also reduces immune cell activity, including CD8+, CD4+, and natural killer (NK) Cells [3][5]. These immune shifts are both markers of sleep deprivation and mechanisms by which GBM can evade detection. Created in BioRender. Chan, N. (2025) https://BioRender.com/uf6m14a.

Other markers with altered expression levels in sleep-deprived studies include IL-1α, IL-1β, IL-2, IL-8, IL-13, IL-15, IL-18, TNF-β, IFN-α, IFN-β, macrophage inhibitory protein (MIP)-1β, corticosterone, and homocysteine. [1] [3]. In multiple rodent models, sleep loss has been linked to an increase in IL-1, IL-6, IL-15, IL-17, and TNF-α [5]. In summary, sleep

deprivation influences the balance between pro- and anti-inflammatory responses, leading to chronic inflammation. Sleep deprivation also impairs the anti-tumor response by reducing T-cell activity. Adequate sleep enhances the activity of T-cells by increasing the expression of integrins on T cells, proteins that facilitate a cell's ability to attach to and destroy infected cells [2]. Therefore, sleep deprivation leads to cytokine and immune alterations that favor tumor development and progression.

The correlation between sleep deprivation, neuroinflammation, and immune suppression must be further studied as sleep deprivation cases continue to increase. While chronic sleep deprivation is associated with elevated pro-inflammatory cytokines (e.g., IL-6, TNF-α) and impaired immune cells (e.g., T cells, NK cells), the specific molecular mechanisms, including downstream transcription factors and alterations in cytokine receptor signaling, are not fully understood. Nevertheless, sleep deprivation - induced immune imbalances creates the immunosuppressive and pro-tumor environment that accelerates GBM progression. Future studies could investigate whether sleep quality or quantity is more impactful on immune regulation as addressing these concerns could lead to targeted interventions involving sleep hygiene and sleep therapies.

Understanding Glioblastoma: The Interactions Between Genetics, Neuroinflammation, and Immune Suppression

Glioblastoma (GBM) is the most aggressive and lethal form of primary brain cancer and is associated with poor survival outcomes. This resistance is in large part due to glioblastoma's highly immunosuppressive tumor microenvironment (TME), which is a complex of inflammatory cytokines and immunosuppressive molecules. Key immune players, including tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and astrocytes, contribute to favorable tumor conditions. Molecular and cellular pathways play a decisive role in GBM proliferation and progression.

Glioblastoma & Mutations

Glioblastoma (GBM) is the most aggressive and common primary malignant brain tumor that has been difficult to treat due to its location and immunosuppressive microenvironment [3] [6] [7] [8]. Glioblastoma arises from glial cells and accounts for 60 to 70% of all malignant gliomas, 50% of all gliomas that arise in the central nervous system (CNS), and about 15% of all brain tumors [11] [13] [18]. Patients with GBM have a median survival of 14.7 months, with only 6.8% of patients surviving past five years [9] [10] [11] [12], and 75% of affected patients die within two years of their diagnosis [19] [20] Despite only making up 1.4% of annual cancer incidences, GBM accounts for 2.5 % if total cancer deaths and is the leading cause of cancer deaths in ages 15 to 34 [8] [9]. There are an estimated 10,000 new annual cases in the United

States and 100,000 new cases globally [8] [21] [22]. Hence, additional research in the molecular mechanisms involved in GBM proliferation and progression is required.

Genetic Alteration	Functional Alteration
CDK2A-p16INL4a	Cell Cycle Regulation
CDK2A-p16INL4a	Cell Cycle Regulation
EGFR	Cell Growth Regulation
ERBB2	Cell Growth Regulation
PDGFR	Cell Growth Regulation
EMP3	Tumor suppression
RASSF1A	Tumor suppression
BLU	Tumor suppression
TP53	Tumor suppression
PTEN	Tumor suppression
NF1	Tumor suppression
RB1	Tumor suppression
MGMT	DNA Repair
MLH1	DNA Repair
DAPK1	Inhibition of Apoptosis
TIMP3	Inhibition of Apoptosis
CDH1	Inhibition of Apoptosis

Table 1: Glioblastoma progression is influenced by genetic mutations and epigenetic changes that suppress the immune response, including mutations and hypermethylation in oncogenes, tumor suppressor genes, and genes in DNA repair and apoptosis signaling pathways [11] [12] [13] [14] [15].

Neuroinflammation also plays a significant role in GBM progression by activating oncogenic signaling pathways and inducing genetic and epigenetic changes [6] [13] [22]. GBM samples frequently express higher concentrations of pro-inflammatory proteins such as P2X7R, RAGE, NOS2, COX2, and PTX3 [20]. These genetic and epigenetic alterations are central to discerning the complex relationship between neuroinflammation and immunosuppression in GBM. While mutations initiate GBM, sleep deprivation exacerbates these oncogenic pathways fueling neuroinflammation and immune suppression.

The Blood-Brain Barrier and Tumor Microenvironment

The blood-brain barrier (BBB) is essential for maintaining homeostasis, but it becomes compromised in GBM, contributing to immune suppression and inflammation. The BBB maintains normal brain function by preventing the absorption of toxins and pathogens, regulating transport and immune surveillance, and helping to maintain a stable environment [6] [11]. However, when compromised, such as when an individual is sleep deprived, blood vessels in the brain are altered both anatomically and functionally [11]. Hypoxia-inducible factor-a (HIF-A) regulates the expression of inflammatory factors such as vascular endothelial growth factor (VEGF), which disrupts the cellular barrier around blood vessels by creating capillaries with fewer tight junctions [11]. These alterations make the administration of medicine very challenging. Sleep deprivation disrupts BBB integrity increasing susceptibility to GBM proliferation and progression.

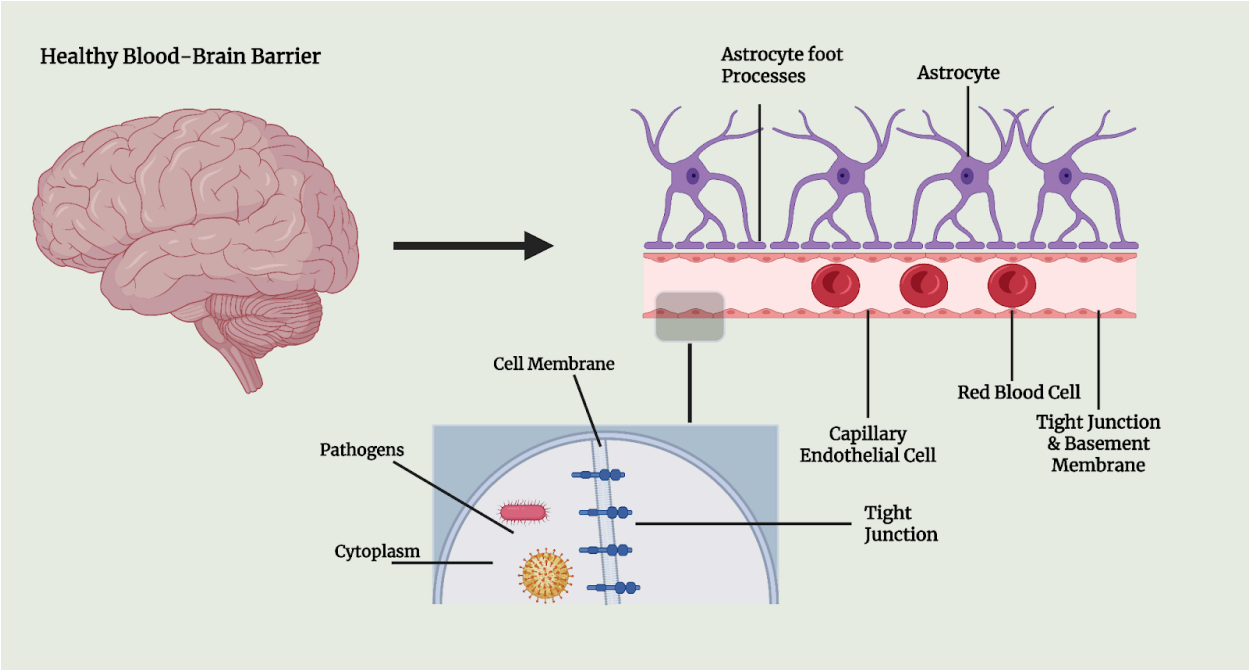

Figure 2: The healthy BBB is a selectively permeable barrier that is secured by endothelial cells linked by tight junctions and surrounded by astrocytes and basement membranes [6] [11]. Created in BioRender. Chan, N. (2025) https://BioRender.com/jx8fcbd.

Similarly, the glioblastoma tumor microenvironment (TME), a complex system, promotes tumor progression and immune suppression. The TME includes brain resident microglia, infiltrating macrophages, collectively known as glioma-associated microglia and macrophages or GAM, and myeloid-derived suppressor cells (MDSCs) [8]. Inflammatory cells are believed to make up between 30% and 50% of the tumor mass [6].

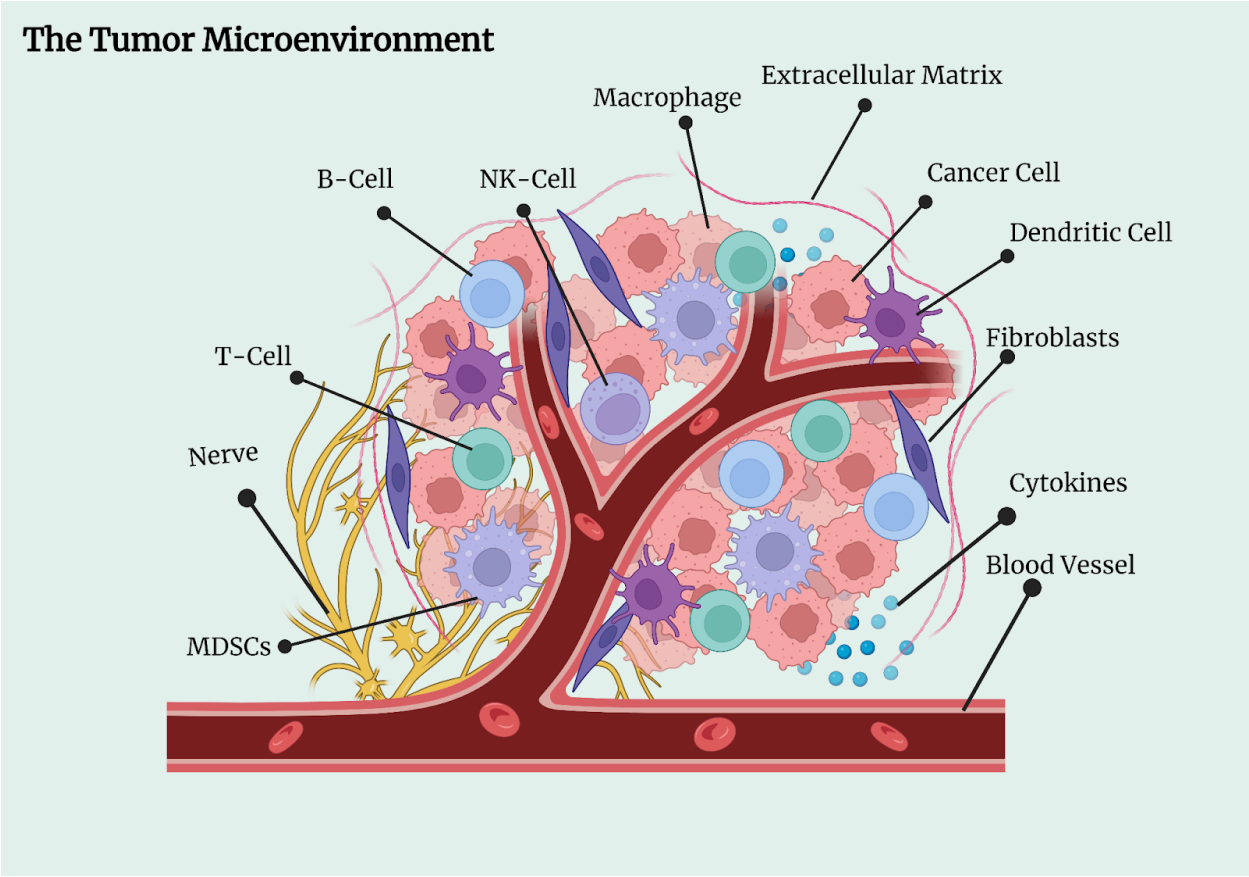

Figure 3: The TME accounts for up to 30% of the tumor's mass and is made up of cancer cells, immune cells, macrophages, dendritic cells, cytokines, fibroblasts, and extracellular matrix components. [2] [6] [8] [9] [11]. Created in BioRender. Chan, N. (2025) https://BioRender.com/17u1jhp.

The GBM TME promotes immunosuppression through the activation of Tregs and MDSCs, reactive astrocytes, microglia, and endothelial cells secrete transforming growth factor

(TGF-β) and metalloproteinases (MMP2 and MMP9) [11]. GBM tumors can change the TME by increasing immunosuppressive cells, including Tregs and MDSCs, that promote inflammation [2] [11]. The activation of these immunosuppressive molecules weakens the anti-glioma immune response [8] [11]. Key components of the tumor microenvironment, tumor-associated macrophages, and MDSCs require further research.

Tumor-Associated Macrophages, Cytokines & Chemokines

Tumor-associated macrophages (TAMs) and MDSCs are pivotal in immunosuppression and tumor progression in the GBM TME. Microglia cells are the resident macrophages of the central nervous system (CNS) and respond to chemokines and cytokines [23]. TAMS accounts for 30% of the tumor volume and are the main inflammatory cell components [6]. Pathological microglia, peripheral macrophages, and monocytes activate and contribute to a disrupted immune response by activating immunosuppressive pathways [11]. MDSCs are immature myeloid cells that express higher levels of immunosuppressive molecules.

In GBM, microglia show increased expression of proinflammatory genes, including SPP1, HLA-DR, TREM2, APOE, CD163, and GPR56, which is elevated [23]. Other inflammatory molecules include let7, tenascin-C (TNC), veriscan, IL-1β, TGF-β, TNF-α, stress-inducible protein (STI-1), prostaglandin E2, IL-6, IL-1β, IL-10, and epidermal growth factor [11]. Interestingly, macrophages are divided into M1 and M2 phenotypes. M1 macrophages exhibit immune supportive and anti-tumor functions, while M2 macrophages are immune suppressive and pro-tumor [6] [23]. Specifically, Gal-1 converts tumor-infiltrating macrophages to the immunosuppressive M2 variants [11]. TAMs are critical components in immune suppression and GBM progression.

Cytokines and chemokines are key components in the promotion of immune suppression in the glioblastoma tumor microenvironment by impairing anti-tumor responses. Cytokines are multifunctional molecules that control angiogenesis, proliferation, and immune cell infiltration in the TME [8] [11]. Cytokines are secreted by immune cells and include pro-inflammatory (IL-6, IL-8, TNF-α) functions and anti-inflammatory (IL-4, IL-10, TGF-β) functions [6]. Inflammatory cytokines significantly increase the proliferation and invasiveness of GBM cells as immune cells fail to recognize tumor cells [7] [11]. Glioma cells express many cytokines, including TGF-β, IL-10, IL-4, IL-6, IL-13, colony-stimulating factor-1 (CSF-1), LDH5, galectin-1 (gal-1), and prostaglandin-E [7] [11] [24]. IL-6 and TGF-β are especially important to glioblastoma progression. High IL-6 expression is associated with poor survival and is produced by malignant cells in response to oncogenic mutations [7] [8]. TGF-β, meanwhile, is involved in the regulation of cell growth and differentiation [8]. TGF-β is expressed at low levels in the brain but is greatly increased in GBM, suppressing CD8+ T cell activity [8]. Likewise, chemokines are small proteins that manage the migration of various cells in the body. Chemokines that are highly expressed in TME include CXCL2, IL-8, and CCL2. The relationship between cytokines and

chemokines facilitates neuroinflammation and immunosuppression, leading to GBM progression. Because cytokines and chemokines are elevated in patients with GBM and those that are chronically sleep deprived, sleep loss directly compounds the immunosuppressive TME, crucial to GBM progression.

T-Cells, Natural Killers & Astrocytes

The GBM TME impairs the immune functions of T-cells and natural killer (NK) cells through exhaustion and suppressed signaling. T cells are the largest group of lymphocytes that infiltrate the TME, preventing glioma immunity [6] [11] [25]. However, low proportions of T-cells are found in most GBM patients [6] [9]. The most common T lymphocytes in GBM are CD8+, CD4+ helper T cells, and regulatory T (Treg) cells [9]. Studies have shown that increased CD8+T cell expression in the TME results in positive outcomes [11]. However, a majority of CD8+ T cells infiltrating the TME are exhausted and ineffective [11]. In GBM, Tregs, a subtype of CD4+ T cells, regulate immune homeostasis by inhibiting the anti-tumor response [6].

