

**DEVELOPMENT OF CHITOSAN NANOCARRIER FOR
NATURAL PRODUCT TARGETED ANTICANCER DRUG
DELIVERY**

LILA VIDYA VINOTHINI A/P MURALITHARAN

SCHOOL OF HEALTH SCIENCES

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NATURAL PRODUCT TARGETED ANTICANCER DRUG
DELIVERY**

by

LILA VIDYA VINOTHINI A/P MURALITHARAN

**Dissertation submitted in partial fulfilment of the
requirements for the degree of Bachelor of Biomedical
Science (Honours)
(Biomedicine)**

January 2026

CERTIFICATE

This is to certify that the dissertation entitled “Development of Chitosan Nanocarrier for Natural Product Targeted Anticancer Drug” is the bona fide record of research work done by Ms. Lila Vidya Vinothini A/P Muralitharan during the period from August 2025 to January 2026 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Health Science (Honours) (Biomedicine).

Main supervisor,

.....
Dr. Yusmazura Zakaria
Biomedicine Lecturer
School of Health
Sciences University
Sains Malaysia Health
Campus 16150 Kubang
Kerian Kelantan,
Malaysia

Date: 16 JANUARY 2026

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

.....
(LILA VIDYA
VINOTHINI A/P
MURALITHARAN)

Date: 14 JANUARY 2026

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Degree Celsius
%	Percentage
±	Plus–minus (measure of variability)
×	Multiplication sign
≤	Less than or equal to
≥	Greater than or equal to
µg/mL	Microgram per millilitre
µL	Microlitre
ANOVA	Analysis of variance
ATR	Attenuated Total Reflectance
cm ⁻¹	Reciprocal centimetre (wavenumber)
CO ₂	Carbon Dioxide
CP	Cisplatin
CSNPs	Chitosan-Based Nanocarriers
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
EDTA	Ethylenediaminetetraacetic Acid
EE%	Encapsulation Efficiency Percentage
ELISA	Enzyme-Linked Immunosorbent Assay
FA	5-Fluorouracil
FBS	Fetal Bovine Serum
FITC	Fluorescein Isothiocyanate
FTIR	Fourier Transform Infrared Spectroscopy
h	Hour
hFOB	Human Fetal Osteoblast
HPV	Human Papillomavirus
IC ₅₀	Half Maximal Inhibitory Concentration
mL	Millilitre
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
<i>n</i>	Sample size
p	p-value

PBS	Phosphate Buffered Saline
pH	Potential of hydrogen
pKa	Negative logarithm of the acid dissociation constant
RPMI	Roswell Park Memorial Institute Medium
RF	Retention Factor
ROS	Reactive oxygen species
rpm	Revolutions per minute
SD	Standard Deviation
SEM	Scanning Electron Microscope /Standard error of mean
SeNPs	Selenium Nanocarriers
SF1	Standardized Anticancer Fraction 1
TLC	Thin Layer Chromatography
TPP	Sodium Tripolyphosphate
UIAM	Universiti Islam Antarabangsa Malaysia
WHO	World Health Organization

