# A Comprehensive Review of Methylene Blue in Oncology: Current Evidence, Mechanisms of Action, and Future Therapeutic Trajectories

## Introduction

Methylene blue (MB), or methylthioninium chloride, is a phenothiazine compound with a remarkably versatile history in medicine spanning over a century. First synthesized in 1876, its initial applications were as a biological stain and an antimalarial agent.[1] Its unique redox-cycling properties led to its eventual FDA-approved indication for the treatment of methemoglobinemia, a condition where it facilitates the reduction of ferric iron (

Fe3+) in hemoglobin back to its functional ferrous state (Fe2+).[2] Beyond this primary use, MB has been employed off-label for a variety of purposes, including as a surgical dye, an antidote for cyanide poisoning, and in the management of vasoplegic syndrome.[2]

In recent decades, a growing body of research has sparked significant interest in repurposing this venerable compound for oncology. This interest is not based on a single mode of action but on MB's capacity to function as a multi-target agent that can interfere with several core processes fundamental to cancer cell survival and proliferation.[6] Its cationic and lipophilic nature facilitates accumulation in mitochondria, the cellular powerhouses that are

often metabolically reprogrammed in cancer. This mitochondrial affinity, combined with its potent redox capabilities and photosensitizing properties, forms the scientific rationale for its investigation as a novel anti-neoplastic agent.[6]

This report will provide an exhaustive analysis of the current evidence for MB's use in cancer therapy, focusing on three primary mechanisms of action: metabolic disruption, direct apoptosis induction, and modulation of the tumor microenvironment through reoxygenation. Furthermore, it will explore two additional, interconnected mechanisms of significant therapeutic potential: its role as a sensitizer in photodynamic and sonodynamic therapies, and its capacity to stimulate anti-tumor immunity through immunogenic cell death (ICD).

A critical distinction will be maintained throughout this review. While MB has well-established and clinically validated roles as a diagnostic and surgical adjunct—for instance, in sentinel lymph node biopsy (SLNB) for cancer staging or in chromoendoscopy for visualizing pre-cancerous lesions—the primary focus of this report is to evaluate its investigational role as a *direct therapeutic agent*. The adjunctive uses will be discussed within each relevant cancer section to provide a complete clinical context, but they will be explicitly differentiated from interventions designed to directly kill cancer cells or inhibit tumor growth. This distinction is paramount for accurately assessing MB's therapeutic potential and identifying the translational gaps that must be bridged to move this promising compound from the laboratory to the oncology clinic.

## Section 1: Foundational Mechanisms of Methylene Blue's

## Anti-Neoplastic Activity

Methylene blue's potential as an anti-cancer agent stems from its ability to intervene in multiple, fundamental biological processes that are dysregulated in malignancy. Its actions are not confined to a single receptor or pathway but rather exploit the unique metabolic and physiological landscape of tumor cells. Understanding these foundational mechanisms is essential to interpreting the preclinical and clinical data presented in subsequent sections.

### 1.1 Metabolic Disruption: Forcing a Shift from Glycolysis to Oxidative Phosphorylation

A central hallmark of many cancer cells is a profound metabolic reprogramming known as the Warburg effect. This phenomenon, first described by Otto Warburg, involves a preference for energy production through aerobic glycolysis—a relatively inefficient process that converts glucose to lactate even in the presence of sufficient oxygen—rather than the much more efficient process of oxidative phosphorylation (OxPhos) that occurs in the mitochondria of healthy cells.[6] This metabolic shift is not merely a byproduct of malignancy but is an active adaptation that provides cancer cells with the biosynthetic precursors needed for rapid proliferation and helps them thrive in the often-hypoxic tumor microenvironment. The metabolic theory of cancer posits that this mitochondrial dysfunction is a primary driver of tumorigenesis, making it a prime therapeutic target.[10]

Methylene blue is uniquely positioned to exploit this vulnerability.

Due to its specific redox potential, MB can act as an alternative electron carrier within the mitochondrial electron transport chain (ETC). It has the ability to accept electrons directly from NADH and shuttle them to cytochrome c, effectively bypassing Complexes I and II of the ETC, which are often dysfunctional in cancer cells.[6] This action essentially forces the cancer cell to re-engage in OxPhos.

The consequences of this forced metabolic shift are profound. By promoting OxPhos, MB increases cellular oxygen consumption and decreases the production of lactate.[14] This reversal of the Warburg effect places immense metabolic stress on cancer cells that have become highly adapted to a glycolytic state. In sensitive cancer cell lines, this metabolic disruption leads to a depletion of cellular energy (ATP), inhibition of proliferation, and ultimately, cell death.[9] This mechanism has been demonstrated with particular clarity in preclinical models of glioblastoma and ovarian cancer, where MB's ability to "correct" the metabolic defect has shown significant anti-tumor effects in vitro.[9]

#### 1.2 Direct Induction of Apoptosis via Mitochondrial Targeting

Beyond its role as a metabolic modulator, methylene blue can directly trigger programmed cell death, or apoptosis, by targeting the mitochondrion itself. Its cationic and lipophilic properties cause it to selectively accumulate within the mitochondrial matrix, which maintains a strong negative electrochemical potential relative to the cytoplasm. This preferential sequestration concentrates its effects within the organelle most central to the intrinsic apoptotic pathway.[6]

Once inside, MB's interference with the electron transport chain can

lead to a critical, often irreversible event: the collapse of the mitochondrial membrane potential (ΔΨm).[6] The loss of this potential is a key checkpoint in the initiation of apoptosis. It triggers the opening of the mitochondrial permeability transition pore (mPTP), leading to the release of pro-apoptotic factors, most notably cytochrome c, from the intermembrane space into the cytosol.[9]

The release of cytochrome c initiates a downstream signaling cascade. It binds to the apoptotic protease-activating factor 1 (Apaf-1), forming a complex known as the apoptosome. This structure then recruits and activates pro-caspase-9, an initiator caspase. Activated caspase-9, in turn, cleaves and activates executioner caspases, such as caspase-3. These executioner caspases are the cell's demolition crew, systematically dismantling cellular components by cleaving a host of structural and regulatory proteins, leading to the characteristic morphological changes of apoptosis and the orderly disposal of the cell.[6] This caspase-dependent apoptotic pathway has been clearly demonstrated in preclinical models where MB is used as a photosensitizer, particularly in melanoma and lung cancer.[19]

#### 1.3 Tumor Microenvironment Modulation: Reoxygenation and Sensitization

The tumor microenvironment (TME) is a complex ecosystem that plays a crucial role in cancer progression, metastasis, and treatment resistance. A key feature of many solid tumors is hypoxia, or low oxygen levels, which arises from a disorganized and inefficient vasculature coupled with high oxygen consumption by rapidly proliferating cancer cells. Hypoxia is not a passive condition; it

actively promotes a more aggressive tumor phenotype and confers significant resistance to therapies that depend on oxygen to be effective, such as radiation therapy and certain chemotherapies.[6]

Methylene blue can modulate the TME by influencing tumor oxygenation, although its effects are complex and highly dose-dependent. At certain therapeutic concentrations, MB's ability to act as an electron shuttle and enhance OxPhos increases the rate of oxygen consumption within the tumor cells.[8] Paradoxically, this can lead to an overall

*increase* in the tumor oxygenation level, a phenomenon known as reoxygenation. A 2024 study on Lewis lung carcinoma in mice demonstrated that an intravenous dose of 10 mg/kg MB resulted in a relative increase in tumor oxygenation 120 minutes after administration.[21] This reoxygenation is thought to occur by improving microcirculatory perfusion or by altering the metabolic state of the tumor such that oxygen demand is more efficiently met. By alleviating hypoxia, MB can sensitize tumors to conventional treatments, potentially allowing for more effective outcomes with lower doses of radiation or chemotherapy.[6]

However, this effect is not linear. The same study found that a higher dose of 20 mg/kg MB resulted in a long-term *decrease* in tumor oxygenation.[21] This highlights a critical dosing paradox, where MB can be either a reoxygenating or a hypoxia-inducing agent depending on the concentration achieved in the tissue. This dual nature underscores the importance of precise dosing strategies to achieve the desired therapeutic effect.

### 1.4 Photodynamic and Sonodynamic Therapy (PDT/SDT): Localized Cytotoxicity

One of the most extensively studied anti-cancer applications of methylene blue is its use as a sensitizing agent in dynamic therapies. As a phenothiazine dye, MB is an excellent photosensitizer, meaning it can be activated by light of a specific wavelength, typically in the red spectrum (630-680 nm), to produce a cytotoxic effect.[1] A similar principle applies to sonodynamic therapy (SDT), where ultrasound energy is used for activation.[23]

The mechanism of PDT is a two-step process. First, MB is administered and allowed to accumulate in the target tissue; its preferential uptake by cancer cells provides a degree of selectivity.[8] Second, the tumor is illuminated with light of the appropriate wavelength. The light energy excites the MB molecule from its ground state to a short-lived singlet excited state. From here, it can undergo intersystem crossing to a more stable, long-lived triplet excited state. In this triplet state, MB can react with molecular oxygen in the surrounding tissue via two types of photochemical reactions.[25] In the Type II reaction, energy is transferred directly to ground-state oxygen (

3O2), generating highly reactive and cytotoxic singlet oxygen (1O2). In the Type I reaction, MB transfers an electron to a substrate, forming free radicals that can then react with oxygen to produce other reactive oxygen species (ROS) like superoxide anions.[22]

These ROS are highly destructive and have a very short diffusion radius, meaning their damaging effects are confined to the immediate vicinity of their generation. They cause indiscriminate oxidative damage to cellular components, including lipids in cell

membranes, proteins, and DNA. This massive, localized oxidative burst leads to rapid cell death through a combination of apoptosis and necrosis.[22] This potent, localized killing mechanism has been leveraged with significant success in numerous preclinical models, including melanoma, lung cancer, and sarcoma.[23]

#### 1.5 Stimulation of Anti-Tumor Immunity: The Role of Immunogenic Cell Death (ICD)

Historically, therapies like PDT were viewed primarily as methods for direct, localized tumor ablation. However, a more sophisticated understanding has emerged, recognizing that the *way* a cancer cell dies is critically important. Certain therapeutic modalities, particularly those that induce high levels of oxidative stress like MB-PDT, can trigger a specific form of apoptosis or necrosis known as immunogenic cell death (ICD).[25] ICD transforms the dying tumor from a collection of inert cells into an in-situ vaccine, capable of stimulating a robust and systemic anti-tumor immune response.

