CHAPTER. 1
INTRODUCTION

Cancer is an intricate medical condition that contributes significantly to morbidity and mortality globally. It is defined by unregulated proliferation of cells as well as the capacity to attack and spread to distant areas. In developed countries, one in three individuals receives a cancer diagnosis during their lifetime (Bhat *et al*., 2024). Although it predominantly affects the elderly, advancements in medical science and public health have also increased the susceptibility of children to cancer.

Chemotherapy, radiation therapy, and surgery are the main methods used to treat cancer (Riaz *et al*., 2023). Treatment selection depends on patient-specific characteristics, tumor kind, and stage. Chemotherapy, either as monotherapy or combined with other modalities, remains a cornerstone despite its challenges. Unlike bacterial infections, treating cancer is complicated due to the biochemical similarities between normal and cancerous human cells, making selective targeting difficult (Anand *et al*., 2023).

In recent years, emerging therapies such as immunotherapy and targeted molecular treatments have begun to complement traditional approaches, offering improved specificity and reduced systemic toxicity. These advancements focus on harnessing the immune system or inhibiting specific oncogenic pathways, enhancing the efficacy of treatment while minimizing damage to normal tissues (Bai *et al*., 2023).

1.1 Global Burden of Cancer

Cancer continues to be a major global health challenge, responsible for nearly 10 million deaths in 2020 and approximately 9.7 million cancer-related deaths in 2022, according to the World Health Organization and GLOBOCAN estimates. The rising burden of cancer is driven by factors such as population aging, unhealthy lifestyles, environmental exposures, and disparities in access to early detection and treatment, particularly in low- and middle-income countries, where over 70% of cancer deaths occur. The most commonly diagnosed cancers globally include breast, lung, colorectal, and prostate cancers, with breast cancer now being the most frequently diagnosed, especially among women in both developed and developing regions. Lung cancer remains the leading cause of cancer-related mortality, followed by colorectal, liver, stomach, and breast cancers (Sung *et al.*, 2021;, Filho *et al.*, 2024; Cao *et al.*,

2024; Begum *et al.*, 2025).In men, prostate and colorectal cancers are more prevalent, while cervical cancer continues to be a significant cause of mortality among women in resource-limited settings (Sung *et al.*, 2021).