PENGHASILAN NANOPEMBAWA KITOSAN UNTUK PENGHANTARAN UBAT ANTIKANSER BERSASAR BAGI PRODUK SEMULA JADI

ABSTRAK

Kanser kekal sebagai cabaran kesihatan utama di peringkat global, dan rawatan konvensional seperti kemoterapi sering dikaitkan dengan ketoksikan yang tinggi serta sasaran khusus yang terhad. Produk semula jadi, termasuk *Clinacanthus nutans*, telah menunjukkan potensi antikanser. Walau bagaimanapun, aplikasi terapeutiknya dibatasi oleh kebolehlarutan dan kestabilan yang rendah. Kajian ini bertujuan untuk membangunkan sistem nanopembawa berasaskan kitosan bagi penghantaran fraksi antikanser piawai (SF1) yang diasingkan daripada daun *C. nutans*. SF1 telah diekstrak, dipencilkan dan ditulenkan menggunakan teknik kromatografi sebelum dienkapsulasi ke dalam nanopembawa kitosan melalui kaedah gelasi ionik menggunakan natrium tripolifosfat sebagai agen penghubung silang. Pencirian fizikokimia nanopartikel telah dijalankan menggunakan mikroskopi elektron imbasan (SEM) dan spektroskopi inframerah transformasi Fourier (FTIR). Aktiviti ketoksikan in vitro bagi nanopembawa kitosan dan nanopembawa kitosan bermuatan SF1 telah dinilai menggunakan ujian MTT terhadap titisan sel kanser manusia SiHa dan MCF-7, dengan titisan sel osteoblast, hFOB digunakan sebagai kawalan sel normal. Keputusan kajian mengesahkan kejayaan enkapsulasi SF1 ke dalam nanopartikel kitosan, yang mempamerkan ciri morfologi yang sesuai serta bukti interaksi kimia. Ujian ketoksikan menunjukkan bahawa kedua-dua nanopembawa kitosan dan nanopembawa kitosan bermuatan SF1 menghasilkan kesan ketoksikan tidak selektif terhadap titisan sel kanser dan sel normal yang diuji. Nilai julat IC_{50} adalah antara $1.53 \pm 0.06 \mu\text{g/mL}$ hingga ke $5.44 \pm 0.02 \mu\text{g/mL}$. Walau bagaimanapun,

keputusan ini merupakan pemerhatian awal secara in vitro, dan kajian lanjut diperlukan untuk menjelaskan selektiviti, mekanisme tindakan, serta potensi terapeutik keseluruhan sistem nanopembawa yang dibangunkan. Walaupun kajian ini belum mencapai pemilihan sel yang spesifik, penemuan ini menyediakan asas bagi pengoptimuman sistem nanopembawa bersasar pada masa hadapan, serta membimbing reka bentuk strategi untuk meningkatkan spesifisiti dan keberkesanan terapeutik.

DEVELOPMENT OF CHITOSAN NANOCARRIER FOR NATURAL PRODUCT TARGETED ANTICANCER DRUG DELIVERY

ABSTRACT

Cancer remains a major global health challenge, and conventional treatments such as chemotherapy are often associated with high toxicity and limited target specificity. Natural products, including *Clinacanthus nutans*, have shown anticancer potential. However, their therapeutic application is restricted by poor solubility and stability. This study aimed to develop a chitosan-based nanocarrier system for the delivery of a standardized anticancer fraction (SF1) isolated from *C. nutans* leaves. SF1 was extracted, isolated, and purified using chromatographic techniques before being encapsulated into chitosan nanocarriers via the ionic gelation method using sodium tripolyphosphate as a crosslinking agent. Physicochemical characterization of the nanocarriers was carried out using scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). The *in vitro* cytotoxicity of chitosan nanocarrier and SF1-loaded chitosan nanocarrier was evaluated using the MTT assay against human cancer cell lines SiHa and MCF-7, with osteoblast cells, hFOB serving as the normal cell line. The results confirmed successful encapsulation of SF1 within chitosan nanocarriers, which exhibited suitable morphological characteristics and evidence of chemical interactions. Cytotoxicity assays demonstrated that both chitosan nanocarrier and SF1-loaded chitosan nanocarrier induced non-selective cytotoxic effects in the tested cancer cell lines and normal cell lines. The IC_{50} values ranged from $1.53 \pm 0.06 \mu\text{g/mL}$ to $5.44 \pm 0.02 \mu\text{g/mL}$. However, these findings represent preliminary *in vitro* observations, and further studies are required to clarify selectivity, underlying mechanisms, and the overall therapeutic potential of the developed

