RESEARCH ARTICLE

Editorial Process: Submission:05/22/2025 Acceptance:09/28/2025 Published:10/19/2025

Metronomic Chemotherapy: A Novel Approach in Cancer Treatment

Mangal Charan Murmu[1], Hari Kumar Kuragayala[2], Balaji Nagarajan[3], Kavitha Raja[4], Shamina S[5], Tabinda Hasan[6], Rajkumar Krishnan Vasanthi[7], Kumaraswamy Dabburu[8], Somenath Ghosh[9], Sita Rama Chowdary Raparla[10]*

Abstract

Background: Systemic chemotherapy remains a cornerstone of cancer treatment, traditionally relying on maximum tolerable dosing (MTD) of cytotoxic agents to achieve remission. While MTD-based regimens have successfully treated certain malignancies such as pediatric acute lymphoblastic leukemia, their toxicity to healthy cells poses significant health risks and limitations. Metronomic chemotherapy (MCT), characterized by the frequent administration of low-dose chemotherapeutics without prolonged breaks, has emerged as a promising alternative with distinct antiangiogenic, immunomodulatory, and tumor dormancy-inducing properties. **Methods:** This review critically examines current literature on MCT, including preclinical and clinical studies, to assess its therapeutic efficacy, safety profile, and integration into standard cancer care. Emphasis is placed on evaluating combination therapies, patient selection criteria, resistance mechanisms, and nanoformulation advancements. The review also explores how MCT compares to other treatment modalities, such as immunotherapy. **Results:** Findings suggest that MCT offers significant advantages over conventional MTD regimens, including reduced toxicity, improved patient tolerance, and enhanced modulation of the tumor microenvironment. Studies show promising outcomes in various cancer types, particularly when MCT is combined with other therapeutic approaches. Nanoformulations further enhance drug delivery and effectiveness, while ongoing clinical trials continue to validate its role in cancer management. **Conclusion:** Metronomic chemotherapy presents a novel and effective strategy in oncology, with the potential to overcome limitations of traditional chemotherapy. By consolidating current evidence and clinical insights, this review supports the broader adoption of MCT and offers recommendations for optimizing its use in future cancer treatment protocols.

Keywords: Antiangiogenic Effects- Drug Resistance- Immunomodulation- MCT- Nano formulations

Asian Pac J Cancer Prev, **26 (10)**, 3849-3855

Introduction

Systemic chemotherapy remains the cornerstone of conventional cancer treatment in humans. Cytotoxic chemotherapeutic drugs interfere with the cell cycle through one or more mechanisms. These therapies, which are often provided at the MTD, attempt to kill all cancer cells. MTD chemotherapy has led to complete remission and, in some cases, a cure for various chemotherapy-sensitive diseases, particularly hematological cancers like pediatric acute lymphoblastic leukemia [1]. After cancer recurrence and micrometastases emerged due to radiation therapy and surgery, the introduction of combination chemotherapy demonstrated significant potential benefits. This method, which combines targeted medicines with traditional chemotherapy drugs such as platinum compounds and taxanes, has shown synergistic results [2]. Chemotherapy is a successful cancer treatment method, but it has limitations like high dosages, poor absorption, side effects, and drug resistance. Traditional chemotherapy protocols use cytotoxic drugs in cycles, with drug-free intervals for recovery. Metronomic chemotherapy (MCT)

*[1]Professor Pediatric, Government Medical College, Sundargarh, Odisha, India. [2]Clinical Team Manager, Thermofisher Scientific, Mason, Ohio, USA. [3]Principal Scientist, Apicore LLC, 148 Princeton Hightstown Rd, East Windsor, New Jersey, USA. [4]Post-Doctoral Research Fellow, Al Jalila Foundation Research Center, Mohammad Bin Rashid University of Medicine and Health Sciences, Dubai. [5]Associate Professor and Head, Department of Biochemistry, RVS College of Arts and Science Sulur Coimbatore, India. [6]Department of anatomy, College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, KSA. [7]Faculty of Health and Life Sciences, INTI International University, Nilai, Negeri Sembilan, Malaysia. [8]Professor of Pharmacology, Bridgetown International University, Barbados. [9]Assistant Professor, Department of Zoology, Dr. Harisingh Gour Central University, Sagar, India. [10]Principal Clinical Research Associate, ThermoFisher Scientific, 168 Third Avenue, Waltham, MA, 02451, USA. *For Correspondence: Sitarama.raparla@gmail.com*