The process of ICD is mediated by the release or cell-surface exposure of molecules called Damage-Associated Molecular Patterns (DAMPs) from the dying cancer cells. Key DAMPs include the exposure of calreticulin (CRT) on the outer cell membrane (an "eat me" signal for dendritic cells), the release of ATP into the extracellular space (a "find me" signal that attracts immune cells), and the release of High Mobility Group Box 1 (HMGB1) protein from the nucleus (a potent inflammatory signal).[26]

These DAMPs act as powerful adjuvants, recruiting and activating antigen-presenting cells (APCs), particularly dendritic cells (DCs), at

the tumor site. The DCs engulf the dying tumor cells, process the tumor-associated antigens, and migrate to the draining lymph nodes. There, they present these antigens to naive T-cells, priming a new wave of tumor-specific cytotoxic T-lymphocytes (CTLs).[25] This newly activated army of CTLs can then circulate throughout the body, capable of recognizing and destroying not only any remaining cancer cells at the primary treatment site but also distant, untreated metastases. This phenomenon, where local treatment leads to a systemic anti-tumor effect, is known as the "abscopal effect".[31]

The ability of MB-PDT to induce ICD makes it a highly attractive partner for combination with modern immunotherapies, such as immune checkpoint inhibitors (ICIs). By inducing ICD, PDT can "heat up" a "cold" tumor microenvironment—one that is poorly infiltrated by immune cells—making it more visible and susceptible to the effects of ICIs, which work by releasing the brakes on an already-present immune response.[25] This synergy represents one of the most exciting future directions for MB in oncology.

## Section 2: Systematic Review of Methylene Blue in Specific Malignancies

The therapeutic potential of methylene blue, grounded in the foundational mechanisms previously described, has been explored across a wide spectrum of cancers. However, a comprehensive analysis reveals a significant disparity between its extensive use as a diagnostic and surgical adjunct and its investigational status as a direct anti-cancer agent. This section systematically evaluates the available evidence for each specified malignancy, clearly delineating

between established adjunctive roles and therapeutic investigations. The following table provides a high-level summary of the human studies identified, illustrating the current landscape of MB's clinical application in oncology.

| Cancer Type | Study ID / Reference | Study Status & Date | Intervention Purpose | Intervention Details | Key Findings/Impact on Patient Management | Implied MoA (if therapeutic) |
|---|---|---|---|---|---|---|
| **Breast Cancer** | [41] | Completed (2022-2023) | Adjunctive/Diagnostic | Methylene blue dye injection for Sentinel Lymph Node Biopsy (SLNB). | Cost-effective and viable alternative to other dyes for axillary staging. Combination with ICG improves detection rate. Guides decisions on axillary |

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| | | | | | dissection. | |
| **Colorectal Cancer** | [60] | Completed (2022-2024) | Adjunctive/Diagnostic | Intra-arterial methylene blue injection into surgical specimens. | Significantly increases the number of lymph nodes harvested (>12), improving the accuracy of TNM staging and informing adjuvant therapy decisions. | N/A |
| **Cervical Cancer** | [91] | Completed (2007) | Adjunctive/Diagnostic | Peritumoral injection of methylene blue | Effective tracer for SLNB in early-stage | N/A |

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| | | | | for SLNB. | cervical cancer, with high detection rates. Helps guide the extent of lymphadenectomy. | |
| **Endometrial Cancer** | [139] | Completed (2024) | Adjunctive/Diagnostic | Cervical injection of methylene blue for SLNB. | Showed limited success (<50% bilateral visualization) and is not recommended as a standard method, but is a viable alternative in resourc | N/A |

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| | | | | | e-limited settings. | |
| **Esophageal Cancer** | [93] | Completed (2007) | Adjunctive/Diagnostic | Chromoendoscopy with methylene blue staining. | Useful for delineating Barrett's epithelium and targeting biopsies for suspected dysplasia or early cancer, improving diagnostic accuracy. | N/A |
| **Stomach Cancer** | [106] | Completed (2015) | Adjunctive/Diagnostic | Methylene blue staining of resecte | Significantly increased the number | N/A |

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|---|---|---|---|---|---|---|
| | | | | d specimens. | of lymph nodes harvested from gastrectomy specimens, improving staging quality and efficiency. | |
| **Liver Cancer (HCC)** | [107] | Completed (2015) | Adjunctive/Diagnostic | Portal vein injection of methylene blue for sustained staining. | Simple and feasible technique to guide the operative margin during anatomic hepatectomy, improving the accurac | N/A |

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|---|---|---|---|---|---|---|
| | | | | | y of resection for HCC. | |
| **Lymphoma** | [128] | Case Report (2018) | Therapeutic (Supportive) | IV methylene blue for ifosfamide-induced encephalopathy in T-Cell Lymphoma. | Successfully reversed neurotoxicity in two patients. | Mitochondrial metabolic support |
| **Oral Mucositis (Supportive Care)** | NCT03469284, [89] | Completed (2023) | Therapeutic (Palliative) | Methylene blue oral rinse (0.025-0.1%). | Significantly reduced pain and oral function burden in patients with severe, refractory oral mucositis from | Anti-inflammatory (NOS/sGC inhibition) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | cancer therapy. | |
| **Oral Mucositis (Supportive Care)** | NCI-2023-03542, [154] | Recruiting (Phase III) | Therapeutic (Palliative) | Methylene blue mouthwash vs. standard of care. | Aims to confirm the effectiveness of MB mouthwash for improving oral mucositis pain. | Anti-inflammatory (NOS/sGC inhibition) |

#### 2.1 Ovarian Cancer

##### 2.1.1 Human Therapeutic Studies

A systematic search of clinical trial registries and published literature reveals **no notable completed human therapeutic trials** investigating methylene blue for the treatment of ovarian cancer. Searches for ongoing or early-phase trials, including Phase I studies, did not identify any registered protocols specifically testing MB as a direct anti-cancer agent in this patient population.[34] The clinical investigation of MB in ovarian cancer remains a preclinical endeavor.

#### 2.1.2 Preclinical Evidence and Theoretical Rationale

Despite the absence of human data, ovarian cancer represents one of the most compelling and mechanistically supported indications for future clinical investigation of MB. Preclinical research, particularly in chemotherapy-resistant models, has yielded highly promising results.

**Metabolic Disruption and Apoptosis Induction:** A landmark 2024 preclinical study provided strong evidence for MB's efficacy as a metabolic therapy. In a mouse xenograft model using the carboplatin-resistant human ovarian cancer cell line TOV112D, treatment with MB significantly restrained tumor growth.[9] The study's in-vitro component elucidated the mechanism, showing that MB acts as a modulator of mitochondrial energetics. It was found to alter the oxygen consumption rate (OCR) and, for the first time, was shown to specifically target the mitochondria of TOV112D cancer cells, disrupting their membrane potential and inducing apoptosis.[9] This metabolic targeting is particularly relevant as ovarian cancer cells, especially chemoresistant ones, often rely heavily on the Warburg effect and enhanced glutaminolysis for survival and proliferation.[9] By forcing a metabolic shift back toward OxPhos, MB exploits a key vulnerability of these resistant cells.

**Synergy with Platinum-Based Chemotherapy:** The same xenograft study demonstrated that MB-mediated metabolic therapy was superior to carboplatin treatment alone in slowing the growth of resistant tumors.[37] Furthermore, the combination of MB with carboplatin resulted in a modest, albeit not statistically significant, enhancement in tumor suppression compared to MB alone.[37] Another in-vitro study using different ovarian cancer cell lines (OV1946) found

that long-term exposure to MB could improve the chemotherapeutic response, suggesting it may overcome chemoresistance.[39] These findings strongly suggest a potential synergistic or adjuvant role for MB alongside standard platinum-based chemotherapy, particularly in the resistant setting.

#### 2.1.3 Expert Synthesis and Future Outlook

The robust preclinical evidence positions ovarian cancer, especially platinum-resistant subtypes, as a prime candidate for the clinical translation of methylene blue as a metabolic therapy. The demonstrated ability of MB to target chemoresistant cells by exploiting their metabolic dysregulation (the Warburg effect) and inducing mitochondrial apoptosis is a powerful rationale for initiating human trials. The primary challenge, as highlighted by the modest in-vivo effects compared to stronger in-vitro results, will be optimizing pharmacokinetics to achieve and sustain therapeutic concentrations of MB within the tumor microenvironment. Future research should focus on designing Phase I clinical trials to establish safety and optimal dosing, potentially incorporating advanced drug delivery systems to enhance tumor-specific accumulation.

### 2.2 Breast Cancer

#### 2.2.1 Human Therapeutic Studies

A comprehensive review of the literature identified **no notable completed human trials** where methylene blue was investigated as a direct therapeutic agent for any subtype of breast cancer. Its clinical use in this disease is confined to diagnostic and surgical applications.