NK cells recognize and destroy tumor cells by detecting the presence of receptor ligands [25]. Despite only making up about 2% of the TME, NKs are critical in the antitumor response [6]. NK cells play an important inhibitory role in the metastasis of GBMs, regulating T cell-mediated immune responses [19]. However, NK cells are hindered by the TME, limiting their effectiveness [6].

Astrocytes, the most abundant glial cells in the brain, make up nearly 50% of all brain cells [11] [26]. Astrocytes secrete factors that help maintain the tight junctions in the BBB [11] [26]. They also promote the release of degradative enzymes, cytokines, and chemokines [11]. When the CNS is impacted by tumors, astrocytes undergo reactive changes called astrogliosis and facilitate GBM proliferation [18] [26]. Reactive astrocytes were detected by increased glial fibrillary acidic protein (GFAP) expression levels [18] [26]. Thus, astrocytes support glioma pathogenicity by promoting immunosuppression, regulating immune cells, and contributing to the TME [26].

Glioblastoma is a highly lethal brain tumor that is characterized by genetic mutations, chronic neuroinflammation, and immune suppression. Key contributors to GBM progression include MDSCs, TAMs, T-regs, astrocytes, cytokines, and chemokines. Additionally, BBB disruption and the TME promote neuroinflammation and immunosuppression. Advancing our understanding of these mechanisms, particularly the roles of other critical molecules along with strategies to restore BBB integrity, will be essential for developing targeted and effective GBM therapies.

Targeting Glioblastoma: The Surgical Approach

While glioblastoma (GBM) remains one of the most challenging cancers to treat, advances in surgical techniques have begun to alleviate this problem. Traditional approaches,

including surgical interventions, only provide limited effectiveness due to GBM's highly invasive and immunosuppressive nature. As a result, research has focused on the development of advanced surgical techniques.

The Surgical Approach

Surgical interventions are a vital component in glioblastoma management through their ability to reduce tumor size and alleviate symptoms. Surgery can be a lifesaving treatment for glioblastoma, improving quality of life [16]. The goal of surgery is to reduce as much tumor mass as possible, relieve symptoms, and obtain brain tissue for pathological analysis [13] [21] [27]. Historically, surgery was the initial therapeutic approach for tumor debulking, including the use of cytoreductive surgery ranging from lobectomies to hemispherectomies [21] [28]. Currently, common techniques include craniotomy and biopsies for histopathological analysis [13]. Despite surgery's importance, GBM remains a dangerous disease; therefore, improvements in surgical intervention are necessary.

Advanced surgical techniques have led to improved outcomes. These techniques include fluorescence-guided surgery and intraoperative magnetic resonance imaging (iMRI) [13] [27]. Fluorescence-guided surgery utilizes fluorescent dyes (ie, 5-aminolevulinic acid), selectively taken up by tumor cells and visualized under a special microscope [13]. 5-ALA is a photosensitizing agent that selectively accumulates in GBM cells [21] [28]. Specifically, the tumor appears red, where normal tissue does not express fluorescence, helping surgeons differentiate tumors from healthy tissue in real time [21] [27]. This technique increases the rate of complete resection and improves patient outcomes and survival [28]. A meta-analysis of 20 studies, including 565 patients who underwent 5-ALA-guided resection, had a mean overall survival gain of 6.2 months [27]. Intraoperative magnetic resonance imaging (iMRI) is another critical technique utilizing a specialized MRI machine for real-time imaging during surgery [13] [28]. iMRI allows for the precise visualization of tumor boundaries and areas that may be confused with normal brain tissue [28]. In summary, the use of advanced techniques, including intraoperative MRI and fluorescence-guided surgery, has led to safer and more effective surgical resections [21]. Like many of these potential therapeutic strategies, surgery has its limitations.

Surgical procedures can induce inflammation through the disruption of the blood-brain barrier. Globally, millions of patients undergo surgery that involves extensive tissue damage [16]. These procedures, including GBM, are associated with systemic inflammation and can lead to major complications and even death [16]. Surgical trauma can trigger endogenous factors, or damage-associated molecular patterns (DAMPs), which can activate immune cells. [16]. When activated, these cells contribute to systemic inflammation facilitated through molecules like IL-1β and IL-18 [16]. Anesthesia and surgery can reduce tight junction protein expression in the brain, leading to increased migration of CCR2+ and other inflammatory macrophages into the brain [16]. Lastly, GBM can infiltrate surrounding brain tissue, making a complete surgical removal of the tumor impossible [13] [28]. Thus, the limitations of surgery can increase the risk

of immunosuppression and inflammation, a side effect that can be mitigated through sleep therapies.

Alternative Approaches: Immunotherapy, Pharmacology & Lifestyle Management

Given the limitations of surgery, research has been conducted on immunotherapies, including checkpoint inhibitors, chimeric antigen receptor T-cell (CAR-T) therapy, and oncolytic viruses, that aim to bolster the body's anti-tumor immune response. Similarly, pharmacological strategies targeting neuroinflammation and lifestyle changes addressing sleep deprivation and diet are also considered.

Immunotherapy

Immunotherapy strategies have provided some breakthroughs in targeting glioblastoma tumor cells, but have been limited by the tumor microenvironment. Some immunotherapies include peptide vaccines, dendritic cell therapy, adoptive T cell therapy, CAR-T cells, oncolytic viruses, and immune checkpoint inhibitors [6] [8]. The specific use of immune checkpoint inhibitors and CAR-T-cell therapy can be employed to attack GBM cells [13]. Other methods include programmed death ligands (PD-L1), cytotoxic T-lymphocyte antigen 4 (CTLA-4), HSC transplantation, gene therapies and virotherapies, dendritic cell vaccines, and high-density lipoprotein nanoparticle vaccines [6] [11].

Looking closer, anti-vasculature therapy blocks the VEGF/VEGFR signaling pathways, but has failed to demonstrate significant benefit in patients [6]. Alternatively, oncolytic virus therapy infects cancer cells with antigens that can lyse the tumor cells. These viruses can activate macrophages, enhancing the infiltration of T-cells into the TME, leading to reduced immunosuppression [6]. Oncolytic virus therapy, likewise, is limited by safety and efficacy tests [6].

Lastly, CAR-T cell therapy is a novel and promising immunotherapeutic strategy. CAR-T Cells are T cells that have been removed from patients and modified to have tumor antigen-binding receptors that are specific. They are then reinserted, increasing T Cells' ability to recognize and destroy cancer cells [6] [11] [29]. However, CAR-T Cell therapy is limited by the heterogeneity of target antigen expression and difficulty in maintaining the activity of injected CAR-T cells [6] [25] [30]. Given these challenges, the pharmacological approach must also be considered.

The Pharmacological Approach

Addressing sleep deprivation through pharmacological means can lead to improved immune function and reduced inflammation [2]. Physicians can prescribe sedative-hypnotic

drugs: benzodiazepines and non-benzodiazepines used to improve sleep [2]. Furthermore, orexin agonists and antagonists can be implemented to manage sleep cycles [2] [3]. Moreover, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) has been shown to reduce the production of pro-inflammatory cytokines, chemokines, and prostaglandins in addition to inhibiting macrophages and microglia [13].

Similarly, corticosteroids, including dexamethasone and prednisone, are widely used to reduce neuroinflammation in GBM patients [13] [31] [32]. High doses of corticosteroids have been shown to rapidly decrease tumor-associated edemas and improve clinical symptoms [21]. Corticosteroids inhibit the production of inflammatory mediators such as prostaglandins and cytokines, suppressing inflammation and restoring BBB integrity, but can also induce hyperglycemia, weight gain, infections, myopathy, diabetes, immunosuppression, and osteoporosis [13] [31] [32]. As a result, current studies are evaluating alternatives to dexamethasone, which can enable some patients to reduce or discontinue corticosteroid doses. [31]. Hence, addressing the main cause of GBM, sleep deprivation, must also be considered.

Lifestyle Management

While Immunotherapies and pharmacological approaches may alleviate symptoms, they must be considered alongside lifestyle interventions. The management of sleep deprivation and maintenance of a balanced lifestyle can help reduce immune suppression and inflammation associated with GBM development. Experts have recommended routine screening for sleep disorders and sleep interventions [2].

Likewise, GBM cells grown in high-glucose media replicate significantly faster than those grown in normal media [10]. Thus, the Ketogenic diet (KD), characterized by a high fat-to-carb ratio, has been proven in managing many neurological conditions [10]. KD slows tumor growth and increases survival due to changes in immune response, gene expression, and amount of reactive oxygen species [10]. In fact, 44% of ketogenic diet studies reported improved health-related quality of life, a fundamental component of GBM management [33].

Calorie restriction or short-term fasting can also have a major impact on glioma survival. Calorie restriction has been proven to extend life span in yeast, mice, and primates by selectively protecting normal cells over cancer cells [10]. Lastly, the consumption of caffeine, such as through coffee or tea, has been associated with a lower risk of glioma by inhibiting tumor invasion and migration [10].

In addition to diet change, supplements and exercise can also be crucial in maintaining sufficient immune function, preventing the proliferation of GBM. For instance, forms of vitamin A, especially retinoic acid, have been shown to inhibit the proliferation of tumor cells in some human GBM cell lines [10]. Likewise, melatonin, an antioxidant produced by the pineal gland, has an anticancer effect on many cancer types [3] [10]. In GBM mouse studies, exercise significantly reduces tumor proliferation, and up to 41% of glioma patients complete recommended exercise during treatment [10] [33].

Sleep Therapies

Interventions aimed at improving sleep quality and duration are thus the centerpiece in connecting multiple therapeutic strategies and reducing GBM proliferation and progression. In fact sleep intervention have been promising in their objective of reducing oncogenic pathways. For instance, therapies such as Cognitive behavior therapy for insomnia (CBT-I) have been effective in treating insomnia and by extension is associated with improvements in immune function [2] [34]. CBT-I is a multicomponent intervention that targets sleep disturbance using sleep hygiene, sleep restriction, stimulus control, cognitive restructuring, and relaxation strategies [34] [35]. Furthermore, treatments like continuous positive airway pressure (CPAP) therapy for obstructive sleep apnea not only alleviate sleep fragmentation but also reduce levels of inflammatory markers, which are crucial in GBM growth [2]. Ultimately, restoring adequate sleep duration and quality can enhance the activity of immune cells, bolstering immune surveillance against cancer [2]. These insights highlight the strong association between sleep, immune function, and GBM, underscoring the importance of addressing sleep disorders in cancer prevention and treatment [2].

Sleep therapies can be combined with other therapies including cancer screening, immunotherapy and lifestyle management. Oncology clinics can adopt approaches that include sleep specialists who collaborate with oncologists to assess and manage sleep-related issues [2]. Similarly, integrating sleep management strategies such and pharmacological treatments into immunotherapy protocols may help optimize immune function and enhance treatment efficacy [2]. Lastly the benefits of lifestyle changes can be enhanced through adequate sleep. Diets rich in fruits, vegetables, lean proteins, and whole grains can enhance sleep quality by providing essential nutrients that support the body's natural sleep–wake cycle [2]. Therefore, sleep interventions is not only a promising yet overlooked therapeutic, but it also enhances the efficacy of other strategies.

In summary, treating GBM requires a multifaceted approach that addresses both immunological and physical components. Surgical interventions remain an effective treatment for reducing the tumor mass but may increase the risk of neuroinflammation. Immunotherapy, checkpoint inhibitors, CAR-T cell therapy, and oncolytic viruses are promising, but are limited by the suppressive nature of the TME and fragile BBB. Pharmacological interventions, including corticosteroids and NSAIDS, may also inadvertently induce immune suppression. Lastly, lifestyle changes, including improved sleep habits, may be difficult to implement in modern societal structures. Hence, given the drawbacks of each approach alone, a combined approach would be most effective in addressing glioblastoma and sleep deprivation. Future research should test the effectiveness of combinative therapies and research how surgery can bypass neuroinflammatory limitations.

Conclusion

The complex relationship between sleep deprivation and glioblastoma (GBM) proliferation has been largely overlooked. Chronic sleep deprivation promotes a pro-tumor microenvironment through increased neuroinflammation and immune suppression, which is fundamental in GBM progression. Moreover, the investigation of surgical intervention has resulted in mixed results in which surgery can both alleviate symptoms and exacerbate neuroinflammation [16], consequently warranting a consideration of a combinative therapeutic strategy. Given the severity of GBM and limitations of individual treatment, a combined approach involving surgery, immunotherapy, pharmacology, and lifestyle changes could overcome these individual limitations.

Sleep plays a vital role in immune regulation, and chronic sleep deprivation leads to the increased expression of pro-inflammatory cytokines and a reduction in T and NK cell function [3] [5]. This immune dysregulation can be directly translated to GBM development, as a result linking sleep deprivation with GBM progression.

The severity of GBM is driven by genetic mutations, an immunosuppressive tumor microenvironment, and the impairment of the blood-brain barrier. Key components include Tregs, TAMs, MDSCs, cytokines, and chemokines [23]. Thus, given the role of sleep deprivation in immune regulation, sleep is a main contributor rather than a side factor in GBM progression.

Each therapeutic approach, including surgery, immunotherapy, pharmacology, and lifestyle interventions, has its limitations. Surgery, while critical for tumor debulking and relieving symptoms, can worsen neuroinflammation and disrupt the blood-brain barrier. [13] [16] [27]. Likewise, immunotherapies, including CAR-T cell therapy and checkpoint inhibitors, are promising but are largely ineffective as a result of the immunosuppressive TME [6] [25] [30]. Pharmacological interventions, including corticosteroids and NSAIDs, and lifestyle changes (Keto Diet and Sleep Hygiene), may be unfeasible or lack long-term success.

When put together, these findings suggest that a combinative approach should be considered as a method to overcome individual limitations. At the forefront of this approach is addressing sleep deprivation through sleep therapies Likewise, surgery should not be used in isolation but in conjunction with other therapeutic strategies. By connecting sleep deprivation to GBM progression, treatment plans address the interplay between lifestyle, immunity and surgical interventions.

Nevertheless, questions remain. Is the timing or quality of sleep more important in maintaining sufficient immune function? What other key molecules contribute to immune suppression and neuroinflammation that haven’t been discovered/researched? How effective is the combinative approach & which therapies should be combined? How can surgery bypass the neuroinflammatory limitations? And how can we expand studies to human subjects instead of relying solely on rodent models? Future research should prioritize identifying new molecules

related to sleep-induced inflammation and immunosuppression in GBM patients and test effective combinative strategies that can overcome the limitations of each therapeutic strategy.

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EDITOR COMMENTS AND RECOMMENDATION:
The reviewers both commend the student for tackling a challenging and clinically significant question. Reviewer 1 raises several "major issues" that need addressing to strengthen the manuscript, namely reframing the hypothesis, updating some language for clarity and/or precision, including a description of review methods, and editing some figure presentation. The editor recommends that the student focus on these major critiques, with the student and advisor's discretion used for the minor suggestions. Reviewer 2 similarly highlights the lack of clarity in the hypothesis and requests that this be reworked, but is overall incredibly enthusiastic about the manuscript, its quality, and its originality. Overall, the editor recommends a major revision prior to acceptance.

REVIEWER 1:

The topic is relevant and potentially impactful, but the manuscript currently: (i) overstates a causal link between chronic sleep deprivation and GBM progression, (ii) does not actually demonstrate how surgery modifies the "sleep deprivation to neuroinflammation/immune suppression to GBM" pathway, (iii) contains several molecular/epidemiologic inaccuracies, and (iv) relies on broad, secondary sources rather than a focused synthesis of primary studies on sleep in GBM patients and peri-operative sleep/inflammation. A clearer review question, corrected terminology/numbers, and an explicit review methodology are needed.

Strengths:
Conceptual bridge (sleep-immunity-tumour axis): The paper sketches the roles of cytokines, GAM/TAMs, T-cell exhaustion, and BBB dysfunction in an immunosuppressive GBM TME. That overview is useful for readers and is thematically consistent across sections.

Clinical context: The surgical section recognises modern techniques (5-ALA, iMRI) and is directionally correct about benefits/limitations.

Practical angle: Flagging CBT-I/CPAP/sleep hygiene as potentially integrable into oncology workflows could become a distinctive contribution if tied to GBM-specific evidence.

