Cancer Pathogenesis and Therapy xxx (xxxx) xxx

Contents lists available at ScienceDirect

Cancer Pathogenesis and Therapy

journal homepage: www.journals.elsevier.com/cancer-pathogenesis-and-therapy

Review article

Methionine restriction for cancer therapy: From preclinical studies to clinical trials

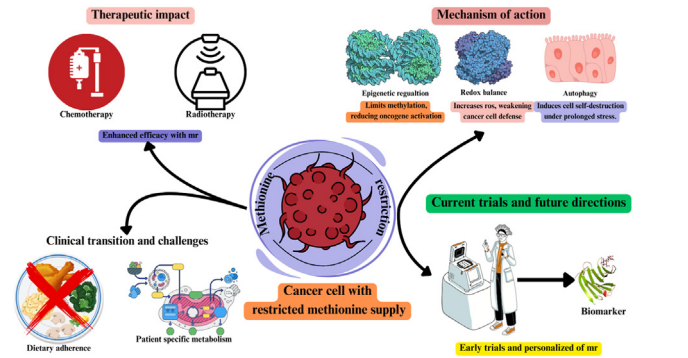
Nagaraju Bandaru [a,*], Shaik Mohammad Noor [b], Maha Lakshmi Kammili [b], Mohan Gandhi Bonthu [a], Alluri Pavani Gayatri [c], Perli Kranti Kumar [a]

[a] Department of Pharmacology & Pharmaceutical Analysis, School of Pharmaceutical Sciences, Sandip University, Nashik, Maharashtra 422213, India
[b] College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddesweram, Andhra Pradesh 520002, India
[c] Department of Pharmacology, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra 422213, India

HIGHLIGHTS

- Preclinical studies have shown that methionine restriction (MR) reduces cancer cell proliferation via different mechanisms.
- MR lowers sulfur-containing metabolite levels, reduces oxidative stress, and enhances the immune response.
- Clinical trials suggest that MR, when combined with conventional treatments, may sensitize tumors to chemo/radiotherapy.
- MR disrupts methionine-dependent pathways, thereby reducing cancer cell survival.
- The therapeutic potential of MR lies in its ability to synergize with other therapies, enhancing overall antitumor efficacy.

GRAPHICAL ABSTRACT



Methionine restriction (MR) as a treatment approach for cancer. The multifaceted role of MR in cancer treatment is shown in the diagram. Mechanistically, MR controls redox reactions by increasing reactive oxygen species production, limiting methylation to control epigenetic processes, and triggering autophagy in response to extended stress. MR also improves the effectiveness of traditional treatments such as radiation and chemotherapy. Clinical difficulties, such as patient-specific metabolism and dietary compliance, are emphasized. Biomarker identification and early personalized healthcare trials are part of the shift to clinical applications, opening the door for further studies and treatment development.

ARTICLE INFO

Managing Editor: Peng Lyu

Keywords:
Cancer treatment
Clinical studies
Methionine metabolism
Preclinical studies

ABSTRACT

Methionine restriction (MR) has shown significant promise in cancer therapy because it targets the unique methionine dependency of many tumors. However, despite extensive research on MR, a clear synthesis of preclinical findings and their translation into clinical settings is lacking. This review aims to address this gap by consolidating existing evidence, identifying challenges, and highlighting opportunities for advancing MR as a viable cancer treatment strategy. Preclinical studies have revealed that MR effectively hinders cancer cell proliferation, triggers cell cycle arrest, and enhances the effectiveness of standard treatments, including chemotherapy and radiotherapy. Mechanistically, MR disrupts critical cancer pathways by influencing epigenetic regulation, redox balance, and autophagy. Moreover, animal models have demonstrated notable tumor suppression and extended survival, underscoring the therapeutic potential of MR. Early-phase clinical trials are now examining MR in combination with established therapies, reporting positive preliminary results regarding safety and tolerability, and investigating biomarkers for predicting patient responsiveness. These findings suggest the

* Corresponding author. Department of Pharmacology & Pharmaceutical Analysis, School of Pharmaceutical Sciences, Sandip University, Nashik, Maharashtra 422213, India
E-mail address: bnrajupharma@gmail.com (N. Bandaru).

https://doi.org/10.1016/j.cpt.2025.01.002
Received 13 September 2024; Received in revised form 5 December 2024; Accepted 2 January 2025

Please cite this article as: Bandaru N et al., Methionine restriction for cancer therapy: From preclinical studies to clinical trials, Cancer Pathogenesis and Therapy, https://doi.org/10.1016/j.cpt.2025.01.002

utility of MR as a complementary treatment strategy, particularly for tumors resistant to conventional therapies. The outcomes of this study underscore the importance of further research to refine MR protocols, understand long-term effects, and identify optimal patient groups. Furthermore, combining MR with immunotherapies, targeted treatments, and advanced modalities such as chimeric antigen receptor (CAR)-T cell therapy may offer new therapeutic pathways. Additionally, the development of MR-mimetic drugs and targeted supplements can improve patient compliance and broaden the therapeutic applicability of MR. Large-scale clinical trials are essential to evaluate the efficacy of MR across diverse cancer types, focusing on sustainability and safety over extended periods. If successful, MR can transform cancer therapy by exploiting metabolic vulnerabilities in cancer cells, providing a novel and less toxic treatment option for challenging malignancies.

Introduction

Cancer is an abnormal cell type that can spread throughout the body and rapidly increase in number, which can cause death. According to the World Health Organization (WHO), cancer is the second most common cause of death, and it is estimated that there will be 10 million cancer-related deaths in 2020 worldwide. According to the International Agency for Cancer Research (IARC), there were 19.3 million new cancer cases in 2020; 10 million fatal cases are expected to occur by 2030.[1] In the United States in 2023, there were 609,820 deaths associated with cancer and approximately 1,958,310 new cancer cases.[2] Hippocrates (460–370 Before Christ [BC]) coined the term Karakinos (cancer), meaning crab, to describe cancer because the disease sticks to the body stubbornly, like a crab.[3] As per WHO, 2020 reports, there were 2.26 million breast cancer, 2.21 million lung cancer, 1.93 million colorectal cancer, 1.41 million prostate cancer, 1.20 million skin cancers (apart from melanoma), and 1.09 stomach cancer cases worldwide. Moreover, approximately 400,000 children per year are diagnosed with cancer.

The hallmarks of cancer provide a framework for the knowledge of cancer complexities, encompassing the following: sustaining proliferative signaling, evading growth suppressors, cell death resistance, enabling replicative immortality, angiogenesis induction, and activating invasion and metastasis.[4] Current therapies rely on the type of cancer, including pharmacological and radiotherapeutic management.[5] Standard chemotherapy is commonly used to treat cancer. These drugs kill fast-growing cells but may cause side effects. Normal cells, such as those found in hair follicles, bone marrow, and the digestive and reproductive systems, can be damaged by chemotherapy.[6] In cancer therapy, tumor recurrence is mostly caused by innate, acquired, or multidrug resistance (MDR).[7–9] In recent years, cancer therapy has evolved from non-specific cytotoxic agents to selective mechanism-based treatments.[10]

Nutrition plays a crucial role in cancer management, with amino acids such as methionine being essential for tumor growth.[11] In 1950, Sugimura et al.[12] worked on male Sprague–Dawley rats and found that tumor-bearing rats fed with methionine deficiency showed slower tumor growth than those fed with other amino acid deficiencies. Unlike normal cells, cancer cells cannot divide when methionine is substituted by homocysteine (Hcy). Despite being able to produce methionine from Hcy, cancer cells depend on external methionine because of altered metabolic flux needs; this is called the Hoffman effect.[13] This effect is brought about by the high demand for transmethylation in cancer cells. Moreover, methionine dependence may affect oncogenic transformation and therapeutic efficacy in several types of human cells.[14] This review examines the methionine metabolism in various cancers and the effects of MR on cancer cell growth, both in preclinical and clinical studies.

Methionine cycle and the one-carbon metabolic network

Methionine is an essential human amino acid. It is a sulfur-containing amino acid that supports cell viability and growth via three main metabolic pathways. The L-methionine (L-Met) cycle produces S-adenosyl methionine (SAM), a crucial methyl donor for DNA, RNA, and protein methylation. SAM is then converted into S-adenosylhomocysteine (SAH) and L-homocysteine (L-Hcy). L-Met cannot be produced in the body and must originate from a food source. High levels of methionine (300 mg) are mostly present in animal products, such as meat, poultry, eggs, fish, and dairy products.

Methionine functions as a protein component and is connected to other critical metabolic pathways, including those involved in detoxification (glutathione [GSH]), nuclear activity (polyamines), epigenetics (SAM), and phospholipids in cellular membranes. The methionine cycle indirectly influences nucleotide production and is associated with folate metabolism. Methionine synthase (MS) remethylates L-Hcy to L-Met using N5-methyl tetrahydrofolate and cobalamin, which supports the folate and methionine cycle. The L-Met salvage pathway recycles L-Met from polyamine synthesis byproducts, such as S-methyl-5-thioadenosine, converting it through a series of reactions back to L-Met.[15] Further, the transculturation pathway converts L-Hcy to cystathionine and then to cysteine, which is essential for GSH synthesis and redox balance [Figure 1].

Methionine metabolism in cancer biology

The functions of methionine relevant to cancer biology include GSH formation, polyamine synthesis, and methyl group donation.

Glutathione formation

Methionine, a cysteine precursor, is essential for GSH formation.[16,17] GSH is an important thiol antioxidant that scavenges reactive oxygen species (ROS), forming oxidized glutathione (GSSG). Low GSH levels and a reduced GSH/GSSG ratio indicate oxidative stress, which can activate the phosphatidylinositol 3-kinase (PI3K) pathway and promote cancer cell growth.[18] The progression of cancer and inflammation can be caused by chronic oxidative stress.[19] Interestingly, dietary MR in rats increases blood GSH levels despite reducing hepatic GSH levels because of adaptations in sulfur amino acid metabolism.[20,21] MR enhances antioxidant ability and reduces oxidative stress by decreasing ROS production.[22]

Polyamine synthesis

SAM is a decisive methylation cofactor and aminopropyl group donor in the synthesis of polyamines.[23] Polyamine synthesis-related enzymes are often overexpressed in cancer.[24] High intracellular concentrations of polyamines (typically in the millimolar range) require substantial SAM levels to maintain homeostasis during cell proliferation.[25] Polyamines preserve chromatin structure, regulate ion channels by modulating their activity and gating mechanisms, and support membrane stability. The primary enzyme involved in polyamine biosynthesis, S-adenosylmethionine decarboxylase (SAMDC), generates decarboxylated SAMs (dcSAMs). Polyamine synthesis results in dcSAM generation, the aminopropyl group donor for spermine and spermidine synthase, via SAM decarboxylation. dcSAM is then converted to 5′-deoxy-5′-methylthioadenosine (MTA), which is recycled through the methionine salvage pathway to recover adenine and methionine.