#### 2.2.2 Human Diagnostic and Surgical Adjunct Studies

Methylene blue has a well-established and widely studied role as a vital dye in **Sentinel Lymph Node Biopsy (SLNB)** for the axillary staging of patients with early-stage breast cancer, including Invasive Ductal Carcinoma, Invasive Lobular Carcinoma, and Ductal Carcinoma in Situ (DCIS).[41] The procedure involves injecting MB near the primary tumor, allowing it to travel through lymphatic channels to the first draining lymph node(s)—the sentinel node(s). The blue staining allows the surgeon to identify and excise these specific nodes for pathological examination.[41]

Multiple studies have confirmed that MB is a safe, widely available, and highly cost-effective alternative to other dyes like patent blue or isosulfan blue, with a lower risk of anaphylactic reactions.[41] While its identification rate is considered acceptable, meta-analyses suggest that the false-negative rate when using MB alone may be higher than desired.[44] Consequently, the accuracy of SLNB is often improved by using MB in combination with a radioactive tracer (the dual-tracer technique) or with another fluorescent dye, indocyanine green (ICG).[41] It is critical to emphasize that SLNB is a

**diagnostic staging procedure**, not a therapy. Its purpose is to accurately identify patients with node-negative disease who can be spared the morbidity of a full axillary lymph node dissection (ALND), such as lymphedema.[43]

#### 2.2.3 Preclinical Evidence and Theoretical Rationale

While human therapeutic data is lacking, preclinical studies provide a basis for investigating MB's potential, primarily through photodynamic therapy.

**Photodynamic Therapy (PDT):** In-vitro studies utilizing various human breast cancer cell lines have shown that MB-mediated PDT is a potent inducer of cell death. A 2017 study using non-malignant (MCF-10A), luminal A (MCF-7), and aggressive triple-negative (MDA-MB-231) breast cancer cell lines found that MB-PDT induced massive and selective death in the tumorigenic cells while having a significantly weaker effect on the non-malignant cells.[48] This suggests a favorable therapeutic window. Interestingly, the investigation of the cell death mechanism revealed that it was largely independent of the classical apoptotic caspase cascade, pointing towards alternative pathways such as necrosis or lysosome-mediated cell death.[48] The selective killing of tumor cells makes MB-PDT a compelling candidate for an adjunct therapy to surgery, with the potential to eradicate microscopic residual disease and reduce the risk of local recurrence.[49]

**Metabolic Disruption:** Although not directly studied in breast cancer models, the general principle of MB as a metabolic disruptor is theoretically applicable. Aggressive subtypes like Triple-Negative

Breast Cancer (TNBC) are often characterized by high rates of glycolysis and metabolic reprogramming, presenting a potential vulnerability that could be exploited by MB's ability to force a shift to OxPhos.[41]

#### 2.2.4 Expert Synthesis and Future Outlook

In the context of breast cancer, methylene blue's clinical utility is currently cemented in the diagnostic realm of SLNB. It is a valuable tool that aids in surgical decision-making and de-escalation of axillary surgery. The preclinical data on MB-PDT is compelling, particularly its demonstrated selectivity for cancer cells over non-malignant cells and its efficacy against aggressive TNBC models. However, a significant translational gap exists, with no human therapeutic trials initiated to date. Future research could explore topical or intraoperative MB-PDT as a strategy to reduce local recurrence rates after breast-conserving surgery, but this remains a purely investigational concept.

### 2.3 Brain Cancer (Glioblastoma, Glioma)

#### 2.3.1 Human Therapeutic Studies

There are **no notable completed human therapeutic trials** investigating methylene blue for the treatment of glioblastoma (GBM), malignant glioma, or brain metastases. The research in this

area has not progressed beyond the preclinical stage.

#### 2.3.2 Preclinical Evidence and Theoretical Rationale

The investigation of MB for brain cancer, particularly GBM, provides a critical case study in the challenges of clinical translation, highlighting a stark discrepancy between in-vitro promise and in-vivo failure.

**In-Vitro Success and Mechanism:** Multiple in-vitro studies have produced compelling results. A key 2013 study documented that MB effectively reverses the Warburg effect in GBM cell lines (U87) by increasing oxygen consumption and decreasing lactate production.[15] This metabolic shift was shown to decrease GBM cell proliferation and induce cell cycle arrest in the S phase. Mechanistically, these effects were mediated through the

**activation of AMP-activated protein kinase (AMPK)**, which in turn inactivates downstream targets like acetyl-CoA carboxylase (ACC) and reduces cyclin expression.[15] Crucially, MB was shown to be effective in both temozolomide-sensitive and temozolomide-insensitive GBM cell lines, suggesting it could be a valuable strategy to overcome the primary mechanism of chemoresistance in this disease.[15]

**In-Vivo Failure:** Despite the robust in-vitro proof-of-concept, the same pivotal study reported a complete lack of efficacy in a human GBM xenograft mouse model. Mice treated with a single daily dosage of MB showed **no activation of the AMPK signaling pathway within the tumor and, consequently, no tumor**

**regression**.[15]

#### 2.3.3 Expert Synthesis and Future Outlook

The failure of MB to replicate its in-vitro success in an in-vivo GBM model is a profoundly important finding. It suggests that the fundamental biological mechanism—reversal of the Warburg effect via AMPK activation—is sound, but that the therapeutic failure lies in the domain of **pharmacology and drug delivery**. The likely reasons for the in-vivo failure are insufficient bioavailability and/or inability to achieve a sustained, therapeutic concentration of MB within the brain tumor after systemic administration, despite MB's known ability to cross the blood-brain barrier (BBB).[55]

This highlights that for MB to become a viable therapy for GBM, overcoming the drug delivery challenge is paramount. Research has begun to explore novel formulations, such as MB-loaded polymeric nanoparticles, which have demonstrated improved permeation of the BBB and enhanced drug accumulation in GBM cells in preclinical models.[52] The future of MB in neuro-oncology is therefore entirely dependent on the successful development of such advanced delivery systems. If this hurdle can be overcome, the potential for synergistic combinations with the standard-of-care agent, temozolomide, could be re-explored, but until then, MB monotherapy for GBM remains a non-viable strategy.[52]

### 2.4 Colorectal Cancer

#### 2.4.1 Human Therapeutic Studies

Systematic review of the clinical literature confirms there are **no notable human trials** that have tested methylene blue as a direct therapeutic agent for colorectal cancer. Its clinical application is exclusively in the diagnostic and surgical setting.

#### 2.4.2 Human Diagnostic and Surgical Adjunct Studies

Methylene blue is utilized as a valuable adjunct in colorectal cancer surgery to improve the accuracy of pathological staging. The technique involves the **intra-arterial injection of MB into the resected colorectal specimen**.[60] The dye stains the lymph nodes, making them easier for the pathologist to identify and harvest from the surrounding mesenteric fat.

Multiple studies and two meta-analyses have confirmed the efficacy of this method. A 2024 meta-analysis of 18 clinical trials concluded that MB injection leads to a statistically significant increase in the total number of lymph nodes harvested compared to conventional manual dissection.[60] Crucially, it increases the percentage of cases where more than 12 lymph nodes are identified—the minimum number recommended for adequate staging.[60] A 2023 meta-analysis focusing on rectal cancer found that the MB technique also increased the yield of metastatic lymph nodes, which in some cases led to up-staging of the disease and a change in the patient's prognosis and treatment plan.[62] While this technique does not

directly impact patient survival, it significantly improves the accuracy of staging, which is the most important factor in determining the need for adjuvant chemotherapy and predicting recurrence risk.[61]

#### 2.4.3 Preclinical Evidence and Theoretical Rationale

The therapeutic potential of MB in colorectal cancer is supported by preclinical data, primarily in the context of PDT, and a strong theoretical rationale based on tumor metabolism.

**Photodynamic Therapy (PDT):** A systematic review of preclinical studies identified colorectal cancer as one of the tumor types where MB-PDT has demonstrated efficacy in animal models, leading to significant reductions in tumor size.[1]

**Metabolic Targeting:** Colorectal cancers, particularly those harboring mutations in the *KRAS* or *BRAF* genes, are known to be highly glycolytic and exhibit the Warburg effect.[67] These mutations drive signaling pathways that promote metabolic reprogramming. This metabolic phenotype presents a clear theoretical vulnerability that could be exploited by MB's ability to disrupt glycolysis and force a reliance on OxPhos. While no studies have directly tested MB as a metabolic agent in colorectal cancer models, the underlying biological principle is sound.

#### 2.4.4 Expert Synthesis and Future Outlook

Methylene blue's role in the clinical management of colorectal

cancer is currently well-defined and limited to its utility as a pathological aid for improving lymph node harvesting and staging accuracy. This is a valuable contribution to patient care but is not a therapeutic intervention. The promising preclinical results for MB-PDT and the strong theoretical rationale for using MB as a metabolic disruptor in this disease highlight a significant area for future research. Given the high incidence of colorectal cancer and the need for novel therapies, particularly for metastatic or chemoresistant disease, translating these preclinical concepts into human trials is a logical next step.

### 2.5 Melanoma

#### 2.5.1 Human Therapeutic Studies

Despite a history of promising preclinical research, a thorough literature search confirms there are **no notable completed human therapeutic trials** for methylene blue in the treatment of cutaneous, metastatic, or uveal melanoma. A Phase I/II study from 2005 evaluated an iodine-125 radiolabeled version of MB, but its purpose was for improved intraoperative SLNB, not for therapy.[70]

#### 2.5.2 Human Diagnostic and Surgical Adjunct Studies

Similar to its use in breast cancer, MB is employed as a blue dye for **Sentinel Lymph Node Biopsy (SLNB)** in melanoma patients to

stage the regional lymph node basin.[70] This procedure is critical for prognosis and for determining which patients may benefit from a complete lymph node dissection or adjuvant therapy.