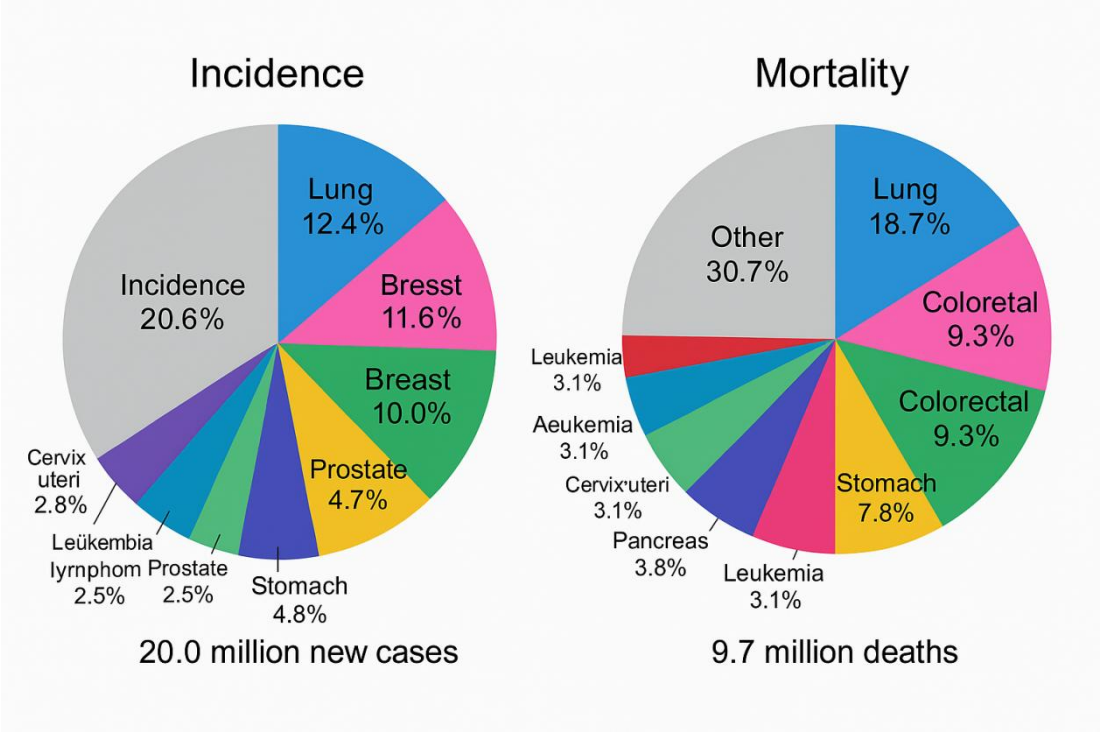

Figure 1.1. Global distribution of new cancer cases and cancer-related deaths by type (Sung *et al.*, 2021)

1.1.1 L-Methionase Impact on Global Burden of Cancer

L-methionase (methionine γ-lyase) is a promising anticancer enzyme that targets methionine-dependent tumor cells by depleting extracellular methionine. This induces selective cancer cell death while sparing normal cells. Its tumor-specific action, broad-spectrum efficacy, and low toxicity make it a strong candidate for reducing the global cancer burden. Recent advances in recombinant production and PEGylation have improved its stability and reduced immunogenicity, increasing its clinical potential. L-methionase may also enhance the effectiveness of chemotherapy and immunotherapy, especially in resource-limited settings (Qoura, Pokrovsky, & Balakin, 2024).

Table 1.1 Methionine Dependency and Therapeutic Potential of L-Methionase Across Various Cancer Types.

Cancer type	Methionine Dependency	L-Methionase Potential Impact
Breast Cancer	High	Inhibits proliferation and enhances sensitivity to chemotherapy
Lung Cancer	High	Induces cell cycle arrest and apoptosis
Colon Cancer	Moderate to High	Reduces tumor growth and improves efficacy of conventional therapies
Prostate Cancer	Moderate	Slows tumor progression and reduces methionine supply
Liver Cancer	High	Enhances therapeutic response, especially in combination treatments
Brain Cancer	High (e.g., glioblastoma)	Targets methionine-addicted gliomas; impairs methylation processes

1.2 Pathogenesis of Cancer

Understanding cancer's biological behavior is essential for evaluating the effectiveness and limitations of current anticancer drugs and identifying potential challenges for future therapies (Ahmed *et al*., 2023).

1.2.1 Uncontrolled Proliferation

Cancer cells develop a capability to multiply constantly, whereas normal cells have a limited capacity for division. Some tumors, such as plasma cell neoplasms, grow slowly, whereas others like Burkitt's lymphoma, exhibit rapid proliferation (Tiwari, 2024). Cancer cells bypass regulatory mechanisms of cell division, primarily due to:

- Inactivation of tumor suppressor genes
- Conversion of proto-oncogenes to oncogenesDisruption of growth factors, cell-cycle regulators, apoptotic pathways, and angiogenesis (Dakal *et al*., 2024)