This recycling is crucial for two reasons: dcSAM can inhibit DNA methyltransferases and other methyltransferases[26] while MTA acts as a protein arginine N-methyl transferase5 (PRMT5) inhibitor,[27] a protein

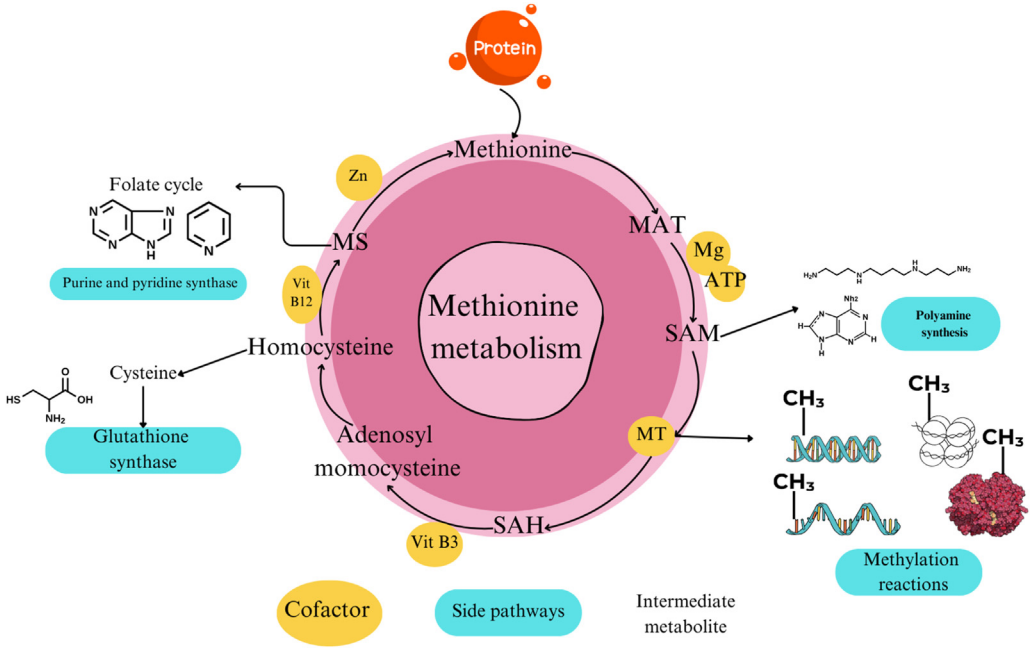


Figure 1. The methionine metabolism pathway and its interconnected biochemical processes. Methionine, an essential amino acid, is converted into SAM with the help of ATP and magnesium. SAM is a crucial methyl donor for methylation reactions that modify DNA, RNA, and proteins. After methyl donation, SAM is converted into SAH, which is hydrolyzed to Hcy. Hcy can be remethylated to methionine via vitamin B12 and zinc or converted to cysteine, which is important for glutathione synthesis, an antioxidant pathway. SAM is also involved in polyamine synthesis, which is essential for cell growth. Low levels of L-Met lead to reduced SAM levels, resulting in several effects consistent with the inhibition of β-Hcy methyltransferase and methylene tetrahydrofolate reductase along with preventing cystathionine β-synthase activation to support L-Met cycle flow. The accumulation of 5-MHTF that inhibits glycine N-methyltransferase direct the utilization of SAN toward DNA methylation. However, an upregulation in MAT expression suggests the regulation of SAM production to support cellular levels, particularly for maintaining proper epigenetic regulation and cell proliferation. ATP: Adenosine triphosphate; Hcy: Homocysteine; L-Met: L-methionine; MAT: Methionine Adenosyltransferase; MG: Magnesium; MHTF: Methylene Tetrahydrofolate; MS: Methionine synthase; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; SAM: S-adenosylmethionine; Vit B12: Vitamin B12; Vit B13: Vitamin B13: ZN: Zinc.

often overexpressed in most cancers and is associated with the survival of individuals.[28] Tumors often show deletions in the methylthioadenosine phosphorylase (*MTAP*) gene, which is next to the tumor suppressor gene *CDKN2A*. The deletion of *MTAP* leads to elevated levels of MTA, which inhibits *PRMT5*, thereby rendering tumors with these deletions more susceptible to *PRMT5* inhibition.[29] Polyamines also regulate the transcriptional and translational stages of protein synthesis and are crucial for cell growth and proliferation. A depletion of polyamines disrupts the cell cycle, inducing apoptosis.[30,31] Elevated polyamine levels correlate with increased tumor growth. Polyamine metabolism inhibitors, such as alpha-difluoromethyl ornithine, can disrupt the cell cycle and DNA synthesis in cancer cells.[32] Polyamine also synthesis relies on methionine, and MR might suppress cancer cell growth by lowering polyamine production, thereby offering a potential therapeutic strategy.

Deoxyribonucleic acid methylation

DNA methylation is a highly prominent epigenetic modification that occurs on Cytosine-phosphate-Guanine (CpG) dinucleotides and affects approximately 70% of cytosine bases.[33] The hypermethylation of CpG islands within gene promoter regions can aberrantly silence transcription, leading to the downregulation of tumor suppressor gene expression.[34] CpG island hypermethylation is a hallmark of various types of cancer.[35,36] SAM acts as a multi-purpose methyl donor for catecholamines, proteins, phospholipids, histones, and DNA and RNA methylation.[37] It has been shown that in the presence of SAM, the methylation of biomarkers, including *TFP12* (Tumor Formation Prevention 12), *SEPT9* (Septin 9), *GSTP1* (Glutathione S-Transferase Pi 1), and *MGMT* (O6-Methylguanine-DNA Methyltransferase) affects tumor growth or suppression.[38]

DNA methylation is reversible. Dietary MR could potentially change methylation patterns, affecting cancer development and progression. Aging is associated with global DNA hypomethylation; however, specific gene regions may be hypermethylated. As suggested, DNA methyltransferases sense methionine metabolism, which could alter methylation patterns and impact the life span of an organism.[39] It is interesting to note that, in rodent studies, the effects of dietary MR on global DNA methylation vary with age. The livers of older mice (1-year-old at the beginning of the intervention) showed increased global DNA methylation after 12 weeks of dietary MR, whereas the livers of younger mice (6 weeks old at the beginning of the intervention) did not show any change in global DNA methylation.[40] These studies suggest that DNA methylation is crucial for cancer cell development, and dietary MR offers a promising approach for changing methylation patterns, potentially influencing cancer progression and health outcomes, with age-dependent effects.

Methionine restriction

Previous studies have shown that MR suppresses tumor growth in rats and several human cancer cell lines. Normal cells, which can remethylate

Hcy to generate methionine, are not harmed by this limitation compared to a targeted approach in cancer therapy.[41] Cancer cells are methionine-addicted; hence, there is a unique opportunity to target this dependency with methionine-reducing therapies, such as recombinant methioninase (rMETase), which is known for its antiproliferative effects and ability to inhibit tumor growth. However, this raises the question of whether this approach could selectively kill cancer cells and avoid killing normal tissues.

A study published in 1974 investigated the effects of MR on various cell types, including rat and mouse cancer cell lines, and normal human and animal cells.[42] This study showed that methionine-depleted media significantly impaired the growth of malignant cells while leaving normal cell growth unchanged. This differential effect is due to the capacity of a typical cell to recycle Hcy via MS, which cancer cells lack [Table 1].[63] Consequently, MR has the potential to selectively target cancer cells without affecting normal cells.

Methionine restriction influences oncogenic pathways

MR in preclinical studies or cell culture media offers metabolic benefits, such as reduced adiposity, enhanced insulin sensitivity, and reduced inflammation and oxidative stress, while contributing to lifespan extension. The effects of MR are mediated by various mechanisms, including decreased oxidative stress and inflammation, modulation of autophagy, and a reduction in both cancer incidence and mortality. Studies suggest that MR affects oncogenic pathways by limiting the availability of methionine, which is essential for cell proliferation and DNA methylation in cancer cells. Importantly, MR is effective only when the non-essential amino acid cysteine is excluded from the diet; adding cysteine reverses the positive metabolic and antioxidant effects of MR.[64]

One case reported a 63-year-old female diagnosed with metastatic pancreatic cancer in October 2023. The patient was started on FOLFIRINOX (A chemotherapy regimen consisting of FOLinic acid (leucovorin), Fluorouracil (5-FU), IRINotecan, and OXaliplatin) as first-line chemotherapy in combination with MR, which included 250 units of oral-rMETase twice daily and a low-methionine diet. Five months after the start of combination therapy, the tumor size of the patient decreased by 40%, her liver metastases regressed, and her CA19-9 (Carbohydrate Antigen 19-9) blood marker level decreased by 86%. These findings suggest that MR, consisting of o-rMETase, a low-methionine diet, and standard chemotherapy, may offer a highly effective strategy for managing advanced inoperable cancers.[65]

Methionine metabolism in cancer and normal cells

Methionine is an essential amino acid that plays crucial roles in various metabolic pathways, including protein synthesis, epigenetics, detoxification, and cellular membrane formation. In normal cells, methionine can be synthesized from its metabolic precursor Hcy, allowing these cells to obtain the necessary levels even upon dietary MR. This flexibility in methionine metabolism supports consistent cellular functions, such as DNA methylation, without relying solely on external methionine sources.[66]

However, methionine metabolism differs in cancer cells. Unlike normal cells, most cancer cells exhibit a unique dependency on external methionine, known as methionine dependence or the Hoffman effect. Although cancer cells can convert Hcy to methionine, they require higher methionine levels due to the increased demand for metabolites derived from methionine, such as SAM, which is critical for DNA and histone methylation. This heightened methionine requirement supports rapid cell proliferation and extensive epigenetic modifications that drive oncogenesis and tumor progression.[13]

Studies have shown that methionine dependence correlates with increased transmethylation activity in cancer cells, leading to genome-wide hypomethylation, a factor associated with genomic instability and tumor aggressiveness. This correlation emphasizes the distinct metabolic demands of cancer cells, where methionine overuse contributes to the hallmark characteristics of cancer, such as accelerated growth and disrupted epigenetic regulation.[67]