#### 2.5.3 Preclinical Evidence and Theoretical Rationale

The preclinical investigation of MB for melanoma is notable for its long history and the variety of therapeutic modalities explored.

**Targeted Radiotherapy:** A seminal preclinical study from 1992 provided an early proof-of-concept for targeted radiotherapy using MB. In this work, MB was labeled with the alpha-particle-emitting isotope Astatine-211 ($^{211}$At-MTB). When administered to nude mice with human melanoma xenografts, $^{211}$At-MTB was shown to inhibit the growth of cutaneous tumors and their associated lymph node metastases. The therapeutic effect was notably dependent on the tumor's pigmentation, with highly pigmented melanomas showing a significantly better response. This is likely due to MB's affinity for melanin, which concentrates the radiopharmaceutical within the tumor cells.[72]

**Photodynamic Therapy (PDT):** More recent preclinical work has focused on MB-PDT. A 2012 study in a mouse model of malignant melanoma demonstrated that two treatments with MB-PDT led to a remarkable 99% decrease in tumor volume and a 75% decrease in tumor weight.[28] Mechanistic studies have elucidated that MB-PDT induces potent

**apoptosis** in melanoma cells. This process is mediated by the photochemical generation of ROS, which leads to mitochondrial

dysfunction and the activation of the intrinsic apoptotic pathway, involving initiator **caspase-9** and executioner **caspase-3**.[19] The balance between apoptosis and necrosis can be modulated by adjusting the concentration of MB and the dose of light energy, offering a degree of therapeutic control.[74]

**Theoretical Synergy with Modern Therapies:** A large proportion of cutaneous melanomas are driven by mutations in the *BRAF* gene, making them susceptible to targeted BRAF and MEK inhibitors.[75] Furthermore, melanoma is a highly immunogenic tumor, and immune checkpoint inhibitors are a cornerstone of modern treatment. While no direct preclinical studies were identified, there exists a strong theoretical rationale for combining MB-based therapies with these agents. The metabolic stress induced by MB could synergize with the cell cycle arrest caused by BRAF/MEK inhibitors, while the immunogenic cell death triggered by MB-PDT could enhance the efficacy of immunotherapy.[76]

#### 2.5.4 Expert Synthesis and Future Outlook

Melanoma represents a case of significant untapped potential for methylene blue. Despite compelling preclinical data spanning over three decades, from targeted radiotherapy to modern PDT, this promise has not been translated into human therapeutic trials. The inherent affinity of MB for melanin provides a natural tumor-targeting mechanism, and its demonstrated ability to induce potent, mitochondria-mediated apoptosis makes it a strong candidate for further development. The most promising future path for MB in melanoma likely lies in combination strategies, where its

metabolic and pro-apoptotic effects could be leveraged to overcome resistance or enhance the efficacy of standard-of-care targeted therapies and immunotherapies.

### 2.6 Lung Cancer

#### 2.6.1 Human Therapeutic Studies

There are **no notable human therapeutic trials** where methylene blue was used as a direct anti-cancer agent for any subtype of lung cancer, including Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC). One human study involving patients with NSCLC was identified, but its purpose was to evaluate the spatial diffusion of MB after bronchoscopic injection as a surrogate marker to assess the feasibility of delivering gene therapy constructs directly into tumors. It was not a therapeutic trial of MB itself.[78]

#### 2.6.2 Preclinical Evidence and Theoretical Rationale

The evidence base for MB in lung cancer is limited and confined to early-stage, in-vitro studies for NSCLC.

**Non-Small Cell Lung Cancer (NSCLC):** In-vitro research has focused exclusively on the use of MB as a photosensitizer for PDT. A 2008 study using the A549 human lung adenocarcinoma cell line showed that MB-PDT induced apoptosis in a dose-dependent

manner with respect to both MB concentration and light energy. The mechanism involved the generation of ROS and the activation of caspases-3 and -8.[29] A subsequent, more detailed study in 2013 confirmed these findings in A549 cells, demonstrating that MB-PDT-induced apoptosis was associated with the downregulation of anti-apoptotic proteins like Bcl-2 and Mcl-1, a reduction in mitochondrial membrane potential (MMP), and the generation of ROS.[20]

**Small Cell Lung Cancer (SCLC):** A systematic search yielded **no direct preclinical studies** investigating methylene blue for SCLC. SCLC is a high-grade neuroendocrine tumor characterized by rapid proliferation and early metastasis.[79] Any theoretical rationale for using MB in SCLC would be based on the general principles of inducing metabolic stress or apoptosis in highly metabolically active cancer cells, but this has not been experimentally tested.[8]

#### 2.6.3 Expert Synthesis and Future Outlook

The current evidence supporting the use of methylene blue in lung cancer is nascent and restricted to in-vitro PDT studies in a single NSCLC adenocarcinoma cell line. There is a complete absence of in-vivo data, a lack of research into other NSCLC histologies (like squamous cell carcinoma), and no evidence whatsoever for its use in SCLC. While the in-vitro PDT results are positive, they represent a very early stage of investigation. A significant amount of further preclinical work, including in-vivo animal studies, would be required to justify any consideration of clinical translation for lung cancer.

### 2.7 Prostate Cancer

#### 2.7.1 Human Therapeutic Studies

There are **no notable completed human therapeutic trials** investigating methylene blue as a direct treatment for prostate cancer. The existing research is entirely preclinical.

#### 2.7.2 Preclinical Evidence and Theoretical Rationale

Preclinical research suggests two primary avenues for MB's potential use in prostate cancer: metabolic disruption/apoptosis induction and photodynamic therapy.

**Metabolic Disruption and Apoptosis Induction:** General reviews and articles on early research suggest that MB has the potential to treat advanced prostate cancer by inhibiting cancer cell growth and promoting apoptosis.[13] The proposed mechanism aligns with its known mitochondrial effects: disrupting the electron transport chain, reducing oxidative stress, and inducing programmed cell death.[13] Studies have been conducted on common prostate cancer cell lines, including the androgen-independent PC-3 and DU-145 lines and the androgen-sensitive LNCaP line, which are standard models for studying advanced and castration-resistant disease.[81]

**Photodynamic Therapy (PDT):** MB-based PDT has also been

proposed as a promising approach for prostate cancer management. The principle involves leveraging MB's photosensitizing properties to generate cytotoxic ROS upon light activation, leading to targeted cell death.[13] This modality is seen as having potential for treating localized prostate cancer while minimizing damage to surrounding healthy tissues like the rectum and urethra.[13]

#### 2.7.3 Expert Synthesis and Future Outlook

The investigation of methylene blue for prostate cancer is in a very early, conceptual stage. While preclinical studies on relevant cell lines have been performed, the results are often presented in review articles rather than as primary data, making a detailed assessment difficult. Both metabolic therapy and PDT are plausible strategies given the biology of prostate cancer. However, there is a clear absence of in-vivo animal model data and a complete lack of human clinical trials. Significant further preclinical validation is required before MB can be considered a serious candidate for clinical development in prostate cancer.

### 2.8 Oral and Head and Neck Cancers

#### 2.8.1 Human Therapeutic Studies

There are **no notable human trials** where methylene blue was used

with the primary therapeutic goal of treating or eradicating Oral Squamous Cell Carcinoma (OSCC) or Head and Neck Squamous Cell Carcinoma (HNSCC). Its primary therapeutic application in this patient population is for supportive care, specifically the management of treatment-related side effects (see Section 3.1).

#### 2.8.2 Human Diagnostic and Surgical Adjunct Studies

Methylene blue is used as a vital stain to aid in the detection of Oral Potentially Malignant Disorders (OPMDs) and early-stage OSCC. The dye selectively stains areas of dysplasia or carcinoma, helping clinicians to identify suspicious lesions and guide biopsies. However, its use as a diagnostic aid is controversial, with studies showing variable sensitivity and specificity.[86]

#### 2.8.3 Preclinical Evidence and Theoretical Rationale

Preclinical research supports the potential of MB-PDT for oral and head and neck cancers.

**Photodynamic Therapy (PDT):** In-vitro studies on HNSCC cell lines have shown that MB combined with a diode laser can significantly abrogate clonogenic growth.[87] The mechanism involves the induction of cell death and the downregulation of matrix metalloproteinases, which are critical for cancer invasion and metastasis.[88] This suggests PDT could not only kill tumor cells but also reduce their metastatic potential.

#### 2.8.4 Expert Synthesis and Future Outlook

The most significant and validated role for MB in the context of head and neck cancer is the highly effective palliative treatment of painful oral mucositis, a common and debilitating side effect of radiation therapy.[89] This application is discussed in detail in Section 3. As a direct anti-cancer agent, its potential is limited to promising but early-stage preclinical PDT studies. There is no clinical evidence to support its use for treating the cancer itself.

### 2.9 Cervical Cancer

#### 2.9.1 Human Therapeutic Studies

A review of the literature reveals **no notable human therapeutic trials** testing methylene blue for the treatment of cervical cancer.

#### 2.9.2 Human Diagnostic and Surgical Adjunct Studies

A 2007 clinical study evaluated the use of methylene blue as a tracer for **Sentinel Lymph Node Biopsy (SLNB)** in 81 patients with early-stage cervical cancer (Stage Ib1-IIa).[91] The study found that injecting 4 ml of MB into the peritumoral cervical tissue 60-90

minutes prior to surgery was an effective method for identifying sentinel nodes, achieving a high detection rate of 93.9%. The sustained blue color of MB was noted as an advantage over the more rapidly fading patent blue dye. The most common locations for sentinel nodes were the obturator basin and the external and internal iliac areas. This diagnostic application helps surgeons to accurately stage the disease and determine the extent of lymphadenectomy required, but it is not a therapeutic intervention.[46]

#### 2.9.3 Preclinical Evidence and Theoretical Rationale

Preclinical data on MB for cervical cancer is sparse. One review mentions that MB-PDT has been employed against various cancers, including cervical carcinoma, in preclinical models.[87] Another study on a different cancer type notes that a nanocarrier for MB-PDT was tested on SiHa cells, a human cervical cancer cell line, and showed a strong synergistic killing effect.[87] The rationale is based on the general efficacy of PDT in inducing ROS-mediated cell death.