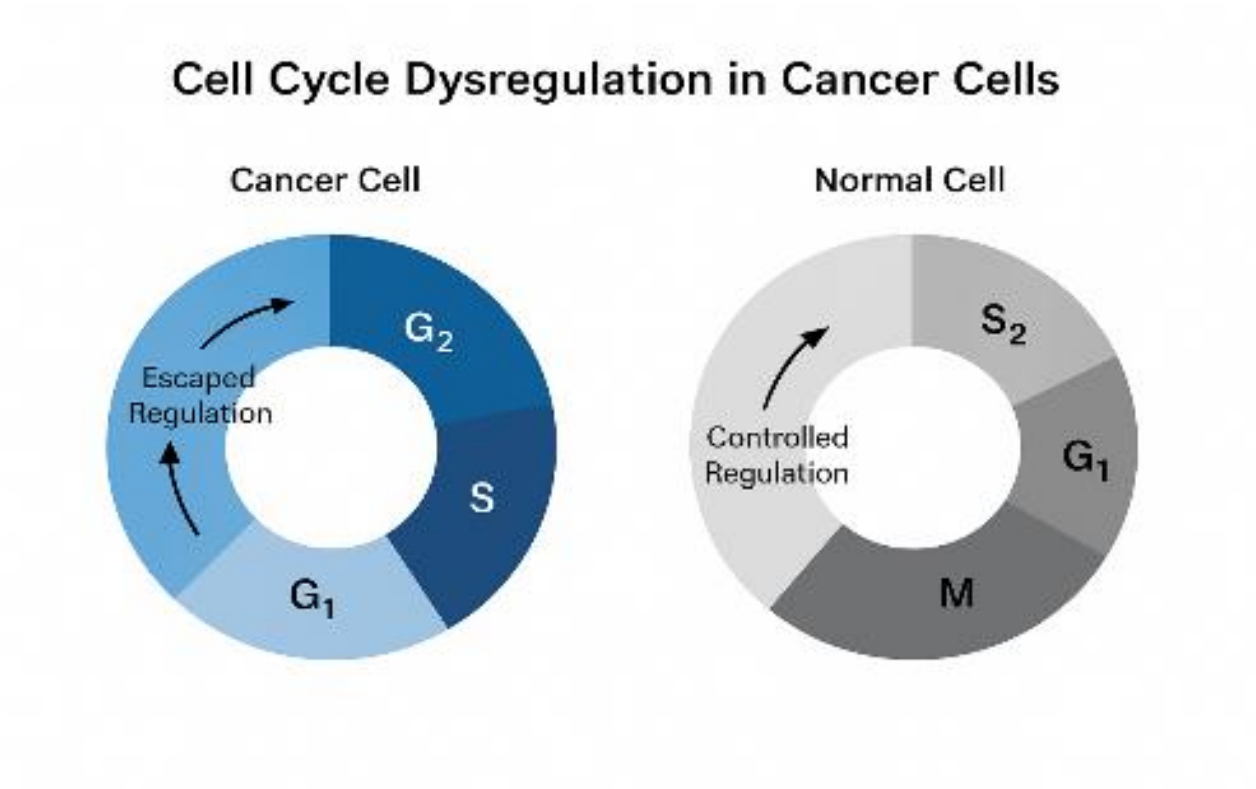

Figure 1.2 Cell Cycle Dysregulation in Cancer Cells (Hanahan & Weinberg, 2011; Vermeulen *et al*., 2003)

1.2.2 Differentiation and Loss of Function

Stem cells differentiate into specialized cells as part of normal tissue development. The degree of differentiation in cancer varies and is correlated with prognosis; tumors that are poorly differentiated multiply more quickly and have a worse prognosis compared with those which is well-differentiated (Al-Ostoot *et al*., 2024).

1.2.3 Invasiveness

Normal cells remain confined to their tissue of origin, regulated by survival factors and adhesion molecules. Cancer cells lose these restrictions, acquiring mutations and secreting enzymes like metalloproteinases that degrade the extracellular matrix, enabling tissue invasion (Al Hussein Al Awamlh & Chang, 2023).

1.2.4 Metastasis

The most common cause of cancer-related mortality is still metastasis, which is the development of additional tumors from the beginning tumor cells. Genetic and epigenetic alterations allow cancer cells to detach, migrate, and establish tumors in distant organs. Tumor-induced angiogenesis further supports metastasis (Mei *et al*., 2023).

Table 1.2 Differences Between Normal and Cancer Cells

Characteristics	Normal Cells	Cancer Cells
Cell division	Regulated and limited	Uncontrolled and continuous
Cell differentiation	Well-differentiated and specialized	Poorly differentiated or undifferentiated
Tissue invasion	Remain within tissues boundaries	Invade surrounding tissues and organs

1.3 Drugs Used in Cancer Chemotherapy

Chemotherapy remains one of the most widely used systemic treatments for cancer. It involves the use of cytotoxic agents to destroy rapidly dividing cells, a hallmark of malignant tumors.

Table 1.3 Classification of Chemotherapeutic Agents with Mechanisms

Class	Example Drug	Mechanism of Action	Reference
Cytotoxic Drugs	Cyclophosphamide	Alkylates DNA, causing cross-linking and inhibition of replication	(Hussein et al., 2024)
Antimetabolites	Methotrexate	Inhibits folate pathway, blocking DNA and RNA synthesis	(Anand et al., 2023)
Cytotoxic Antibiotics	Doxorubicin	Intercalates into DNA and inhibits topoisomerase II	(Braatz et al., 2022)
Plant Alkaloids	Vincristine	Disrupts microtubule formation, arresting mitosis	(Riaz et al., 2023)
Hormonal Agents	Tamoxifen	Binds estrogen receptors, blocking hormone-driven tumor growth	(Muneer et al., 2020)
Targeted Therapies	Imatinib	Inhibits BCR-ABL tyrosine kinase in chronic myeloid leukemia	(Rudd, 2023)

Despite its effectiveness, chemotherapy often affects normal proliferating cells such as those in the bone marrow, gastrointestinal tract, and hair follicles, leading to significant side effects.