Comparative effectiveness of methionine restriction on different cancer types

MR has shown diverse therapeutic effects in multiple cancer models and human studies, with varying efficacies depending on the cancer type, MR sensitivity, and the mechanistic pathways involved. MR has shown promising results in breast cancer cell culture by inhibiting the growth, migration, and invasion of aggressive breast cancer cells such as MDA-MB-231(M.D. Anderson-Metastatic Breast 231), thereby affecting focal adhesion kinase (*FAK*) phosphorylation and matrix metalloproteinase (MMP) activity. Similarly, MR inhibited tumor growth in nude mice injected with breast cancer cells by upregulating the expression of cell cycle inhibitors. In colon cancer, MR increases oxidative stress, induces cell cycle arrest, and promotes the apoptosis of *p53*(Tumor Protein 53)-deleted cells. Animal models treated with MR also showed a >80% reduction in early colon cancer marker levels, highlighting the potential of MR in colon cancer prevention and therapy. Prostate cancer cell lines respond to MR by selectively inhibiting *FAK* and *ERK* phosphorylation, suggesting interference with tumor proliferation signaling pathways.[68]

For gastric cancer, MR improves the efficacy of chemotherapeutic drugs, such as 5-fluorouracil (5-FU), by modulating folate metabolism and tumor marker activity, leading to significant tumor reduction in both xenograft models and patient studies.[58] Triple-negative breast cancer (TNBC) also shows responsiveness to MR in cell cultures and animal models, where MR inhibits growth, induces apoptosis through GCN2(General Control Nonderepressible 2) and PERK-independent pathways, and reduces lung metastasis rates.[62] In metastatic melanoma and glioma, MR combined with chemotherapeutic agents, such as cystemustine, produced moderate results, offering limited survival benefits but proving to be well-tolerated, showing that MR could complement existing treatments.[50] Gastrointestinal cancers have also been treated with MR, with clinical trials showing considerable tumor shrinkage when combined with 5-FU, as the effects of MR on plasma methionine reduction alter tumor cell metabolism.[64] MR also enhances sensitivity to chemotherapy by affecting redox and nucleotide metabolism in colorectal cancer models, including patient-derived xenografts (PDXs).[64] Human studies have similarly demonstrated the ability of MR to stabilize colorectal cancer when combined with chemotherapy regimens such as FOLFOX(Folinic acid [leucovorin calcium], fluorouracil, and oxaliplatin).[58]

Overall, the efficacy of MR depends on the cancer type and context, with certain cancers, such as breast, colon, and gastrointestinal cancers, responding robustly to MR through significant effects on cell growth, migration, and chemotherapy resistance. For more resistant cancers, such as melanoma and glioma, MR shows limited standalone effectiveness but holds potential as an adjunct therapy, particularly for cancers with metabolic vulnerabilities to methionine.[69]

Biomarker prediction of methionine restriction response in personalized medicine

Identifying biomarkers for predicting the response to MR has opened new avenues in personalized cancer treatment, enabling the development of more precise and effective therapy choices. Methionine dependency, a hallmark of many tumor cells, has allowed researchers to explore MR as a strategy for targeting cancer metabolism. Biomarkers such as MET-positron emission tomography (PET) imaging have shown promise, offering a noninvasive approach to assess methionine metabolism directly within tumors. This imaging technology has been proven to have superior sensitivity in identifying methionine-addicted cancers, particularly when compared to standard imaging techniques such as fluorodeoxyglucose (FDG)-PET. MET-PET can effectively visualize active tumor sites, allowing clinicians to predict which patients might benefit from methionine-restriction-based therapies and to check the response over time.[70]

Table 1
Effects of methionine restriction in cancer specimens and human studies.

Cancer models	Effect of methionine restriction	Reference
Cell culture model		
Human breast cancer cell line	Methionine restriction significantly impairs the growth of MDA-MB-231-CD63-GFP cells while promoting increased exosome production and secretion.	Li et al.[43]
Peritoneal-metastatic cancers from patient-derived samples	Tumors with high sensitivity to rMETase, particularly colorectal cancer and pseudomyxoma, showed significant responses to methionine restriction therapy. Pancreatic and ovarian cancers also show responsiveness but to a lesser degree.	Hoshiya et al.[44]
p53-deleted colon cancer cells	SAMe overcomes uL3-mediated drug resistance by cell cycle arrest induction in the S-phase, inhibiting autophagy, increasing reactive oxygen species, and promoting apoptosis in *p53*-deleted colon cancer cells.	Hens et al.[45]
Human TNBC and mouse fibroblast cell lines	In TNBC cells, MR caused apoptosis and growth inhibition in a manner that was independent of *PERK* and *GCN2*.	Komninou et al.[46]
Human prostate cancer cell lines	In PC-3 cells, MR suppresses *FAK* and *ERK* phosphorylation but not protein expression.	Jeon et al.[47]
MDA-MB-231 and Hs 578T cell lines	Methionine deficiency attenuated invasion and migration. It also attenuated matrix metalloproteinase phosphorylation, MMP-2 and MMP-9 activity, and mRNA expression; it elevated TIMP-1 expression; it attenuated urokinase plasminogen activator, It also attenuated matrix metalloproteinase phosphorylation; it attenuated MMP-2 and MMP-9 activity and mRNA expression; it elevated the TIMP-1expression; it attenuated urokinase plasminogen activator; and it attenuated the expression of plasminogen activator inhibitor 1.	Komninou et al.[48]
p53-deficient colon cancer cells	S-adenosyl-L-methionine overcomes uL3-mediated drug resistance in colon cancer cells with *p53* deletions. It enhanced reactive oxygen species, triggered apoptosis, hindered autophagy, and induced cell-cycle arrest in the S-phase.	Mosca et al.[49]
Animal studies		
Walker tumors implanted subcutaneously in Sprague–Dawley rats	Restricting methionine inhibited the growth of tumors.	Thivat et al.[50]
Human gastric cancer xenografts in nude mice	Methionine deficiency altered intratumoral folate metabolism, which enhanced the anticancer efficacy of 5-FU.	Epner et al.[51]
Human pre-malignant epithelial breast cell lines implanted into mice	Methionine restriction increases the levels of cell cycle inhibitors in nude mice, reducing the formation of breast tumors.	Hoffman[52]
F344 rats treated with azoxymethane to induce colon cancer	Methionine restriction inhibits the development of colonic tumors during the post-initiation phases of carcinogenesis, partially due to the inhibition of cell proliferation.	Kubota et al.[53]
4T1-mouse TNBC model	Animals on a methionine-deprived diet had fewer lung metastases than those on a controlled diet.	Ji et al.[54]
F344 rat model	MR reduced the levels of early colon cancer markers by >80% in rats and decreased colon cell growth by 12%, potentially preventing tumor development.	Sinha et al.[55]
Chemoresistant RAS-driven colorectal cancer PDXs and *KRASG12D*$^{+/-}$*TP53*$^{-/-}$ soft tissue sarcomas	MR led to therapeutic responses by altering one-carbon metabolism, affecting redox and nucleotide metabolism and enhancing sensitivity to chemotherapy and radiation.	Meyskens et al.[56]
Human studies		
Cohort of 20 patients with metastatic melanoma and two with recurrent glioma ($n = 22$)	MR showed no impact on survival but was well-tolerated (no signs of toxicity or nutritional issues).	Lu et al.[57]
Patients with advanced gastric cancer ($n = 14$)	Total parenteral nutrition deficient in methionine synergized with 5-FU on gastric cancer progression TS activity.	Ahn et al.[58]
Metastatic solid tumors in adults phase-I clinical trial with eight patients	Enteral MR was both safe and bearable. Within 2 weeks, there was a notable 58% decrease in plasma methionine levels, accompanied by either steady or elevated levels of serum albumin and pre-albumin. The only adverse impact noted was a weekly weight loss equivalent to approximately 0.5% of the whole body mass index.	Fu et al.[59]
Gastrointestinal tract cancers phase I clinical trial	MR TPN AO-90(Antioxidant 90) combined with 5-FU resulted in a more significant tumor reduction compared to MR TPN with methionine in patients with resected gastric cancer.	Hu et al.[60]
Melanoma and glioma in Phase II trial	With a median overall survival of 4.6 months and median disease-free survival of 1.8 months, dietary MR in combination with cystemustine was well-tolerated. Plasma methionine levels were reduced by 40%.	
Metastatic colorectal cancer	Combining dietary MR with a FOLFOX regimen reduced plasma methionine levels by 58% and led to a partial response in three out of four patients and disease stabilization in one.	
Case of a 55-year-old male with recurrent locally advanced rectal cancer	Administered a treatment regimen comprising a low-methionine diet and oral recombinant methioninase. The treatment was associated with stable CEA levels and stable tumor size even without standard chemotherapy. This diet may be effective for the stabilization of long-term disease in rectal cancer.	Lu and Epner[61]
Human feeding study	MR produced similar effects on systemic metabolism as seen in mice, suggesting its potential to influence tumor cell metabolism and cancer outcomes.	Strekalova et al.[62]

This table summarizes the outcomes of MR in various cancer models, including cell culture, animal studies, and human clinical trials. MR exhibits diverse therapeutic effects, including growth inhibition, apoptosis induction, and enhanced efficacy of chemotherapeutic agents. The results of human studies highlight the safety, tolerability, and potential role of MR in synergy with standard treatments. The data presented herein include references to specific cancer types, the mechanism of action of MR, and notable findings from each study. 4T1: A murine mammary adenocarcinoma cell line; 5-FU: 5-Fluorouracil; AO-90: A human breast cancer cell line; CEA: Carcinoembryonic antigen: *ERK*: Extracellular signal-regulated kinase; F344: A rat strain; FAK: Focal adhesion kinase; GCN2: General control nonderepressible 2; Hs 578T: A human breast cancer cell line; *KRASG12D*$^{+/-}$: A specific mutation in the KRAS gene linked to cancer; "G12D" refers to glycine-to-aspartic acid at codon 12; MDA-MB-231: A human triple-negative breast cancer (TNBC) cell line; MDA-MB-231-CD63-GFP: A genetically modified MDA-MB-231 cell expressing green fluorescent protein (GFP) tagged to CD63; MMP: Matrix metalloproteinase; MR: Methionine restriction; mRNA: Messenger ribonucleic acid; p53: Tumor protein 53,; PC-3: A human prostate cancer cell line; PDX: Patient-derived xenograft; PERK: Protein kinase RNA-like endoplasmic reticulum kinase; RAS: A family of proteins (HRAS, NRAS, KRAS) involved in cell signaling and oncogenesis; rMETase: Recombinant methioninase; SAMe: S-adenosylmethionine; S-phase: The DNA synthesis phase of the cell cycle during which DNA replication; TIMP-1: Tissue inhibitor of metalloproteinases-1; TNBC: Triple-negative breast cancer; *TP53*$^{-/-}$: A knockout mouse model lacking functional p53; TPN: Total parenteral nutrition; TS: Thymidate synthase; uL3: A ribosomal protein.