nanocarrier system. Although selectivity was not achieved in this study, these findings provide a foundational platform for the future optimization of targeted nanocarrier systems, guiding the design of strategies to enhance selectivity and therapeutic efficacy.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cancer remains one of the leading causes of death worldwide and continues to place a heavy burden on global healthcare systems, societies, and economies. Despite major advancements in medical research and treatment strategies, cancer is still considered a serious threat to human health. It is estimated that by 2030, approximately 13.1 million people will die from cancer-related causes (Grewal & Salar, 2024a). In 2022, around 20 million new cancer cases were reported globally, with 9.7 million deaths recorded. The number of new cancer cases is expected to rise to more than 35 million by 2050, representing a 77% increase compared to 2022. This increase is mainly due to population growth, ageing populations, and greater exposure to risk factors such as smoking, alcohol consumption, obesity, and air pollution. While countries with a high Human Development Index (HDI) are predicted to experience the largest increase in absolute case numbers, low- and middle-HDI countries are expected to face the highest percentage increase. According to the World Health Organization (2024), cancer-related deaths in these regions are projected to almost double by 2050.

At present, cancer treatment mainly involves surgery, chemotherapy, and hormone therapy. Although these approaches have shown effectiveness in treating certain types of cancer, their clinical use is often limited. One of the major challenges is toxicity, as these treatments do not specifically target cancer cells and may also damage healthy tissues. In addition, resistance to chemotherapy and hormone therapy frequently develops over time due to genetic mutations, changes in cellular signalling pathways, and tumour heterogeneity. As a result, long-term treatment outcomes may be compromised. Patients

undergoing these therapies often experience severe side effects, including neurotoxicity, gastrointestinal complications, haematological disorders, and cardiovascular problems. These limitations clearly indicate the need for more targeted and safer therapeutic approaches in cancer treatment (Zafar et al., 2025).

Natural products have long been an important source of anticancer agents. Several commonly used anticancer drugs, such as irinotecan, vincristine, etoposide, and paclitaxel, are derived from plants. Other examples include bleomycin, which originates from marine sources, as well as actinomycin D and mitomycin C, which are produced by microorganisms. Compounds like camptothecin and Taxol further highlight the significant role of natural substances in cancer therapy. Although synthetic chemical libraries have gained popularity in recent years, natural products continue to attract attention in drug discovery due to improvements in extraction methods, analytical techniques, and new therapeutic concepts (Huang et al., 2021). However, many natural compounds face limitations such as poor solubility, low bioavailability and limited cellular uptake, which restrict their clinical application.

Recent developments in nanotechnology have introduced new possibilities for cancer treatment, particularly in the area of targeted drug delivery including for natural products delivery. Nanocarrier-based systems are designed to improve drug solubility, bioavailability, and distribution within the body while reducing systemic toxicity. These systems can enhance drug accumulation at tumour sites and protect therapeutic agents from premature degradation. In addition, active targeting strategies using specific ligands or external stimuli allow better control over drug release, both in terms of location and timing. Compared to conventional free-drug formulations, nanotechnology-based

approaches offer clear advantages in improving treatment effectiveness (Di Stefano, 2023; Liu et al., 2024).

Among the various nanomaterials studied, chitosan has gained considerable attention as a potential nanocarrier for cancer therapy. Chitosan is a natural polysaccharide derived from chitin and is known for its biocompatibility, biodegradability, and low toxicity. The presence of amino functional groups allows chitosan to interact effectively with drugs and biological membranes through electrostatic interactions. This property enhances cellular uptake and improves drug retention at the target site. Chitosan nanocarriers also exhibit mucoadhesive properties, pH-sensitive solubility, and increased drug permeability, which contribute to improve bioavailability. Several *in vitro* and *in vivo* studies have reported promising results for chitosan-based delivery systems in transporting anticancer agents and immunomodulators, reducing systemic toxicity, and enhancing therapeutic efficacy (Guadarrama-Escobar et al., 2023).