Over the years, chemotherapeutic drugs have been classified based on their mechanisms of action, targeting specific stages of the cell cycle or interfering with essential cellular functions. Understanding these drug categories is crucial for designing appropriate treatment regimens and minimizing toxicity.

1.4 Enzyme Therapy of Cancer

Enzyme therapy is an evolving and targeted approach in cancer treatment that exploits the catalytic efficiency and specificity of enzymes to disrupt metabolic pathways essential for cancer cell survival. Unlike conventional chemotherapeutic drugs, which often harm healthy proliferating cells, therapeutic enzymes can act with high selectivity, reducing systemic toxicity and improving patient outcomes. A prominent and clinically validated example is *L*-asparaginase, which is widely used in treating acute lymphoblastic leukemia (ALL). It works by depleting circulating asparagine, an amino acid that leukemic cells are unable to synthesize sufficiently, thus inducing selective cytotoxicity (Wang *et al.*, 2021). Similarly, arginine deiminase has been explored in arginine-dependent tumors such as hepatocellular carcinoma and melanoma, demonstrating promising anticancer effects in preclinical and clinical studies (Pokrovsky *et al.*, 2022; Tsai *et al.*, 2015; Ni *et al.*, 2008; Fernandes *et al.*, 2017).

L-Methionase (methionine γ-lyase), an enzyme that degrades methionine into α-ketobutyrate, ammonia, and methanethiol. This enzyme targets the unique metabolic defect known as methionine dependency, which is common across various cancer types. Many malignant cells rely heavily on external methionine for proliferation, while normal cells can synthesize it via the methionine salvage pathway. By depleting methionine, L-methionase selectively inhibits tumor growth, arrests the cell cycle, and enhances the sensitivity of cancer cells to chemotherapeutic and radiotherapeutic agents (Qoura, Pokrovsky, & Balakin, 2024).

Advances in recombinant technology have allowed for the large-scale production and PEGylation of L-methionase, which significantly improves its pharmacokinetics and reduces immune recognition. As a result, L-methionase is currently under investigation in preclinical and early clinical studies as a promising adjunct to conventional therapies, especially in cancers like breast, colon, lung, and glioblastoma.

In summary, enzyme-based therapies, particularly those exploiting amino acid dependencies such as L-methionase, represent a promising avenue for the development of safer and more effective cancer treatments, paving the way for more personalized and precision-driven oncological care.

1.5 L-Methionase as an Anticancer Enzyme

L-methionase, also known as methionine γ-lyase (MGL), is a pyridoxal 5′-phosphate (PLP)-dependent catalytic enzyme the breakdown of L-methionine into α-ketobutyrate, ammonia, and methanethiol. It is absent in mammalian cells but naturally found in bacteria and fungi, making it an attractive agent for selective cancer therapy (Sharma, Singh, & Kanwar, 2014).

1.5.1 Mechanism of L-Methionase in Cancer Therapy

The pyridoxal 5′-phosphate (PLP)-dependent enzyme *L*-methionase, also known as methioninase, catalyzes the α,γ-elimination process of *L*-methionine, which produces α-ketobutyrate, methanethiol, and ammonia. The therapeutic potential of *L*-methionase lies in its ability to deplete both extracellular and intracellular methionine pools, creating a state of methionine starvation that selectively targets cancer cells without harming normal tissues (Javia *et al.*, 2024, Li *et al.*, 2020 , Bes *et al.*, 2015; Machover *et al.*, 2019), and ; Kolasani *et al.*, 2014).

Cancer cells exhibit a heightened dependence on methionine due to their increased methylation needs for DNA, RNA, and protein synthesis, essential for their rapid growth and survival (Hoffman & Yano, 2019).

In contrast, normal cells possess adaptive mechanisms to survive under low methionine conditions, utilizing homocysteine to regenerate methionine through the enzyme methionine synthase. This distinct metabolic vulnerability of tumor cells provides a strategic window for selective therapeutic intervention.