Specific genetic biomarkers in tumor tissues, such as upregulated methionine cycle-related genes, have been associated with stemness and chemoresistance in certain cancers. For example, four genes, *SDHAF2*, *MRPS34*, *MRPL11*, and *COX8A*, are linked to methionine dependency in intrahepatic cholangiocarcinoma (ICC) and influence the responses to transarterial chemoembolization (TACE) in patients with high methionine cycle activity. The regulation of immune checkpoints, such as *PD-L1* (Programmed Death-Ligand 1) expression via methionine-derived SAM, is another biomarker of interest, as increased *PD-L1* expression in certain cancers suggests the potential for combining MR with immune checkpoint inhibitors to enhance immunotherapy outcomes.[71] In liver cancer, hepatocyte nuclear factor 4α (*HNF4α*) and its regulation of sulfur amino acid metabolism can also predict response to MR, as its presence or absence dictates cancer cell sensitivity to MR.

Research on resected peritoneal-metastatic cancers, including colorectal, pancreatic, and ovarian cancers, and pseudomyxomas treated with rMETase showed varying degrees of sensitivity to MR. Colorectal cancer and pseudomyxoma exhibited the highest responsiveness, whereas pancreatic and ovarian cancers only showed moderate responses. These findings suggest that patients with at least 40% sensitivity to rMETase, as determined by a histoculture drug response assay (HDRA), may benefit from MR therapy, particularly in combination with a low-methionine diet. Additionally, investigations on the antitumor immune effects of methionine restriction diet (MRD) have revealed that MRD enhances the number and cytotoxic activity of tumor-infiltrating $CD8^+$(Cluster of Differentiation 8 Positive) T cells in murine models, thereby inhibiting tumor growth. This effect is mechanistically linked to methionine-derived SAM, which promotes N6-methyladenosine (m6A) methylation and the translation of immune checkpoints, such as *PD-L1* and *VISTA* (V-Domain Immunoglobulin Suppressor of T Cell Activation), in tumor cells. Furthermore, the depletion of YTH domain-containing family protein 1 (*YTHDF1*) in MRD models synergizes with programmed cell death protein 1 (PD-1) blockade, restoring $CD8^+$ T cell infiltration and improving tumor control. Clinically, *YTHDF1* expression has been correlated with poor outcomes in immunotherapy, positioning methionine metabolism and *YTHDF1* as promising targets in developing personalized cancer immunotherapy strategies.[41,43]

These biomarkers provide a more refined approach to cancer treatment by allowing clinicians to personalize MR strategies based on a patient's unique molecular profile. By tailoring MR-based treatments to individual biomarker patterns, personalized medicine can not only improve patient outcomes but also minimize unnecessary side effects, offering a promising new strategy for targeting the metabolic vulnerabilities of cancer.

Prostate cancer is a prominent cause of death among men and currently has limited preventive options. MR has been shown to inhibit prostate cancer development, particularly in severe lesions in mouse models.[72] The mechanisms of MR in prostate cancer include the reduction of polyamine production, control of the insulin/insulin-like growth factor-1 (IGF-1) axis, and inhibition of cancer cell growth, which is vital for tumor cell dissemination. MR also targets thymidylate synthase (TS), a key enzyme in nucleotide synthesis, enhancing the chemotherapeutic efficacy of 5-FU by lowering TS activity and expression.[73] Additionally, MR induces prostate cancer cell apoptosis by impairing mitochondrial integrity and affecting oncogenic pathways such as Raf(Rapidly Accelerated Fibrosarcoma) and Akt (Protein Kinase). However, the effects of MR can vary among different prostate cancer cell lines, indicating the need for personalized treatment approaches.[51]

Breast cancer, particularly TNBC, poses treatment challenges due to limited therapeutic options. Studies on MR have focused on inhibiting tumor progression in TNBC models and enhancing the effectiveness of TRAIL-R2 (Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Receptor 2) agonists, such as lexatumumab, and upregulating TRAIL-R2 expression. MR also reduced cell migration and invasion in TNBC cells.[74] Nevertheless, MR activation of the integrated stress response through kinases such as GCN2 and PERK may require further optimization to improve their efficacy.[75]

MR has shown promise in the prevention and treatment of colorectal cancer. In PDX models of colorectal cancer, MR inhibited tumor growth and enhanced the efficacy of 5-FU chemotherapy. In rat models of colon carcinogenesis, MR significantly reduced the formation of aberrant crypt foci, which are precursors of colon cancer, demonstrating its preventive potential.[76]

Methionine restriction and dietary interventions

MR has also emerged as a valuable adjunct to chemotherapy, utilizing the unique metabolic dependence of cancer cells to enhance treatment efficacy. Cancer cells often show a heightened reliance on methionine, making them vulnerable to methionine-depleting therapies. By incorporating MR into traditional chemotherapy, researchers aim to exploit this vulnerability and improve therapeutic outcomes. Previous studies have shown that reducing methionine levels can selectively impair tumor growth without significantly affecting normal cells. This selective action can be synergistic with chemotherapy as it may enhance the sensitivity of cancer cells to standard chemotherapeutic agents. For instance, combining methionine-free diets with drugs such as cystemustine and 5-FU has shown promising results in reducing

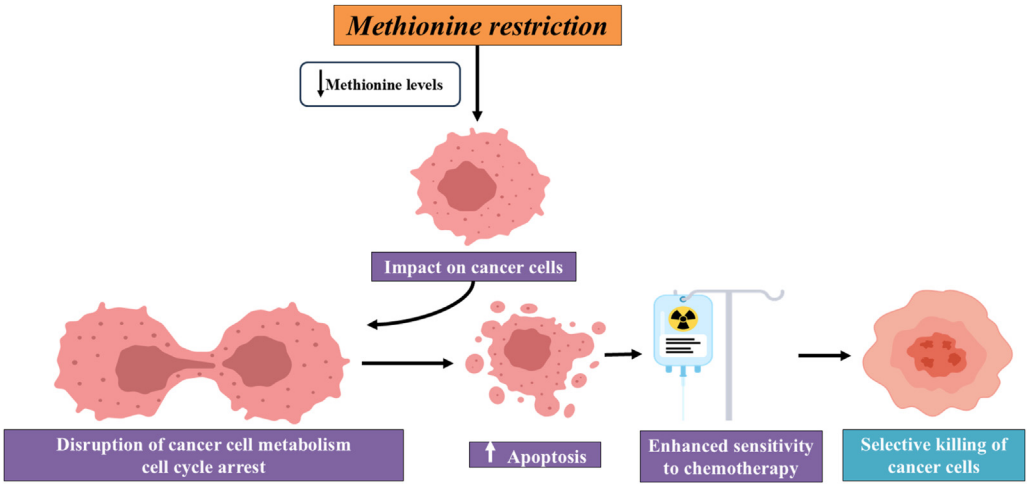


Figure 2. Mechanisms of methionine restriction in cancer therapy. MR and METase are effective at reducing plasma methionine levels, enhancing chemotherapy sensitivity, and inducing tumor suppression in various cancers. Clinical studies have revealed that MR is generally well-tolerated with minimal side effects, such as mild weight loss. In combination with therapies such as radiation and chemotherapy (e.g., FOLFOX and cystemustine), MR shows promising outcomes, including tumor stabilization, apoptosis induction, and improved drug efficacy. Preclinical studies support this potential by revealing mechanisms such as S/G2-phase cell cycle arrest and immune modulation. FOLFOX: Folinic acid (leucovorin calcium), fluorouracil, and oxaliplatin; METase: Methioninase; MR: Methionine restriction; S/G2: S phase.G2 phase.

tumor size and improving patient responses [Figure 2].[77] MR has also appeared to be a promising strategy for enhancing the effectiveness of chemotherapy by targeting the unique metabolic needs of cancer cells. Methionine is a crucial amino acid on which many cancer cells rely, making them particularly vulnerable to treatments that reduce their availability. Cancer cells often have an increased dependence on methionine compared with normal cells. This differential dependency allows MR to impair tumor growth while sparing normal cells, which can be compensated using alternative metabolic pathways. The combination of MR with conventional chemotherapeutic drugs can improve treatment outcomes. For instance, methionine-free diets paired with chemotherapeutic agents such as cystemustine and 5-FU have shown the potential to reduce tumor size more effectively and improve patient responses than chemotherapy alone.[66]

Recent clinical trials explored the integration of MR into various chemotherapeutic regimens. These studies showed that MR enhanced the sensitivity of cancer cells to chemotherapy, leading to improved tumor control and potentially better overall treatment efficacy. In a phase I clinical trial, patients with diverse types of non-skin cancers were placed on an MRD for a period extending around their radiation therapy sessions. The study found that the average methionine level dropped to 18.8 μmol/L in nine patients. However, the trial was eventually stopped because it was challenging to keep patients on a diet and enroll more participants. Importantly, no severe side effects were reported, suggesting that MRD can be safely integrated with radiotherapy (RT). Another study focused on patients with metastatic melanomas and recurrent gliomas. These patients followed a methionine-free diet for 1 day while receiving cystemustine, a chemotherapeutic drug. The study included 22 patients and found that the median overall survival was approximately 4.6 months, and disease progression was relatively slow for some patients. In a trial involving patients with gastrointestinal tract cancers, a specialized amino acid formulation (AO-90) lacking methionine was tested along with 5-FU, a common chemotherapy drug.

This approach led to a significant reduction in the size and growth rate of the treated tumors, highlighting the potential benefits of combining MR with traditional chemotherapy. For patients with metastatic colorectal cancer, one study evaluated a methionine-free diet in combination with a 5-FU-based FOLFOX chemotherapy protocol. The diet led to a reduction in plasma methionine levels to 58%. Moreover, three out of four patients showed partial responses to the treatment, while one had stable disease. Laboratory studies using rMETase, an enzyme that depletes methionine, showed that targeting methionine in various tumor cell lines made them more sensitive to chemotherapy drugs like doxorubicin and cisplatin. This is because methionine depletion arrests cancer cells in a particular cell cycle phase (S/G2-phase). Studies on melanoma cells showed that methionine deprivation stress (MDS) led to increased cancer cell death and made cancer cells more sensitive to drugs such as temozolomide (TMZ) and cisplatin. MDS was also found to reduce the ability of cancer cells to divide and grow, which could potentially enhance the effectiveness of chemotherapy.[78] A phase II clinical trial that combined a methionine-free diet with cystemustine in patients with metastatic melanoma and glioma revealed a decline in the activity of *MGMT*, a protein that helps tumors resist chemotherapy, and varied patient responses, including long-term disease stabilization [Table 2].[61] In non-small cell lung cancer (NSCLC) models, MR during early tumor development improved the effectiveness of carboplatin, a chemotherapeutic drug, and altered the tumor environment to make it less favorable for tumor growth. In animal

Table 2
Methionine restriction, the use of methioninase in cancer therapy, and side effect mitigation.