Major issues to address:
1) The central hypothesis is asserted, not demonstrated:
Across the abstract/introduction/conclusion, the manuscript implies a causal arc "chronic sleep deprivation to neuroinflammation/immune suppression to accelerated GBM," with surgery as a modulator. Yet, it cites almost no GBM-patient studies linking measured sleep (actigraphy/PSG/OSA) to inflammatory markers (IL-6, TNF-α, CRP) or outcomes (EOR-adjusted

PFS/OS). The text mostly strings together (a) generic sleep-inflammation studies and (b) GBM immunology papers, then infers the bridge. This should be reframed as a question-driven review with explicit evidence tables for each link in the chain; where GBM-patient data are absent, label that gap clearly.

2) Molecular table and terminology errors:
The “Genetic Alteration/Functional Alteration” table contains factual and nomenclature issues that need correction: duplicated and misspelled CDKN2A/p16^INK4a (“CDK2A-p16INL4a,” repeated twice), EMP3 is labelled “tumor suppression” despite frequent association with adverse biology in GBM, and apoptosis labels for TIMP3/CDH1 are oversimplified; elsewhere HIF-1α is written as “HIF-A.” Please fix gene symbols, expand functional categories beyond one-liners, and cite primary sources for each entry.

3) Epidemiology needs verification/softening:
The manuscript states GBM is “the leading cause of cancer deaths in ages 15-34” and gives global/US incidence totals without primary registry attribution. As written, these lines are overconfident and likely inaccurate for the 15-34 age band. Either provide specific registry-grade citations (with years, geography) or soften the language.

4) Surgery is not analytically connected to sleep:
The peri-operative section correctly notes BBB disruption, DAMP signalling, and macrophage trafficking after surgery, but it does not show any data on peri-operative sleep architecture/circadian disruption (pre/post craniotomy), nor link those changes to inflammatory markers or relapse dynamics in GBM. Add a dedicated “Peri-operative sleep-inflammation axis” sub-section synthesising what is known in neuro-oncology (or closely related neurosurgical cohorts) and specify what is unknown.

5) “Combined therapy” is enumerated, not integrated:
Lists of immuno/pharmaco/lifestyle options read as declarative catalogues. Please develop a mechanistic matching logic (e.g., why CBT-I ± CPAP for patients with OSA/fragmentation and elevated IL-6; how to balance steroid-sparing with oedema control; where melatonin fits as a chronobiotic). Frame tangible clinical scenarios and potential synergies/risks.

6) Balance of evidence (diet/caffeine/NSAIDs/steroids):
Claims about ketogenic diets, caffeine, NSAIDs, and corticosteroids are stated strongly relative to a mixed evidence base (and with safety trade-offs). Please separate preclinical vs clinical findings, report effect sizes/quality, and include contraindications (e.g., bleeding risk for NSAIDs; potential survival impact of high-dose steroids despite anti-oedema benefit).

7) No review methods:
As it stands, this is a narrative review with no search strategy, inclusion/exclusion criteria, time window, or bias assessment. Add a brief Methods section describing databases, terms, date range, selection flow, and how you assessed study quality; mark narrative content as such.

8) Clarity and presentation:
Figures should have self-contained captions (no raw URLs), consistent abbreviations on first use (GBM/Glioblastoma, TME/GAM/TAM, sleep deprivation vs restriction vs fragmentation), and careful copy-editing to remove duplicates/typos (see gene table).

<u>Additions that would substantially strengthen the paper:</u>
Re-structure around a five-link causal chain:
A. Chronic sleep deprivation to B. systemic inflammation/HPA axis (IL-6/TNF-α/CRP) to C. BBB/microglia/monocyte trafficking to D. TME immune landscape (TAM/MDSC/Tregs, T-cell exhaustion) to E. clinical outcomes (growth/recurrence/survival). Build a table for each link with GBM-patient data where available, then preclinical. Flag explicit "evidence gaps."

Peri-operative module:
Synthesise data on sleep disturbance before/after GBM resection (insomnia, fragmentation, OSA), time-course of IL-6/TNF-α/CRP and monocyte shifts in the first 2-4 weeks, and associations with EOR, oedema, and steroid exposure. Propose measurement standards (actigraphy + inflammatory panels).

Concrete study designs:
Pilot RCT: CBT-I (or stepped-care CBT-I) 2-3 weeks pre-op + 4 weeks post-op vs usual care; primary: IL-6/TNF-α/CRP and actigraphy; secondary: quality of life and PFS adjusting for EOR.
Prospective cohort: GBM patients with PSG/actigraphy, cytokine panels, and, where feasible, paired immunophenotyping of blood and tumour; correlate with growth/recurrence.
Mechanistic preclinical: chronic sleep fragmentation models assessing microglial polarisation/monocyte infiltration in orthotopic GBM.

Missing summary tables/figures:
"Sleep interventions to immune/inflammatory effects to hypothesised GBM impact," covering CBT-I, CPAP, melatonin, light therapy, physical activity, and hypnotics (with cautions re: cognition/falls/interactions).
"Candidate biomarkers" to stratify/monitor (IL-6, sTNFR, CRP, actigraphy indices, circadian markers) and suggested clinical use cases.

Minor but required edits:
Avoid categorical phrasing like “sleep is a main contributor” to GBM progression; reframe as a potential modifier of the immune microenvironment/BBB with currently indirect evidence.

Standardise abbreviations at first mention (GAM, MDSC, CBT-I, CPAP) and keep them consistent across text/figures.

In the surgery section (5-ALA/iMRI), provide effect sizes and study types when claiming improved outcomes; otherwise, soften the language.

Revise the caffeine/ketogenic diet paragraphs to clearly separate GBM-specific clinical data from broader oncology or preclinical findings, and to note feasibility/tolerability constraints explicitly.

Bottom line:
This manuscript raises an underexplored and worthwhile question (how sleep may modulate the immune micro-landscape in GBM and how surgery could intersect with that axis), but as written, it is a broad narrative rather than a targeted, evidence-based synthesis focused on GBM patients or peri-operative effects. Implementing the structural changes above, correcting factual/nomenclature issues, adding a methods section, and proposing concrete study designs would substantially elevate the work and make it genuinely useful to neuro-oncology clinicians and investigators.

Overall Recommendation: Major revisions required.

REVIEWER 2:

I had the pleasure of reading and reviewing the paper titled “The Sleep-Cancer Mystery: An Investigation of How Surgery Affects the Association Between Sleep Deprivation and Glioblastoma Through Neuroinflammation and Immunosuppressive Modulation.” I thought the student chose a very interesting topic to research and they examined the issue quite thoroughly. This is clearly an important topic since glioblastoma is so difficult to treat, and therefore any methods to prevent it or to improve treatment options are desperately needed. The paper does feel original and significant, since there is not a lot of research around the role of sleep in glioblastoma progression. The argument is well-organized and relatively easy to follow, and the ideas are clearly presented for the most part - although the hypothesis in the abstract is perhaps a bit convoluted. There are some small errors, like the first two mutations in the table

are duplicates of each other and the section title should be “Targeting Glioblastoma” rather than “Targeting Glioblastoma: The Surgical Approach.”

The authors cited plenty of research and used appropriate methods, although I would have liked to see some searches from clinicaltrials.gov or some other clinical data to support how well some therapies work in comparison to others. They did engage with the literature but there is more literature available than just the classic scientific journals! If they are interested in combination therapies, it is particularly important to dig into things like checkpoint inhibitors in combination with other cancer therapies, and I think even if there isn’t a lot of data available in glioblastoma with certain combinations there may be examples of those same combinations being used in other kinds of cancer. The student connected their findings to their main hypothesis but the story rambled a bit. It would be good to add something like a graphical abstract that can help ground your reader in their core claim and how everything they are discussing relates back to that. The grammar and language was clean and professional as expected of a research paper.

Based on all of this (a well-researched paper that is presenting a mostly coherent story on an important and unique topic), I would recommend accepting this paper with minor revisions. These revisions should include a more clear statement of the hypothesis and the mechanisms it is linking together, which specific kinds of combinations would be useful and any examples of clinical data relevant to the central claims.

The Sleep-Cancer Mystery: An Investigation of How Surgery Affects the Association Between Chronic Sleep Deprivation and Glioblastoma Progression Through Neuroinflammation and Immunosuppressive Modulation.

[redacted]

Abstract

Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumor in adults. GBM progression is influenced by cytokines, chemokines, tumor-associated macrophages (TAMs), and other immune molecules that can lead to an immunosuppressive tumor microenvironment (TME) and infiltrate the blood-brain barrier. Chronic sleep deprivation is a pervasive and dangerous condition that is associated with systemic immunosuppression and neuroinflammation. Numerous studies have found links between sleep deprivation and elevated markers of systemic inflammation and impaired anti-tumor immunity; however, the direct impact on GBM progression remains poorly defined. This review examines whether and how chronic sleep deprivation contributes to neuroinflammation and immunosuppression that may facilitate GBM progression and whether surgical interventions can modulate these effects. While prior studies have explored individual therapeutic strategies, including surgery and immunotherapy, results have been limited. Rather than asserting causality, this review synthesizes evidence across five mechanistic links: (1) Chronic sleep deprivation and inflammatory biomarkers (Interleukin (IL)-6, Tumor Necrosis Factor Alpha (TNF-α), and C-Reactive Proteins (CRP)), (2) systemic neuroinflammation to Blood Brain Barrier (BBB) disruption, microglial activation and monocyte trafficking, (3) neuroimmune changes to TME remodelling, (4) TME remodeling and clinical outcomes, and (5) clinical treatments of surgical modulation and its effects on the BBB, TME and neuroinflammation. The current literature suggests there is a strong association between sleep deprivation and systemic neuroinflammation, and between neuroinflammation and GBM proliferation, but direct GBM-patient studies connecting measured sleep quality (actigraphy, polysomnography (PSG), obstructive sleep apnea (OSA)) to inflammatory markers remain scarce. Given the challenging nature of directly addressing sleep deprivation and surgery's possible linkage to increased inflammation, combinative therapy could be more effective. By clarifying the current evidence and critical gaps, this review highlights the need for integrated clinical and translational studies examining how sleep therapy and combinative surgical-immunotherapeutic strategies could improve GBM outcomes. The implications of this review can offer a greater understanding of the association between sleep deprivation and the proliferation and progression of GBM, and whether a combination therapy or surgery alone is the most feasible and effective approach in addressing this prevalent and dangerous disease.

Introduction

Chronic sleep deprivation is a widespread and severe condition that can contribute to impaired immune function and neuroinflammation, promoting a pro-TME [1] [2] [3]. Cytokines and other immune molecules influence this response and have made the treatment of GBM, a common form of brain cancer, very challenging [2] [3].

Sleep is essential for maintaining homeostasis, supporting immune, neural, hormonal, and metabolic function [1] [2]. Sleep deprivation, the reduced sleep below physiological needs, affects an estimated 50–70 million Americans across all ages and socioeconomic groups, and has become a growing issue [2] [3] [4].

Chronic sleep deprivation is associated with impaired immune responses, including lower cytotoxic activity (reduced CD8+ T cells and natural killer (NK) cells) and increased pro-tumor inflammation [3]. More specifically, sleep deprivation can contribute to neuroinflammation, which is marked by proinflammatory cytokines [2] [5], promoting tumor-favorable conditions [1] [5] [6]. Furthermore, sleep deprivation weakens antitumor immunity and decreases T-cell activity and impairs the blood-brain barrier, leading to an influx of inflammatory agents [2] [3]. These immune alterations are connected to mechanisms implicated in cancer progression, but their specific relevance to GBM remains underexplored.

Patients with GBM, the most common and aggressive primary malignant brain tumor in adults [3] [6] [7] [8], have a survival time of fewer than 15 months [9] [10] [11] [12]. The severity of GBM can be attributed to its anatomical location and the disruption of the blood-brain barrier [11]. GBM proliferation is complex and driven by epigenetic alterations and mutations in oncogenes, tumor suppressor genes, and DNA repair pathways [11] [12] [13] [14] [15]. Neuroinflammation promotes tumor growth, invasion, and therapy resistance by altering the TME [13]. The TME is composed of tumor cells, immune cells (microglia, macrophages, T cells, NK cells), stromal cells, and the extracellular matrix [2] [6] [8] [9] [11]. Glioma-associated microglia/macrophages (GAMs) release TGF-β, IL-6, IL-10, and other molecules to inhibit T cells [11]. Furthermore, CD8+ cells and NK cells, key to the anti-tumor response, are suppressed or exhausted in the GBM TME [6].

Evidence from non-GBM populations indicates that sleep deprivation increases neuroinflammatory signaling and BBB permeability [3] [5]; however, few studies have directly assessed whether chronic sleep deprivation correlates with inflammatory markers (IL-6, TNF-α, CRP) or survival outcomes such as progression-free survival (PFS) and overall survival (OS)

Multiple therapeutic strategies exist to combat GBM, including immunotherapy, pharmacological means, lifestyle and sleep management, and surgical interventions. [2] [6] [8] [13] Surgery is especially vital in GBM treatment and can relieve symptoms by reducing tumor mass [13]. Surgery, however, can disrupt the BBB by increasing the infiltration of inflammatory macrophages and decreasing the integrity of tight junctions [16]. Given these contrasting effects,

surgical intervention may serve as a modulator in the broader connection between sleep, neuroinflammation, and immune suppression.

Despite advances in neuroscience and oncology, treatment options for GBM remain uncertain and are only marginally effective. Recent studies have suggested that sleep deprivation plays a role in increasing neuroinflammation and immunosuppression facilitated through proinflammatory cytokines, macrophages, microglia, and T-cells [3] [5] [6]. While previous studies have associated sleep deprivation with neuroinflammation and immune suppression, and others have found a correlation between neuroinflammation/immune suppression and GBM progression, a direct association has yet to be identified.

This review, therefore, aims to answer these two key questions:

1. What evidence connects sleep deprivation with systemic neuroinflammatory and immunosuppressive markers that connect to GBM proliferation?
2. Can surgical or combinative interventions mitigate neuroimmune dysregulation associated with chronic sleep deprivation?

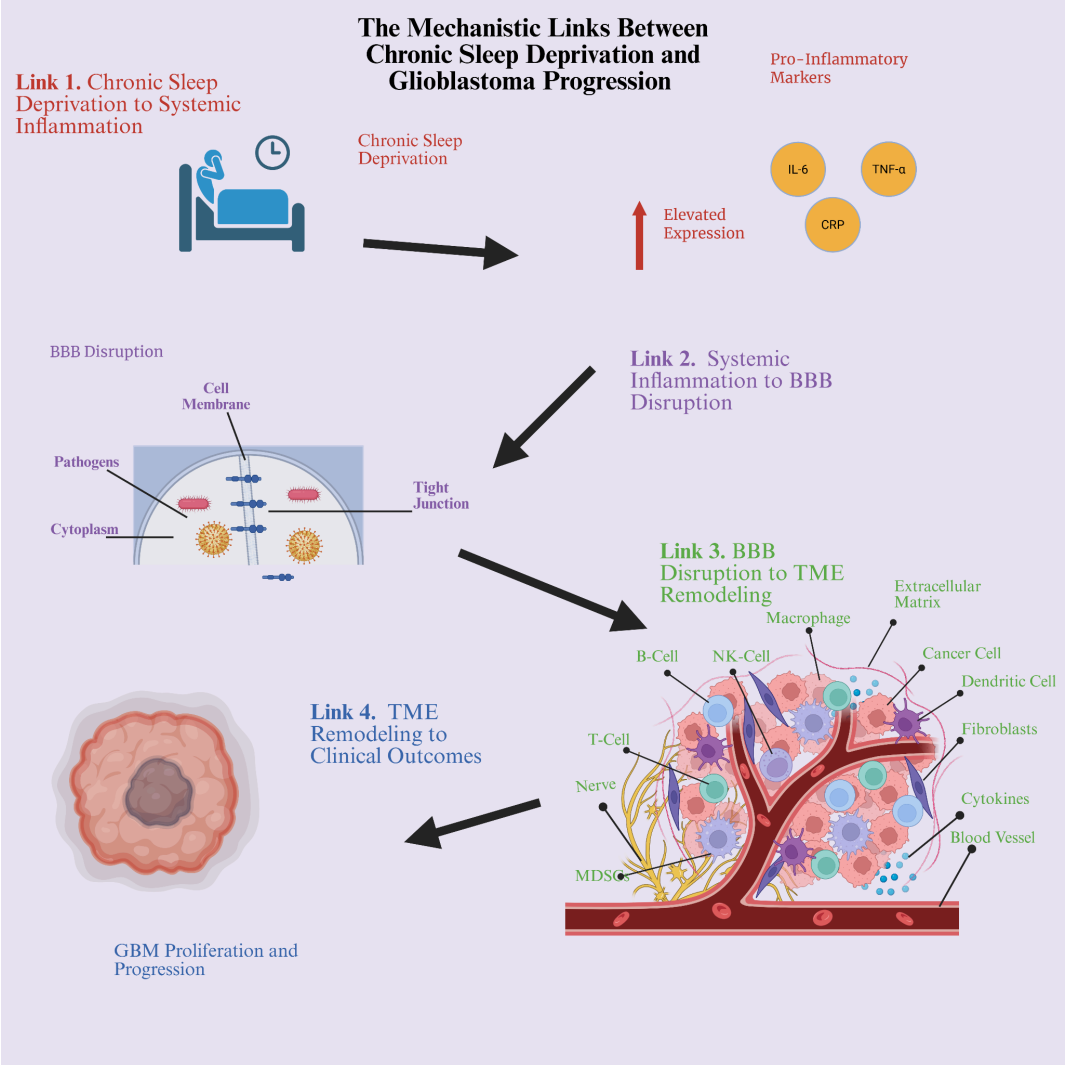

Figure 1 (Chan, 2025): A graphical abstract illustrating the key mechanistic links of this review.