is an innovative strategy that uses small doses of standard agents, offering reduced side effects, lower costs, and simplified treatment protocols. Both methods have their advantages and challenges. The approach demonstrated superior efficacy in eradicating drug-resistant breast cancer cells compared to conventional treatment methods [3]. Similarly, prolonged tumor regression was achieved by administering frequent low doses of vinblastine in combination with an anti-VEGF receptor 2 antibody (DC101) [4]. MCT was initially developed to combat drug resistance by redirecting attention from the direct targeting of tumor cells to disrupting the tumor's blood supply, with a particular emphasis on endothelial cells. Its effectiveness is attributed to the activation of innate and adaptive immune responses, the induction of tumor dormancy, and chemotherapy-induced dependency in cancer cells, collectively known as the "4D effect." Although MCT is a promising cancer treatment, resistance may still develop due to an incomplete understanding of its underlying mechanisms, as indicated by both preclinical and clinical trials. Consequently, MCT has been developed as an alternative to conventional treatment methods to address these challenges. This technique entails administering smaller doses of chemotherapy medications continuously and spaced, with no lengthy rest intervals. This method is novel in its capacity to target low toxicity tumors and move the focus to tumor endothelial cells [5]. The insights gained from preclinical research and clinical experience would be instrumental in reshaping cancer treatment guidelines. The rise of nanotechnology over the past two decades has significantly impacted medical treatments. Nano-drug delivery systems, including those used in metronomic chemotherapy, have shown promise in overcoming several challenges by enhancing therapeutic effectiveness while minimizing toxicity to healthy cells, thanks to selective tumor deposition, improved permeation, accumulation, and active cellular uptake.

Mechanism of Action

Antiangiogenic Effects

Metronomic drug delivery has been demonstrated to improve the in-vitro efficacy of various anticancer compounds, including cyclophosphamide (CPM) and taxanes, by disrupting angiogenesis [6]. Invivo studies suggest that MCT has anti-angiogenic effects by inhibiting apoptosis, reducing endothelial cell proliferation, and preventing endothelial cell migration. MCT reduces the quantity and survival of bone marrow-derived endothelial progenitor cells by increasing the production of thrombospondin-1, a critical angiogenesis inhibitor [7]. High-risk malignancies often experience relapse or metastasis, despite chemotherapy causing tumor regression. Angiogenesis, the formation of new blood vessels, is crucial for tumor progression. Vascular endothelial cells undergo proliferation, allowing progenitor cells to circulate and contribute to new blood vessel formation [8]. The regulation of angiogenesis balances stimulatory and inhibitory factors, with key promoters including receptor tyrosine kinase ligands like VEGF, FGF, and PGF. Conventional chemotherapy kills cancer cells by inducing DNA damage or interfering with essential cell division proteins but lacks specificity in targeting tumor cells. Cyclophosphamide has been proven to have anti-angiogenic and anti-tumor properties in drug-resistant malignancies, particularly when delivered at MTD, causing death in endothelial cells within the tumor's blood arteries [9]. This anti-angiogenic action, however, does not translate into therapeutic advantages since endothelial cells may repair themselves during rest periods. A more successful strategy might entail delivering cyclophosphamide in low, frequent doses of MCT, which targets endothelial cells rather than cancer cells and inhibits tumor angiogenesis. MCT has been linked to reduced circulating endothelial progenitor cells (CEPs), necessary for vasculogenesis-dependent tumor development. Metronomic treatment is expected to have anti-angiogenic effects by raising TSP-1 expression and lowering CEP viability [10]. It also suppresses endothelial cell growth and migration while inducing apoptosis. Hypoxia-inducible factor (HIF)-1α, a key transcription factor in angiogenesis and tumor progression processes, could potentially be a target for anti-angiogenic therapies. The topoisomerase I inhibitors, topotecan and camptothecin, have been demonstrated to lower HIF-1α activity [11]. A preliminary study was reported to evaluate whether oral metronomic topotecan could inhibit HIF-1α expression in tumor tissue samples [12]. Metronomic chemotherapies, which involves continuous low-dose drug administration, aims to reduce toxicity while targeting tumor angiogenesis and progression. HIF-1α plays a crucial role in tumor adaptation to hypoxia, promoting survival, metastasis, and resistance to therapy. By suppressing HIF-1α expression, metronomic topotecan could enhance treatment efficacy and reduce tumor aggressiveness. The study's findings suggest a potential therapeutic benefit, highlighting the need for further research to explore its clinical applications in improving cancer treatment outcomes while minimizing adverse effects.