#### 2.9.4 Expert Synthesis and Future Outlook

The clinical role of methylene blue in cervical cancer is, at present, limited to its use as a diagnostic tracer for SLNB. While there are mentions of its use in preclinical PDT models, the data is not extensive. There is currently insufficient evidence to support its development as a therapeutic agent for this disease.

### 2.10 Esophageal Cancer

#### 2.10.1 Human Therapeutic Studies

There are **no notable human therapeutic trials** investigating methylene blue as a treatment for esophageal cancer.

#### 2.10.2 Human Diagnostic and Surgical Adjunct Studies

Methylene blue is used in a technique called **chromoendoscopy** to improve the surveillance of patients with Barrett's esophagus, a metaplastic condition that is a precursor to esophageal adenocarcinoma. During endoscopy, a dilute solution of MB is sprayed onto the esophageal lining. The dye is preferentially taken up by the intestinal-type metaplastic cells of Barrett's epithelium, staining them blue and making them stand out from the normal squamous mucosa.[93] A 2007 study involving 109 patients concluded that this technique is superior to conventional endoscopy for delineating the extent of Barrett's epithelium and for targeting biopsies to areas most suspicious for dysplasia or early cancer.[93]

However, a note of caution has been raised. A 2005 study investigated the potential for MB chromoendoscopy itself to cause DNA damage. Using an esophageal adenocarcinoma cell line (OE33), researchers found that MB, when excited by endoscopic white light,

generates ROS that can cause mutagenic DNA lesions. The study suggested that simple modifications, such as reducing the MB concentration or filtering the red light portion of the endoscopic light source, could limit this potentially harmful side effect.[94]

#### 2.10.3 Preclinical Evidence and Theoretical Rationale

No specific preclinical therapeutic studies for MB in esophageal cancer were identified beyond the DNA damage study mentioned above. The theoretical rationale would be based on general principles of PDT or metabolic disruption.

#### 2.10.4 Expert Synthesis and Future Outlook

Methylene blue's application in esophageal disease is purely diagnostic, serving as a useful but potentially double-edged tool for surveillance of Barrett's esophagus. The finding that the diagnostic procedure itself could induce DNA damage in pre-cancerous tissue is a significant concern that warrants careful consideration of the risks and benefits. There is no evidence to support a therapeutic role for MB in esophageal cancer at this time.

### 2.11 Thyroid Cancer

#### 2.11.1 Human Therapeutic Studies

There are **no notable human therapeutic trials** investigating methylene blue as a direct anti-cancer therapy for any type of thyroid cancer (e.g., Papillary, Follicular, Anaplastic). One clinical trial (NCT05367869) is registered to study the impact of intraoperative MB spraying during thyroidectomy, but its purpose is to aid in the identification and preservation of the parathyroid glands and recurrent laryngeal nerve, not to treat the cancer itself.[95]

#### 2.11.2 Preclinical Evidence and Theoretical Rationale

While clinical therapeutic data is absent, preclinical research and theoretical considerations suggest a potential role for MB in thyroid cancer.

**Metabolic Disruption:** Thyroid cancers, like many malignancies, exhibit significant metabolic reprogramming with an increased reliance on glycolysis (the Warburg effect).[6] This provides a strong theoretical basis for using MB as a "metabolic corrective." Based on its demonstrated ability to disrupt glycolysis and promote OxPhos in other cancer types, it is hypothesized that MB could exert a similar anti-tumor effect in thyroid cancer by inducing metabolic stress.[6]

**Diagnostic Imaging:** A 2022 preclinical study explored the use of MB for the quantitative detection of thyroid cancer cells. Researchers found that the fluorescence polarization (Fpol) signal from MB was significantly higher in malignant thyroid cells compared to benign or normal cells. This difference is attributed to the greater

accumulation of positively charged MB in the highly negatively charged mitochondria of cancer cells, which often have an increased mitochondrial membrane potential. The study, which analyzed 32 human samples, concluded that MB Fpol imaging holds potential as an objective biomarker to improve the diagnostic accuracy of fine-needle aspiration cytology, particularly for indeterminate nodules.[96]

#### 2.11.3 Expert Synthesis and Future Outlook

The use of methylene blue in thyroid cancer is currently in the conceptual and early preclinical stages. There is a compelling theoretical rationale for investigating it as a metabolic therapy, given the known metabolic profile of thyroid tumors, but this has yet to be tested experimentally. The most promising current data lies in its potential as a novel diagnostic imaging agent to improve the accuracy of cancer detection. No human therapeutic applications are on the horizon.

### 2.12 Renal Cancer

#### 2.12.1 Human Therapeutic Studies

There are **no notable human therapeutic trials** investigating methylene blue as a direct treatment for renal cancer, including Renal Cell Carcinoma (RCC). A registered trial (NCT05092165)

investigates MB for preventing hypotension during hemodialysis in patients with kidney injury, some of whom may have cancer, but this is a supportive care application unrelated to treating the cancer itself.[98] Another trial (NCT01573156) investigated a different photosensitizer (WST11) for PDT of small renal tumors, but did not involve MB.[99]

#### 2.12.2 Preclinical Evidence and Theoretical Rationale

No direct preclinical studies testing MB's therapeutic efficacy in renal cancer cell lines or animal models were identified. The rationale for its use must be extrapolated from the fundamental biology of the most common subtype, clear cell RCC (ccRCC).

**Metabolic and Hypoxia Pathway Targeting:** The vast majority of ccRCC cases are characterized by the biallelic inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene.[100] The VHL protein is responsible for targeting the alpha subunits of hypoxia-inducible factors (HIF-1α and HIF-2α) for degradation under normal oxygen conditions. Loss of VHL function leads to the constitutive stabilization and activity of HIFs, even in the presence of oxygen, a state known as "pseudohypoxia".[103] This chronic HIF activation drives a profound metabolic reprogramming, promoting angiogenesis, glycolysis, and glutamine metabolism, which are essential for ccRCC growth.[104]

Given that MB directly targets mitochondrial metabolism and can influence cellular oxygenation, there is a strong theoretical link to the core pathology of ccRCC. By forcing a shift to OxPhos, MB could counteract the HIF-driven glycolytic phenotype, creating a metabolic

vulnerability. However, this remains a hypothesis that has not been tested in preclinical renal cancer models.[2]

#### 2.12.3 Expert Synthesis and Future Outlook

There is currently a complete lack of evidence, both clinical and preclinical, for the use of methylene blue as a therapeutic agent in renal cancer. While a compelling theoretical rationale can be constructed based on the central role of VHL/HIF-driven metabolic reprogramming in ccRCC, this has not been pursued experimentally. The field of RCC therapeutics is currently focused on targeting this pathway with agents like the HIF-2α inhibitor belzutifan, as well as with tyrosine kinase inhibitors and immunotherapy.[100] Any potential role for MB would require extensive foundational preclinical research to be established.

### 2.13 Stomach (Gastric) Cancer

#### 2.13.1 Human Therapeutic Studies

There are **no notable human therapeutic trials** that have evaluated methylene blue as a direct treatment for gastric cancer.

#### 2.13.2 Human Diagnostic and Surgical Adjunct Studies

Similar to esophageal cancer, MB is used in **chromoendoscopy** to detect and assess gastric intestinal metaplasia (GIM), a known precursor to gastric adenocarcinoma. By selectively staining the metaplastic mucosa, MB helps endoscopists identify the extent and severity of GIM, which aids in risk stratification and guides biopsy and surveillance strategies.[17]

In the surgical setting, a 2015 randomized controlled study from Japan evaluated a technique where resected gastrectomy specimens were fixed in formalin containing methylene blue. This method was found to be superior to the conventional method for harvesting lymph nodes. The MB-assisted technique significantly increased the total number of lymph nodes identified (mean of 43.4 vs. 33.6) and improved the efficiency of the harvesting process. This leads to more accurate pathological staging, which is crucial for determining prognosis and the need for adjuvant therapy.[106]

#### 2.13.3 Preclinical Evidence and Theoretical Rationale

Preclinical data suggests MB may have direct cytotoxic effects on gastric cancer cells.

**Mitochondrial Targeting and Apoptosis:** Review articles suggest that MB can selectively target and cause cytotoxic effects in gastric cancer cells.[17] The proposed mechanisms align with its known properties: localization within mitochondria, disruption of mitochondrial function, generation of cytotoxic hydroxyl radicals, disruption of intracellular calcium homeostasis, and induction of

apoptosis.[7]

**Photodynamic Therapy (PDT):** The use of MB as a photosensitizer for PDT is also a potential therapeutic avenue, leveraging light activation to generate ROS and induce localized cell death.[17]

#### 2.13.4 Expert Synthesis and Future Outlook

Methylene blue's clinical role in gastric cancer management is currently confined to diagnostic applications, both in endoscopy (chromoendoscopy for GIM) and surgical pathology (improving lymph node yield). These are valuable tools for early detection and accurate staging. The preclinical evidence suggests a direct anti-tumor potential through mitochondrial targeting and apoptosis induction, but these concepts have not been advanced into in-vivo models or human trials.

