Chapter 01

Introduction

1 Chapter 01: Cancer, its epidemiology, causes & treatment

1.1 Introduction

Cancer poses a multifaceted challenge in the field of clinical practice, marked by its widespread occurrence, substantial impact on health, and notable mortality rates. It encompasses a diverse range of diseases affecting various body parts and is alternatively termed as malignant tumors or neoplasms. A key characteristic of cancer is the accelerated development of abnormal cells that exceed their normal confines, leading to infiltration into adjacent tissues and the potential spread to other organs - a phenomenon known as metastasis (1). The main reason for fatality in cancer cases is the extensive metastases. It has the potential to manifest in virtually every part of the human body, impacting individuals of all ages and genders.

The transformation of a normal cell into a cancerous one occurs due to the interplay between an individual's genetic factors and external carcinogenic agents, including but not limited to tobacco, food contaminants, water pollutants, ultraviolet radiation, and specific viral or bacterial infections. Additionally, lifestyle-related elements such as excessive alcohol consumption, an unhealthy diet, and a lack of physical activity significantly increase the risk of cancer development (2).

The human body is comprised of over trillions of cells, which serve as the fundamental units of life. In a general process, normal cells undergo development and division to generate new cells according to the body's requirements. Eventually, these cells die naturally as they age or incur damage, and fresh cells take their place. In a healthy individual, there exists an appropriate balance of each cell type, regulated by the cells themselves through the production of signals that guide their division and cessation. If a malfunction occurs in the transmission of these signals, cells may start proliferating uncontrollably, leading to abnormalities. This

abnormal cell behavior results in instances where they disregard control signals, allowing old or damaged cells to persist instead of perishing, and new cells to form when unnecessary, consequently leading to the formation of tumors. These cells lose their capacity to recognize their own boundaries and spread throughout the body to locations where they are not supposed to be. The process through which cells undergo division is termed cell division.

Cells possess a central command hub known as the nucleus, housing the genetic material called deoxyribonucleic acid (DNA). This DNA comprises genes that regulate cellular division and functions based on the body's requirements. The DNA serves as the blueprint for all the cell's activities. In a human cell, the DNA is organized into 46 distinct segments referred to as chromosomes, collectively containing over 10,000 genes. Each of these genes instructs cells to produce a unique protein through a sequence of biochemical reactions (3).

Specific genes guide the cell in the synthesis of structural proteins, constituting the foundational components. On the other hand, distinct genes prompt the cell to generate hormones, growth factors, or cytokines, which exit the cell and engage in communication with other cells. Another category of proteins, known as regulatory proteins, is produced by certain genes. These proteins oversee the function of other proteins or signal additional genes regarding when to activate or deactivate (4).

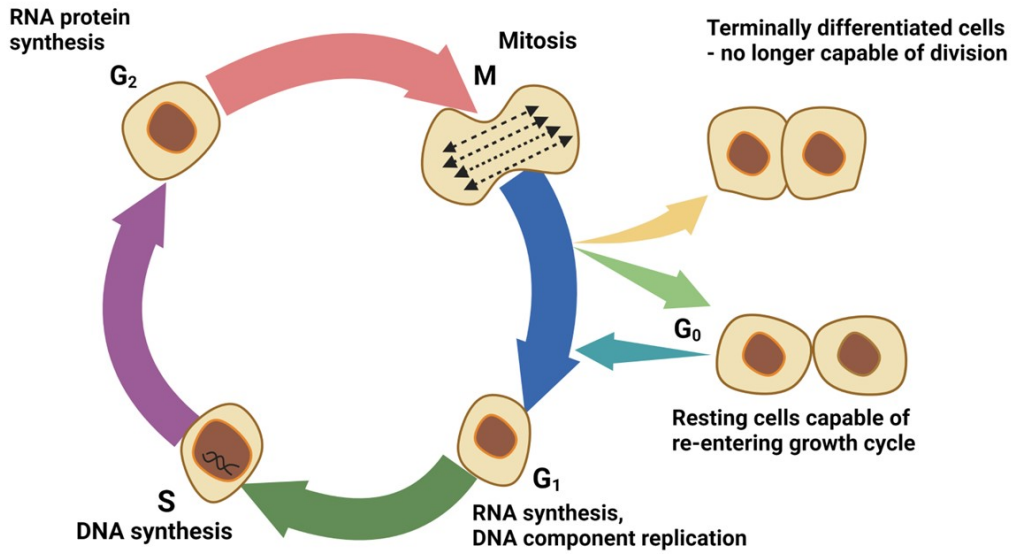

Figure 1 Diagram representing phases of cell cycle and events occurring at each phase