Immunomodulation

Cancer cells elude immune detection and elimination to circumvent immunosurveillance, particularly by evading recognition by macrophages, natural killer (NK) cells, and T and B lymphocytes. This immune evasion is facilitated by mechanisms such as reducing their immunogenicity or the secretion of immunosuppressive molecules, including adenosine, prostaglandin E2, transforming growth factor-beta (TGF-β), and VEGF-A. These immunosuppressive agents impede the function of dendritic cells (DC), obstruct T-cell infiltration into the tumor microenvironment, and directly prevent the activation of effector T cells while simultaneously promoting the activity of regulatory T (Treg) cells. A well-functioning immune system is essential for the efficacy of chemotherapy, and both innate and adaptive immune responses are essential for the clearance of early-stage malignancies. However, conventional chemotherapy using MTD depletes lymphocytic cells, impairing immune function. In contrast, MCT, with its lower, more frequent dosing, has been shown to enhance host immune responses through various immunomodulatory mechanisms. For example, low-dose cyclophosphamide inhibits Treg cell function by down-regulating forkhead box P3 expression by MCT. In contrast, low-dose gemcitabine selectively depletes Treg cells, improving survival in pancreatic tumor-bearing mice [13]. Studies have shown that ultra-low doses of paclitaxel can drive the transformation of immunosuppressive myeloid-derived suppressor cells (MDSCs) into immunostimulatory dendritic cells (DCs) in vitro. This process occurs independently of Toll-like receptor (TLR)4 activation [14]. While chemotherapy is often linked to lymphopenia and neutropenia, its impact on regulatory T (Treg) cells appears moderately compatible with metronomic treatment strategies. Regulatory T cells (Treg), characterized by the CD4+ CD25+ Foxp3+ markers, suppress antigen-specific immune reactions by secreting cytokines and engaging in direct cell-to-cell interactions, and this results in the inhibition of tumor-targeting immune cells, including CD8+ cytotoxic T lymphocytes, CD4+ T helper cells, and broader effector cells such as NK and NKT cells [15]. One potential approach to enhance the immune response against tumor-related antigens is to selectively block or diminish regulatory T cell (Treg) function. Tregs are crucial in maintaining immune homeostasis by suppressing excessive immune activation. However, in the tumor microenvironment, they contribute to immune evasion by inhibiting cytotoxic T-cell activity, thereby allowing tumor progression. Targeting Tregs can be achieved through multiple strategies, including the use of monoclonal antibodies against Treg-specific markers such as CTLA-4, CD25, or FoxP3 [16]. Small-molecule inhibitors and immune checkpoint blockade can also disrupt Treg-mediated immunosuppression, promoting a more robust antitumor response. Selective depletion of Tregs through low-dose cyclophosphamide or metronomic chemotherapy has shown potential in preclinical and clinical studies. While Treg-targeted therapies offer a promising avenue for cancer immunotherapy, careful modulation is necessary to avoid autoimmune side effects and maintain a balanced immune response in the Figure 1.