Key Mechanisms Involved:

- **Tumor-Specific Methionine Dependence:** Cancer cells demand higher methionine levels compared to normal cells to sustain excessive methylation reactions necessary for oncogenic transformation and proliferation (Hoffman & Yano, 2019).

- **Epigenetic Impact:** Methionine depletion disrupts the methyl group supply for epigenetic modifications. This alteration can reverse aberrant hypermethylation patterns, leading to the reactivation of genes that inhibit tumors and suppression of oncogenic pathways (Xia *et al*., 2020).
- **Cell Cycle Arrest:** When methionine level decrease, cancer cells are unable to progress through the cell cycle efficiently, resulting in arrest predominantly at the G2 phase. This makes them more susceptible to damage by S-phase and mitosis-specific chemotherapeutic agents (Shin Ly & Kunnath, 2021).
- **Sensitization to Chemotherapy:** Methionine starvation impairs DNA repair, stress response mechanisms, and redox balance in cancer cells, thereby increasing their vulnerability to conventional chemotherapy and radiation therapies.

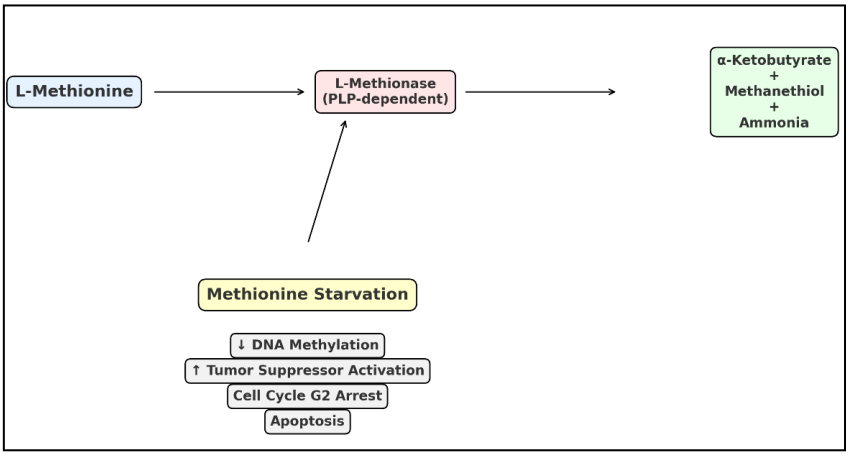

Figure 1.3 Mode of Action of L-Methionase Anti-Cancer enzyme

1.6 Advantages and Challenges of L-Methionase Therapy

1.6.1 Advantages

- **Tumor Selectivity:**

  L-methionase specifically targets methionine-dependent cancer cells, sparing normal cells that can Convert homocysteine into methionine. (Sharma, Singh, & Kanwar, 2014).

- **Broad-Spectrum Efficacy:**

  L-methionase exhibits anticancer activity against a variety of tumor types, including lung, colon, melanoma, kidney, and brain cancers (Tan, Xu, & Hoffman, 2010).

- **Low Toxicity:**

  Preclinical studies have shown minimal side effects and toxicity toward normal tissues, suggesting A good safety profile (Javia *et al*., 2024).

- **Potential for Universal Cancer Therapy:**

  Given that methionine dependence is a general metabolic defect across various cancer types, L-methionase holds promise as a broad-spectrum therapeutic or an adjunct to conventional chemotherapies (Qoura *et al*., 2024).

1.6.2 Challenges

- **Immune Response:**

  As L-methionase is of bacterial origin, its use may elicit an immune response, leading to reduced efficacy after repeated administrations (Qoura *et al*., 2024).

- **Delivery Systems:**

  Achieving efficient and targeted delivery of L-methionase remains challenging. Current strategies under investigation include PEGylation, nanoparticle delivery, and erythrocyte encapsulation (Bes, Bourgeaux, & Godfrin, 2015).

- **Incomplete Serum Methionine Depletion:**

  Some tumor cells can survive because It's challenging to entirely depletion serum methionine, even with restricted diets and enzyme therapy (Gay, Bes, & Godfrin, 2015).