1.2 Problem statement

Cancer remains one of the most challenging diseases to treat due to its complex biology, high recurrence rate, and resistance to therapy. Standard cancer therapies like chemotherapy and radiotherapy are non-specific and detrimental to both malignant and healthy cells, frequently leading to significant adverse effects and diminished patient outcomes. Moreover, several cancers are challenging to diagnose in their initial stages due to non-specific or asymptomatic manifestations, facilitating disease progression and metastasis prior to effective intervention (Chakraborty & Rahman, 2012).

Emerging evidence indicates that tumour recurrence and treatment failure are significantly correlated with the existence of cancer stem cells (CSCs), a minor

subpopulation of tumour cells characterized by self-renewal capability, drug resistance, and quiescent behavior. CSCs are not the main cause of therapy failure but provide a supporting rationale for developing strategies that improve the selectivity and efficacy of anticancer treatments (Chakraborty & Rahman, 2012).

Natural products have known as alternative sources of anticancer agents owing to their varied bioactivities and comparatively low toxicity. *Clinacanthus nutans* is a medicinal plant known to exhibit various biological activities and poses anticancer properties. Different extracts of *C. nutans* have been demonstrated to impede cancer cell proliferation, induce apoptosis, and interfere with cell cycle progression (Lin et al., 2021). A standardized anticancer fraction (SF1) extracted from *C. nutans* leaves has shown strong cytotoxic effects and antiproliferative effects against cervical cancer cells especially. It also selectively targets cancer cells and has less effect on normal cells, which makes it a promising safer anticancer agent (Ismail et al., 2024). Nevertheless, the therapeutic application of SF1 may be limited by factors such as low solubility, poor stability and limited cellular uptake.

Nanotechnology advancements have created novel avenues to address these challenges via targeted drug delivery systems that enhance drug stability, enable controlled release, and facilitate tumour-specific accumulation. Nanocarrier-based delivery systems have shown that they can improve the effectiveness of drugs while lowering their toxicity and resistance (Abbas & Bashir, 2025). Chitosan nanocarriers stand out among these systems because they are biocompatible, biodegradable, non-toxic, and better choice for drug encapsulation through ionic gelation (Bashir et al., 2022).

Although both nanotechnology-based delivery systems and *C. nutans* extracts have been examined independently to the best of our knowledge there is a notable lack of research focusing on the encapsulation of the standardized SF1 fraction within chitosan

nanocarriers. Moreover, there is still inadequate comprehensive physicochemical characterization and comparative analysis of the anticancer efficacy between free SF1 and SF1-loaded chitosan nanocarriers against human cancer cell lines. This absence of comparative in vitro data constrains the comprehension of whether nano-encapsulation can enhance the therapeutic potential of SF1.

Thus, there is a clear need to develop a chitosan-based nanocarrier system for the administration of SF1, encompassing its formulation, physicochemical characterization, and in vitro cytotoxicity evaluation. Filling this gap could give us useful information about how chitosan nanocarriers could work as a natural product-based targeted anticancer drug delivery system. Therefore, this research aims to develop a chitosan-based nanocarrier system to improve the delivery and therapeutic effectiveness of natural anticancer agents, addressing key limitations in current cancer treatments.

1.3 Scope of study

The objective of this study is to develop a chitosan-based nanocarrier system capable of transporting a natural anticancer fraction extracted from the leaves of *Clinacanthus nutans*, the SF1. This research focuses on the isolation and purification of a standardized anticancer fraction (SF1) through established chromatographic techniques. This study exclusively utilizes the chloroform extract of *C. nutans* leaves.

This formulation work of this study is about developing chitosan nanocarriers using the ionic gelation method and sodium tripolyphosphate (TPP) as the cross-linking agent. In order to develop a stable nanocarrier system, SF1 is encapsulated in chitosan nanocarriers in a controlled setting.

The characterization of the synthesized nanocarriers is limited to the assessment of surface morphology, particle structure, and functional group interactions. Scanning electron microscopy (SEM) is used to observe the size and shape of the nanocarriers. Next, Fourier-transform infrared spectroscopy (FTIR) is used to verify the ionic crosslinking and SF1 encapsulation.