Oncogenic Dormancy

Tumor dormancy is a complex phenomenon where tumor cells remain in a non-proliferative state for extended periods before reactivating to cause disease progression or relapse. This dormant state can be maintained through mechanisms like cell cycle arrest, angiogenic dormancy, or immune surveillance. Stress signals can trigger this quiescent state, allowing tumor cells to evade treatments. Angiogenic dormancy balances cell proliferation and apoptosis [17]. Without adequate blood vessel formation, oxygen and nutrient supply remain insufficient, limiting tumor growth and maintaining dormancy. The tumor microenvironment plays a crucial role in regulating this process through anti-angiogenic factors such as thrombospondin-1. Immune surveillance also contributes to cancer dormancy by restraining tumor progression at subclinical levels. Cytotoxic CD8+ T lymphocytes, natural killer (NK) cells, and other immune components continuously monitor and eliminate cancerous cells before they establish a growing tumor mass [18]. However, immune evasion strategies, such as immune checkpoint upregulation, can eventually disrupt this balance, leading to tumor reactivation and disease recurrence. MCT aims to induce dormancy by exerting anti-angiogenic effects. The evasion of immune surveillance can trigger an angiogenic switch, as the immune system modulates cancer angiogenesis through pro- and anti-angiogenic mechanisms [19]. Due to its immunomodulatory effects, MCT may enhance anti-cancer immunity while strengthening its anti-angiogenic properties.

Overcoming Drug Resistance

Although chemotherapy is administered at the MTD, tumors frequently contain clones that exhibit resistance to the drug. Consequently, high-dose chemotherapy exerts selective pressure on the heterogeneous tumor cell population, thereby facilitating the survival of the most drug-resistant clones. On the other hand, MCT is less likely