Cells undergo division from a parent cell to daughter cells through the mitosis process, constituting a sequence of events known as the cell cycle. The cell cycle comprises the mitosis phase (M phase) and a synthetic phase termed the S phase, during which DNA synthesis occurs. Positioned between the M phase and the S phase is the G1 phase, where RNA, proteins, and enzymes necessary for DNA synthesis in the S phase are generated. Following the S phase is the G2 phase, which readies the cell for the M phase. The G0 phase serves as the resting phase, during which cells are non-dividing. Multiple checkpoints at each stage of the cell cycle ensure proper progression. These normal cells are highly specialized and carry out specific functions with precision (5).

1.2 Epidemiology

Frequently identified mutations in lung cancer patients that are the focus of treatment efforts include EGFR, ALK, and PD-L1. In India, EGFR and ALK mutations are frequently observed, while PD-L1 mutations are not commonly reported. The significance of molecular testing has

increased as various biomarkers are being specifically examined to diagnose individuals with lung cancer. Surgical procedures, radiotherapy, systemic chemotherapy, and personalized molecular-targeted therapy contribute to extending the overall survival (OS) of individuals with NSCLC. Despite notable enhancements in clinical outcomes due to chemotherapy and molecular-targeted therapies, achieving prolonged disease control remains a challenge for the majority of NSCLC patients (6).

The data on cancer incidence and mortality in GLOBOCAN 2020, generated by the International Agency for Research on Cancer, indicates that globally, around 19.3 million new cancer cases (excluding nonmelanoma skin cancer) and nearly 10.0 million cancer-related deaths (excluding nonmelanoma skin cancer) occurred in 2020. The most frequently diagnosed cancer is now female breast cancer, surpassing lung cancer, with approximately 2.3 million new cases (11.7%). Other common cancers include lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach (5.6%) cancers. Lung cancer remained the leading cause of cancer deaths, accounting for about 1.8 million deaths (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast (6.9%) cancers. The overall incidence of cancer was 2 to 3 times higher in transitioned countries compared to transitioning countries for both genders, but mortality varied by less than 2 times for men and minimally for women. Notably, death rates for female breast and cervical cancers were significantly higher in transitioning countries compared to transitioned countries (15.0 vs 12.8 per 100,000 and 12.4 vs 5.2 per 100,000, respectively).

The anticipated global cancer incidence is projected to reach 28.4 million cases in 2040, marking a 47% increase from 2020. The surge is more pronounced in transitioning countries, expected to rise between 64% and 95%, compared to transitioned countries, where the increase is estimated to be between 32% and 56%. This escalation is primarily attributed to demographic shifts, though it could be further intensified by heightened risk factors associated with

globalization and economic growth. It is imperative to focus on establishing a sustainable infrastructure in transitioning nations for the effective dissemination of cancer prevention measures and the provision of cancer care. This strategic approach is crucial for the global management of cancer (7).

1.2.1 Incidence

1.2.1.1 International status

Annually, the American Cancer Society provides estimates for new cancer cases and deaths in the United States, aggregating the latest information on population-based cancer occurrence and outcomes. This data is derived from incidence records collected by central cancer registries and mortality statistics gathered by the National Center for Health Statistics. For the year 2023, it was anticipated that there would be 1,958,310 new cancer cases and 609,820 cancer-related deaths in the United States (8).

In 2040, the worldwide incidence of new cancer cases is anticipated to reach 27.5 million, and cancer-related deaths are projected to be 16.3 million, primarily attributed to population growth and aging. Nonetheless, it is likely that the future burden of cancer will be significantly higher due to the increasing prevalence of risk factors such as smoking, an unhealthy diet, physical inactivity, and fewer pregnancies. Cancers linked to these factors, including lung, breast, and colorectal cancers, are already increasing in economically transitioning countries. This trend is expected to persist unless widespread preventive measures are implemented (7).

1.2.1.2 National status

As per the Global Cancer Observatory (GLOBOCAN) assessments, there were 19.3 million new cases of cancer reported globally in 2020. India held the third position in this regard, following China and the United States of America. According to GLOBOCAN projections, the

number of cancer cases in India is expected to reach 2.08 million in 2040, reflecting a 57.5% increase from the figures recorded in 2020 (7, 9).

The prevalence of cancer cases in India is on the rise. Breast cancer stands out as the most common among the top five cancers in females, followed by cervical, ovarian, and uterine cancers. In males, three sites - lung, mouth, and tongue are predominantly associated with tobacco-related cancers. To alleviate the future burden of cancer, proactive and preventable measures must be implemented.