The biological evaluation solely examines in vitro cytotoxicity and anticancer property as determined by using the MTT assay. The effects of free SF1 and SF1-loaded chitosan nanocarriers are evaluated on specific human cancer cell lines, such as SiHa and MCF-7. The osteoblast cell line is used as the control cell line in this study. There are no studies looking into mechanisms or using animal models. Consequently, the findings of this study provide preliminary in vitro data concerning the potential of chitosan nanocarriers as a delivery system for SF1.

1.4 Study objectives

1.4.1 General objective

The general objective of the present study is to develop a targeted nanocarrier-based delivery system using chitosan nanocarriers for a SF1 derived from *Clinacanthus nutans*.

1.4.2 Specific objectives

The specific objectives of this study are outlined as follows:

- i. To isolate and purify the standardized anti-cancer fraction (SF1) from *Clinacanthus nutans* leaves using chromatographic techniques.
- ii. To synthesize and optimize chitosan-based nanocarriers and encapsulate the SF1

using ionic gelation method.

- iii. To characterize the physicochemical properties of the developed chitosan nanocarrier, including particle size and functional group modification using scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR).
- iv. To determine the cytotoxicity and anticancer activity against selected human cancer cell lines, comparing the free compound with the chitosan nanocarrier.

1.5 Hypothesis

1.5.1 Null Hypotheses

The null hypotheses of this study are outlined as follows:

- i. SF1 cannot be effectively encapsulated into chitosan nanocarriers using the ionic gelation method.
- ii. The SF1-loaded chitosan nanocarriers do not exhibit significantly different physicochemical properties compared to unloaded nanocarriers.
- iii. There is no significant difference in cytotoxicity and anticancer activity between SF1-loaded nanocarriers and free SF1 against the tested cancer cell lines.

1.5.2 Alternative Hypotheses

The alternative hypotheses of this study are outlined as follows:

- i. SF1 can be successfully encapsulated into chitosan nanocarriers via ionic gelation.
- ii. SF1-loaded chitosan nanocarriers exhibit significantly enhanced physicochemical properties compared to unloaded nanocarriers.

- iii. SF1-loaded chitosan nanocarriers show significantly higher cytotoxicity and anticancer activity against human cancer cell lines.

1.6 Significance of study

This study is significant as it addresses the need for more effective and safer anticancer drug delivery system. Currently, there are a lot of anticancer drugs on the market, but their clinical use is often limited due to poor targeting capacity towards cancer cells and shows serious side effects on healthy cells. This study investigates a novel approach to enhance the delivery of anticancer agents through the development of a chitosan-based nanocarrier system.

A standardized anticancer fraction (SF1) originated from *Clinacanthus nutans* leaves is used because it is a natural product that is known to be less toxic and more effective than the synthetic ones. However, their applications are limited due to low solubility and low cellular uptake. Encapsulation of SF1 into chitosan nanocarriers might help overcome the problems by making it more stable and able to interact better with cancer cells.

This research provides significant insights into the formulation and characterization of SF1-loaded chitosan nanocarriers via the ionic gelation technique from a scientific and academic standpoint. The comparative analysis of free SF1 and SF1-loaded nanocarriers offers initial evidence regarding the benefits of nanocarrier-based delivery systems in enhancing anticancer efficacy.

Moreover, the results of this study may establish a basis for subsequent research on targeted drug delivery, encompassing mechanistic analyses and in vivo investigations. Overall, this study supports the use of nanotechnology and natural products in the creation

of possible cancer treatments. It may also help improve plant-based drug delivery systems.