Table 1.4 Advantages vs Challenges of L-Methionase Therapy

Advantages	Challenges
High tumor selectivity	Risk of immune response
Broad applicability across cancers	Need for advanced delivery methods
Low toxicity to normal cells	Incomplete serum methionine depletion
Potential universal cancer therapy	Repeated administration limitations

1.7. Clinical Status and Future Developments

Recent advances in preclinical and translational research have established a solid foundation for the clinical development of L-methionase-based cancer therapies. Significant Improvements have been made in areas such as enzyme production, formulation optimization, and strategic planning for clinical trials.

- **Recombinant L-methionase (rMETase) Production and PEGylation**:

  Recombinant methioninase (rMETase) may now be produced on a huge scale because to the effective cloning and amplification of L-methionase in Escherichia coli. To enhance its therapeutic potential, PEGylation techniques have been employed, which significantly improve the pharmacokinetic profile of rMETase by prolonging its serum half-life and reducing immunogenic responses (Tan *et al*., 1997).

- **Preparation for Early-Phase Clinical Trials**:

  The initial steps toward clinical application have commenced, with preparations underway for Phase I clinical trials. These trials are designed to assess the pharmacokinetics, safety, and initial effectiveness of L-methionase, particularly when administered in combination with standard chemotherapy or radiotherapy regimens (Hoffman, 2015).

- **Innovative Delivery Systems**:

  To overcome challenges associated with immune recognition and to enhance targeted delivery, biotechnology firms are actively developing novel delivery platforms. Strategies such as nanoparticle encapsulation and erythrocyte-encapsulated L-methionase aim to protect the enzyme from immune surveillance and enable sustained release at tumor sites (Gay, Bes, & Godfrin, 2015).

- **Development of Oral Formulations**:
  Promising advances have also been made toward the development of oral formulations of recombinant methioninase. Oral delivery offers a more patient-friendly administration route and could facilitate the integration of L-methionase into broader oncological treatment protocols (Pokrovsky *et al*., 2023).

1.8 Comparison with Other Enzyme Therapies

Enzyme-based therapies have emerged as innovative approaches for selectively targeting metabolic dependencies unique to cancer cells.

Several enzymes have been developed or investigated to exploit specific amino acid dependencies in tumors, demonstrating varying degrees of clinical success. A comparison of key enzyme therapies is summarized in (Table 1.5)

Table 1.5 Comparison of Various Enzymes

Therapy	Target	Mechanism	Cancer Types	Status
L-Asparaginase	Asparagine	Hydrolyzes and depletes asparagine	Acute Lymphoblastic Leukemia (ALL)	Approved
Arginine Deiminase	Arginine	Depletes extracellular arginine	Melanoma, Hepatocellular Carcinoma (HCC)	Investigational
L-Methionase	Methionine	Catalyzes breakdown of methionine	Broad (lung, colon, melanoma, brain)	Preclinical/Early Clinical
L-Glutaminase	Glutamine	Depletes extracellular glutamine	Pancreatic, Colorectal, Lung Cancers	Investigational

1.9 Market Value of Anticancer Enzymes

The global anticancer enzyme market is growing rapidly due to rising cancer rates and a shift toward precision medicine. Enzymes like L-asparaginase, arginase, and ribonuclease are gaining prominence for their tumor-targeting capabilities.

L-asparaginase dominates the market, especially in leukemia treatment. North America holds the largest market share, followed by Europe and Asia-Pacific. Increased R&D and biotech investments are further boosting market expansion.

Table 1.6 Market value of various anti-cancer enzymes

Enzyme	Primary Application	Market Size (USD Million)	CAGR (%)	Forecast Year	References
L-asparaginase	Acute lymphoblastic leukemia	$1,575.62	16.1	2028	Bhatt, R., Sharma, R., & Chauhan, M. (2021)
Arginine deiminase	Hepatocellular carcinoma, melanoma	~$300	~9.5	2028	Rehman, T., Fatima, S., & Qureshi, M. A. (2021)
L-methionase	Breast, lung, colon cancers	~$500	~12.4	2030	Qoura, F., Pokrovsky, V. S., & Balakin, K. V. (2024)