Disease	Formulation of METase	No. of patients/cell lines	Dose in treatment	In conjunction with other chemotherapy drugs	Disease progression monitoring	Reference
Non-skin cancer malignancies	MRD	Nine patients	MRD: extended to 2 weeks following RT from 2 weeks before RT	None	Adverse events of grade ≥3 were not reported. Methionine levels are average at 18.8 μmol/L and average at 16.8 μmol/L. Due to the trial's delayed accrual and challenges sticking to the diet, the trial was closed.	Durando et al.[79]
Metastatic melanoma and recurrent glioma	One-day MET-free diet	22 patients	60 mg/m^3 of cystemustine every 2 weeks; 1-day methionine-free diet	Cystemustine	Median OS: 4.6 months; median TTP: 1.8 months; outcomes: three cases of SD, 19 cases of PD; two long-duration stabilizations (7 and 29 months)	DuCote et al.[80]
Gastrointestinal tract cancers (phase I)	AO-90 (MR TPN lacking MET and CYS)	–	5-FU	None (control TPN had MET)	Significant cancer reduction was noted in the AO-90 group, indicating resected tumors; the effect was nil in the control group.	Lin et al.[81]
Melanoma or glioma (phase II)	MRD	22 individuals (20 with recurrent glioma and two with metastatic melanoma)	60 mg/m^2 of cystemustine for every 2 weeks	Cystemustine	Median disease-free survival: 1.8 months; median OS: 4.6 months; two patients had stabilizations in longer duration; depletion of plasma MET: 40%	
Metastatic solid tumors (phase I)	MR medical food	Eight patients	MRD for 17.3 weeks on average	None	Levels of plasma MET dropped by 58% within 2 weeks (from 21.6 to 9.0 μmol/L); side effect: weight loss of approximately 0.5 kg/ week.	
Metastatic colorectal cancer (feasibility study)	MRD	Four patients	FOLFOX regimen	Leucovorin calcium (folinic acid), fluorouracil, and oxaliplatin	Plasma MET concentration was reduced by 58% on the first day of the MRD; three patients showed partial response and one with SD	

(*continued on next page*)

Table 2 (*continued*)

Disease	Formulation of METase	No. of patients/cell lines	Dose in treatment	In conjunction with other chemotherapy drugs	Disease progression monitoring	Reference
Various human tumor cell lines (*in vitro* study)	rMETase, PEG-rMETase	21 tumor cell lines	rMETase: targeted methionine depletion	Doxorubicin, cisplatin, 5-fluorouracil	rMETase-induced S/G2-phase blockage made cancer cells extremely sensitive to chemotherapy. The S/G2-phase block was found using FUCCI imaging. This approach enhances the effectiveness of chemotherapy.	Lee et al.[82]
Different cell lines from human tumors (lung, colon, kidney, melanoma, CNS, prostate)	rMETase, PEG-rMETase	21 cell lines	Not specified	None	IC50 values, S/G2 phase blockage using FUCCI imaging	Khamisipour et al.[83]
Prostate cancer (cell-lines PC-3, DU-145, and LNCaP)	MR	Three cell lines	Methionine-free medium	None	Growth rates, cell cycle phase distribution, apoptosis, levels of cyclin-dependent kinases	
Non-skin cancer malignancies (pilot study)	MRD	Nine patients	Not specified	Standard-of-care definitive radiation therapy	Dietary adherence, acute and late toxicities grade $\geq$3, and plasma methionine levels	
Metastatic colorectal cancer	MRD	11 patients	Methionine-free diet (3 consecutive days)	FOLFOX6 regimen	Plasma methionine concentration, patient response (PR, SD)	Henry et al.[84]
Metastatic colorectal cancer	MET-free diet + FOLFOX6 regimen	11 patients	Median of three 2-week cycles of MET-free diet and FOLFOX6 regimen	FOLFOX regimen	Plasma MET concentration reduction, feasibility, and patient response evaluation	Kuczynski et al.[85]
Melanoma	MDS	Melanoma cell lines	Not specified	Temozolomide, carmustine, cisplatin, and radiation	Apoptosis, mitotic activity loss, gene expression changes	
Metastatic melanoma and glioma	MET-free diet + cystemustine	Six patients	MET-free diet for four cycles while taking cystemustine (60 mg/m^2)	Cystemustine (nitrosourea)	MGMT activity in PBMCs and reduction of plasma MET levels	Vanneman and Dranoff[10]
KRAS/Lkb1 mutant NSCLC	MR	Mouse model and human cell lines	MR during early tumorigenesis; carboplatin treatment	Carboplatin; combination with immunotherapy	Tumor progression, carboplatin efficacy, TIME, T cell proliferation, *CBS* levels, apoptosis, lineage switching	Cellarier et al.[66]

MR and methioninase demonstrate effectiveness in reducing plasma methionine levels, enhancing chemotherapy sensitivity, and inducing tumor suppression across various cancers. Clinical studies reveal that MR is generally well-tolerated with minimal side effects, such as mild weight loss. In combination with therapies like radiation and chemotherapy (e.g., FOLFOX, cystemustine), MR shows promising outcomes, including tumor stabilization, apoptosis induction, and improved drug efficacy. Preclinical studies support its potential by revealing mechanisms like S/G2-phase cell cycle arrest and immune modulation. 5-FU: 5-Fluorouracil; AO-90: A human breast cancer cell line.; CBS: Cystathionine β-synthase; CNS: Central nervous system; CYS: Cysteine; DU-145: A human prostate cancer cell line; FOLFOX: Folinic acid (leucovorin calcium), fluorouracil, and oxaliplatin; FUCCI: Fluorescent ubiquitination-based cell cycle indicator; IC50: The half-maximal inhibitory concentration; *KRAS/Lkb1*: KRAS is an oncogene, while LKB1 (also known as STK11) is a tumor suppressor gene.; LNCaP: A human prostate cancer cell line; MDS: Methionine deprivation stress; MET: Methionine; METase: Methioninase; MRD: Methionine-restricted diet; MR: Methionine restriction, MGMT: O6-methylguanine-DNA methyltransferase; No.: Number; NSCLC: Non-small cell lung cancer; OS: Overall survival; PBMC: Peripheral blood mononuclear cells.; PC-3: Prostate Cancer-3 PD: Progressive disease; PEG: Polyethylene glycol; RT: Radiotherapy; rMETase: Recombinant methioninase; S/G2: A phase in the cell cycle, representing the transition from the S-phase. G2-phase; SD: Stable disease; TIME: Tumor immune microenvironment; TPN: Total parenteral nutrition; TTP: Time to progression.

models, D-methionine has been tested for its ability to protect against the side effects of cisplatin, a common chemotherapeutic drug. Specifically, it helped reduce appetite loss, weight loss, and kidney damage caused by cisplatin.

Collectively, these studies show that MR or manipulation could potentially improve cancer treatment outcomes by making tumors more responsive to chemotherapy and reducing the side effects associated with treatment. However, further research is required to fully understand the advantages and useful applications of these strategies in clinical settings.

Translational potential

Several studies have investigated the feasibility and safety of MR in humans. A study involving patients with advanced metastatic cancer found that MR could be safely implemented without adverse effects on nutritional status. Another study showed that MR enhanced the effectiveness of 5-FU in individuals with advanced gastric cancer. However, a phase II clinical trial confirmed that while MR was possible, it did not show clinically meaningful effects on survival.[14] These findings suggest that MR has potential as a complementary approach to the treatment of cancer. However, further large-scale clinical trials are necessary to establish the best regimens and efficacy of this approach across different cancer types.

Mechanism of methionine metabolism and signaling in cancer

Methionine metabolism plays pivotal roles in cancer cell survival, proliferation, and immune evasion. Cancer cells often rely heavily on exogenous methionine due to the Hoffman effect, in which cells cannot proliferate when methionine is replaced by Hcy, even if they synthesize methionine internally.[13] Methionine is converted to SAM by *MAT2A* (Methionine Adenosyltransferase 2A), which provides a primary methyl donor for critical methylation processes, affecting global DNA methylation and gene regulation. In cancer, methionine extends to the regulation of the cell cycle and DNA methylation patterns, in which methionine-derived SAM acts as a primary methyl donor. This donation supports methylation reactions essential for maintaining global DNA hypomethylation, paired with the hypermethylation of tumor suppressor genes, favoring tumorigenicity.[86] Altered methylation patterns, often including the hypermethylation of tumor suppressor genes and hypomethylation elsewhere, contribute to tumor progression and tumor cell survival. In addition, methionine scarcity in the cellular environment

activates the SAM checkpoint, arrests cancer cells in the G1 (Gap 1) phase, and inhibits cancer growth.

Another aspect of methionine signaling is its influence on immune checkpoint proteins, where methionine availability affects the methylation of m6A sites on *PD-L1* and *VISTA* messenger ribonucleic acids (mRNAs). Moreover, reduced dietary methionine levels decrease DNA methylation modifications, particularly at key immune-related gene promoters, resulting in higher cytotoxic $CD8^+$ T cell infiltration and enhanced antitumor immunity.[87] MR has also been shown to suppress tumor growth and improve the response to various anticancer therapies.[88] This metabolic dependency of cancer cells on methionine opens new therapeutic avenues that target methionine metabolism and SAM synthesis to curb cancer proliferation and modulate immune responses.

Challenges of methionine restriction in clinical applications

We have shown several studies confirming MR as a promising therapeutic approach in oncology, particularly for gastric cancer, because of its potential to target the unique methionine dependency of certain cancer cells and bypass cancer heterogeneity. However, despite its potential benefits, MR presents several clinical challenges that require further investigation to establish safe and effective protocols. For instance, long-term dietary restriction of methionine may lead to adverse health effects such as bone-related disorders, growth impairment, and hyperhomocysteinemia, which limit the practicality of sustained MR as a standalone treatment. This necessitates careful monitoring and dose adjustments.[89] Conversely, high levels of methionine supplementation, while beneficial in conditions such as hepatic steatosis and insulin resistance, can trigger adverse metabolic responses, including hyperhomocysteinemia, weight loss, and increased cholesterol levels. These side effects illustrate the narrow therapeutic window of methionine intake, where both deficiency and excess pose potential health risks, making it difficult to establish a safe, standardized dose for therapeutic use. In addition, the interaction between methionine and cardiovascular health is crucial for MR therapy. Excessive methionine intake or insufficient levels of B vitamins (B6, B12, riboflavin, and folate) can increase plasma Hcy concentration, elevating the risk of cardiovascular disease (CVD). Consequently, any MR therapy must consider an individual's baseline methionine and vitamin intake to avoid exacerbating Hcy levels, especially in at-risk populations.[90] Thus, although MR shows potential as a novel metabolic therapy for cancer treatment, careful consideration of its clinical applications, including dosage, duration, and patient-specific factors, is essential to maximize therapeutic benefits while minimizing risks.