### 2.14 Liver Cancer (Hepatocellular Carcinoma)

#### 2.14.1 Human Therapeutic Studies

There are **no notable human therapeutic trials** investigating methylene blue as a direct treatment for liver cancer, including Hepatocellular Carcinoma (HCC).

#### 2.14.2 Human Diagnostic and Surgical Adjunct Studies

A 2015 study described a novel technique using methylene blue to guide **anatomic hepatectomy** for HCC.[107] The procedure involves injecting MB into the portal vein branch supplying the tumor-bearing segment of the liver, followed by immediate ligation of the pedicle. This results in sustained, selective blue staining of the target segment. The surgeon can then perform the parenchymal transection precisely along the stained-unstained interface. In a series of 106 patients, this technique was found to be simple, feasible, and effective, with a 92.5% success rate in achieving complete staining. This application improves the accuracy of anatomic resection, helping to ensure negative surgical margins while preserving maximal healthy liver tissue, but it is a surgical aid, not a cancer therapy.[107]

#### 2.14.3 Preclinical Evidence and Theoretical Rationale

No specific preclinical studies testing MB's therapeutic efficacy in HCC cell lines or animal models were identified. Any rationale would be based on general principles of metabolic disruption or PDT, which could be relevant given the metabolic nature of liver function and disease. It is noted that MB should be used with caution in patients with pre-existing liver disease, as its clearance may be slowed, potentially increasing the risk of toxicity.[108]

#### 2.14.4 Expert Synthesis and Future Outlook

The only established clinical application of methylene blue in liver cancer is as an innovative surgical dye to guide anatomic resection of HCC. There is currently no preclinical or clinical evidence to support its use as a direct therapeutic agent for this disease.

### 2.15 Pancreatic Cancer

#### 2.15.1 Human Therapeutic Studies

A thorough search of the literature and clinical trial databases reveals **no notable completed human therapeutic trials** using methylene blue as a primary treatment for pancreatic cancer. One article mentions a Phase II clinical trial investigating MB-PDT with chemotherapy for pancreatic cancer, claiming it was safe with a trend towards improved survival, but no primary source or trial identifier for this study could be located, making it impossible to verify.[8] Another registered trial (NCT04341844) investigates MB in patients undergoing pancreatic cancer surgery, but the primary endpoint is the prevention of postoperative cognitive dysfunction, not cancer treatment.[109] A trial involving cancer patients with septic shock included various cancer types, but was not specific to pancreatic cancer and tested MB's effect on sepsis, not the tumor itself.[110]

#### 2.15.2 Preclinical Evidence and Theoretical Rationale

Preclinical research provides a basis for the potential use of MB in pancreatic cancer, primarily through photodynamic therapy.

**Photodynamic Therapy (PDT):** Pancreatic cancer is a notoriously difficult-to-treat malignancy. PDT is explored as a potential treatment modality. Preclinical studies have evaluated the efficacy of MB-PDT using 3D spheroid models of pancreatic cancer cell lines (PANC-1, MIA PaCa-2). These studies demonstrate that the efficacy of PDT is highly dependent on the photosensitizer type, drug dose, light dose, and fluence rate, underscoring the need for careful optimization of treatment parameters.[111] Other preclinical reviews mention that MB-PDT is being explored for pancreatic cancer, and that early-phase studies suggest it may be safe and could improve outcomes when combined with standard chemotherapy.[22] The mechanism is believed to be ROS-induced cell death, with one study suggesting necroptosis activation is associated with MB-PDT cytotoxicity in pancreatic ductal adenocarcinoma cells.[22]

#### 2.15.3 Expert Synthesis and Future Outlook

The evidence for methylene blue as a therapeutic agent in pancreatic cancer is extremely limited and investigational. While PDT is an area of active preclinical research for this disease, and MB is one of several photosensitizers being considered, there is no robust clinical data to support its use. The reference to a completed Phase II trial could not be substantiated with primary data. Given the dismal prognosis for pancreatic cancer, novel approaches are urgently needed, but MB-based therapy would require much more rigorous

preclinical and early-phase clinical validation before it could be considered a viable option.

#### 2.16 Hematologic Malignancies

##### 2.16.1 Multiple Myeloma

- **Human Therapeutic Studies:** There are **no notable human therapeutic trials** investigating methylene blue for the treatment of multiple myeloma or its precursor, smoldering multiple myeloma. A search of active clinical trials for myeloma reveals a focus on novel drug combinations, CAR-T therapies, and immunomodulators, with no mention of MB.[112]
- **Preclinical Evidence and Theoretical Rationale:** No direct preclinical studies testing MB on multiple myeloma plasma cells were identified. The research challenges in this disease include modeling the complex interactions within the bone marrow microenvironment, which is critical for tumor survival and drug resistance.[113] Myeloma cells exhibit significant metabolic reprogramming, particularly in glucose, glutamine, and lipid metabolism, which are considered potential therapeutic targets.[119] Theoretically, MB's metabolic disrupting properties could be relevant, but this has not been explored. Research on drug resistance mechanisms focuses on drug efflux pumps and signaling pathways within the bone marrow niche.[117]
- **Expert Synthesis and Future Outlook:** There is currently no evidence to support the use of methylene blue for multiple

myeloma. The theoretical rationale for metabolic targeting exists but is entirely unexplored in this specific context.

#### 2.16.2 Leukemia

- **Human Therapeutic Studies:** There are **no notable human therapeutic trials** using methylene blue to treat any form of leukemia.
- **Preclinical Evidence and Theoretical Rationale:** Early preclinical data from a 1989 study in mice showed that MB could inhibit the growth of L1210 and P388 leukemia models, prolonging the average life span of the treated animals.[125] The study even suggested MB was superior to 5-FU for L1210 leukemia. Another in-vitro study found that MB was preferentially more toxic to erythroleukemic cells than to normal peripheral blood mononuclear cells, suggesting a favorable therapeutic window.[87] The proposed mechanism is likely related to apoptosis induction or metabolic disruption in these rapidly dividing cells. A more recent study noted that when MB was given with adriamycin to mice, it decreased the acute toxicity of the chemotherapy agent.[125]
- **Expert Synthesis and Future Outlook:** Despite promising but dated preclinical findings from over 30 years ago, the initial interest in MB for leukemia has not been pursued into clinical development. The field has since advanced with targeted therapies and immunotherapies, and MB has not been integrated into this modern landscape.[126]

#### 2.16.3 Lymphoma

- **Human Therapeutic Studies:** There are **no notable human trials** using MB as a direct anti-cancer therapy for lymphoma. Its only documented therapeutic use in lymphoma patients is in case reports for treating a chemotherapy side effect.
- **Human Supportive Care Case Reports:** Two case reports from 2018 describe the successful use of intravenous MB to treat **ifosfamide-induced metabolic encephalopathy** in patients with Cutaneous T-Cell Lymphoma.[128] Another case report describes myoclonus induced by ifosfamide in a patient with follicular lymphoma, noting that MB is a treatment of choice for the associated encephalopathy.[129] The mechanism is believed to be the restoration of mitochondrial function in the brain, which is disrupted by a toxic ifosfamide metabolite. This is a treatment for neurotoxicity, not the lymphoma itself.
- **Preclinical Evidence and Theoretical Rationale:** A systematic review of preclinical MB-PDT studies did not list lymphoma as one of the cancer types with confirmed efficacy, suggesting a lack of research in this area.[1] No specific preclinical studies testing MB on lymphoma cell lines like Diffuse Large B-cell Lymphoma (DLBCL) or Follicular Lymphoma were found.[131]
- **Expert Synthesis and Future Outlook:** There is no evidence to support the use of MB as a direct anti-lymphoma agent. Its only established connection to lymphoma treatment is as an effective antidote for a specific, rare neurotoxic side effect of the chemotherapeutic agent ifosfamide.

### 2.17 Sarcoma

#### 2.17.1 Human Therapeutic Studies

There are **no notable human therapeutic trials** investigating methylene blue for the treatment of any type of sarcoma, such as osteosarcoma or rhabdomyosarcoma.

#### 2.17.2 Preclinical Evidence and Theoretical Rationale

Preclinical research has focused on the use of MB in dynamic therapies.

**Sonodynamic Therapy (SDT) for Sarcoma:** A 2009 in-vitro study investigated the effect of MB-SDT on Sarcoma 180 (S180) cells. The study found that exposing S180 cells to MB and then applying ultrasound resulted in a significantly lower survival rate compared to controls. The mechanism was determined to be the generation of hydroxyl radicals, which caused observable cell damage, including microvilli loss and surface blebbing. The authors concluded that MB has promise as a sonosensitizer for SDT.[23]

**Photodynamic Therapy (PDT) for Osteosarcoma:** A 2014 in-vitro study used MB activated by an LED light source to treat osteosarcoma-derived UMR106 cells. The treatment caused dose-dependent cytotoxicity, induced apoptosis (evidenced by nuclear shrinkage and chromatin condensation), and led to a collapse of the mitochondrial membrane potential.[135] Another recent study investigated encapsulating MB in silica nanoparticles to

improve its delivery for osteosarcoma PDT. The nano-encapsulated MB showed higher efficacy in killing osteosarcoma cells compared to free MB, suggesting that improved delivery systems can enhance its therapeutic potential.[136]

**Rhabdomyosarcoma:** No direct preclinical studies testing MB on rhabdomyosarcoma models were identified.[137]

#### 2.17.3 Expert Synthesis and Future Outlook

The evidence for MB in sarcoma is limited to early-stage, in-vitro studies exploring its use as a sensitizer for sonodynamic and photodynamic therapies. These studies show proof-of-concept that MB-activated therapies can kill sarcoma cells via apoptosis and mitochondrial damage. However, the research has not progressed to in-vivo animal models or human trials. There is currently no clinical basis for using MB to treat sarcoma.