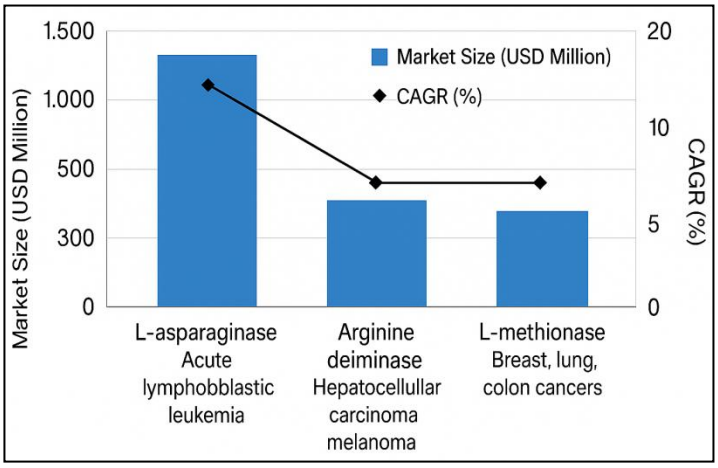

Figure 1.4. The estimated market size (in USD million) and compound annual growth rate (CAGR %) for three major anticancer enzymes L-asparaginase, Arginine deiminase, and L-methionase (Yadav *et al.*, 2020)

1.10 Current Status of L-Methionase

L-methionase is currently undergoing extensive experimental research, with strong potential as a novel anticancer agent. Preclinical studies have demonstrated its efficacy in inhibiting tumor growth by selectively depleting extracellular and intracellular methionine levels, exploiting the methionine dependency exhibited by many cancer cell types (Al-Zahrani & Bukhari, 2019).

The enzyme catalyzes the conversion of methionine into toxic byproducts, such as α-ketobutyrate, methanethiol, and ammonia, thereby impairing cancer cell proliferation and enhancing their sensitivity to other therapeutic interventions. Encouraged by promising preclinical outcomes, preparations for early-phase There are ongoing clinical trials, with researchers and pharmaceutical companies actively exploring its clinical translation.

Nevertheless, number of challenges remain before L-methionase able can be completely integrated into standard oncological treatment regimens. These include the optimization of delivery systems to ensure tumor-specific targeting, the minimization of potential immunogenic responses, and the establishment of consistent therapeutic efficacy across diverse patient populations. Continued research efforts are essential to validate the enzyme's safety profile, determine ideal dosage approaches, and refine combination protocols with existing chemotherapeutic and immunotherapeutic agents.

1.11 Rational Study of L-Methionase

The development of L-methionase as a potential anticancer agent is being pursued through a comprehensive, multidisciplinary research strategy that spans from fundamental biochemical investigations to clinical application. Current research efforts are focused on elucidating the detailed mode of action of the enzyme, optimizing its therapeutic efficacy, and refining its delivery systems.

Preclinical studies have been instrumental in evaluating the efficacy, safety, and pharmacokinetic properties of L-methionase across a variety of cancer cell lines and animal models. These studies provide critical insights into its tumor-selective activity and help define optimal dosing strategies.

As L-methionase advances toward clinical implementation, early-phase clinical trials are being designed to determine their safety and therapeutic potential in human patients. These trials typically progress through Phase I (protection and dosage), Phase II (effectiveness and negative effects), and Phase III (comparison to standard treatments) to ensure rigorous validation.

A key focus of current research also lies in combination therapy approaches, where L-methionase is evaluated alongside chemotherapeutic agents or immunotherapies to target multiple aspects of tumor metabolism and resistance mechanisms simultaneously.

In parallel, biochemical characterization of the enzyme supports efforts to improve its large-scale production, formulation stability, and targeted delivery. Innovative delivery methods, including nanoparticle encapsulation and PEGylation, are under exploration to enhance bioavailability and reduce immunogenicity.

Moreover, patient stratification based on molecular biomarkers or genetic profiles associated with methionine dependency is being integrated into study designs. This personalized approach aims to identify patients that will be most likely to gain from L-methionase therapy, thereby maximizing clinical outcomes and minimizing unnecessary exposure.

Through this structured and rational framework, L-methionase continues to emerge as a promising candidate in the evolving landscape of targeted cancer therapies.

RESEARCH OBJECTIVES

1. Isolation and Screening of L- Methionase producing fungi from Soil.
2. Morphological and Molecular identification of L-Methionase enzyme.
3. Optimization of various production parameters to maximize the L-Methionase production
4. Purification and biochemical characterization of L-Methionase
5. Evaluation of *in vitro* anticancer activity of L-Methionase.