In India, it is anticipated that the number of cancer cases will rise from 1.46 million in 2022 to 1.57 million in 2025. The national crude incidence rate per 100,000 for 2022 averages at 100.4, with rates of 95.6 for males and 105.4 for females. The primary cancer sites continue to be lung cancer in males and breast cancer in females. Among children, lymphoid leukemia, followed by brain neuroblastoma, is the predominant cancer site for both genders. The age-specific incidence rate (ASIR) rises with age, with the female reproductive age group (15-49 years) exhibiting higher rates. Lung cancer is estimated to account for 103,371 cases in 2022, ranking among the top five cancer sites for both males and females. The current estimates for cancer in India show a five percent increase, totaling 1,461,427 cases in 2022 compared to 1,392,179 in 2020 (10).

1.3 Cancer cell biology

Within cancer cells, genetic mutations occur during the cell division process, resulting in the creation of faulty cells. These defective cells subsequently undergo uncontrolled cell division, producing an increasing number of copies of the mutated cell. Mutations are alterations that occur in genes, rendering them incapable of carrying out their normal functions. The occurrence of mutations can be random or attributed to various factors, including environmental influences, chemical exposure, and lifestyle habits (11).

Typically, there are two categories of gene mutations: dominant mutations and recessive mutations. These mutations can impact three primary types of genes: proto-oncogenes, tumor suppressor genes, and DNA repair genes (12).

A dominant mutation arises when there is an abnormality in one gene within a pair. For instance, if a proto-oncogene undergoes mutation, it results in the production of a defective protein. This faulty protein prompts the growth factor receptor to remain consistently active on the cell surface, even in the absence of a growth factor to signal. Moreover, it signals the cell to continually undergo division. This dominant mutation, known as an oncogene (where "onco" refers to cancer), represents a cancer-inducing "gain of function gene."

In the case of recessive mutation, damage occurs to both genes within a pair. An example of this mutation type involves the modification in the functioning of tumor suppressor genes or anti-oncogenes. One such tumor suppressor gene, p53, produces a protein that effectively halts the cell cycle, thereby aiding in the regulation of cell growth. It plays a crucial role in preventing the formation of potentially cancerous cells by either repairing or eliminating faulty cells.

If one of the two p53 genes in the pair undergoes mutation, the other gene remains capable of regulating the cell cycle. However, when both genes undergo transformation, the "off" switch is lost, and cell division is no longer under control (13). Cells that have undergone mutations experience unregulated growth, resulting in the formation of tumors. Anomalous cell division can occur either through the activation of oncogenes or the failure of tumor suppressor genes to express. In reality, for a regular cell to transform into a malignant one, multiple mutations are necessary. In specific instances, both dominant and recessive mutations may occur simultaneously.

Likewise, DNA repair genes, responsible for repairing damaged DNA, may undergo additional changes in other genes if they become mutated. In combination, these mutations can lead to

the development of cancerous cells. These changes are commonly referred to as "drivers" of cancer. Viruses can also instigate irregular cell division. In such instances, even if the genes seem normal, the protein may not function normally due to the presence of a cancer-inducing virus within the cell (14).