Methods

Search Strategy and Databases

A comprehensive literature search was conducted through PubMed, Google Scholar, Scopus, Science.gov, Embase, BioMed Central, and Science Direct. Included entries were published from January 2000 to October 2025. Key words include "glioblastoma," "sleep deprivation," *"circadian rhythm," "neuroinflammation," "blood-brain barrier," "cytokines," "surgery," "immunotherapy,"* and *"tumor microenvironment."* Boolean operators (AND/OR) were used to refine results.

Inclusion Criteria and Study Selection

Studies were included in this review if they:

1. Were peer-reviewed
2. Involved human participants or relevant animal models
3. Investigated at least one of the following: (a) effects of sleep deprivation or circadian disruption on immune or inflammatory signaling, (b) neuroinflammatory and immunosuppressive mechanisms in GBM, or (c) surgical modulation or other therapeutics involved in mitigating the effects of these pathways.

Excluded studies were non-English publications, sources without primary data, and papers that did not include measurable inflammatory, immune, or clinical outcomes.

Quality Assessment

Titles and abstracts were screened for relevance, and full-text articles were reviewed in depth. Study quality was determined based on methodological rigor, including sample size, control design, and clarity of variables. Potential biases and limitations of selected studies were noted in the results and discussion sections. When quantitative data were not feasible due to the nature of the study, results were assessed by mechanistic evidence and research gaps involving sleep deprivation, neuroinflammation, and therapeutic strategies.

Chronic Sleep Deprivation, Inflammation, and Immune Suppression: Investigating Sleep's Crucial Role in Immune Function

Adequate sleep ensures that the immune system is sufficiently regulated, controlling the spread of diseases. New studies highlight how insufficient sleep triggers neuroinflammation, characterized by elevated pro-inflammatory cytokine levels, and the impairment of key immune cells like T-cells and NK cells [1] [2] [3]. Given the rising prevalence of sleep deprivation due to modern lifestyle changes, its immunological consequences must be addressed.

Sleep and Sleep Deprivation

Sleep is a fundamental physiological and neurological process that is crucial for maintaining immune, metabolic, and neural homeostasis [2] [3]. This process is shared among many organisms and is vital for healthy neural and brain functions [3]. Given the importance of sleep in immune function, sleep deprivation should be viewed as a serious health concern.

In contrast, sleep deprivation, the reduction in sleep time from the required amount [3], has become increasingly common in modern society, driven by environmental and lifestyle changes (socioeconomic environment and lifestyle) [2] [3]. Chronic sleep deprivation is excessive daytime sleepiness that occurs nearly every day for at least three months [4]. Sleep deprivation affects an estimated 50 to 70 million Americans of all ages and socioeconomic classes [4]. Alarmingly, the number of people getting sufficient sleep, at least 6 hours of sleep a night, has continued to decrease over the last 25 years [1].

Beyond fatigue, sleep deprivation is associated with an increased risk of cardio/cerebrovascular diseases, accidents, hypertension, stroke, cancer, and neurodegenerative diseases [4] [17]. These associations underscore the necessity of researching sleep deprivation's interactions with the immune system, specifically, inflammatory and anti-tumor functions.

Sleep Deprivation and Immune Regulation

Sleep deprivation disrupts immune regulation, increasing susceptibility to infections and tumor proliferation [4]. Individuals who are consistently sleep deprived exhibit a higher susceptibility to common infections and are more vulnerable to diseases [2] [5]. Past studies have shown that sufficient sleep leads to lower cortisol levels, allowing the immune system to function optimally, reducing the risk of inflammation [2].

Sleep deprivation triggers an inflammatory response characterized by increased levels of proinflammatory cytokines, supporting protumor conditions. Neuroinflammation is an inflammatory response in the brain marked by the release of cytokines, chemokines, and growth factors [1] [6].

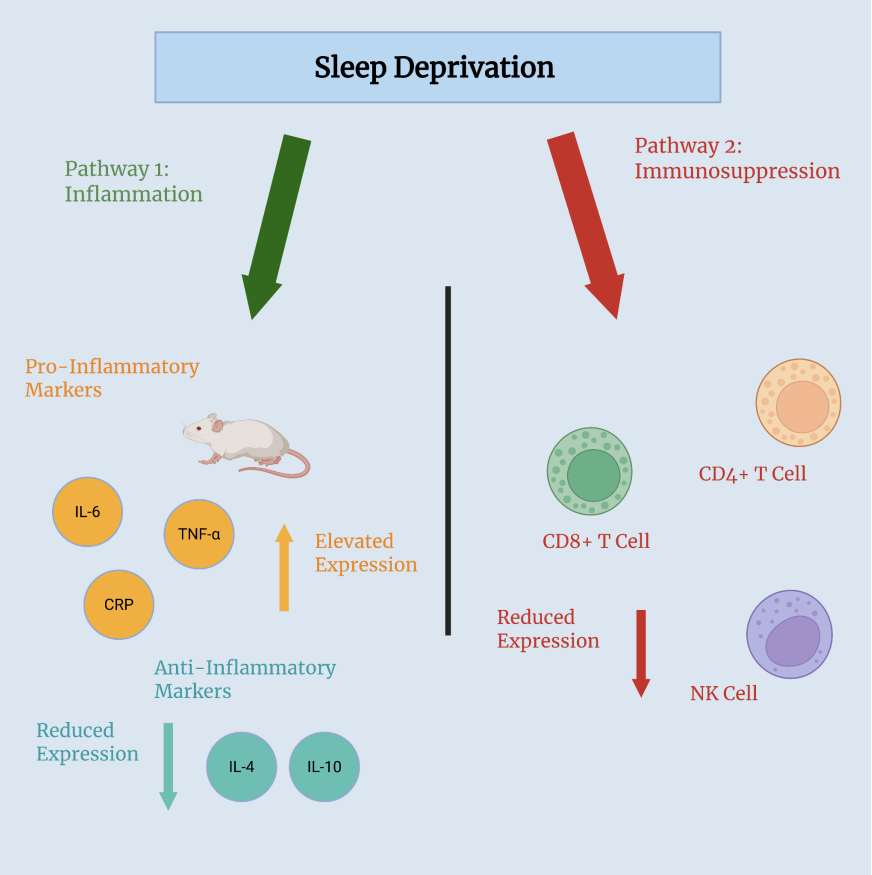

Figure 2 (Chan, 2025): Sleep deprivation has been associated with increased levels of pro-inflammatory markers, including IL-6, TNF-α, and CRP [2] [3] [4] and suppressed levels of anti-inflammatory cytokines IL-10 and IL-4 [5]. Sleep deprivation also reduces immune cell activity, including CD8+, CD4+, and NK Cells [3][5]. These immune shifts are both markers of sleep deprivation and mechanisms by which GBM can evade detection.

Other markers with altered expression levels in sleep-deprived studies include IL-1α, IL-1β, IL-2, IL-8, IL-13, IL-15, IL-18, TNF-β, IFN-α, IFN-β, macrophage inhibitory protein (MIP)-1β, corticosterone, and homocysteine [1] [3]. In multiple rodent models, sleep loss has been linked to an increase in IL-1, IL-6, IL-15, IL-17, and TNF-α [5]. In summary, sleep deprivation influences the balance between pro- and anti-inflammatory responses, leading to chronic inflammation. Chronic sleep deprivation also impairs the anti-tumor response by reducing T-cell activity. Adequate sleep enhances the activity of T-cells by increasing the expression of integrins on T cells, proteins that facilitate a cell's ability to attach to and destroy infected cells [2]. Therefore, sleep deprivation contributes to cytokine and immune alterations that favor tumor development and progression.

The correlation between sleep deprivation, neuroinflammation, and immune suppression must be further studied as sleep deprivation cases continue to increase. While chronic sleep

deprivation is associated with elevated pro-inflammatory cytokines (e.g., IL-6, TNF-α) and impaired immune cells (e.g., T cells, NK cells), the specific molecular mechanisms, including downstream transcription factors and alterations in cytokine receptor signaling, are not fully understood. Nevertheless, sleep deprivation-induced immune imbalances create the immunosuppressive and pro-tumor environment that accelerates GBM progression. Future studies could investigate whether sleep quality or quantity is more impactful on immune regulation, as addressing these concerns could lead to targeted interventions involving sleep hygiene and sleep therapies.

Table 1: Evidence Linking Chronic Sleep Deprivation to Inflammatory and Immune Markers

Study	**Population/ Model**	**Key Inflammatory Markers/**	**Key Immune Markers**	**Limitations/ Gaps**
[2] [5]	Human Volunteers	IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-15, IL-18, TNF-α, TNF-β, IFN-α, IFN-β, IFN-γ	NK, monocytes, T, and B lymphocytes	Short-term studies; limited data on chronic deprivation
[5]	Mouse and Rat Models (Forced Wakefulness)	IL-1α, IL-1β, IL-1, IL-6, IL-17, and TNF-α	NK, T lymphocytes	Inflammatory levels vary by sleep deprivation method

Understanding Glioblastoma: The Interactions Between Genetics, Neuroinflammation, and Immune Suppression

GBM is the most aggressive and lethal form of primary brain cancer and is associated with poor survival outcomes. This resistance is in large part due to GBM's highly immunosuppressive TME, which is a complex of inflammatory cytokines and immunosuppressive molecules. Key immune players, including TAMs, Myeloid-Derived Suppressor Cells (MDSCs), regulatory T cells (Tregs), and astrocytes, contribute to favorable tumor conditions. Molecular and cellular pathways play a decisive role in GBM proliferation and progression.

Glioblastoma & Mutations

GBM is the most aggressive and common primary malignant brain tumor that has been difficult to treat due to its location and immunosuppressive microenvironment [3] [6] [7] [8]. GBM arises from glial cells and accounts for 60 to 70% of all malignant gliomas, 50% of all gliomas that arise in the central nervous system (CNS), and about 15% of all brain tumors [11] [13] [18]. Patients with GBM have a median survival of 14.7 months, with only 6.8% of patients surviving past five years [9] [10] [11] [12], and approximately 75% of affected patients die within two years of their diagnosis [19] [20]. Although GBM represents roughly 1.4% of annual cancer incidences, it accounts for 2.5% of total cancer deaths. There are an estimated 10,000 new annual cases in the United States and 100,000 new cases globally [8] [9] [21] [22]. Hence, additional research in the molecular mechanisms involved in GBM proliferation and progression is required.

Genetic Alteration	Functional Alteration & Biological Role	Representative Sources
CDKN2A/p16^INK4a	A tumor suppressor that inhibits CDk4/6 and halts the cell cycle at G1 phase. In GBM, CDKN2A is deleted or silenced.	[12] [23]
EGFR	The epidermal growth factor receptor is a transmembrane protein that controls cell growth and proliferation. In GBM, EGFR is amplified or mutated, leading to uncontrolled cell proliferation and tumor progression.	[11] [12] [14] [15] [24]
ERBB2	This gene codes for a receptor tyrosine kinase, ERBB2, to form heterodimers, enhancing cell proliferation. In GBM, ERBB2 is amplified or overexpressed, contributing to tumor growth.	[14] [25]
PDGFR	Plantelet-Derived Growth Factor Receptor is a tyrosine kinase that activates intracellular pathways, including MAPK, promoting cell proliferation. In GBM, PDGFR is amplified or overexpressed.	[11] [15] [26]
EMP3	The Epithelial Membrane Protein 3 gene encodes a tetraspan	[12] [27]

	transmembrane protein involved in cell proliferation. In GBM, EMP3 is often overexpressed or epigenetically dysregulated, exhibiting oncogenic behavior.	
RASSF1A	The Ras Association Domain Family 1 gene encodes a RAS effector protein involved in cell cycle regulation and DNA damage response. In GBM, RASSF1A is inactivated by promoter hypermethylation, leading to cell cycle and DNA repair disruption.	[12] [28]
BLU	The BLU gene encodes a transcriptional repressor related to cell cycle regulation and apoptosis. In GBM, BLU is often silenced by promoter hypermethylation, leading to the loss of cell cycle control and apoptosis.	[12] [29]
TP53	The TP53 gene encodes the p53 tumor suppressor protein, which is involved in DNA repair and apoptosis. In the case of GBM, TP53 is functionally inactivated.	[11] [12] [14] [15] [30]
PTEN	The PTEN gene encodes a phosphatase enzyme that functions as a tumor suppressor, promoting genomic stability and apoptosis. In GBM, PTEN is often mutated, deleted, or epigenetically silenced.	[11] [12] [14] [15] [31]
NF1	The NF1 gene encodes for neurofibromin, a tumor suppressor protein expressed in neurons, oligodendrocytes, and Schwann cells. In GBM, NF1 is mutated, leading to	[11] [12] [14] [15] [32]

	uncontrolled tumor proliferation and enhanced survival.	
RB1	The RB1 gene encodes the retinoblastoma protein (pRB), a key tumor suppressor that regulates the cell cycle, specifically the G1 S Phase transition. In GBM, RB1 is often deleted, mutated, or functionally inactivated, leading to G1/S checkpoint loss and tumor proliferation.	[12] [14] [33]
MGMT	The MGMT (methylguanine-DNA methyltransferase) gene encodes a DNA repair enzyme, reversing damage caused by alkylating agents. In GBM, MGMT is silenced through promoter hypermethylation	[11] [12] [34]
MLH1	The MLH1 gene encodes a DNA mismatch repair protein that detects and repairs base-pairing errors during DNA replication. In GBM, MLH1 loss and hypermethylation lead to defective mismatch repair proteins.	[12] [35]
DAPK1	The Death-Associated Protein Kinase 1 gene encodes a calmodulin-dependent serine/threonine kinase regulating cytoskeletal dynamics and apoptosis. In GBM, DAPK1 is silenced, leading to reduced apoptotic signaling.	[12] [36]
TIMP3	The Tissue Inhibitor of Metalloproteinases 3 gene encodes a secreted extracellular matrix (ECM) protein that inhibits matrix metalloproteinases. In GBM, TIMP3 expression is reduced or inactivated, leading to enhanced invasion and	[12] [37]

	metastasis and reduced tumor suppressive activity.	
CDH1	The CDH1 gene encodes E-cadherin, a transmembrane protein fundamental for cell-cell adhesion in epithelial tissues. In GBM, CDH1 is downregulated, disrupting cell adhesion and promoting enhanced invasion and metastasis.	[12] [38]

Table 2: GBM progression is influenced by genetic mutations and epigenetic modifications that regulate cell cycle control, apoptosis, and DNA repair pathways. These alterations facilitate immune evasion and therapeutic resistance.

Neuroinflammation also plays a significant role in GBM progression by activating oncogenic signaling pathways and inducing genetic and epigenetic changes [6] [13] [22]. GBM samples frequently express higher concentrations of pro-inflammatory proteins such as P2X7R, RAGE, NOS2, COX2, and PTX3 [20]. These genetic and epigenetic alterations are central to discerning the complex relationship between neuroinflammation and immunosuppression in GBM. While mutations initiate GBM, sleep deprivation exacerbates these oncogenic pathways, fueling neuroinflammation and immune suppression.

Neuroinflammation, Immunosuppression, and Blood-Brain Barrier Disruption

The Blood-Brain Barrier and Tumor Microenvironment

The BBB is essential for maintaining homeostasis, but it becomes compromised in GBM, contributing to immune suppression and inflammation. The BBB maintains normal brain function by preventing the absorption of toxins and pathogens, regulating transport and immune surveillance, and helping to maintain a stable environment [6] [11]. However, when compromised, such as when an individual is sleep deprived, blood vessels in the brain are altered both anatomically and functionally [11]. Hypoxia-inducible factor-α (HIF-1α) regulates the expression of inflammatory factors such as vascular endothelial growth factor (VEGF), which disrupts the cellular barrier around blood vessels by creating capillaries with fewer tight junctions [11]. These alterations make the administration of medicine very challenging. Sleep deprivation can disrupt BBB integrity, increasing susceptibility to GBM proliferation and

progression.