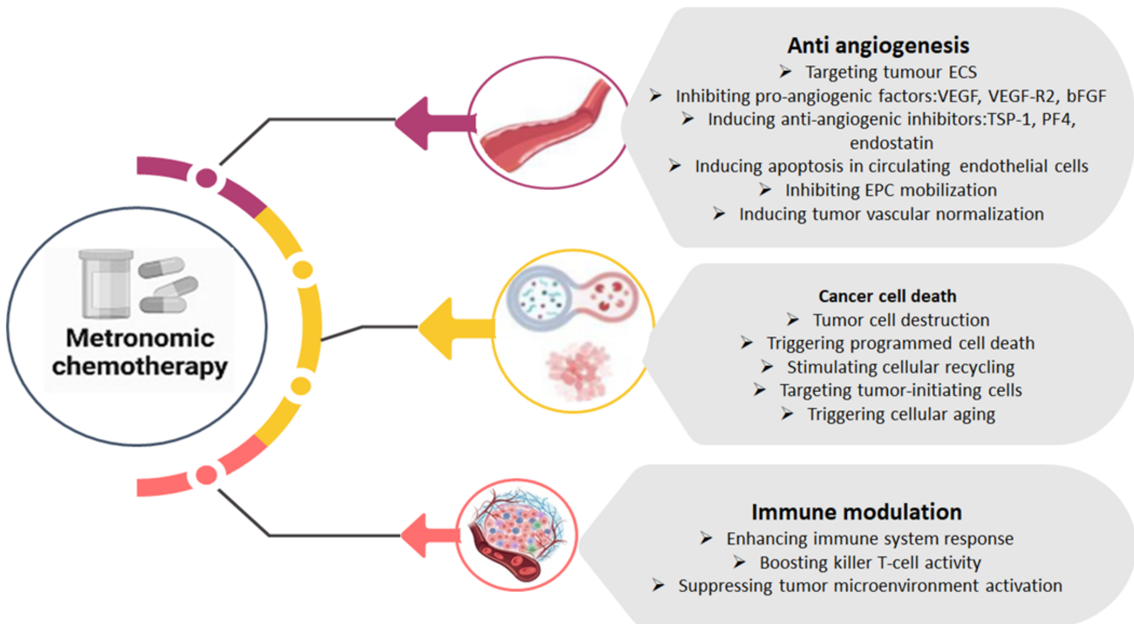

Figure 1. Mechanism of action of MCT

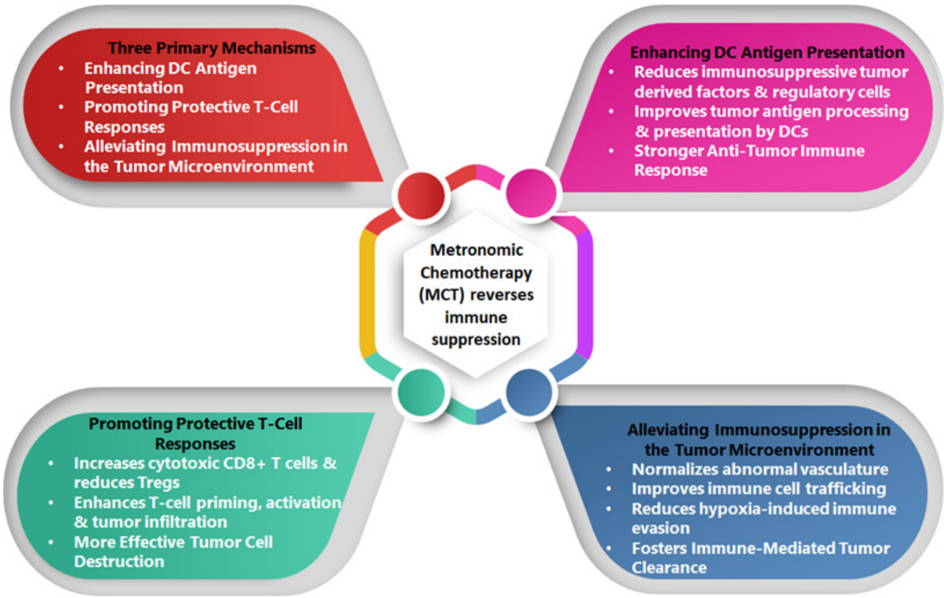

Figure 2. Metronomic Chemotherapy (MCT) Reverses Immune Suppression

to induce acquired drug resistance in endothelial cells due to their genetic stability [20]. This treatment approach has shown potential in eliminating drug-resistant tumors and has demonstrated encouraging clinical outcomes in metastatic and treatment-resistant cancers. As a form of anti-angiogenic therapy, MCT may exhibit resistance mechanisms similar to those observed in other anti-angiogenic treatments. Following an initial therapeutic response, tumors can develop evasive resistance, enabling them to either stimulate neovascularization despite treatment or adapt by becoming less dependent on new blood vessel formation for continued growth. Studies suggest that continuous administration of taxanes leads to stronger anti-tumor responses and a reduced risk of drug resistance compared to periodic dosing in ovarian cancer models. Furthermore, variations in the gene expression profiles of vascular endothelial cells have been noted when comparing long-term MTD chemotherapy with MCT approaches. Studies have explored alterations in protein expression linked to acquired resistance in human prostate cancer cells treated with metronomic cyclophosphamide [21].