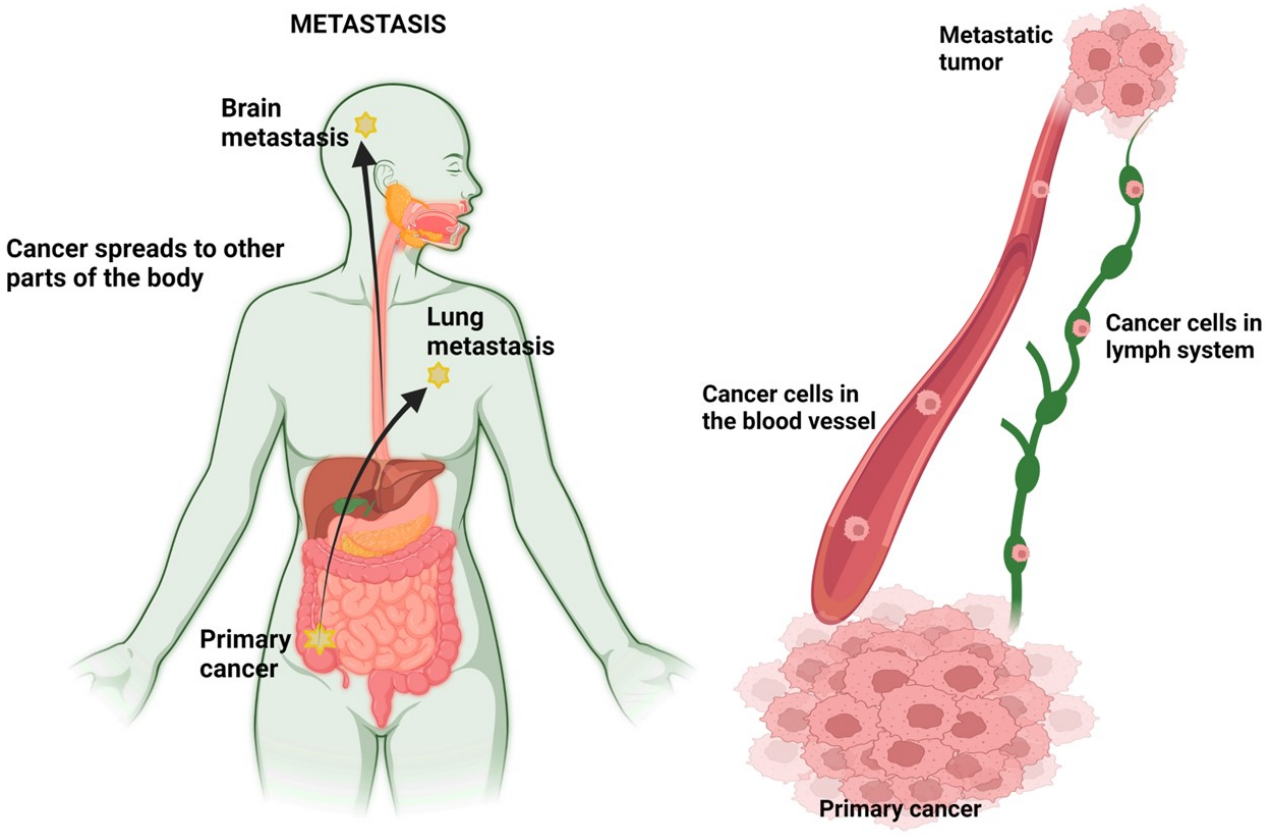

Figure 2 Illustration depicting the process of metastasis originating from primary tumors and resulting in one or more metastatic tumors in various parts of the body.

The factors mentioned above can contribute to the development of tumors, typically of a solid nature. However, certain cancers, like leukemia, do not manifest as solid masses and instead involve the blood. The initial site of cancer development is referred to as the primary tumor, which may remain localized or extend into adjacent tissues, lymph nodes, or the bloodstream, spreading cancer to distant areas. This phenomenon is termed a metastatic tumor and is a primary cause of death in individuals with cancer. Cancers are categorized into six major types

based on the histological site of origin: carcinoma, sarcoma, myeloma, leukemia, lymphoma, and mixed types.

1.4 Hallmarks of Cancer

Hanahan and Weinberg have presented six distinctive features of cancer that serve as an organizational concept, offering a logical structure for comprehending the intricacies of cancer biology. Despite significant advancements in scientific understanding over the past decade, it is now evident and established that an adequate explanation of cancer biology cannot be solely based on elucidating the behavior of cancer cells. It is equally imperative to grasp the contributions of the tumor microenvironment to the process of tumorigenesis. The six hallmarks of cancer encompass sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis (1).

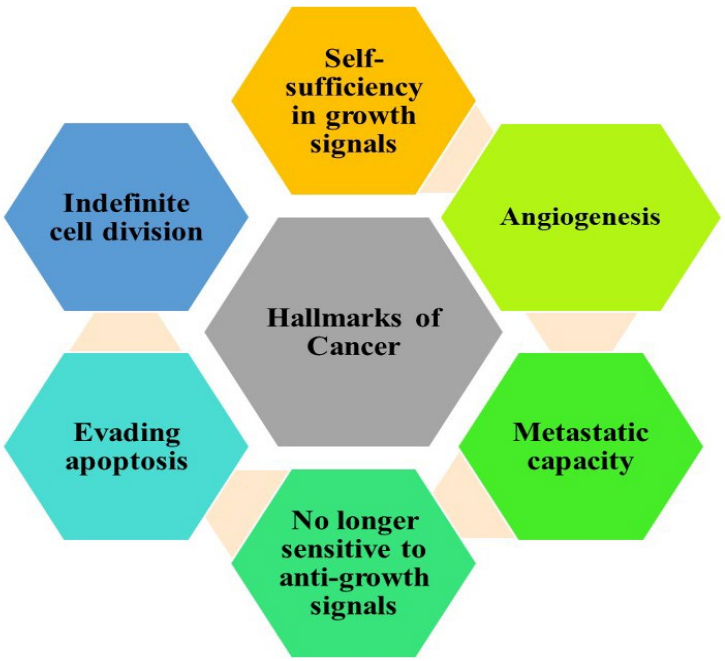

Figure 3 Hallmarks of cancer

In addition to the aforementioned six hallmarks, two additional characteristics were identified as emerging and were put forward along with their facilitating features. Disturbing cellular energetics and evading the immune response are the two emerging hallmarks. These are