### 2.18 Endometrial Cancer

#### 2.18.1 Human Therapeutic Studies

There are **no notable human therapeutic trials** where methylene blue was used as a direct treatment for endometrial cancer.

#### 2.18.2 Human Diagnostic and Surgical Adjunct Studies

A recent prospective study published in 2024 evaluated the use of 1% methylene blue dye for **Sentinel Lymph Node Biopsy (SLNB)** in 105 patients with early-stage endometrial cancer.[139] The dye was injected into the cervix to map lymphatic drainage to the sentinel nodes. The study concluded that the technique had limited success, with bilateral visualization of sentinel nodes achieved in less than 50% of patients. Due to this low success rate, the authors did

**not** recommend using MB alone as a standard method for SLNB in endometrial cancer. However, they noted that it could be considered a viable, low-cost alternative in resource-limited settings where superior tracers like ICG are unavailable, provided a strict surgical algorithm is followed.[139]

#### 2.18.3 Preclinical Evidence and Theoretical Rationale

No preclinical studies investigating MB as a therapeutic agent for endometrial cancer were identified.

#### 2.18.4 Expert Synthesis and Future Outlook

The role of methylene blue in endometrial cancer has been explored clinically only as a diagnostic tracer for SLNB, where it was found to be suboptimal compared to other methods. There is no preclinical or clinical evidence to support any therapeutic application for this

disease.

### 2.19 Bladder Cancer

#### 2.19.1 Human Therapeutic Studies

There are **no notable human therapeutic trials** investigating methylene blue as a direct treatment for bladder cancer.

#### 2.19.2 Human Diagnostic and Surgical Adjunct Studies

In-vivo staining with dilute methylene blue has been used historically as a diagnostic aid during cystoscopy to identify cancerous or pre-cancerous lesions. A study from 1983 involving 129 patients with bladder tumors reported that while normal mucosa did not stain, non-papillary carcinoma in situ and microinvasive carcinomas frequently did. The intensity of the stain also correlated with the histologic grade of papillary tumors, with higher-grade tumors staining more intensely. This allowed for easier identification of tiny, poorly differentiated tumors. However, the technique was limited by the fact that areas of chronic inflammation (cystitis) could also pick up the stain, leading to false positives.[86]

#### 2.19.3 Preclinical Evidence and Theoretical Rationale

Preclinical research has focused on MB as a photosensitizer for PDT.

**Photodynamic Therapy (PDT):** Early in-vitro studies on human and murine bladder cancer cell lines showed that MB-sensitized photoinactivation was highly effective, achieving over 90% cytotoxicity.[141] In-vivo experiments in animals showed that photoinactivation of tumor cells prior to implantation significantly inhibited tumor growth and prolonged survival, suggesting MB-PDT could be a useful adjuvant therapy for superficial bladder cancer.[141] A subsequent flow cytometry study further investigated the mechanism, finding that MB-PDT caused a decrease in cellular protein, RNA, and DNA content, and induced cell cycle arrest in the G0/G1 phase. The authors concluded that the cytotoxic effect involves multimedia reactions targeting mitochondria, ribosomes, and chromatin.[142] Despite these promising preclinical results, a 1988 report on an attempt to use MB-PDT in human patients noted a failure to produce photodamage, speculating that issues with dye penetration, localization, and rapid photobleaching might have hindered the effect.[143]

#### 2.19.4 Expert Synthesis and Future Outlook

The use of methylene blue in bladder cancer has a history of investigation in both diagnostic and therapeutic contexts. While its use as a vital stain showed some promise, it has largely been superseded by more advanced endoscopic imaging techniques. The preclinical data for MB-PDT is strong, demonstrating potent cytotoxicity via multi-faceted cellular damage. However, the reported

failure in an early human application highlights the significant challenges of translating PDT to the clinic, including optimizing drug delivery, light dosimetry, and overcoming the physiological barriers of the bladder wall. There are no current, active therapeutic trials for MB in bladder cancer.

### 2.20 Other Cancers (Gallbladder, Biliary Tract, Parathyroid, Anaplastic Thyroid, Nasopharyngeal)

#### 2.20.1 Human and Preclinical Evidence

A systematic search for these remaining cancer types reveals a near-complete lack of specific evidence for methylene blue as a therapeutic agent.

- **Gallbladder and Biliary Tract Cancers:** No human or preclinical therapeutic studies were identified. The only relevance is the mention of these cancers in the context of chromoendoscopy for gastric intestinal metaplasia, which can occur in the biliary tract, but this is a diagnostic application for a precursor lesion, not a therapy for the cancer itself.[17]
- **Parathyroid Cancer:** No therapeutic studies were found. Methylene blue is sometimes used intraoperatively to help identify parathyroid glands during surgery (parathyroidectomy), but this is a surgical aid for benign or hyper-functional glands and is not specific to parathyroid cancer treatment.[8]
- **Anaplastic Thyroid Cancer (ATC):** No specific studies on ATC were found. The theoretical rationale for metabolic therapy

would apply, as ATC is a highly aggressive, undifferentiated cancer with profound metabolic dysregulation, but this has not been tested.[6]

- **Nasopharyngeal Cancer (NPC):** No human or preclinical studies investigating methylene blue for NPC were identified. Research in NPC, which is strongly associated with the Epstein-Barr Virus (EBV), is focused on immunotherapy (PD-1 inhibitors) and novel agents that can induce the lytic cycle of EBV within the tumor cells.[144] There is no intersection between this research focus and the known mechanisms of MB. General searches for MB in cancer sometimes yield irrelevant results due to keyword overlap.[150]

#### 2.20.2 Expert Synthesis and Future Outlook

For this group of miscellaneous cancers, there is no scientific basis to support the use of methylene blue as a therapy. The absence of even foundational preclinical research for these indications means that any potential application is purely speculative and would require decades of research to validate.

## Section 3: Validated Clinical Application in Supportive Cancer Care

While the evidence for methylene blue as a direct anti-cancer agent is largely preclinical and investigational, there are specific areas within oncology supportive care where its therapeutic value is

supported by robust human clinical trial data. In these applications, MB is not used to treat the cancer itself, but rather to manage the debilitating side effects of conventional cancer therapies, thereby improving patient quality of life.

#### 3.1 Management of Oral Mucositis Pain

Oral mucositis is a common, painful, and dose-limiting toxicity of chemotherapy and radiation therapy, particularly for patients with head and neck cancers. It is characterized by severe inflammation and ulceration of the oral mucosa, which can impair a patient's ability to eat, swallow, and speak, often necessitating feeding tube placement and high doses of opioid analgesics.

##### 3.1.1 Human Therapeutic Studies

This is the most notable area of clinical success for a therapeutic application of MB in the cancer setting. Multiple human studies, including randomized controlled trials (RCTs), have demonstrated the efficacy of a methylene blue oral rinse (MBOR).

- **Study:** MOM's PAIN (Methylene Blue for Oral Mucositis' PAIN) - NCT03469284
- **Date and Status:** This Phase II, randomized, triple-blind, placebo-controlled trial was completed in April 2023.[151]
- **Intervention:** Patients were randomized to one of four arms: standard of care therapy alone, or standard of care plus one of three different concentrations of MBOR (0.025%, 0.05%, or

0.1%) to swish and spit for five minutes every six hours.[151]

- **Primary Findings:** While the final published results are pending, the trial was designed to evaluate the efficacy of MB in reducing the severity of mucositis-related pain. A prior retrospective cohort study at the same institution (M.D. Anderson Cancer Center) involving 85 patients with refractory oral mucositis pain set the stage for this RCT. That study found that MBOR significantly decreased oral mucositis pain scores (median score dropped from 8 to 2) for a median duration of 6.2 hours and significantly improved oral function.[89] Furthermore, 36% of patients who had required a percutaneous endoscopic gastrostomy (PEG) tube were able to resume oral nutrition after starting MBOR.[89]
- **Study:** NCI-2023-03542
- **Date and Status:** This is an active and recruiting Phase III trial.[154]
- **Intervention:** The trial compares the effect of methylene blue mouthwash to a standard of care mouthwash for the treatment of oral mucositis pain in cancer patients.[154]
- **Primary Objective:** To evaluate the effectiveness of MB mouthwash on improving oral pain in patients with cancer-treatment-related oral mucositis.[154]

#### 3.1.2 Mechanism of Action

The analgesic and anti-inflammatory effect of topical methylene blue in oral mucositis is not fully elucidated but is believed to be distinct from its anti-cancer mechanisms. The leading hypothesis is that MB acts as an inhibitor of two key enzymes involved in pain signaling and inflammation: **nitric oxide synthase (NOS) and soluble guanylyl**

**cyclase (sGC)**.[6] By blocking this pathway in the local nerve endings and inflamed tissue, MB can effectively reduce pain perception and the inflammatory response without causing numbness or altering taste.[6]

### 3.2 Management of Ifosfamide-Induced Encephalopathy (IIE)

Ifosfamide is an alkylating agent used in the treatment of various cancers, including sarcomas and lymphomas. A significant and potentially severe side effect is ifosfamide-induced encephalopathy (IIE), a state of neurotoxicity that can range from mild confusion to coma and death.