MCT Versus Immunotherapy

Cancer immunotherapy aims to enhance the immune system's capacity to identify and eliminate cancer cells more efficiently. Its primary objective is to generate a long-lasting population of highly activated T cells specifically targeting tumor cells. Cancer cells can avoid immune detection by reducing the expression of tumor-associated antigens, MHC class I and II proteins, and critical components involved in antigen processing and presentation [22]. The interaction between cancer cells and the immune system leads to the buildup of immunosuppressive cells and cytokines in the tumor microenvironment. Furthermore, tumor cells may express immune checkpoint ligands, further dampening immune responses. To overcome these barriers, integrating immunotherapy with other immunomodulatory approaches is crucial for maximizing treatment efficacy. MCT plays a crucial role in reversing immune suppression through three primary mechanisms: enhancing dendritic cell (DC) antigen presentation, promoting protective T-cell responses, and alleviating immunosuppression within the tumor microenvironment. MCT enhances DC antigen presentation by reducing the immunosuppressive effects of tumor-derived factors and regulatory cells [23]. By decreasing the population of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), MCT allows DCs to process and present tumor-associated antigens more efficiently, initiating stronger anti-tumor immune responses. MCT promotes protective T-cell responses by shifting the immune balance toward cytotoxic CD8+ T cells while reducing the presence of immunosuppressive Tregs [24]. This leads to improved T-cell priming, activation, and infiltration into tumors, enhancing the destruction of malignant cells. MCT fosters a pro-inflammatory cytokine environment that sustains T-cell function and prevents exhaustion. MCT alleviates immunosuppression within the tumor microenvironment by normalizing abnormal vasculature, improving immune cell trafficking, and reducing hypoxia-induced immune evasion [25]. This creates a more favorable setting for immune-mediated tumor clearance. By modulating these key mechanisms, MCT enhances the efficacy of immunotherapy and other anti-cancer treatments, positioning itself as a promising strategy for overcoming tumor-induced immune suppression in the Figure 2.

Nano formulations

Nanotechnology has long been utilized to enhance therapeutic applications, with research in this field advancing significantly as its benefits become increasingly apparent. This technology has seamlessly expanded into radiotherapy, chemotherapy, diagnostics, and imaging, showcasing its potential to improve patient care. Due to their nanoscale size, nanotechnology-based agents play a crucial role in drug formulation, as this dimension is considered a fundamental threshold for nanomedicine development. Currently, most approved anticancer nanomedicines consist of liposomal formulations and drug conjugates (including antibody-based systems, Polymer, and protein) designed to optimize the pharmacokinetics and pharmacodynamics of free drugs, primarily employing passive targeting mechanisms. Various cytotoxic agents, including Pemetrexed (PMX), Etoposide (ETP), Docetaxel (DTX), and Oxaliplatin (OXA), have been incorporated into different nanocarriers—such as nanoemulsions, micelles, and niosomes—to assess their anticancer potential in preclinical studies [26]. MCT, when combined with other cytotoxic drugs like CPM, has shown limited success in treating breast cancer tumors. Long-term treatment with continuous CPM and antiangiogenic drugs can inhibit tumor progression. Oral MCT regimens containing OXA and PMX showed immune-modulating effects in colon cancer rat models. Oral formulations with nanoscale particles showed the most effective anticancer activity. Triptolide-loaded liposomal formulations showed greater anticancer activity [27]. This was demonstrated by a considerable decrease in serum tumor volume, blood vessel density, and endothelial progenitor cells.