Therapeutic approaches involving methionine restriction to treat cancer alone or as part of a combination therapy

MR has emerged as a promising approach for cancer treatment, both as a standalone strategy and in combination with established therapies. Research indicates that the dietary restriction of methionine influences cancer outcomes by altering one-carbon metabolism, a pathway critical for various cancer interventions.[91] This alteration enhances the efficacy of apoptosis-inducing chemotherapy and RT; however, its effects on ferroptosis-targeting therapies and immunotherapy remain poorly understood. For instance, studies show that prolonged methionine deprivation can prevent excessive depletion of GSH by inhibiting cation transport regulator homolog 1 (*CHAC1*) protein synthesis, thereby protecting tumor cells from ferroptosis. Conversely, short-term methionine starvation accelerates ferroptosis through *CHAC1* upregulation, suggesting that the duration of methionine deprivation can significantly affect the tumor cell response. Intermittent methionine deprivation appears particularly beneficial, enhancing tumor cell sensitivity to $CD8^+$ T cell-mediated cytotoxicity and synergizing with checkpoint blockade therapies, leading to improved survival outcomes in patients.[92] In a notable clinical case, combination therapy with doxorubicin and cyclophosphamide followed by docetaxel, when paired with MR, achieved a remarkable complete response in a patient with invasive lobular carcinoma (ILC) of the breast, an outcome expected in <10% of patients receiving standard neoadjuvant chemotherapy alone.[93] Additionally, patients treated with o-rMETase, an enzyme that facilitates methionine degradation, combined with a low-methionine diet, showed promising results for long-term disease stabilization in rectal cancer, with significant decreases in circulating methionine levels and improvements in clinical markers. In patients with high-grade gliomas, MR combined with radiation and TMZ also demonstrated high efficacy, underscoring the versatility of MR in various cancer types.[94] Collectively, these findings support the potential use of MR as a therapeutic modality, either alone or in combination with conventional therapies, to enhance treatment efficacy and improve outcomes in patients with cancer. Further clinical studies with larger patient cohorts are necessary to establish the broader applicability of MR and its mechanisms in augmenting the effects of existing cancer therapies.

Future perspectives

The potential use of MR in cancer therapy is expected to expand considerably, as supported by promising preclinical results and emerging clinical evidence. Key areas for further exploration include advancements in precision medicine to identify patients who are most likely to benefit from MR. Future research should aim to identify genetic and metabolic biomarkers that can predict responsiveness to MR, enabling more personalized treatments. This strategy could enhance therapeutic outcomes and reduce unnecessary dietary restrictions for those unlikely to respond.[64] Moreover, the ability of MR to increase the effectiveness of existing cancer treatments also suggests new opportunities for combination therapies. Studies should examine MR alongside immunotherapies, targeted treatments, and emerging modalities, such as chimeric antigen receptor (CAR)-T cell therapy, potentially yielding more effective and less toxic cancer treatment options. Additionally, the development of MR-mimetic drugs or targeted dietary supplements could boost patient adherence and broaden the usability of MR, making it a feasible and sustainable choice for long-term cancer management.[95]

To establish MR as a mainstream cancer treatment, large-scale clinical trials across different cancer types and stages, particularly treatment-resistant forms, are required. Long-term studies are also crucial to evaluate the safety and effectiveness of MR over time. Beyond treatment, MR may also offer preventive benefits to high-risk individuals. Hence, future research should investigate its role in cancer prevention. Moreover, as MR transitions from a preclinical research topic to real-world clinical applications, interdisciplinary collaboration is essential. By addressing these opportunities, MR could become a foundational element in cancer therapy, offering new possibilities for patients with difficult-to-treat cancers.[96]

Conclusions

In conclusion, this review synthesized evidence from a combination of *in vitro* studies, animal studies, and preliminary human data, highlighting the complex role of MR in cancer management. While much of the current data originates from laboratory models, providing valuable mechanistic insights, there are limitations to the extent of human evidence available. Therefore, we aimed to guide future research with these preliminary findings, which suggest that MR may be a viable adjunctive strategy for cancer treatment.

This review focused on the application of MR across different cancer types and explored both its metabolic implications and therapeutic potential when combined with conventional treatments, such as chemotherapy. Specifically, we emphasized the Hoffman effect, the distinct metabolic vulnerability of methionine-addicted cancer cells, and explored promising combinations such as MR with rMETase and standard

chemotherapies. To date, there have been limited comprehensive analyses of the role of methionine in metabolic reprogramming as a targeted approach, and this review aims to bridge this gap by integrating insights from both preclinical and emerging clinical studies.

Although the preliminary findings regarding MR in cancer treatment are promising, this review acknowledges the exploratory nature of the available data. Most of the anticancer effects of MR have been observed *in vitro* and in animal models, with limited but encouraging results from small-scale human studies. Consequently, while MR presents a novel and potentially effective therapeutic regimen, large-scale, rigorously controlled human trials are necessary to establish its definitive anticancer efficacy and safety profile. This review also aims to stimulate further research, emphasizing the potential of MR as an adjunct therapy while underscoring the need for continued exploration to translate these findings into robust clinical applications.

Authors contribution

Shaik Mohammad Noor and Maha Lakshmi Kammili: data analysis, figure preparation, and manuscript writing. Nagaraju Bandaru: data analysis, supervision, review, and editing. Mohan Gandhi Bonthu and Nagaraju Bandaru: supervision, review, and editing. Nagaraju Bandaru and Alluri Pavani Gay: supervision, data discussion, interpretation, critical manuscript revision and editing, project administration, and funding acquisition. Nagaraju Bandaru and Perli Kranti Kumar: review and editing. All the authors have read and approved the final version of the manuscript.

Ethics statement

None.

Declaration of generative AI and AI-assisted technologies in the writing process

We utilized AI and AI-assisted technologies primarily to develop an initial outline of the manuscript's contents and structure. These tools were employed to analyze existing literature, identify key themes, and organize information logically, ensuring a comprehensive and systematic approach to the writing process. However, the actual content development, interpretation, and final drafting were performed by the research team to maintain academic rigor and ensure accuracy. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Funding

None.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

All authors are thankful to the Principal and Management of the College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Andhra Pradesh, India, and the Management of the School of Pharmaceutical Sciences, Sandip University, Nashik, Maharashtra, India for providing the facilities for this work.

Data availability statement

The datasets used in the current study are available from the corresponding author on reasonable request.

References

1. Ferlay J, Colombet M, Soerjomataram I, et al. Cancer statistics for the year 2020: an overview. *Int J Cancer*. 2021;147:1–20. https://doi.org/10.1002/ijc.33588.
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA A Cancer J Clin*. 2023;73:17–48. https://doi.org/10.3322/caac.21763.
3. Sakthivel R, Devi KP. Antioxidant, anti-inflammatory and anticancer potential of natural bioactive compounds from seaweeds. *Stud Nat Prod Chem*. 2019;63:113–160. https://doi.org/10.1016/B978-0-12-817901-7.00005-8.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144:646–674. https://doi.org/10.1016/j.cell.2011.02.013.
5. World Health Organization. *WHO guidelines for the pharmacological and radiotherapeutic management of cancer pain in adults and adolescents*. Geneva: World Health Organization; 2018. Available from: https://www.who.int/publications/i/item/9789241550390. [accessed on 13-12-2024].
6. Anderson MK, Matey L. Overview of cancer and cancer treatment. In: Olsen MM, LeFebvre KB, Brassil KJ, eds. *Chemotherapy and immunotherapy guidelines and recommendations for practice*. Pittsburgh: Oncology Nursing Society; 2019:25–50. https://moodle.riversideonline.com/pluginfile.php/5950/mod_resource/content/1/Chemotherapy%20and%20Immunotherapy%20Guidelines%20ebook.pdf.
7. Van der Jeught K, Xu HC, Li YJ, Lu XB, Ji G. Drug resistance and new therapies in colorectal cancer. *World J Gastroenterol*. 2018;24:3834–3848. https://doi.org/10.3748/wjg.v24.i34.3834.
8. Johnsson A, Zeelenberg I, Min Y, et al. Identification of genes differentially expressed in association with acquired cisplatin resistance. *Br J Cancer*. 2000;83:1047–1054. https://doi.org/10.1054/bjoc.2000.1420.
9. Zhao Y, You H, Liu F, et al. Differentially expressed gene profiles between multidrug resistant gastric adenocarcinoma cells and their parental cells. *Cancer Lett*. 2002;185:211–218. https://doi.org/10.1016/s0304-3835(02)00264-1.
10. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. *Nat Rev Cancer*. 2012;12:237–251. https://doi.org/10.1038/nrc3237.
11. Tashiro Y, Han Q, Tan Y, et al. Oral recombinant methioninase prevents obesity in mice on a high-fat diet. *In Vivo*. 2020;34:489–494. https://doi.org/10.21873/invivo.11799.
12. Sugimura T, Birnbaum SM, Winitz M, Greenstein JP. Quantitative nutritional studies with water-soluble, chemically defined diets. VIII. The forced feeding of diets each lacking in one essential amino acid. *Arch Biochem Biophys*. 1959;81:448–455. https://doi.org/10.1016/0003-9861(59)90225-5.
13. Kaiser P. Methionine dependence of cancer. *Biomolecules*. 2020;10:568. https://doi.org/10.3390/biom10040568.
14. Mecham JO, Rowitch D, Wallace CD, Stern PH, Hoffman RM. The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. *Biochem Biophys Res Commun*. 1983;117:429–434. https://doi.org/10.1016/0006-291x(83)91218-4.
15. Hoffman RM. Clinical studies of methionine-restricted diets for cancer patients. In: Hoffman RM, ed. *Methionine dependence of cancer and aging. Methods in molecular biology*. 1866. New York: Humana Press; 2019:95–105. https://doi.org/10.1007/978-1-4939-8796-2_9.
16. Hoffman RM. Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: a 40-year odyssey. *Expet Opin Biol Ther*. 2015;15:21–31. https://doi.org/10.1517/14712598.2015.963050.
17. Cavuoto P, Fenech MF. A review of methionine dependency and the role of methionine restriction in cancer growth control and life-span extension. *Cancer Treat Rev*. 2012;38:726–736. https://doi.org/10.1016/j.ctrv.2012.01.004.
18. Sanderson SM, Gao X, Dai Z, Locasale JW. Methionine metabolism in health and cancer: a nexus of diet and precision medicine. *Nat Rev Cancer*. 2019;19:625–637. https://doi.org/10.1038/s41568-019-0187-8.
19. Janssen-Heininger YM, Nolin JD, Hoffman SM, et al. Emerging mechanisms of glutathione-dependent chemistry in biology and disease. *J Cell Biochem*. 2013;114:1962–1968. https://doi.org/10.1002/jcb.24551.
20. Chai EZ, Siveen KS, Shanmugam MK, Arfuso F, Sethi G. Analysis of the intricate relationship between chronic inflammation and cancer. *Biochem J*. 2015;468:1–15. https://doi.org/10.1042/BJ20141337.
21. Lu SC. Glutathione synthesis. *Biochim Biophys Acta*. 2013;1830:3143–3153. https://doi.org/10.1016/j.bbagen.2012.09.008.
22. Maddineni S, Nichenametla S, Sinha R, Wilson RP, Richie Jr JP. Methionine restriction affects oxidative stress and glutathione-related redox pathways in the rat. *Exp Biol Med (Maywood)*. 2013;238:392–399. https://doi.org/10.1177/1535370213477988.
23. Richie Jr JP, Leutzinger Y, Parthasarathy S, Malloy V, Orentreich N, Zimmerman JA. Methionine restriction increases blood glutathione and longevity in F344 rats. *Faseb J*. 1994;8:1302–1307. https://doi.org/10.1096/fasebj.8.15.8001743.
24. Tamanna N, Kroeker K, Braun K, Banh S, Treberg JR. The effect of short-term methionine restriction on glutathione synthetic capacity and antioxidant responses at the whole tissue and mitochondrial level in the rat liver. *Exp Gerontol*. 2019;127:110712. https://doi.org/10.1016/j.exger.2019.110712.
25. Loenen WA. S-adenosylmethionine: Jack of all trades and master of everything? *Biochem Soc Trans*. 2006;34:330–333. https://doi.org/10.1042/BST20060330.
26. Casero RA Jr, Murray Stewart T, Pegg AE. Polyamine metabolism and cancer: treatments, challenges and opportunities. *Nat Rev Cancer*. 2018;18:681–695. https://doi.org/10.1038/s41568-018-0050-3.
27. Pegg AE, Casero Jr RA. Current status of the polyamine research field. In: Pegg A, Casero Jr RA, eds. *Polyamines. Methods in molecular biology*. 720. New York: Humana Press; 2011:3–35. https://doi.org/10.1007/978-1-61779-034-8_1.