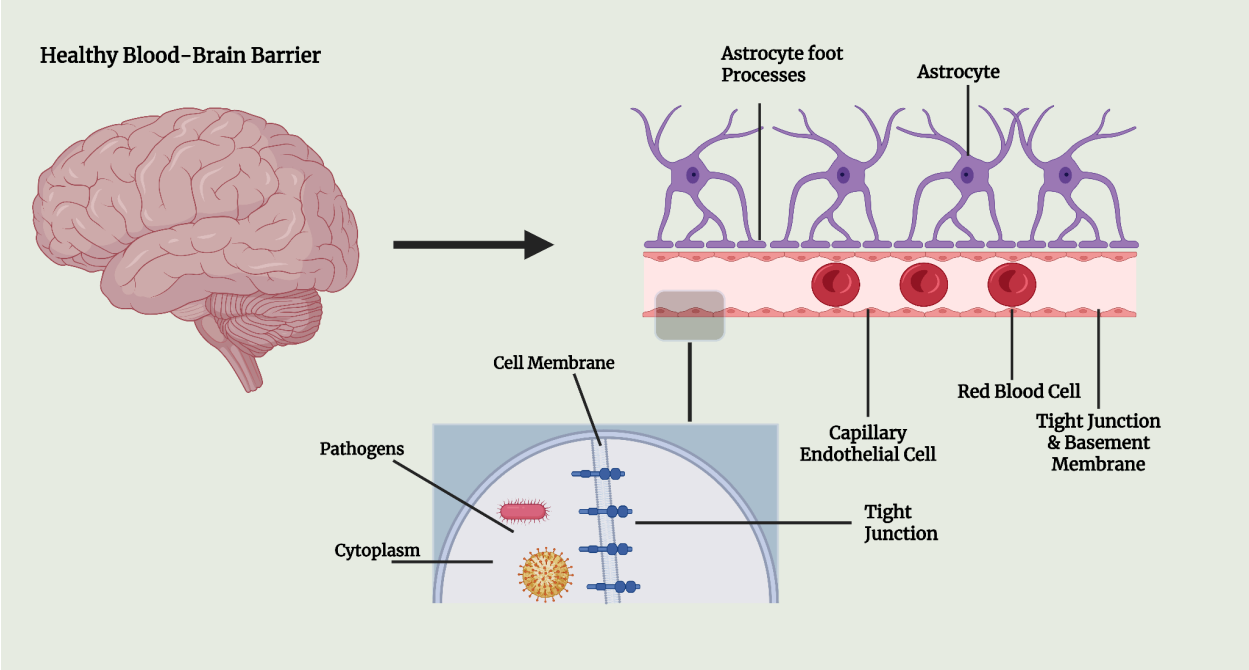

Figure 3 (Chan, 2025): The healthy BBB is a selectively permeable barrier that is secured by endothelial cells linked by tight junctions and surrounded by astrocytes and basement membranes [6] [11].

How Neuroimmune Changes Remodel the Tumor Microenvironment

Similarly, the GBM TME, a complex system, promotes tumor progression and immune suppression. The TME includes brain resident microglia, infiltrating macrophages, collectively known as GAM, and MDSCs [8]. Inflammatory cells are believed to make up between 30% and

50% of the tumor mass [6].

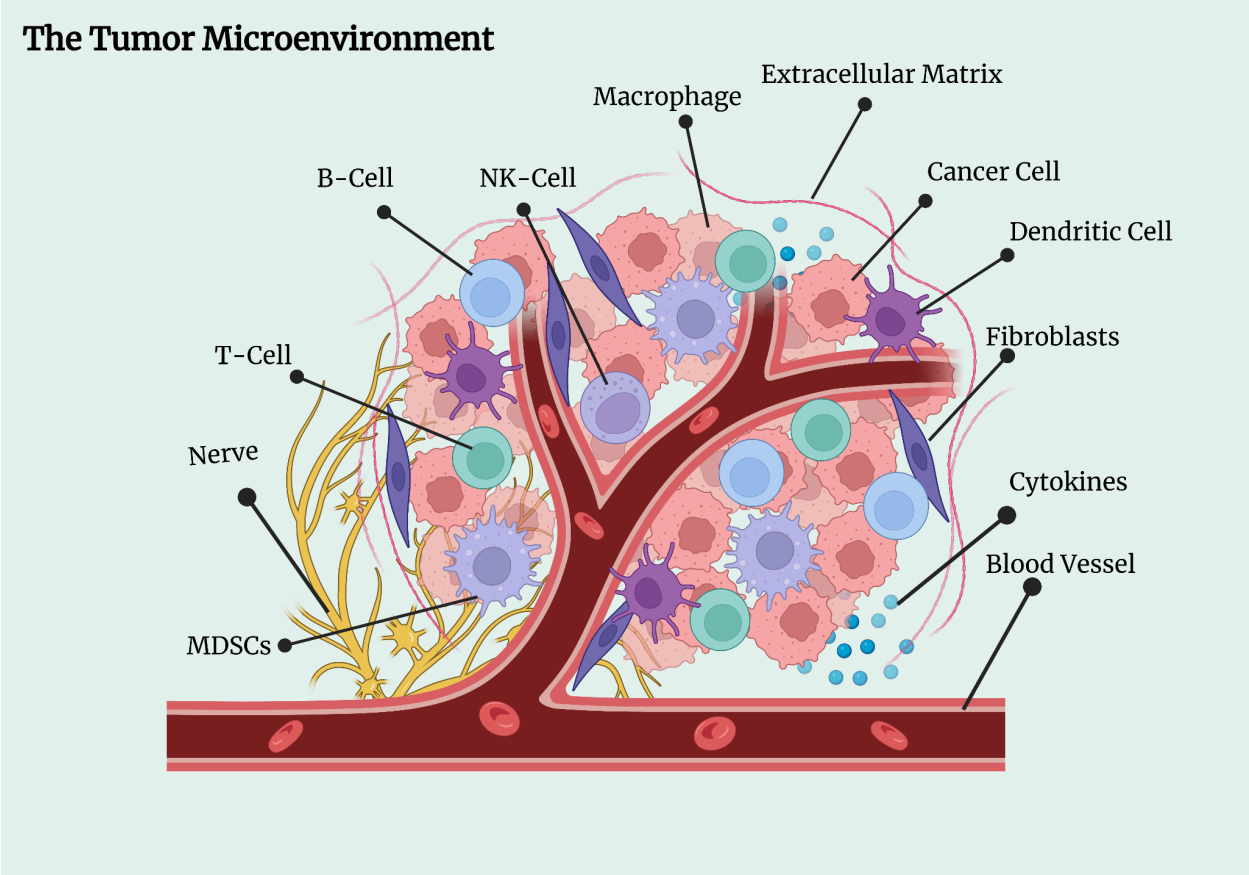

Figure 4 (Chan, 2025): The TME accounts for up to 30% of the tumor's mass and is made up of cancer cells, immune cells, macrophages, dendritic cells, cytokines, fibroblasts, and extracellular matrix components. [2] [6] [8] [9] [11].

Tumor-Associated Macrophages, Cytokines & Chemokines

TAMs and MDSCs are pivotal in immunosuppression and tumor progression in the GBM TME. Microglia cells are the resident macrophages of the CNS and respond to chemokines and cytokines [39]. TAMS accounts for 30% of the tumor volume and are the main inflammatory cell components [6]. Pathological microglia, peripheral macrophages, and monocytes activate and contribute to a disrupted immune response by activating immunosuppressive pathways [11]. MDSCs are immature myeloid cells that express higher levels of immunosuppressive molecules.

In GBM, microglia show increased expression of proinflammatory genes, including SPP1, HLA-DR, TREM2, APOE, CD163, and GPR56, which is elevated [39]. Other inflammatory molecules include let7, tenascin-C (TNC), veriscan, IL-1β, TGF-β, TNF-α, stress-inducible protein (STI-1), prostaglandin E2, IL-6, IL-1β, IL-10, and epidermal growth factor [11]. Interestingly, macrophages are divided into M1 and M2 phenotypes. M1 macrophages exhibit immune supportive and anti-tumor functions, while M2 macrophages are

immune suppressive and pro-tumor [6] [39]. Specifically, Gal-1 converts tumor-infiltrating macrophages to the immunosuppressive M2 variants [11]. TAMs are critical components in immune suppression and GBM progression.

Cytokines and chemokines are key components in the promotion of immune suppression in the GBM TME by impairing anti-tumor responses. Cytokines are multifunctional molecules that control angiogenesis, proliferation, and immune cell infiltration in the TME [8] [11]. Cytokines are secreted by immune cells and include pro-inflammatory (IL-6, IL-8, TNF-α) functions and anti-inflammatory (IL-4, IL-10, TGF-β) functions [6]. Inflammatory cytokines significantly increase the proliferation and invasiveness of GBM cells as immune cells fail to recognize tumor cells [7] [11]. Glioma cells express many cytokines, including TGF-β, IL-10, IL-4, IL-6, IL-13, colony-stimulating factor-1 (CSF-1), LDH5, galectin-1 (gal-1), and prostaglandin-E [7] [11] [40]. IL-6 and TGF-β are especially important to GBM progression. High IL-6 expression is associated with poor survival and is produced by malignant cells in response to oncogenic mutations [7] [8]. TGF-β, meanwhile, is involved in the regulation of cell growth and differentiation [8]. TGF-β is expressed at low levels in the brain but is greatly increased in GBM, suppressing CD8+ T cell activity [8]. Likewise, chemokines are small proteins that manage the migration of various cells in the body. Chemokines that are highly expressed in TME include CXCL2, IL-8, and CCL2. The relationship between cytokines and chemokines facilitates neuroinflammation and immunosuppression, leading to GBM progression. Because cytokines and chemokines are elevated in patients with GBM and those who are chronically sleep deprived, sleep loss can compound the immunosuppressive TME, crucial to GBM progression.

T-Cells, Natural Killers & Astrocytes

The GBM TME impairs the immune functions of T-cells and NK cells through exhaustion and suppressed signaling. T cells are the largest group of lymphocytes that infiltrate the TME, preventing glioma immunity [6] [11] [41]. However, low proportions of T-cells are found in most GBM patients [6] [9]. The most common T lymphocytes in GBM are CD8+, CD4+ helper T cells, and Treg cells [9]. Studies have shown that increased CD8+T cell expression in the TME results in positive outcomes [11]. However, a majority of CD8+ T cells infiltrating the TME are exhausted and ineffective [11]. In GBM, Tregs, a subtype of CD4+ T cells, regulate immune homeostasis by inhibiting the anti-tumor response [6].

NK cells recognize and destroy tumor cells by detecting the presence of receptor ligands [41]. Despite only making up about 2% of the TME, NKs are critical in the antitumor response [6]. NK cells play an important inhibitory role in the metastasis of GBMs, regulating T cell-mediated immune responses [19]. However, NK cells are hindered by the TME, limiting their effectiveness [6].

Astrocytes, the most abundant glial cells in the brain, make up nearly 50% of all brain cells [11] [42]. Astrocytes secrete factors that help maintain the tight junctions in the BBB [11]

[42]. They also promote the release of degradative enzymes, cytokines, and chemokines [11]. When the CNS is impacted by tumors, astrocytes undergo reactive changes called astrogliosis and facilitate GBM proliferation [18] [42]. Reactive astrocytes were detected by increased glial fibrillary acidic protein (GFAP) expression levels [18] [42]. Thus, astrocytes support glioma pathogenicity by promoting immunosuppression, regulating immune cells, and contributing to the TME [42].

The GBM TME promotes immunosuppression through the activation of Tregs and MDSCs, reactive astrocytes, microglia, and endothelial cells secrete transforming growth factor (TGF-β) and metalloproteinases (MMP2 and MMP9) [11]. GBM tumors can change the TME by increasing immunosuppressive cells, including Tregs and MDSCs, that promote inflammation [2] [11]. The activation of these immunosuppressive molecules weakens the anti-glioma immune response [8] [11]. Key components of the TME, TAMs, and MDSCs require further research.

How Neuroimmune Changes Associated with Chronic Sleep Deprivation Affect Clinical Outcomes

The Connection Between Chronic Sleep Deprivation and Glioblastoma Proliferation

A growing body of patient-based research demonstrates that measured sleep loss is associated with inflammatory activation. Sleep supports metabolic regulation, waste removal (glymphatic system), macromolecule synthesis, and immune function [43]. Studies linking sleep deprivation and inflammation focus on the hypothalamus, pineal gland, brainstem (especially the pons), thalamus, and basal forebrain, which regulate circadian rhythm, sleep induction, and NREM/REM control [43]. Existing patient-focused sleep studies show an association between sleep loss and inflammatory markers in clinical cohorts. Studies incorporating clinical trials, twin studies, and the use of PSG support this link [44]. A 2017 cohort of 378 adolescents measured through PSG found that adolescents with short sleep duration had elevated levels of CRP [44]. Similarly, a 2016 analysis of over 50,000 adults and a 2006 22 participant controlled clinical trial found that sleep deprivation was associated with increased IL-6 levels in addition to CRP. [44]. Another 2006 study found through PSG and wrist actigraphy that short sleep duration (under 5 hours) led to prolonged blood oxygen desaturation and increased inflammation (higher CRP, IL-6, and TNF-α levels) [45]. While median OS for adult GBM remained approximately 15 months, the existing literature contains virtually no studies in GBM patients that monitor both objective sleep deprivation and report Extent of Resection (EOR)-adjusted OS and POS. For example, the feasibility study of wearable sleep monitoring in primary brain tumor patients (n=54) demonstrated that obtaining physiologic sleep data (through a Fitbit) is correlated with patient-reported sleep loss, but it did not link sleep metrics to survival outcomes [46]. Moreover, a Mendelian randomisation study reported a potential causal association between shorter sleep

duration and increased GBM risk, but it did not report downstream survival data in diagnosed patients [47]. Lastly, in a circadian clock gene review for brain tumors, circadian desynchronization is associated with poorer OS, but direct evidence in GBM is lacking [48]. Thus, there is a major gap in this field: no large cohort study of GBM patients with baseline and peri-operative sleep monitoring and survival outcomes (OS/PFS).

GBM is a highly lethal brain tumor that is characterized by genetic mutations, chronic neuroinflammation, and immune suppression. Key contributors to GBM progression include MDSCs, TAMs, T-regs, astrocytes, cytokines, and chemokines. Additionally, BBB disruption and the TME promote neuroinflammation and immunosuppression. Advancing our understanding of these mechanisms, particularly the roles of other critical molecules along with strategies to restore BBB integrity, will be essential for developing targeted and effective GBM therapies.

Table 3: Evidence Linking Sleep Loss to Neuroinflammation and Immunosuppression [Markers Associated with GBM Progression]

Study	Model	Population	Sleep Assessment	Key Findings
[49]	Cohort Study	Adolescents from Penn State Child Cohort	Polysomnography	Adolescents with short sleep durations had elevated levels of CRP
[50]	Controlled Clinical Trial	Patients	Polysomnography, PSQI, SF-A	Sleep deprivation was associated with increased IL-6 and CRP levels
[51]	Comparative experimental	Male Long Evans hooded rats	N/A	Increased IL-1α, IL-1β, IL-6, homocysteine, corticosterone, TNF-α, and IL-17A.
[52]	Controlled clinical trial	Young adult college students with Insomnia or No Insomnia	Questionnaires and self-reported sleep diaries	The insomnia group had lower baseline antibody levels than the control group
[53]	Controlled	94 men and	Actigraphy and	Shorter sleep duration

	clinical trial	70 women aged 18– 55 years	self-reported sleep diaries	was associated with a higher risk for the development of a cold.
[54]	Double-blind placebo-controlled study	16 healthy male and female adult participants	Polysomnography	IL-6 levels significantly increased
[55]	Field-based study	56 adolescents aged 14–19 years	Actigraphy and In-Person interviews	Acute illnesses were more frequent in adolescents with shorter sleep durations.

Targeting Glioblastoma

The Surgical Approach

While GBM remains one of the most challenging cancers to treat, advances in surgical techniques have begun to alleviate this problem. Traditional approaches, including surgical interventions, only provide limited effectiveness due to GBM's highly invasive and immunosuppressive nature. As a result, research has focused on the development of advanced surgical techniques.

Surgical interventions are a vital component in GBM management through their ability to reduce tumor size and alleviate symptoms. Surgery can be a lifesaving treatment for GBM, improving quality of life [16]. The goal of surgery is to reduce as much tumor mass as possible, relieve symptoms, and obtain brain tissue for pathological analysis [13] [21] [56]. Historically, surgery was the initial therapeutic approach for tumor debulking, including the use of cytoreductive surgery ranging from lobectomies to hemispherectomies [21] [57]. Currently, common techniques include craniotomy and biopsies for histopathological analysis [13]. Despite surgery's importance, GBM remains a dangerous disease; therefore, improvements in surgical intervention are necessary.