Preclinical and Clinical Studies

Metronomic chemotherapy (MCT) is a promising development in clinical practice, offering patients better quality of life and minimizing side effects. Phase III trials are needed to promote its widespread adoption. Common cytotoxic drugs include CPM, MTX, VRB, and CPB. MCT-based approaches have been explored for advanced prostate cancer, but newer hormonal and anticancer treatments are still being explored. These drugs have received approval for treating castration-resistant prostate cancer (CRPC) due to compelling data from phase III clinical trials [28]. Metronomic cyclophosphamide, when combined with a DNA vaccine in a B16.F10 murine melanoma model, was found to be ineffective in targeting cells with low CD43 expression, which is associated with a memory phenotype [29]. In a tumor model induced by HPV16, the researchers evaluated the synergistic effects of metronomic cyclophosphamide and a peptide vaccine aimed at HPV16E7 [30]. They concentrated on antigen-specific T-cell responses and sought to determine the optimal timing for chemotherapy about vaccination. The results indicated that this combined therapy significantly enhanced long-term tumor management. Furthermore, in a mouse glioma model, the pairing of single-walled carbon nanotube (SWCNT)/CpG-2 immunotherapy with metronomic temozolomide was shown to enhance the anti-tumor response by improving effector cell function and depleting suppressive myeloid cells [31]. A later randomized trial was conducted to evaluate the effectiveness of adding an induction regimen targeting both angiogenesis and anti-tumor immunity to the standard MTD chemotherapy protocol [32]. Their therapeutic approach included Granulocyte colony-stimulating factor, a high-dose cyclooxygenase-2 inhibitor, metronomic cyclophosphamide, a sulfhydryl donor, and an autologous tumor antigen-containing hemoderivative. An additional investigation investigated the efficacy of three distinct cyclophosphamide regimens metronomic, MTD, and a combination of MTD and metronomic when administered with oncolytic adenovirus therapy to patients with resistant solid tumors [33]. All three regimens resulted in improved disease control rates. In addition, patients with treatment-resistant solid tumors were administered oncolytic adenoviruses in conjunction with a low-dose temozolomide pulse. In contrast, metronomic cyclophosphamide was administered at a low dose to reduce regulatory T cells (Tregs). Metronomic cyclophosphamide, celecoxib, and IL-2 were incorporated into DC vaccination for metastatic melanoma, resulting in a two-fold increase in patients who experienced stable disease [34]. Similarly, a combination of survivin-targeted immunotherapy and low-dose cyclophosphamide demonstrated promising immune responses in ovarian cancer patients [35]. Most patients who received DPX-Survivac exhibited CD8+ T cell responses specific to survivin, with stronger immune responses observed in those who received a higher vaccine dose and metronomic cyclophosphamide [36]. These findings highlight the potential of combining MCT with immunotherapy as an effective cancer treatment strategy. Preclinical and clinical research is required to understand the immunomodulatory mechanisms involved in MCT comprehensively.

Challenges

An MCT regimen is employed to ensure continuous drug administration at low doses. Low doses of treatment lead to reduced side effects and could contribute to transitioning cancer care toward managing it as a chronic condition. Multiple assessments have shown a better safety profile and encouraged therapeutic outcomes. Several active clinical trials are exploring the potential of metronomic therapy as a maintenance strategy in various combinations, especially for cancers such as breast, ovarian, and colorectal. Primarily, MCT has been assessed in a maintenance context, often administered alongside standard high-dose treatments. However, no trials have directly compared metronomic therapy as a standalone approach in later treatment stages. Consequently, no conclusive evidence supports the superior efficacy of MCT alone, and definitive clinical proof remains lacking. Further research is necessary to validate its effectiveness in clinical settings. Regarding drug repurposing, the antidiabetic medication metformin has gained considerable research interest, with retrospective studies indicating a survival benefit in diabetic cancer patients. Among its various anticancer mechanisms, metformin has demonstrated antiangiogenic properties and the ability to enhance apoptosis in breast cancer cell lines [37]. Metformin can reduce specific immunosuppressive cells and may exert antagonistic effects when combined with checkpoint inhibitors. MCT persists in its advancement and innovation, regardless of these uncertainties. Clinical trials are expanding to include palliative care and disease management strategies, with a focus on integrating metronomic chemotherapy (MCT) with repurposed non-cancer medications. However, quality of life and cost-effectiveness are significant obstacles to long-term metronomic treatment. No clinical trial has shown significant improvement in quality of life through maintenance therapy. More trials with strong methodological frameworks and randomized designs are needed to demonstrate the clinical relevance of MCT and drug repurposing.

Author Contribution Statement

M.C.M and R.K.V conceptualized the article. H.K.K and T.H was responsible for data collection. B.N and K.D contributed to writing, K.R and S.S contributed to review, and editing, as well as visualization. S.R.C.R and S.G carried out the literature review.

Acknowledgements

None

Conflict of Interest

The authors declare that they have no conflict of interest.

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