CHAPTER 2

LITERATURE

REVIEW

2.1 Cancer and the global burden

Cancer continues to be the foremost cause of mortality worldwide and a significant public health issue. Notwithstanding progress in medical science, contemporary therapies like chemotherapy and radiation frequently exhibit restricted effectiveness and considerable adverse effects. Cancer is a genetic disorder characterized by heightened cellular proliferation, diminished programmed cell death, or a combination thereof. The principal characteristics of cancer progression include accelerated cellular proliferation, tissue invasion, angiogenesis, metastasis, and the progressive deterioration of affected organs (Ghaz-Jahanian et al., 2015a). Abnormal cellular activity, characterized by uncontrolled proliferation and evasion of standard regulatory mechanisms governing cell division and growth, is the fundamental cause of cancer. These malignant cells become autonomous, disregard normal cell cycle signals, and gain the ability to infiltrate adjacent tissues, potentially resulting in lethal consequences. Approximately 90% of global cancer fatalities result from metastasis, characterized by the dissemination of malignant cells to remote organs, highlighting its pivotal role in cancer mortality. Cancer ranks among the foremost causes of mortality globally, with projections indicating approximately 13.1 million fatalities attributed to the disease by 2030 (Grewal & Salar, 2024a).

Cancer denotes a comprehensive classification of diseases, encompassing more than 277 unique types, all defined by the unregulated growth of abnormal cells. Carcinogenesis may transpire in nearly any cell type subsequent to the accumulation of successive genetic

mutations. This multistep process consists of three distinct phases: initiation, promotion, and progression. Initiation is the stage where genetic alterations first occur; promotion is the phase in which malignancy commences and altered cells experience further transformation; progression is marked by accelerated, irreversible cell division and tumour formation. Cancer cells often disseminate to remote organs via metastasis, alongside localized tumour proliferation, which is widely acknowledged as a critical contributor to cancer-related mortality(Naeem et al., 2022a).

Recent global assessments underscore the increasing influence of cancer. The Global Burden of Diseases, Injuries, and Risk Factors Study 2023 reported approximately 18.5 million new cancer diagnoses and 10.4 million cancer-related fatalities globally in 2023. This led to an estimated 271 million disability-adjusted life-years (DALYs), predominantly due to years of life lost. According to the most recent estimates of the global cancer burden that were provided by the World Health Organization, cancers that are diagnosed most frequently are significantly different between the sexes. Cervical cancer is the most common cancer in women in many countries, particularly in sub-Saharan Africa, and although it ranks lower overall, it is still a major public health concern. In 2022, there were about 660,000 new cases and 348,000 deaths related to cervical cancer. This highlights the persistence of cervical cancer in female cancer burden and the importance of research focused on cervical cancer using models like SiHa cells. The most common cancer in men is lung cancer, followed by prostate and colorectal cancers. Men died from lung cancer at a higher rate than any other cancer. These sex-specific patterns show how epidemiological differences in cancer incidence determine clinical priority setting and which experimental models are appropriate for research (World Health Organization: WHO, 2024). Approximately 41.7% of cancer-related fatalities were linked to identifiable environmental and behavioral risk factors. By 2050,

new cancer cases and fatalities are projected to rise by over 60% and 74%, respectively, signifying a significant escalation in the global cancer burden. By mid-century, this trend is anticipated to yield approximately 30.5 million new cancer cases and 18.6 million deaths each year (Luo & Smith, 2025).

The global cancer burden exhibits notable disparities among World Bank income groups. In 2023, age-standardized cancer mortality rates exhibited a U-shaped distribution, with both high-income and low-income nations reporting elevated rates compared to middle-income areas. Although there was a notable global reduction in cancer mortality rates from 1990 to 2023, this decrease predominantly occurred in high-income and upper-middle-income nations. Conversely, during that period, cancer mortality rates rose in low-income and lower-middle-income nations. Forecasts suggest that advancements will decelerate and stabilize, implying that existing cancer control strategies may diminish in efficacy over time, despite a potential gradual decline in global cancer mortality rates until 2050. The findings underscore the pressing necessity for sustained and enhanced global initiatives to tackle the escalating and disparate cancer burden across various economic contexts (Luo & Smith, 2025). According to WHO/IARC estimations (GLOBOCAN 2022), cancer represents a significant and increasing health burden in Malaysia. In 2022, Malaysia documented approximately 51,650 new cancer cases, with a slightly higher incidence in females compared to males. The 5-year prevalence was almost 155,500 individuals, representing a large population of Malaysians with a recent cancer diagnosis requiring continuous care. In the same year, cancer claimed over 31,600 lives, making it one of the nation's top causes of death. In Malaysia, the lifetime chance of getting cancer before the age of 75 is estimated to be 14%, or roughly 1 in 7 people. In general, these statistics highlight on a considerable and ongoing cancer burden in Malaysia. This finding is in line with the World Health Organization's study on