Advanced surgical techniques have led to improved outcomes. These techniques include fluorescence-guided surgery and intraoperative magnetic resonance imaging (iMRI) [13] [56]. Fluorescence-guided surgery utilizes fluorescent dyes (ie, 5-aminolevulinic acid), selectively taken up by tumor cells and visualized under a special microscope [13]. 5-ALA is a photosensitizing agent that selectively accumulates in GBM cells [21] [57]. Specifically, the tumor appears red, where normal tissue does not express fluorescence, helping surgeons

differentiate tumors from healthy tissue in real time [21] [56]. This technique increases the rate of complete resection and improves patient outcomes and survival [57]. A meta-analysis of 20 studies, including 565 patients who underwent 5-ALA-guided resection, had a mean overall survival gain of 6.2 months [56]. In a 2006 multiinstitutional study, a complete resection of malignant glioma was achieved in 65% of 5-ALA-guided resections compared to just 36% of procedures guided by white light [58]. Furthermore, the six-month PFS was observed in 41% of 5-ALA guided resections compared to 2.1% in white light treatments [58]. Another 2019 glioma study found that 5-ALA-guided resection resulted in a 26% higher gross total resection (GTR) rate as well as a 3-month additional OS and 1-month additional PFS, respectively, compared to control groups [59]. However, as the majority of included studies were observational, causal interpretations should be made cautiously [60]. Overall, evidence suggests that 5-ALA may improve EOR and PFS.

iMRI is another critical technique utilizing a specialized MRI machine for real-time imaging during surgery [13] [57]. iMRI allows for the precise visualization of tumor boundaries and areas that may be confused with normal brain tissue [57]. From 3 randomized controlled trials with 384 patients, iMRI outperformed conventional navigation-guided surgery, resulting in a 3.16 times higher rate in GTR and a 1.84 times higher PFS rate [61] [62]. Another study found that GTR rates increased from 30.7% in traditional treatments compared to 71.5% on iMRI [62]. In summary, the use of advanced techniques, including intraoperative MRI and fluorescence-guided surgery, can be associated with safer and more effective surgical resections [21]. Like many of these potential therapeutic strategies, because few randomized trials exist, the survival impact remains less well defined, and thus, iMRI should be viewed as a technique that *can* enhance surgical precision rather than a guaranteed means of improving outcomes [62].

Surgical procedures can induce inflammation through the disruption of the blood-brain barrier. Globally, millions of patients undergo surgery that involves extensive tissue damage [16]. These procedures, including GBM, are associated with systemic inflammation and can lead to major complications and even death [16]. Surgical trauma can trigger endogenous factors, or damage-associated molecular patterns (DAMPs), which can activate immune cells. [16]. When activated, these cells contribute to systemic inflammation facilitated through molecules like IL-1β and IL-18 [16]. Anesthesia and surgery can reduce tight junction protein expression in the brain, leading to increased migration of CCR2+ and other inflammatory macrophages into the brain [16]. Lastly, GBM can infiltrate surrounding brain tissue, making a complete surgical removal of the tumor impossible [13] [57]. Thus, the limitations of surgery can increase the risk of immunosuppression and inflammation, a side effect that can be mitigated through sleep therapies.

Peri-operative Sleep Inflammation Axis

Surgical interventions in GBM involve not only tumor debulking and neuroinflammatory tissue disruption, but also a host of physiological responses, including BBB disruption and DAMP signaling. However, what has been underresearched in many neuro-oncological studies is the sleep-inflammation axis during the perioperative period, specifically, how it may modulate the TME and increase the risk of recurrence.

Prior studies in non-oncological surgical groups have supported that major sleep and circadian disturbances occur prior to and following surgery. Pre-operative studies on aged mice found that sleep loss can trigger neuroinflammation and neuronal damage, potentially worsening outcomes post-surgery [63]. Furthermore, IL-6 and IL-1β levels were elevated after surgery, and were significantly higher when preceded by sleep deprivation [63]. Microglial (IBA1) and astrocytic (GFAP) activation increased after surgery, in addition to increased expression of c-fos and caspase 3, in the sleep-deprived experimental group, indicating that preoperative sleep loss can exacerbate surgical side effects [63]. Sleep deprivation before surgery was associated with increased BBB permeability and reduction of tight junction proteins (occludin, claudin-5) [63] [64].

Likewise, post-operative surgical studies have shown that sleep loss is a frequent and underrecognized effect of surgery. This sleep loss, in turn, is associated with delayed recovery, impaired cognition, heightened pain sensitivity, and cardiovascular risk [65] [66]. Surgery induces systemic and neuroinflammatory responses involving molecules such as TNF-α, IL-1β, and IL-6 [65]. IL-6 is correlated with poor sleep quality, while IL-1β and TNF-α suppress REM sleep [65]. Moreover, sleep deprivation post-operation has been associated with increased pain sensitivity and a bidirectional feedback loop linking pain to increased sleep interruption [65]. In summary, sleep loss post-neural surgery is associated with increased catabolism, recovery delays, prolonged hospitalization, and a worse quality of life [65] [66] [67]. Circadian misalignment is often compounded by the effects of anesthesia, the ICU environment, and light interruptions [67].

Surgical trauma and sleep deprivation can shape postoperative immune responses. Across surgical cohorts, IL-6 rises within 6–12 hours, peaks at 24–48 hours [68] [69]. TNF-α spikes early (around 2 hours post trauma) but stabilizes quickly, while CRP peaks on postoperative days 2–3 [70] [71]. Sleep disturbances are closely tied to foundational surgical and environmental variables. While patients after brain tumor resection commonly experience insomnia, there are no studies to date that have evaluated whether the EOR is associated with longer-term sleep disturbances or prolonged CRP elevation [72] [73] [74]. Finally, steroids, including Dexamethasone, disrupt circadian rhythms, raising neutrophil and monocyte counts [75] [76]. Although these links must be acknowledged, there is a major gap in these factors being measured in GBM sleep studies.

Thus, this review proposes a standardized peri-operative framework measuring sleep, in addition to inflammatory and immune signal peaks.

1) **Pilot Randomized Controlled Trial: Cognitive Behavioral Therapy for Insomnia (CBT-I) Intervention**

   Objective sleep measures:

- Wrist actigraphy for 14 days pre-operatively and 28 days post-operatively
- Optional PSG for baseline OSA Measurements and REM sleep metrics

   Primary Outcomes (Neuroinflammatory/Immune Signaling measurements):

- IL-6, TNF-α, CRP, cortisol
- CCR2+ monocyte counts and lymphocytes

   Secondary Outcomes

- Quality of Life
- PFS
- EOR

2) **Prospective Cohort**

- GBM patients with actigraphy and PSG
- Serial cytokine panels
- Immunophenotyping of blood and tumors

3) **Mechanistic Preclinical Model: Chronic Sleep Fragmentation in Orthopedic GBM**

   Using chronic sleep fragmentation in mice with orthotopic GBM tumors, this model will assess

- Microglial polarization profiles
- Monocyte infiltration and trafficking
- BBB permeability
- Astrocytic activation

Although direct data on GBM craniotomy patients are virtually absent, data from other major surgical populations can be used to support that caniotomes disrupt sleep patterns through pain, hospital environments, and cytokine-induced neuroinflammation. That being said, future studies should study sleep loss and circadian disruption pre- and post-craniotomy in GBM patients, incorporating BBB integrity markers, inflammatory biomarkers, and recovery rate measurements. This proposed framework provides measurable and responsible parameters for evaluating how perioperative sleep deprivation can reshape the neuroimmune landscape and influence GBM outcomes.

Alternative Approaches: Immunotherapy, Pharmacology & Lifestyle Management

Given the limitations of surgical resection, GBM management increasingly depends on mixed states that intersect inflammatory, immune, and metabolic pathways. Rather than acting independently, immunotherapies, including checkpoint inhibitors, chimeric antigen receptor T-cell (CAR-T) therapy, and oncolytic viruses, that aim to bolster the body's anti-tumor immune response, pharmacological strategies, and lifestyle and sleep changes can be combined to reduce neuroinflammation, restore circadian rhythm, and improve immune surveillance.

Immunotherapy in the Neuroimmune Context

Immunotherapy strategies have provided some breakthroughs in targeting GBM tumor cells, but have been limited by the TME. Some immunotherapies include peptide vaccines, dendritic cell therapy, adoptive T cell therapy, CAR-T cells, oncolytic viruses, and immune checkpoint inhibitors [6] [8]. The specific use of immune checkpoint inhibitors and CAR-T-cell therapy can be employed to attack GBM cells [13]. Other methods include programmed death ligands (PD-L1), cytotoxic T-lymphocyte antigen 4 (CTLA-4), HSC transplantation, gene therapies and virotherapies, dendritic cell vaccines, and high-density lipoprotein nanoparticle vaccines [6] [11].

Checkpoint inhibitors, including ICIs (anti-CTLA-4, anti-PD-1, and anti-PD-L1), have revolutionized GBM treatments. Checkpoint inhibitors work by blocking inhibitory pathways that suppress T-Cell activation, promoting antitumor immune responses [77]. Anti-vasculature therapy specifically blocks the VEGF/VEGFR signaling pathways, reducing angiogenesis, but has failed to demonstrate significant clinical benefits in improving progression-free or overall survival [6]. Past mouse studies have shown a synergy between checkpoint inhibitors and radiotherapy, specifically radiation therapy followed by checkpoint inhibitors [77]. Furthermore, Laster interstitial thermal therapy (LITT), a minimally invasive surgical treatment, is a strong candidate for combination therapy with checkpoint inhibitors [77]. Future studies should move forward with combination therapies including checkpoint inhibitors, suggesting synergies with vaccines, oncolytic viruses, and CAR-T cell therapy [77].

CAR-T cell therapy is a novel and promising immunotherapeutic strategy. CAR-T Cells are T cells that have been removed from patients and modified to have tumor antigen-binding receptors that are specific. They are then reinserted, increasing T Cells' ability to recognize and destroy cancer cells [6] [11] [78]. Mouse and vivo models show tumor-killing abilities; however, CAR-T Cell therapy in a clinical context is limited by the heterogeneity of target antigen expression and difficulty in maintaining the activity of injected CAR-T cells [6] [41] [79]. Preclinical trials have shown that checkpoint inhibitors, blocking PD1, could help overcome CAR-T cell exhaustion and enhance the activation and efficacy of this treatment option [77].

Similarly, Oncolytic Virus Therapy infects cancer cells with antigens that can lyse the tumor cells in preclinical trials. But in clinical settings, oncolytic viral therapies face safety and

delivery constraints in clinical trials [6]. These viruses can activate macrophages, enhancing the infiltration of T-cells into the TME, leading to reduced immunosuppression [6]. That being said, studies have tested combining checkpoint inhibitors with oncolytic virotherapies [77]. Mouse GBM models demonstrated increased tumor-infiltrating CD8+ T cells after combined treatment. [77].

Neuroimmune interventions that modulate systemic neuroinflammation, such as increasing sleep duration or reducing corticosteroid dependence, can enhance immunotherapeutic success and checkpoint inhibitor efficacy [80]. For instance, improved sleep is associated with reduced IL-6, TNF-α, and CRP, cytokines linked to neuroinflammation [81]. Preclinical human studies suggest that combining CBT-I or continuous positive airway pressure (CPAP) therapy for OSA with immune checkpoint therapy may enhance immunity in GBM patients with sleep deprivation [82]. Similarly, oncolytic viral therapy can synergize with sleep-based chronotherapy to restore melatonin rhythms, optimizing interferon-mediated antiviral signaling and macrophage polarization [83] [84] [85]. This approach reframes immunotherapy not as an isolated intervention, but as one component of a coordinated circadian-immune approach in mitigating the effects of GBM.

Pharmacological Coordination

Pharmacologically based sleep interventions can lead to improved immune function and reduced inflammation [2]. Physicians can prescribe sedative-hypnotic drugs: benzodiazepines and non-benzodiazepines used to improve sleep [2]. Furthermore, orexin agonists and antagonists can be implemented to manage sleep cycles [2] [3]. Their use must be balanced against risks of dependence, cognitive impairment, and next-day sedation, especially in neuro-oncologic populations.

Nonsteroidal Anti-inflammatory Drugs (NSAIDs) decrease prostaglandin synthesis and inhibit microglial activation, reducing the production of pro-inflammatory cytokines, chemokines [13]. However, the incorporation of NSAIDs into GBM care must consider clinical contraindications, including gastrointestinal toxicity and perioperative bleeding risk [86] [87]. While preclinical models support anti-inflammatory and antitumor effects, human survival data remain mixed and insufficient.

Corticosteroids, including dexamethasone and prednisone, are widely used to reduce neuroinflammation in GBM patients [13] [88] [89] [90]. High doses of corticosteroids have been shown to rapidly decrease tumor-associated edema and improve clinical symptoms [21]. Corticosteroids inhibit the production of inflammatory mediators such as prostaglandins and cytokines, suppressing inflammation and restoring BBB integrity, but can also induce hyperglycemia, weight gain, infections, myopathy, diabetes, immunosuppression, and osteoporosis [13] [39] [88] [90] [91].

Therefore, corticosteroids such as dexamethasone can reduce vasogenic edema but could at the same time also impair immune activation and limit the effectiveness of immunotherapies

[91]. As a result, current studies are evaluating alternatives to dexamethasone, which can enable some patients to reduce or discontinue corticosteroid doses [88] [91]. Furthermore, limiting steroid use through gradual tapering supported by NSAIDs, or sleep-targeted anti-inflammatory agents, should be a clinical priority. The use of orexin receptor agonists or short-term hypnotics to establish sleep structures could reduce cytokine load and indirectly lower the steroid requirement for edema management [92]. Additionally, melatonin could serve as both a chronobiotic to realign circadian rhythms and as an antioxidant and immunomodulator **[**93**] [**94]. Hence, the use of corticosteroids and other pharmacological medications must take into account synergies and antagonisms with other therapeutic strategies in managing GBM.

Lifestyle and Metabolic Modulation

While immunotherapies and pharmacological approaches may alleviate symptoms, they must be considered alongside lifestyle interventions. The management of sleep deprivation and maintenance of a balanced lifestyle can help reduce immune suppression and inflammation associated with GBM development. Lifestyle interventions complement molecular therapies by addressing metabolic substrates of GBM. Experts have recommended routine screening for sleep disorders and sleep interventions [2].

Preclinical GBM Mouse models demonstrate robust tumor growth reduction and increased survival on the ketogenic diet (KD), characterized by a high fat-to-carb ratio, has [10]. Preclinical models showed that GBM cells grown in high-glucose media replicate significantly faster than those grown in normal media [10]. KD slows tumor growth and increases survival due to changes in immune response, gene expression, and amount of reactive oxygen species [10]. Thus, KD remains a promising but not sound approach to mitigating the effects of GBM.

Although human evidence remains limited, small-scale clinical studies show that approximately 44% of ketogenic diet studies reported improved health-related quality of life, a fundamental component of GBM management [95]. In fact, case studies show that KD, when combined with chemotherapies and immunotherapies, can lead to additive effects [96]. A single-arm phase 1 trial at the Cedars-Sinai Medical Center found that GBM patients on the Keto diet had a median PFS of 12.9 months compared to the baseline 6.9 months, and a median OS of 29.4 months compared to 14.6 months [97]. Furthermore, a 2024 patient trial found that the keto diet reduces tumor glucose uptake by 22% and resulted in a 38-month mean survival compared to just 20 months in the control group [98].

KD implementation in GBM patients can be challenging due to strict nutrient requirements, gastrointestinal side effects, and weight loss [99] [100]. Thus, while promising, KD remains only a possible adjunct opposed to a validated therapy.

In rodent and primate models, calorie restriction and short-term fasting can also have a major impact on glioma survival [10]. Calorie restriction has been proven in preclinical glioma studies to extend life span in yeast, mice, and primates by selectively protecting normal cells

over cancer cells [10]. That being said, clinical data are sparse, and feasibility concerns, including weight loss and cachexia, limit broad application.

Observational studies link caffeine to reduced glioma risk and inhibition of tumor invasion/migration, but causal inference is limited [10]. Meanwhile, excessive intake worsens sleep and overall health, undermining circadian therapeutic goals [10]. That being said, excessive caffeine intake can worsen insomnia, elevate stress responses, and disrupt circadian rhythms [101] [102] [103]. Thus, caffeine use should be considered cautiously.

Epidemiological studies have found that consuming coffee and tea every day is associated with a 54% lower risk of glioma [104]. Another study found that every one cup of coffee per day decreases the risk of glioma by 3 % [105].