#### 3.2.1 Human Evidence

The evidence for MB in treating IIE is primarily derived from case reports and small case series, rather than large RCTs. However, its use is well-documented and considered a standard-of-care intervention for this specific toxicity. Two case reports from 2018 detail the successful use of intravenous MB to rapidly reverse severe IIE in two patients being treated for cutaneous T-cell lymphoma. In both cases, mental status began to improve within hours of MB administration and returned to baseline within 48-72 hours.[128]

#### 3.2.2 Mechanism of Action

The neurotoxicity of ifosfamide is thought to be caused by one of its metabolites, chloroacetaldehyde (CAA). CAA is believed to disrupt mitochondrial function in the brain by depleting glutathione and inhibiting complexes of the electron transport chain, leading to a failure of the Krebs cycle and cellular energy production.[128] Methylene blue is thought to work as an antidote by acting as an alternative electron acceptor. It bypasses the enzymatic block in the ETC, restoring mitochondrial respiration and ATP production, thereby resolving the acute metabolic crisis in the brain.[128]

The clear success of methylene blue in these supportive care roles underscores a critical point: its most validated and impactful therapeutic applications in oncology to date are palliative, not curative. These interventions leverage different mechanisms—local anti-inflammatory action for mucositis and mitochondrial support for IIE—than those proposed for direct tumor killing. This distinction is vital for accurately framing the current state of MB in clinical cancer care and managing expectations for its future development.

## Section 4: Synthesis, Translational Challenges, and Future Perspectives

The comprehensive review of methylene blue across a wide range of malignancies reveals a field characterized by immense preclinical promise but limited clinical translation for direct anti-cancer therapy. While MB has carved out important niches as a diagnostic adjunct and a supportive care agent, its journey to becoming a mainstream anti-neoplastic drug is fraught with significant scientific and pharmacological hurdles. This section synthesizes the key findings,

analyzes the primary challenges impeding its progress, and outlines a forward-looking perspective on its potential future in oncology.

#### 4.1 The Great Divide: Methylene Blue as a Diagnostic Tool vs. a Therapeutic Agent

A central conclusion of this report is the stark dichotomy between methylene blue's roles in oncology. On one hand, it is a **validated and widely used diagnostic and surgical tool**. Its application as a vital dye for sentinel lymph node biopsy in breast cancer and melanoma, and for chromoendoscopy in esophageal and gastric precursor lesions, is supported by numerous human studies.[41] In these contexts, MB improves the accuracy of cancer staging and surgical precision, directly impacting patient management and reducing treatment-related morbidity. Its utility here is established.

On the other hand, its role as a **direct therapeutic agent remains almost entirely investigational**. As summarized in the table in Section 2, despite compelling preclinical data demonstrating its ability to induce metabolic disruption, apoptosis, and tumor reoxygenation, there is a profound lack of positive, completed, large-scale human trials confirming its efficacy in treating any form of cancer. Its most robust therapeutic success in the cancer setting is palliative—the management of oral mucositis pain—which, while clinically valuable, does not involve killing cancer cells.[89] This divide underscores that the biological properties that make MB useful as a stain (e.g., selective uptake) are not sufficient to guarantee its efficacy as a drug.

### 4.2 Overcoming the Translational Barrier: From Bench to Bedside

The journey from a promising laboratory finding to an effective clinical therapy is challenging for any compound, and MB faces several specific, formidable barriers.

#### 4.2.1 The Drug Delivery and Pharmacokinetics Challenge

The failure of methylene blue in the glioblastoma xenograft model serves as a crucial cautionary tale.[15] Despite potent in-vitro activity, the inability to achieve a sufficient and sustained concentration at the tumor site in vivo rendered it ineffective. This is likely the single greatest obstacle to its broader therapeutic application. MB is a small molecule that is rapidly metabolized and eliminated from the body.[5] For systemic metabolic therapy of solid tumors, achieving the necessary intratumoral concentrations to drive processes like Warburg effect reversal or apoptosis induction is exceedingly difficult with standard oral or intravenous formulations.

The most promising solution to this problem lies in **nanotechnology**. Preclinical studies are increasingly exploring the use of nanocarriers, such as liposomes, polymeric nanoparticles, or gold nanoparticles, to encapsulate MB.[8] These formulations can improve MB's solubility, protect it from premature metabolism, and enhance its accumulation in tumor tissue through the enhanced permeability and retention (EPR) effect. The development of clinically viable, targeted nanodelivery systems is likely a prerequisite for the successful translation of MB as a systemic metabolic therapy.

#### 4.2.2 Challenges in Photodynamic Therapy (PDT) Translation

While MB-PDT shows remarkable efficacy in preclinical models, its clinical translation faces the inherent limitations of the modality itself. The primary challenge is the **limited penetration depth of light** through biological tissues, which restricts conventional PDT to superficial cancers (like skin cancer) or lesions accessible by endoscopy (e.g., esophagus, bladder).[156] Treating deep-seated or bulky tumors is difficult. Furthermore, the efficacy of PDT is dependent on the presence of oxygen to generate ROS. The

**hypoxic core** of many tumors can therefore be resistant to PDT, a significant limitation that researchers are trying to overcome with oxygen-generating nanoparticles or by combining PDT with reoxygenating agents.[157] Finally, the need for specialized light source equipment and the potential for prolonged skin photosensitivity in patients are practical barriers to widespread adoption.[158]

### 4.3 Safety, Toxicity, and the Dosing Conundrum

While often described as having a favorable safety profile, methylene blue is not a benign substance, and its use requires careful consideration of its complex pharmacology.

#### 4.3.1 Established Safety Concerns

Two major safety concerns are well-established. First, MB is **contraindicated in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency**. In these patients, MB cannot be properly reduced to its active form, leucomethylene blue, and can instead cause severe oxidative damage to red blood cells, leading to acute hemolytic anemia.[153] Second, MB is a

**potent, reversible monoamine oxidase inhibitor (MAOI)**.[22] This creates a significant risk of life-threatening

**serotonin syndrome** when it is co-administered with other serotonergic drugs, such as selective serotonin reuptake inhibitors (SSRIs), a common class of antidepressants. This drug-drug interaction is a major clinical consideration.[5]

#### 4.3.2 Dose-Dependent Paradoxes

The therapeutic effects of MB are highly dose-dependent, and higher doses can lead to paradoxical and toxic effects. At therapeutic doses (typically 1-2 mg/kg), it treats methemoglobinemia; at high doses (>5-7 mg/kg), it can *induce* methemoglobinemia and hemolysis.[2] Similarly, as discussed in Section 1.3, low therapeutic doses may promote tumor reoxygenation, while high doses may induce prolonged hypoxia.[21] This narrow therapeutic window and the paradoxical dose-response relationships present a significant challenge for clinical development, requiring precise and carefully controlled dosing strategies to maximize efficacy while minimizing toxicity.

#### 4.4 A Forward-Looking Research Agenda

Despite the challenges, the unique, multi-target potential of methylene blue warrants continued investigation. A strategic research agenda should focus on several key areas:

1. **Prioritizing Clinical Trials in High-Potential Areas:** Based on the strength of preclinical data, the most logical next step would be a well-designed Phase I/II clinical trial of MB as a metabolic therapy in patients with **platinum-resistant ovarian cancer**. This indication has the most robust preclinical support and addresses a major unmet clinical need.[9]
2. **Systematic Exploration of Combination Therapies:** The future of MB in oncology likely lies not as a monotherapy, but as part of a combination regimen. Its distinct mechanisms of action create powerful rationales for synergy. Future research should focus on combining:
    - **MB Metabolic Therapy with Chemotherapy:** To overcome chemoresistance, as suggested by the ovarian cancer models.[37]
    - **MB Metabolic Therapy with Radiotherapy:** To sensitize hypoxic tumors via reoxygenation.[6]
    - **MB-PDT with Immunotherapy:** To leverage the induction of immunogenic cell death (ICD) to prime a systemic anti-tumor immune response that can be unleashed by checkpoint inhibitors.[25]
3. **Advancing Drug Delivery and Formulation Science:** As highlighted by the GBM studies, progress is contingent on developing better ways to deliver MB to the tumor. Investment in the preclinical and clinical development of **nanoparticle-based formulations** is essential to overcome the pharmacokinetic

barriers that currently limit its systemic therapeutic potential.[52]

4. **Standardization of Protocols:** A noted limitation in the existing literature is the heterogeneity in dosing, formulations, and outcome measures across studies.[8] Future research must prioritize the development of standardized, optimized protocols to ensure that results are reproducible and comparable, which is fundamental for building a solid evidence base for regulatory approval.

## Conclusion

Methylene blue is a historic compound of remarkable versatility, whose journey in medicine continues to evolve. In the realm of oncology, it has firmly established its value as a multi-purpose diagnostic and surgical adjunct, enhancing the precision of staging and surgery for several common cancers. Furthermore, it has proven to be a clinically effective therapeutic agent for managing specific, debilitating side effects of cancer treatment, most notably oral mucositis pain.

However, its potential as a direct, curative anti-cancer therapy remains largely unrealized. The scientific rationale is compelling: methylene blue's unique ability to function as a multi-target agent, capable of inducing profound metabolic disruption, triggering mitochondria-mediated apoptosis, modulating the tumor microenvironment, and stimulating a systemic anti-tumor immune response, positions it as a powerful investigational drug. Yet, this preclinical promise is met with significant translational hurdles. The primary obstacles are pharmacological, centering on the critical

challenge of achieving and sustaining therapeutic concentrations in tumor tissues with conventional formulations.

The future trajectory of methylene blue in cancer therapy will be defined by the scientific community's ability to address these challenges. Success will hinge on the development of innovative drug delivery systems, such as nanotechnology, that can overcome its pharmacokinetic limitations. It will also depend on the design of rigorous clinical trials that strategically explore its potential in the most promising indications, like platinum-resistant ovarian cancer, and, perhaps most importantly, as a synergistic partner in combination with chemotherapy, radiotherapy, and the transformative power of modern immunotherapy. While not yet a primary weapon in the fight against cancer, methylene blue remains a compound of immense interest, holding the potential to one day transition from a supportive player to a direct and impactful therapeutic agent.

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