the rising worldwide cancer burden and the growing demand for services related to prevention, early detection, and treatment (World Health Organization: WHO, 2024).

2.2 Cancer therapy and limitation of conventional chemotherapy

Traditional cancer therapies, encompassing surgery, radiation, chemotherapy, and targeted therapy, continue to be the foundation of clinical cancer management. These methods are well-established and extensively employed in long-term clinical environments due to their efficacy in eliminating tumour cells or inhibiting tumour proliferation, thereby reducing the overall tumour burden. Chemotherapy exerts its antitumour effects through various mechanisms, including DNA damage, inhibition of DNA replication, and disruption of mitotic processes to eradicate cancer cells. Conventional therapies, such as chemotherapy, not only exert direct cytotoxic effects but also induce immunogenic cell death (ICD), potentially activating innate and adaptive immune responses to enhance tumour eradication (Li et al., 2025).

Notwithstanding these advantages, numerous substantial drawbacks often constrain the therapeutic effectiveness of conventional chemotherapy. Chemotherapy reactions are generally transient, as numerous patients encounter treatment resistance and tumour recurrence shortly thereafter, despite frequently observed high initial response rates. The heterogeneity of cancer tissue complicates treatment outcomes, as varying tumour cell populations may exhibit differential responses to chemotherapy, resulting in partial tumour eradication and disease progression (Li et al., 2025). Moreover, genetic mutations, alterations in intracellular signaling pathways, and tumour adaptive responses may lead to chemoresistance, significantly diminishing the effectiveness of prolonged treatment (Zafar et al., 2025).

The lack of specificity in conventional chemotherapy is a significant drawback that may inadvertently harm healthy, rapidly dividing cells. The myriad side effects of chemotherapy stem from its non-specific toxicity, encompassing gastrointestinal issues, hematologic toxicities, neurotoxicity, and broader systemic effects that adversely affect patient quality of life and complicate treatment adherence. These toxicities often necessitate dosage reduction or discontinuation of treatment, thereby compromising therapeutic outcomes(Zafar et al., 2025).

Chemotherapy can alter the tumour microenvironment by enhancing tumour immunogenicity and reducing immunosuppressive cell populations; however, it often proves inadequate as a solitary treatment for various cancer types. Clinical data indicates that, especially in advanced or metastatic cancer cases, chemotherapy alone often does not provide long-term survival advantages or enduring tumour control. This has resulted in heightened interest in integrated treatment approaches aimed at overcoming resistance mechanisms and improving therapeutic efficacy (Li et al., 2025).

The aim of cancer therapies is to extend patient lifespan while concurrently improving quality of life by reducing unintentional and detrimental treatment-associated side effects (Ghaz-Jahanian et al., 2015a). Nonetheless, chemotherapy-based combination strategies present difficulties. Combination therapies often correlate with heightened toxicity, although synergistic effects have been observed in specific clinical contexts. The efficacy of therapy is significantly influenced by variables including medication dosage, treatment duration, patient health, and illness stage. The difficulty of enhancing traditional chemotherapy methods is underscored by instances where the integration of chemotherapy with alternative treatment modalities has not yielded significant improvements in overall survival (Li et al., 2025; Zafar et al., 2025).