Preclinical evidence shows that exercise can be crucial in maintaining sufficient immune function, preventing the proliferation of GBM [10]. In GBM mouse studies, exercise significantly reduces tumor proliferation, and up to 41% of glioma patients complete recommended exercise during treatment [10] [95]. Clinical evidence supports improvements in GBM outcomes, though direct survival benefits remain unproven. Furthermore, exercise is associated with enhanced antitumor immunity and immunotherapeutic efficacy [106]. Preclinical models demonstrate that combining exercise with immune checkpoint inhibitors can lead to reduced tumor volume and increased apoptosis [106].

Vitamin A, especially retinoic acid, has been shown to inhibit the proliferation of tumor cells in some human GBM cell lines [10]. Likewise, melatonin, an antioxidant produced by the pineal gland, has an anticancer effect on many cancer types [3] [10]. Human clinical evidence is limited, however, and safety as well as dosing requires consideration [10].

Integrating these approaches within sleep optimization frameworks may result in addictive effects. For instance, early-day exercise and time-restricted feeding reinforce circadian rhythm stability, enhancing melatonin secretion and improving responsiveness to CBT-I or CPAP [107] [108]. Moreover, Vitamin A supplements and caffeine, when appropriately used, can modulate oxidative stress and glial activation, supporting neuroimmune restoration [109].

Sleep Therapy as the Central Bridge

Interventions aimed at improving sleep quality and duration are thus the centerpiece in connecting multiple therapeutic strategies and reducing GBM proliferation and progression. In fact, sleep interventions have been promising in their objective of reducing oncogenic pathways. For instance, therapies such as CBT-I have been effective in treating insomnia and, by extension, are associated with improvements in immune function [2] [110]. CBT-I is a multicomponent intervention that targets sleep deprivation using sleep hygiene, sleep restriction, stimulus control, cognitive restructuring, and relaxation strategies [110] [111]. When paired with treatments like CPAP, therapy for OSA not only alleviates sleep deprivation but also reduces levels of inflammatory markers, which are crucial in GBM growth [2]. Ultimately, restoring adequate sleep duration and quality can enhance the activity of immune cells, bolstering immune

surveillance against cancer [2]. These insights highlight the strong association between sleep, immune function, and GBM, underscoring the importance of addressing sleep disorders in cancer prevention and treatment [2].

Sleep therapies can be combined with other therapies, including cancer screening, immunotherapy, and lifestyle management. Oncology clinics can adopt approaches that include sleep specialists who collaborate with oncologists to assess and manage sleep-related issues [2]. Similarly, integrating sleep management strategies and pharmacological treatments into immunotherapy protocols may help optimize immune function and enhance treatment efficacy [2]. Diets rich in fruits, vegetables, lean proteins, and whole grains can enhance sleep quality by providing essential nutrients that support the body's natural sleep–wake cycle [2]. Therefore, sleep interventions are not only a promising yet overlooked therapeutic, but they also enhance the efficacy of other strategies. Sleep interventions are foundational in recalibrating the immune and endocrine systems upon which other therapies depend, thus improving quality of life and therapeutic responsiveness, especially when coordinated with immunotherapy or pharmacological drugs.

In summary, integrated therapy for GBM requires optimizing multiple therapies in tandem, involving both immunological and physical components. Surgical interventions remain an effective treatment for reducing the tumor mass but may increase the risk of neuroinflammation. While immunotherapies benefit from cytokine stabilization achieved through sleep and metabolic regulation, pharmacological drugs can be regulated to minimize immune disturbances. Lifestyle inventions can reinforce circadian and inflammatory homeostasis. This mechanistic synergy links sleep, immune modulation, surgery, and metabolism. Future research should test the effectiveness of combinative therapies and discover new and more effective synergies.

Conclusion

The complex relationship between sleep deprivation and GBM proliferation has been largely overlooked. Chronic sleep deprivation is associated with a pro-TME through increased neuroinflammation and immune suppression, which is fundamental in GBM progression.

Sleep plays a vital role in immune regulation, and chronic sleep deprivation contributes to increased expression of pro-inflammatory cytokines and a reduction in T and NK cell function [3] [5]. The severity of GBM is driven by genetic mutations, an immunosuppressive TME, and the impairment of the blood-brain barrier. Key components include Tregs, TAMs, MDSCs, cytokines, and chemokines [23]. Thus, sleep deprivation is strongly hypothesized to contribute to GBM progression through immune regulation.

Current evidence strongly supports that chronic sleep deprivation promotes systemic inflammation and immune suppression, and that these processes contribute to GBM progression.

However, direct clinical studies linking measured sleep duration to inflammatory biomarkers or survival metrics are largely absent.

Moreover, the investigation of surgical intervention has resulted in mixed results in which surgery can both alleviate symptoms and exacerbate neuroinflammation [16], consequently warranting a consideration of a combinative therapeutic strategy. Given the severity of GBM and limitations of individual treatment, a combined approach involving surgery, immunotherapy, pharmacology, and lifestyle changes could overcome these individual limitations.

Each therapeutic approach, including surgery, immunotherapy, pharmacology, and lifestyle interventions, has its limitations. Surgery, while critical for tumor debulking and relieving symptoms, can worsen neuroinflammation and disrupt the blood-brain barrier. [13] [16] [27]. Likewise, immunotherapies, including CAR-T cell therapy and checkpoint inhibitors, are promising but are largely ineffective as a result of the immunosuppressive TME [6] [41] [51]. Pharmacological interventions, including corticosteroids and NSAIDs, and lifestyle changes (KD and Sleep Hygiene), may be unrealistic or lack long-term success.

When put together, these findings suggest that a combinative approach should be considered as a method to overcome individual limitations. At the forefront of this approach is addressing sleep deprivation through sleep therapies. Likewise, surgery should not be used in isolation but in conjunction with other therapeutic strategies. By connecting sleep deprivation to GBM progression, treatment plans address the interplay between lifestyle, immunity, and surgical interventions.

Nevertheless, questions remain. Is the timing or quality of sleep more important in maintaining sufficient immune function? What other key molecules contribute to immune suppression and neuroinflammation that haven't been discovered/researched? How can surgical advancements bypass current neuroinflammatory limitations? Future research should prioritize

1. GBM-patient studies incorporating objective sleep metrics (actigraphy/PSG/OSA) and immune profiling (IL-6, TNF-α, CRP).
2. Evaluate combinative strategies integrating sleep management, surgery, and immunotherapy to address GBM proliferation and progression.

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November 22nd, 2025

Dear Peer Reviewer,

Thank you for reviewing and giving me the opportunity to revise and resubmit my manuscript, *The Sleep-Cancer Mystery: An Investigation of How Surgery Affects the Association Between Chronic Sleep Deprivation and Glioblastoma Progression Through Neuroinflammation and Immunosuppressive Modulation*. I greatly appreciated the feedback and organized it along with a summary of my revisions in a table. I believe my revised manuscript adequately addresses the comments.

Reviewer's Feedback	**My Response**
The central hypothesis is asserted, not demonstrated: Across the abstract/introduction/conclusion, the manuscript implies a causal arc, "chronic sleep deprivation to neuroinflammation/immune suppression to accelerated GBM," with surgery as a modulator. Yet, it cites almost no GBM-patient studies linking measured sleep (actigraphy/PSG/OSA) to inflammatory markers (IL-6, TNF-α, CRP) or outcomes (EOR-adjusted PFS/OS). The text mostly strings together (a) generic sleep-inflammation studies and (b) GBM immunology papers, then infers the bridge. This	In response, the manuscript has been revised to shift from an asserted causal narrative to a question-driven review. ● **Reframed Hypothesis and Scope**: The abstract, introduction, and conclusion have been rewritten to pose the hypothesis as a research question rather than a proven causal statement. The paper now emphasizes investigating whether chronic sleep deprivation contributes to neuroinflammation and immune suppression that may, in turn, influence GBM progression. ● **Evidence Structure Clarified**: To address this concern, I added a section with evidence directly derived from GBM patient studies (e.g., those measuring actigraphy/PSG and inflammatory biomarkers such as IL-6, TNF-α, and CRP). I also directly stated the gap in the field linking sleep loss to survival outcomes (EOR-adjusted PFS/OS) in GBM patient studies. ● **Revised Abstract and Conclusion**: Both the abstract and conclusion have been edited to reflect uncertainty and ongoing inquiry rather than assertion. Specifically, the conclusion now highlights critical research gaps, including a lack of patient-based studies, and reframes the discussion around future research priorities.

should be reframed as a question-driven Review with explicit evidence tables for each link in the chain; where GBM-patient data are absent, label that gap clearly.	• **Surgical and Combinative Modulation Clarified**: The discussion now presents surgery as a potential modulator of neuroinflammatory pathways rather than a proven solution, stressing the need for additional clinical data. • **Proposed Evidence Tables** – The revised manuscript incorporates evidence tables summarizing key findings for mechanistic links, to support inferred and missing data visually.
Molecular table and terminology errors: The "Genetic Alteration/Functional Alteration" table contains factual and nomenclature issues that need correction: duplicated and misspelled CDKN2A/p16^INK4a ("CDK2A-p16INL4a," Repeated twice, EMP3 is labelled "tumor suppression" despite frequent association with adverse biology in GBM, and apoptosis labels for TIMP3/CDH1 are oversimplified; elsewhere HIF-1α is written as "HIF-A." Please fix gene symbols, expand functional categories beyond one-liners, and cite primary sources for each entry.	**Correction of Gene Nomenclature and Duplications:** • The previously duplicated and misspelled gene *CDK2A-p16INL4a* has been corrected to the proper symbol *CDKN2A/p16^INK4a* and now appears only once in the table. • All gene symbols throughout the manuscript have been standardized to conform to Nomenclature guidelines. 1. **Revision of Functional Categories:** The Genetic Alteration/Functional Alteration table has been revised to expand functional descriptions. Each entry now includes specific roles in GBM and normal conditions. 2. **Correction of Functional Misclassifications:** Functional descriptions for *EMP3, TIMP3,* and *CDH1* have been revised to avoid oversimplified labels. 3. **Addition of Primary Literature Citations:** Each table entry is now supported by primary literature citations 4. **Terminology Consistency:** Throughout the manuscript, gene and protein names, molecular pathways, and abbreviations have been reviewed for consistency and accuracy.
Epidemiology needs verification/softening:	**Softening and verification of epidemiology statements:**

The manuscript states GBM is "the leading cause of cancer deaths in ages 15-34" and gives global/US incidence totals without primary registry attribution. As written, these lines are overconfident and likely inaccurate for the 15-34 age band. Either provide a specific registry-grade citations (with years, geography) or soften the language.	• I removed the overconfident claim that GBM is "the leading cause of cancer deaths in ages 15–34," as this statement may not be accurate for this age group. • I clarified incidence and mortality statistics by citing peer-reviewed sources without asserting age-specific mortality claims. • Global and US case numbers were presented with caution, noting that they are estimates.
Surgery is not analytically connected to sleep: The peri-operative section correctly notes BBB disruption, DAMP signalling, and macrophage trafficking after surgery, but it does not show any data on perioperative sleep architecture/circadian disruption (pre/post craniotomy), nor link those changes to inflammatory markers or relapse dynamics in GBM. Add a dedicated "Peri-operative sleep-inflammation axis" sub-section synthesising what is known in neuro-oncology (or closely related	1) **Addition of a Dedicated Subsection:** A new subsection synthesizes evidence on perioperative sleep changes, circadian rhythm disruption, and associated inflammatory pathways following neurosurgery. The section also clarifies that while sleep loss and circadian misalignment are known to exacerbate neuroinflammation and impair immune surveillance, specific studies in GBM patients remain limited. 2) **Clarification of Knowledge Gaps and Future Directions:** The revision delineates what remains unknown, mainly the absence of studies measuring peri-operative sleep (polysomnography or actigraphy) alongside inflammatory markers in GBM. It proposes that future research should explore whether these findings can be extended to GBM patients.

neurosurgical cohorts) and specify what is unknown.	
"Combined therapy" is enumerated, not integrated: Lists of immuno/pharmaco/lifestyle options read as declarative catalogues. Please develop a mechanistic matching logic (e.g., why CBT-I ± CPAP for patients with OSA/fragmentation and elevated IL-6; how to balance steroid-sparing with oedema control; where melatonin fits as a chronobiotic). Frame tangible clinical scenarios and potential synergies/risks.	1. **Cross Therapy Integration:** I rewrote the section to demonstrate how each therapeutic method (immunotherapy, pharmacology, lifestyle/sleep interventions) may interact through shared inflammatory and metabolic pathways rather than listing them sequentially. 2. **Clinical Scenarios** I introduced examples of patient-level decision-making, such as balancing steroid-sparing regimens and considering melatonin both as a chronobiotic and antioxidant for patients with circadian dysregulation. 3. **Therapeutic Synergies and Risks:** The revised manuscript discusses synergistic potential (eg, how increased sleep duration can improve immunotherapy efficacy). I also highlight clinical risks, such as increased immunosuppression when combining corticosteroids and immunotherapies. 4. **Sleep Focused Framing:** I reframed the section to emphasize sleep therapy as a unifying bridge that links neuroinflammatory modulation, immune regulation, and metabolic equilibrium.
Balance of evidence (diet/caffeine/NSAIDs/steroids): Claims about ketogenic diets, caffeine, NSAIDs, and corticosteroids are stated strongly relative to to a mixed evidence base (and with safety trade-offs).	• I have clarified the distinction between preclinical and clinical findings. For example, ketogenic diets and calorie restriction show effects on tumor growth in GBM mouse models, whereas clinical evidence for survival benefit remains limited and mixed. Effect sizes and quality of evidence are now explicitly reported where available. • The discussion of caffeine intake now acknowledges the predominantly observational human data supporting reduced glioma risk, emphasizing the limited association.

Please separate preclinical vs clinical findings, report effect sizes/quality, and include contraindications (e.g., bleeding risk for NSAIDs; potential survival impact of high-dose steroids despite anti-oedema benefit).	• NSAID use is described with both anti-inflammatory potential and clinical contraindications, including bleeding risk and gastrointestinal toxicity. • Corticosteroid benefits for vasogenic edema are balanced against their adverse effects, including immunosuppression and interference with immunotherapy.. • I emphasized the synergistic potential of sleep-based interventions, immunotherapies, and pharmacologic strategies in modulating neuroinflammation and enhancing immune surveillance. • Preclinical and clinical evidence supporting CBT-I, CPAP therapy, melatonin, and other circadian-modulating interventions is now distinguished and referenced. • All pharmacological interventions are now discussed in the context of potential trade-offs and contraindications. • Lifestyle interventions, including diet, exercise, and supplement use, are contextualized and focused on human applicability.
No review methods: As it stands, this is a narrative review with no search strategy, inclusion/exclusion criteria, time window, or bias assessment. Add a brief Methods section describing databases, terms, date range, selection flow, and how you assessed study quality; mark narrative content as such.	• **Databases searched:** PubMed, Scopus, and Google Scholar. • **Search terms:** combinations of *"glioblastoma," "sleep deprivation," "neuroinflammation," "surgery," "immunotherapy,"* and related cytokine and BBB keywords (e.g., *IL-6, TNF-α, CRP, microglia, TME*). • **Date range:** Publications between 2000 and 2025. • **Inclusion criteria:** Peer-reviewed human and relevant animal studies that examined 1) sleep loss or circadian disruption and neuroinflammatory or immunosuppression; 2) GBM biology involving cytokine signaling, macrophage or T-cell activity; or 3) surgical effects on inflammation or BBB integrity. • **Exclusion criteria:** Non-English articles and studies lacking primary data.

	• **Quality and bias assessment:** Each included study was evaluated for methodological rigor based on sample size, design, and acknowledgements of limitations; potential biases are discussed where relevant.
Clarity and presentation: Figures should have self-contained captions (no raw URLs), consistent abbreviations on first use (GBM/Glioblastoma, TME/GAM/TAM, sleep deprivation vs restriction vs fragmentation), and careful copy-editing to remove duplicates/typos (see gene table).	1) All figure captions have been rewritten to be fully self-contained. Captions now describe the context, summarize key findings, and define all abbreviations used within the figure. Raw URLs have been removed and replaced with appropriate in-text references. 2) I have standardized the usage of abbreviations throughout the manuscript. Specifically, on first appearance, I now provide both the full term and abbreviation, for example, glioblastoma (GBM) and tumor microenvironment (TME). 3) Terminology referring to sleep disturbance has been clarified and changed to the standard term sleep deprivation. 4) I conducted a line-by-line copy-editing process to remove typographical errors, duplicated text, and inconsistent formatting. The gene table has been edited to remove repeats.
Re-structure around a five-link causal chain: A. Chronic sleep deprivation to B. systemic inflammation/HPA axis (IL-6/TNF-α/CRP) to C. BBB/microglia/monocyte trafficking to D. TME immune landscape (TAM/MDSC/Tregs, T-cell exhaustion) to E. clinical outcomes (growth/recurrence/survival	The manuscript is now organized into five sequential mechanistic modules, each with its own subsection. I included three tables throughout the sections due to the fact that there was a lack of studies in some of the links, and there was a significant amount of overlap in studies. **A → B: Chronic Sleep Deprivation to Systemic Inflammation/HPA Axis Activation** I present evidence that chronic sleep loss elevates IL-6, TNF-α, and CRP.