28. Lin H, Wang M, Zhang YW, et al. Discovery of potent and selective covalent protein arginine methyltransferase 5 (PRMT5) inhibitors. *ACS Med Chem Lett*. 2019;10:1033–1038. https://doi.org/10.1021/acsmedchemlett.9b00074.
29. Lattouf H, Poulard C, Le Romancer M. PRMT5 prognostic value in cancer. *Oncotarget*. 2019;10:3151–3153. https://doi.org/10.18632/oncotarget.26883.
30. Morgan DM. Polyamines. An overview. *Mol Biotechnol*. 1999;11:229–250. https://doi.org/10.1007/BF02788682.
31. Thomas T, Balabhadrapathruni S, Gardner CR, Hong J, Faaland CA, Thomas TJ. Effects of epidermal growth factor on MDA-MB-468 breast cancer cells: alterations in polyamine biosynthesis and the expression of p21/CIP1/WAF1. *J Cell Physiol*. 1999;179:257–266. https://doi.org/10.1002/(SICI)1097-4652(199906)179:3<257::AID-JCP3>3.0.CO;2-4.
32. Battaglia V, DeStefano Shields C, Murray-Stewart T, Casero Jr RA. Polyamine catabolism in carcinogenesis: potential targets for chemotherapy and chemoprevention. *Amino Acids*. 2014;46:511–519. https://doi.org/10.1007/s00726-013-1529-6.
33. Ray RM, Zimmerman BJ, McCormack SA, Patel TB, Johnson LR. Polyamine depletion arrests cell cycle and induces inhibitors p21(Waf1/Cip1), p27(Kip1), and p53 in IEC-6 cells. *Am J Physiol*. 1999;276:C684–C691. https://doi.org/10.1152/ajpcell.1999.276.3.C684.
34. Kulis M, Esteller M. DNA methylation and cancer. *Adv Genet*. 2010;70:27–56. https://doi.org/10.1016/B978-0-12-380866-0.60002-2.
35. Paz MF, Fraga MF, Avila S, et al. A systematic profile of DNA methylation in human cancer cell lines. *Cancer Res*. 2003;63:1114–1121.
36. Landgraf BJ, McCarthy EL, Booker SJ. Radical S-adenosylmethionine enzymes in human health and disease. *Annu Rev Biochem*. 2016;85:485–514. https://doi.org/10.1146/annurev-biochem-060713-035504.
37. Fukushige S, Horii A. DNA methylation in cancer: a gene silencing mechanism and the clinical potential of its biomarkers. *Tohoku J Exp Med*. 2013;229:173–185. https://doi.org/10.1620/tjem.229.173.
38. Parkhitko AA, Jouandin P, Mohr SE, Perrimon N. Methionine metabolism and methyltransferases in the regulation of aging and lifespan extension across species. *Aging Cell*. 2019;18:e13034. https://doi.org/10.1111/acel.13034.
39. Mattocks DA, Mentch SJ, Shneyder J, et al. Short term methionine restriction increases hepatic global DNA methylation in adult but not young male C57BL/6J mice. *Exp Gerontol*. 2017;88:1–8. https://doi.org/10.1016/j.exger.2016.12.003.
40. Inubushi S, Kunihisa T, Mizumoto S, et al. Methionine restriction increases exosome production and secretion in breast cancer cells. *Cancer Genomics Proteomics*. 2023;20:412–416. https://doi.org/10.21873/cgp.20393.
41. Kubota Y, Sasaki M, Han Q, Hozumi C, Tsunoda T, Hoffman RM. Efficacy of recombinant methioninase on late-stage patient cancer in the histoculture drug response assay (HDRA) as a potential functional biomarker of sensitivity to methionine-restriction therapy in the clinic. *Cancer Diagn Progn*. 2024;4:239–243. https://doi.org/10.21873/cdp.10314.
42. Fu YM, Yu ZX, Li YQ, et al. Specific amino acid dependency regulates invasiveness and viability of androgen-independent prostate cancer cells. *Nutr Cancer*. 2003;45:60–73. https://doi.org/10.1207/S15327914NC4501_8.
43. Li T, Tan YT, Chen YX, et al. Methionine deficiency facilitates antitumour immunity by altering m6A methylation of immune checkpoint transcripts. *Gut*. 2023;72:501–511. https://doi.org/10.1136/gutjnl-2022-326928.
44. Hoshiya Y, Kubota T, Inada T, Kitajima M, Hoffman RM. Methionine-depletion modulates the efficacy of 5-fluorouracil in human gastric cancer in nude mice. *Anticancer Res*. 1997;17:4371–4375.
45. Hens JR, Sinha I, Perodin F, et al. Methionine-restricted diet inhibits growth of MCF10AT1-derived mammary tumors by increasing cell cycle inhibitors in athymic nude mice. *BMC Cancer*. 2016;16:349. https://doi.org/10.1186/s12885-016-2367-1.
46. Komninou D, Leutzinger Y, Reddy BS, Richie Jr JP. Methionine restriction inhibits colon carcinogenesis. *Nutr Cancer*. 2006;54:202–208. https://doi.org/10.1207/s15327914nc5402_6.
47. Jeon H, Kim JH, Lee E, et al. Methionine deprivation suppresses triple-negative breast cancer metastasis in vitro and in vivo. *Oncotarget*. 2016;7:67223–67234. https://doi.org/10.18632/oncotarget.11615.
48. Komninou D, Malloy VL, Zimmerman JA, Sinha R, Richie JP. Methionine restriction delays aging-related urogenital diseases in male Fischer 344 rats. *Geroscience*. 2020;42:287–297. https://doi.org/10.1007/s11357-019-00129-4.
49. Mosca L, Pagano M, Pecoraro A, et al. S-adenosyl-l-methionine overcomes uL3-mediated drug resistance in p53 deleted colon cancer cells. *Int J Mol Sci*. 2020;22:103. https://doi.org/10.3390/ijms22010103.
50. Thivat E, Farges MC, Bacin F, et al. Phase II trial of the association of a methionine-free diet with cystemustine therapy in melanoma and glioma. *Anticancer Res*. 2009;29:5235–5240. https://pubmed.ncbi.nlm.nih.gov/20044642/.
51. Epner DE, Morrow S, Wilcox M, Houghton JL. Nutrient intake and nutritional indexes in adults with metastatic cancer on a phase I clinical trial of dietary methionine restriction. *Nutr Cancer*. 2002;42:158–166. https://doi.org/10.1207/S15327914NC422_2.
52. Hoffman RM. Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. *Biochim Biophys Acta*. 1984;738:49–87. https://doi.org/10.1016/0304-419x(84)90019-2.
53. Kubota Y, Han Q, Hamada K, et al. Long-term stable disease in a rectal-cancer patient treated by methionine restriction with oral recombinant methioninase and a low-methionine diet. *Anticancer Res*. 2022;42:3857–3861. https://doi.org/10.21873/anticanres.15877.
54. Ji M, Xu X, Xu Q, et al. Methionine restriction-induced sulfur deficiency impairs antitumour immunity partially through gut microbiota. *Nat Metab*. 2023;5:1526–1543. https://doi.org/10.1038/s42255-023-00854-3.
55. Sinha R, Cooper TK, Rogers CJ, et al. Dietary methionine restriction inhibits prostatic intraepithelial neoplasia in TRAMP mice. *Prostate*. 2014;74:1663–1673. https://doi.org/10.1002/pros.22884.
56. Meyskens Jr FL, Simoneau AR, Gerner EW. Chemoprevention of prostate cancer with the polyamine synthesis inhibitor difluoromethylornithine. *Recent Results Cancer Res*. 2014;202:115–120. https://doi.org/10.1007/978-3-642-45195-9_9.
57. Lu S, Chen GL, Ren C, Kwabi-Addo B, Epner DE. Methionine restriction selectively targets thymidylate synthase in prostate cancer cells. *Biochem Pharmacol*. 2003;66:791–800. https://doi.org/10.1016/s0006-2952(03)00406-4.
58. Ahn JY, Lee JS, Min HY, Lee HY. Acquired resistance to 5-fluorouracil via HSP90/Src-mediated increase in thymidylate synthase expression in colon cancer. *Oncotarget*. 2015;6:32622–32633. https://doi.org/10.18632/oncotarget.5327.
59. Fu YM, Zhang H, Ding M, et al. Selective amino acid restriction targets mitochondria to induce apoptosis of androgen-independent prostate cancer cells. *J Cell Physiol*. 2006;209:522–534. https://doi.org/10.1002/jcp.20766.
60. Hu B, Luo W, Hu RT, Zhou Y, Qin SY, Jiang HX. Meta-analysis of prognostic and clinical significance of CD44v6 in esophageal cancer. *Medicine (Baltimore)*. 2015;94:e1238. https://doi.org/10.1097/MD.0000000000001238.
61. Lu S, Epner DE. Molecular mechanisms of cell cycle block by methionine restriction in human prostate cancer cells. *Nutr Cancer*. 2000;38:123–130. https://doi.org/10.1207/S15327914NC381_17.
62. Strekalova E, Malin D, Good DM, Cryns VL. Methionine deprivation induces a targetable vulnerability in triple-negative breast cancer cells by enhancing TRAIL receptor-2 expression. *Clin Cancer Res*. 2015;21:2780–2791. https://doi.org/10.1158/1078-0432.CCR-14-2792.
63. Jonsson WO, Margolies NS, Anthony TG. Dietary sulfur amino acid restriction and the integrated stress response: mechanistic insights. *Nutrients*. 2019;11:1349. https://doi.org/10.3390/nu11061349.
64. Wanders D, Hobson K, Ji X. Methionine restriction and cancer biology. *Nutrients*. 2020;12:684. https://doi.org/10.3390/nu12030684.
65. Sato M, Han Q, Hozumi C, et al. First-line chemotherapy in combination with oral recombinant methioninase and a low-methionine diet for a stage IV inoperable pancreatic-cancer patient resulted in 40% tumor reduction and an 86% CA19-9 biomarker decrease. *Anticancer Res*. 2024;44:3885–3889. https://doi.org/10.21873/anticanres.17215.
66. Cellarier E, Durando X, Vasson MP, et al. Methionine dependency and cancer treatment. *Cancer Treat Rev*. 2003;29:489–499. https://doi.org/10.1016/s0305-7372(03)00118-x.
67. Hoffman RM. Is the Hoffman effect for methionine overuse analogous to the Warburg effect for glucose overuse in cancer?. In: Hoffman R, ed. *Methionine dependence of cancer and aging. Methods in molecular biology*. 1866. New York: Humana Press; 2019:273–278. https://doi.org/10.1007/978-1-4939-8796-2_21.
68. Kubota Y, Sato T, Han Q, et al. [11C] Methionine-PET imaging as a cancer biomarker for methionine addiction and sensitivity to methionine-restriction-based combination chemotherapy. *In Vivo*. 2024;38:253–258. https://doi.org/10.21873/invivo.13432.
69. Huang L, Xu D, Qian Y, et al. A gene signature is critical for intrahepatic cholangiocarcinoma stem cell self-renewal and chemotherapeutic response. *Stem Cell Res Ther*. 2022;13:292. https://doi.org/10.1186/s13287-022-02988-9.
70. Mizuta K, Kang BM, Han Q, et al. Expression of PD-L1 is increased by methionine restriction using recombinant methioninase in human colorectal cancer cells. *Cancer Genomics Proteomics*. 2024;21:395–398. https://doi.org/10.21873/cgp.20457.
71. Xu Q, Li Y, Gao X, et al. HNF4α regulates sulfur amino acid metabolism and confers sensitivity to methionine restriction in liver cancer. *Nat Commun*. 2020;11:3978. https://doi.org/10.1038/s41467-020-17818-w.
72. Jeon Y, Kim H, Jang ES, et al. Expression profile and prognostic value of glypican-3 in post-operative South Korean hepatocellular carcinoma patients. *APMIS*. 2016;124:208–215. https://doi.org/10.1111/apm.12491.
73. Rajanala SH, Ringquist R, Cryns VL. Methionine restriction activates the integrated stress response in triple-negative breast cancer cells by a GCN2- and PERK-independent mechanism. *Am J Cancer Res*. 2019;9:1766–1775. https://pmc.ncbi.nlm.nih.gov/articles/PMC6726988/.
74. Goseki N, Yamazaki S, Shimojyu K, et al. Synergistic effect of methionine-depleting total parenteral nutrition with 5-fluorouracil on human gastric cancer: a randomized, prospective clinical trial. *Jpn J Cancer Res*. 1995;86:484–489. https://doi.org/10.1111/j.1349-7006.1995.tb03082.x.
75. Thivat E, Durando X, Demidem A, et al. A methionine-free diet associated with nitrosourea treatment down-regulates methylguanine-DNA methyl transferase activity in patients with metastatic cancer. *Anticancer Res*. 2007;27:2779–2783. https://pubmed.ncbi.nlm.nih.gov/17695447/.
76. Halpern BC, Clark BR, Hardy DN, Halpern RM, Smith RA. The effect of replacement of methionine by homocystine on survival of malignant and normal adult mammalian cells in culture. *Proc Natl Acad Sci U S A*. 1974;71:1133–1136. https://doi.org/10.1073/pnas.71.4.1133.
77. Kokkinakis DM, Liu X, Chada S, et al. Modulation of gene expression in human central nervous system tumors under methionine deprivation-induced stress. *Cancer Res*. 2004;64:7513–7525. https://doi.org/10.1158/0008-5472.CAN-04-0592.
78. Mattes MD, Koturbash I, Leung CN, Wen S, Jacobson GM. A phase I trial of a methionine restricted diet with concurrent radiation therapy. *Nutr Cancer*. 2024;76:463–468. https://doi.org/10.1080/01635581.2024.2340784.
79. Durando X, Farges MC, Buc E, et al. Dietary methionine restriction with FOLFOX regimen as first line therapy of metastatic colorectal cancer: a feasibility study. *Oncology*. 2010;78:205–209. https://doi.org/10.1159/000313700.
80. DuCote TJ, Song X, Naughton KJ, et al. EZH2 inhibition promotes tumor immunogenicity in lung squamous cell carcinomas. *Cancer Res Commun*. 2024;4:388–403. https://doi.org/10.1158/2767-9764.CRC-23-0399.

81. Lin MT, Ko JL, Liu TC, Chao PT, Ou CC. Protective effect of d-methionine on body weight loss, anorexia, and nephrotoxicity in cisplatin-induced chronic toxicity in rats. *Integr Cancer Ther*. 2018;17:813–824. https://doi.org/10.1177/1534735417753543.
82. Lee SY, Jeong EK, Ju MK, et al. Induction of metastasis, cancer stem cell phenotype, and oncogenic metabolism in cancer cells by ionizing radiation. *Mol Cancer*. 2017;16:10. https://doi.org/10.1186/s12943-016-0577-4.
83. Khamisipour G, Jadidi-Niaragh F, Jahromi AS, Zandi K, Hojjat-Farsangi M. Mechanisms of tumor cell resistance to the current targeted-therapy agents. *Tumour Biol*. 2016;37:10021–10039. https://doi.org/10.1007/s13277-016-5059-1.
84. Henry DH, Viswanathan HN, Elkin EP, Traina S, Wade S, Cella D. Symptoms and treatment burden associated with cancer treatment: results from a cross-sectional national survey in the U.S. *Support Care Cancer*. 2008;16:791–801. https://doi.org/10.1007/s00520-007-0380-2.
85. Kuczynski EA, Sargent DJ, Grothey A, Kerbel RS. Drug rechallenge and treatment beyond progression—implications for drug resistance. *Nat Rev Clin Oncol*. 2013;10:571–587. https://doi.org/10.1038/nrclinonc.2013.158.
86. Lauinger L, Kaiser P. Sensing and signaling of methionine metabolism. *Metabolites*. 2021;11:83. https://doi.org/10.3390/metabo11020083.
87. Pandit M, Kil YS, Ahn JH, et al. Methionine consumption by cancer cells drives a progressive upregulation of PD-1 expression in CD4 T cells. *Nat Commun*. 2023;14:2593. https://doi.org/10.1038/s41467-023-03943-w.
88. Ji M, Xu Q, Li X. Dietary methionine restriction in cancer development and antitumor immunity. *Trends Endocrinol Metabol*. 2024;35:400–412. https://doi.org/10.1016/j.tem.2024.01.009.
89. Navik U, Sheth VG, Khurana A, et al. Methionine as a double-edged sword in health and disease: current perspective and future challenges. *Ageing Res Rev*. 2021;72:101500. https://doi.org/10.1016/j.arr.2021.101500.
90. Ligthart-Melis GC, Engelen MPKJ, Simbo SY, et al. Metabolic consequences of supplemented methionine in a clinical context. *J Nutr*. 2020;150:2538S–2547S. https://doi.org/10.1093/jn/nxaa254.
91. Gao X, Sanderson SM, Dai Z, et al. Dietary methionine influences therapy in mouse cancer models and alters human metabolism. *Nature*. 2019;572:397–401. https://doi.org/10.1038/s41586-019-1437-3.
92. Xue Y, Lu F, Chang Z, et al. Intermittent dietary methionine deprivation facilitates tumoral ferroptosis and synergizes with checkpoint blockade. *Nat Commun*. 2023;14:4758. https://doi.org/10.1038/s41467-023-40518-0.
93. Kubota Y, Han Q, Masaki N, et al. Elimination of axillary-lymph-node metastases in a patient with invasive lobular breast cancer treated by first-line neo-adjuvant chemotherapy combined with methionine restriction. *Anticancer Res*. 2022;42:5819–5823. https://doi.org/10.21873/anticanres.16089.
94. Han Q, Tan Y, Hoffman RM. Oral dosing of recombinant methioninase is associated with a 70% drop in PSA in a patient with bone-metastatic prostate cancer and 50% reduction in circulating methionine in a high-stage ovarian cancer patient. *Anticancer Res*. 2020;40:2813–2819. https://doi.org/10.21873/anticanres.14254.
95. Orgeron ML, Stone KP, Wanders D, Cortez CC, Van NT, Gettys TW. The impact of dietary methionine restriction on biomarkers of metabolic health. *Prog Mol Biol Transl Sci*. 2014;121:351–376. https://doi.org/10.1016/B978-0-12-800101-1.00011-9.
96. Wanders D, Forney LA, Stone KP, Hasek BE, Johnson WD, Gettys TW. The components of age-dependent effects of dietary methionine restriction on energy balance in rats. *Obesity (Silver Spring)*. 2018;26:740–746. https://doi.org/10.1002/oby.22146.