). Build a table for each link with GBM-patient data where available, then preclinical. Flag explicit "evidence gaps."	A new comparative table distinguishes GBM-patient data from preclinical sleep-loss Rodent models, and measures sleep deprivation on inflammatory and immune markers. **B → C: Systemic Inflammation to BBB Disruption, Microglial Activation, and Monocyte Trafficking** This section integrates data on how systemic cytokine elevations impair BBB integrity. Patient-side data was practically non-existent, so I combined data with the C-D and D-E link table. **C → D: Neuroimmune Changes to TME Remodeling (TAMs, MDSCs, Tregs, T-cell Exhaustion)** I map how BBB disruption and changes in key immune molecules reshape the GBM TME. **D → E: TME Remodeling to Clinical Outcomes** ● This section ties TME alterations to tumor progression to survival (OS/PFS). Key evidence gaps: mainly consist of minimal data linking sleep metrics to OS/PFS in GBM ● A new table synthesizes data from the B-E Links focused on the connection between chronic sleep deprivation and GBM acceleration, delineated by immune markers. There was a significant gap in the data, so I was unable to extract data involving BBB disruption or survival outcomes.
Peri-operative module: Synthesise data on sleep disturbance before/after GBM resection (insomnia, fragmentation,	**Integrated Pre- and Post-Resection Sleep Disturbance:** I synthesized data on preoperative insomnia, postoperative sleep loss, and the prevalence of OSA in patients. This includes evidence from wearable sleep-monitoring studies, postoperative sleep deprivation papers, and sleep-wake disruption in ICU settings.

OSA), time-course of IL-6/TNF-α/CRP and monocyte shifts in the first 2-4 weeks, and associations with EOR, oedema, and steroid exposure. Propose measurement standards (actigraphy + inflammatory panels).	**Added a Time-Course Summary of IL-6, TNF-α, and CRP Shifts** I incorporated data showing IL-6, TNF-α, and CRP changes over the first few weeks post-surgery. **Linked Sleep Deprivation and Neuroinflammation to EOR, Edema, and Steroid Use** **Proposed Standardized Measurement Frameworks** I recommended using actigraphs and neuroinflammatory measurements
Concrete study designs: Pilot RCT: CBT-I (or stepped-care CBT-I) 2-3 weeks pre-op + 4 weeks post-op vs usual care; primary: IL-6/TNF-α/CRP and actigraphy; secondary: quality of life and PFS adjusting for EOR. Prospective cohort: GBM patients with PSG/actigraphy, cytokine panels, and, where feasible, paired immunophenotyping of blood and tumour; correlate with growth/recurrence. Mechanistic preclinical: chronic sleep fragmentation models assessing microglial polarisation/monocyte infiltration in orthotopic GBM.	I edited the manuscript to include explicit and feasible study designs that operationalize the perioperative sleep inflammation axis in GBM. 1) Pilot Controlled Trial (CBT-I Pre and Post Operation): I included a pilot RCT framework testing Cognitive Behavioral Therapy for Insomnia for 2 weeks pre-operatively and 4 weeks post-operatively, compared with normal care. Primary outcomes include IL-6, TNF-α, CRP, and actigraphy-based parameters, while secondary outcomes include quality of life, PFS, and EOR measurements. 2) Prospective Cohort Design: I added a cohort design for GBM patients, including wrist actigraphy, optional baseline PSG, cytokine profiling, and paired blood-tumor immunophenotyping at surgery 3) I also included a Mechanistic preclinical section, which quantifies microglial polarization (M1/M2 states) and monocyte infiltration.

Avoid categorical phrasing like "sleep is a main contributor" to GBM progression; reframe as a potential modifier of the immune microenvironment/BBB with currently indirect evidence.	I have revised the manuscript to avoid categorical or causal phrasing such as "sleep is a main contributor to GBM progression". I instead describe sleep deprivation as a potential modifier of physiological and immunological pathways (BBB integrity and TME) that could lead to GBM progression.
Standardize abbreviations at first mention (GAM, MDSC, CBT-I, CPAP) and keep them consistent across text/figures.	I have standardized all abbreviations throughout the manuscript and figures. Key terms, including GAM, MDSC, CBT-I, and CPAP, are now defined when first mentioned and consistently abbreviated thereafter. I also reviewed all figures and tables to ensure abbreviations match and are consistent.
In the surgery section (5-ALA/iMRI), provide effect sizes and study types when claiming improved outcomes; otherwise, soften the language.	In response, I revised the surgical interventions section to provide effect sizes and study types, specifically randomized trials, cohort studies, and meta-analyses. To measure outcomes, I cited sources that provide quantitative measures, including the extent of resection and progression-free survival differences. That being said, I softened the language to avoid overstating benefits and framed these technological advances as instruments that can enhance operational success.
Revise the caffeine/ketogenic diet paragraphs to clearly separate GBM-specific clinical data from broader oncology or preclinical findings, and to note feasibility/tolerability constraints explicitly.	**Clear separation of GBM-specific clinical data from broader oncology or preclinical findings:** ● I restructured the subsection to ensure that each lifestyle or metabolic intervention distinguishes between GBM-specific clinical data (where available), Preclinical GBM findings (from mouse and cell lines), and broader oncology evidence ● For the ketogenic diet, I reorganized the paragraph so that all GBM-specific clinical observations are separated from preclinical evidence. ● For caffeine, I clarified that existing findings stem mostly from observational associations, not trials, but I still separated these GBM-specific findings from preclinical models.

	Stated Feasibility and Tolerability Constraints ● For the ketogenic diet, I added statements on feasibility, side effects, and concerns regarding weight loss. ● For the caffeine paragraph, I explicitly note that higher intakes may worsen insomnia and circadian disruption, interfering with sleep therapy.

November 22nd, 2025

Dear Peer Reviewer,

Thank you for reviewing and giving me the opportunity to revise and resubmit my manuscript, *The Sleep-Cancer Mystery: An Investigation of How Surgery Affects the Association Between Chronic Sleep Deprivation and Glioblastoma Progression Through Neuroinflammation and Immunosuppressive Modulation*. I greatly appreciated the feedback and organized it along with a summary of my revisions in a table. I believe my revised manuscript adequately addresses the comments.

Reviewer's Feedback	**My Response**
The hypothesis in the abstract is perhaps a bit convoluted. Include a more clear statement of the hypothesis and the mechanisms it is linking together,	1. **Reframed Hypothesis and Scope**: The abstract, introduction, and conclusion have been rewritten to pose the hypothesis as a research question rather than a proven causal statement. The paper now emphasizes investigating whether chronic sleep deprivation contributes to neuroinflammation and immune suppression that may, in turn, influence GBM progression. 2. **Revised Abstract**: Both the abstract and conclusion have been edited to reflect uncertainty and ongoing inquiry rather than assertion. 3. **Reorganizing the Paper**: The manuscript is now organized into five sequential mechanistic modules, each with its own subsection. I detail this new mechanism linkage in the Abstract/Hypothesis. The specific link is provided below: ● A → B: Chronic Sleep Deprivation to Systemic Inflammation/HPA Axis Activation ● B → C: Systemic Inflammation to BBB Disruption, Microglial Activation, and Monocyte Trafficking ● C → D: Neuroimmune Changes to TME Remodeling (TAMs, MDSCs, Tregs, T-cell Exhaustion) ● D → E: TME Remodeling to Clinical Outcomes

There are some small errors, like the first two mutations in the table are duplicates of each other, and the section title should be "Targeting Glioblastoma" rather than "Targeting Glioblastoma: The Surgical Approach."	1. The previously duplicated and misspelled gene *CDK2A-p16INL4a* has been corrected to the proper symbol *CDKN2A/p16^INK4a* and now appears only once in the table. 2. All gene symbols throughout the manuscript have been standardized to conform to nomenclature guidelines. 3. Throughout the manuscript, gene and protein names, molecular pathways, and abbreviations have been reviewed for consistency and accuracy. 4. The section title "Targeting Glioblastoma: The Surgical Approach" has been retitled "Targeting Glioblastoma", and a new neurosurgery subheading has been added.
I would have liked to see some searches from clinicaltrials.gov or some other clinical data to support how well some therapies work in comparison to others. Include examples of clinical data relevant to the central claims.	**Incorporation of ClinicalTrial.gov searches and additional clinical data** In response to the request for more clinical evidence and outcome data, I incorporated new sources from clinicaltrial.gov and other studies, including: ● Checkpoint Inhibitors (anti-PD-1–PD-1/PD-L1, anti-CTLA-4) ● Combinative immunotherapy trials (checkpoint inhibitors + radiotherapy, checkpoint inhibitors + oncolytic viruses, checkpoint inhibitors + LITT) ● Metabolic interventions (ketogenic diet, caffeine) ● Sleep and circadian interventions (melatonin, CPAP, CBT-I) ● Perioperative immunomodulative trials When available, I report sample size, primary outcomes, and PFS/OS to ensure claims are supported by quantitative evidence. Specifically, PFS/OS data have been incorporated into the 5-ALA and iMRI trials, immunotherapies, and ketogenic diet studies. **Expanded Beyond Literature Sources** I noted that the review should incorporate sources beyond classic oncology journals, so I included: ● Perioperative medical studies ● Neuroimmunology studies linking IL-6, TNF-α, CRP, microglial activation, and circadian disruption to surgical outcomes

	● Sleep medicine sources (CBT-I, CPAP, OSA studies) ● Chronotherapy sources ● Lifestyle-medicine trials **Integrated Sleep Optimization as a therapeutic bridge** ● Sleep Interventions (CBT-I, CPAP, melatonin, orexin modulators) are now presented as bridging to enhance immunotherapy responsiveness, surgical outcomes, and alter pharmacological requirements. ● I utilize actigraphy, PSG, and cytokine tracking in a perioperative framework. ● I propose a pilot clinical trial design incorporating sleep metrics. **Other Changes** ● Incorporated clinical data from other cancers, where combinative therapies show promising results, to use as scaffolding for GBM ● Clarified which combinations are likely to be synergistic based on new clinical data ● Added clinical outcomes for the ketogenic and caffeine sections ● Added effect sizes, study types, and trial outcomes for the surgery section
There is more literature available than just the classic scientific journals. If they are interested in combination therapies, it is particularly important to dig into things like checkpoint inhibitors in combination with other cancer therapies, and I think	1) In the surgical section, I maintained the key role of resection and perioperative neuroinflammation, but expanded the framing to incorporate new perioperative medicine, anesthesiology, and neuroimmunology sources. I incorporate data from immune-surgery studies illustrating how BBB disruption, DAMP signaling, cytokine secretion, and sleep deprivation affect surgical outcomes. 2) In the therapeutic sections, specifically the immunotherapy section, I added a new discussion on combination therapies. For instance, the synergy of checkpoint inhibitors with radiotherapy, chemotherapy, oncolytic viruses, and metabolic or circadian interventions. Although combination immunotherapy trials in GBM have been limited, evidence

even if there isn't a lot of data available in glioblastoma with certain combinations, there may be examples of those same combinations being used in other kinds of cancer.	from other cancer studies demonstrates improvements in treatment outcomes. I used their other cancer studies as a scaffold to support combination strategies in GBM. 3) Specifically, in regards to checkpoint inhibitors, I added sections describing how radiotherapy, Laser interstitial thermal therapy, oncolytic viruses, circadian alignment, and melatonin supplementation can improve GBM outcomes.
The story rambled a bit. It would be good to add something like a graphical abstract that can help ground your reader in your core claim and how everything they are discussing relates back to that.	● In response to this feedback, I added a graphical abstract that summarizes my main argument throughout the paper. ● This graphical abstract illustrates each mechanistic link between sleep deprivation and glioblastoma proliferation. ● A → B: Chronic Sleep Deprivation to Systemic Inflammation ● B → C: Systemic Inflammation to BBB Disruption ● C → D: Neuroimmune Changes to TME Remodeling (TAMs, MDSCs, Tregs, T-cell Exhaustion) ● D → E: TME Remodeling to Clinical Outcomes ● I also added a brief caption to contextualize the graphical abstract.
Which specific kinds of combinations would be useful?	In revising the manuscript, I have now added examples of mechanistically logical and clinically relevant combinative strategies. These suggested combinations focus on incorporating sleep hygiene and optimization to maximize the effects of other therapeutic strategies. 1) First, in the surgical and peri-operative sections, I highlight combinations that integrate sleep hygiene/optimization with surgical recovery, drawing from perioperative medical studies. These studies incorporate actigraphy and post-operative CBT-I inflammation tracking, demonstrating the potential for reduced neuroinflammation and enhanced recovery. This combination

	can be further enhanced by immunotherapy, which can modulate cytokine levels. 2) In the immunotherapy section, I provide data from other cancer types where immune checkpoint inhibitors are more common. Some potential combinations include checkpoint inhibitors and circadian/sleep interventions, oncolytic virus therapy and circadian/sleep interventions, and oncolytic virus therapy and checkpoint inhibitors. 3) In the pharmacological section, I specify combinations that can reduce neuroinflammation while minimizing the antagonism that corticosteroids have with immunotherapies. Sleep promoters, including orexin modulators and short-term hypnotics, could reduce the cytokine load and allow for lower dexamethasone doses. Furthermore, sleep interventions can be combined with NSAIDs to optimize the reduction of IL-6 and CRP levels. 4) In the metabolic and lifestyle sections, I outline combinations supported by preclinical and cross-cancer studies, including Exercise and Immunotherapies, as well as KD and Chemotherapy/Immunotherapies.

Thank you for sharing the revised manuscript and the detailed responses from the author. After carefully reviewing the revised submission, **I recommend accepting the paper for publication with minor edits.**

The author has demonstrated exceptional responsiveness to the previous feedback. The shift from asserting a causal link to framing the review as a hypothesis-driven investigation ("The Sleep-Cancer Mystery") significantly strengthens the scientific integrity of the piece. The addition of the "Peri-operative Sleep Inflammation Axis" and the proposed study designs address the critical lack of direct GBM-patient data highlighted in the first round of reviews.

However, to ensure the manuscript is fully polished for publication, I have compiled the following critical feedback that the author should address in the final minor revisions:
1. Clarification of Table 3: While Table 3 ("Evidence Linking Sleep Loss to Neuroinflammation...") is a welcome addition, it risks confusing the reader. The table title implies a link to GBM progression, yet the data cited stems from "adolescents," "healthy adults," and "rats".
Action: The author should explicitly label a column or add a footnote clarifying that these are non-GBM populations used as a proxy to establish the inflammatory mechanism. Alternatively, the table title should be adjusted to "Evidence Linking Sleep Loss to Systemic Inflammation (General Population and Models)" to avoid misleading the reader about the existence of GBM-specific sleep data.
2. Integration of the "pilot RCT" section: Currently, the section outlining the "Pilot Randomized Controlled Trial" and "Prospective Cohort" is formatted almost like a grant application or a protocol list (using bullet points for primary/secondary outcomes). This breaks the narrative flow of a review paper.
Action: This section should be rewritten into prose. Instead of listing "Primary Outcomes: IL-6, TNF-a...", the author should describe the proposed study design in paragraph form, explaining why these specific markers were chosen based on the mechanisms discussed earlier in the text.
3. Refining the "Lifestyle" synergy: The section on "Lifestyle and Metabolic Modulation" has improved, but still feels slightly disjointed from the core "sleep" argument. For example, the paragraph on the ketogenic diet discusses tumour growth but barely touches on how KD interacts with sleep architecture or circadian rhythms.
Action: To fully integrate this into the paper's central thesis, the author needs to explicitly connect metabolic interventions back to sleep. For instance, does the KD influence sleep quality in GBM patients? If the evidence is scarce, it should be stated as a potential area for the combined therapeutic approach that the author advocates for in the conclusion.
4. Visuals and captions: Figure 1 (Graphical Abstract) is helpful, but Figure 2 is somewhat generic.
Action: Ensure that the caption for Figure 2 explicitly states that this mechanism is derived from general physiological studies and is being extrapolated to the GBM context, reinforcing the paper's hypothesis-driven nature.

The manuscript has improved dramatically. With these final adjustments to clarity and flow, it will be a valuable contribution to the field.