described as the modification of cellular metabolism to support the proliferation of cancer cells and the evasion of immunological destruction by T and B lymphocytes, natural killer cells, and macrophages.

1.5 Lung cancer

Lung cancer stands as the leading cause of cancer-related fatalities globally, constituting approximately 18% of all cancer-related deaths. It is the most frequently diagnosed cancer subtype in males worldwide. In the United Kingdom, lung cancer results in around 35,000 annual deaths, with a survival rate of only 10%. The prevalence of lung and oral cancers, particularly those linked to tobacco use, is highest in the Indian subcontinent, encompassing countries such as Bangladesh, Bhutan, Maldives, Nepal, Pakistan, and Sri Lanka. Smoking and tobacco consumption represent the primary risk factors for the development of lung carcinoma, contributing to about 66% of lung cancer deaths (15). The link between smoking and lung cancer was established during the 1950s. Cigarettes contain over 60 carcinogens, as demonstrated in laboratory studies, which have the potential to induce cancers. In addition to active smoking, passive smoking also, to a lesser extent, leads to the development of lung cancer. The International Agency for Research on Cancer (IARC) has identified approximately 50 different carcinogens in cigarette smoke, affecting both the central and peripheral alveolar regions. These carcinogens can instigate mutations in genes responsible for cell division, growth, and apoptosis. Consequently, significant alterations such as tumor suppressor gene (TSG) inactivation, telomerase activation, overexpression of the epidermal growth factor receptor (EGFR), angiogenesis, and the induction of apoptosis contribute to the development of lung carcinoma (16). The onset of lung cancer begins with a rapid increase in the number of cells, termed hyperplasia. Following hyperplasia, cells lose their ability to differentiate, entering a stage known as dysplasia. Carcinoma *in situ* represents the subsequent stage of dysplasia, where cells become cancerous but remain non-invasive. The overall five-year

survival rate for lung cancer patients is approximately 17.8%. The prevalence and mortality rates are notably high in developed countries and are consistently on the rise in developing countries. Various genetic alterations, such as the overexpression of EGFR, inactivation of tumor suppressor genes, telomerase activation, among others, contribute to these alterations and are recognized as the "key attributes" of lung cancer.

1.5.1 Types of lung cancer

World Health Organization (WHO) has categorized lung cancer into two main groups based on histology, diagnosis, and clinical implications.

1.5.1.1 Non-small cell lung cancer (NSCLC)

NSCLC originates from lung epithelial cells, spanning from the central bronchi to the terminal alveoli. The histological classification of NSCLC is associated with the location of its origin, reflecting variances in respiratory tract epithelium from the bronchi to the alveoli. NSCLC is subsequently subdivided into three distinct categories (17).

1.5.1.1.1 Adenocarcinoma

Adenocarcinoma comprises 40% of all cases of non-small cell lung cancer (NSCLC). Typically arising in type II alveolar cells with secretory functions, it represents the most prevalent form of lung cancer across various age groups among both smokers and nonsmokers. Predominantly found at the periphery of the lungs, this occurrence is possibly linked to the presence of filters in cigarettes, which obstruct larger particles from reaching deeper lung areas. Adenocarcinoma is characterized by a less aggressive nature and is more likely to be identified before spreading to distant organs from the lungs (18).

1.5.1.1.2 Squamous-cell carcinoma

Comprising approximately 25-30% of non-small cell lung cancer (NSCLC), this type originates from bronchial epithelial cells, specifically premature squamous cells within the airways. There is a significant association between squamous cell cancer and cigarette smoking (19).

1.5.1.1.3 Large-cell carcinoma

Large cell carcinoma, a rare variant of non-small cell lung cancer, is identified as undifferentiated and/or large cell lung cancer based on the microscopic appearance of its cells. This subtype constitutes approximately 10-15% of cases within the broader category of NSCLC (20).

1.5.1.2 Small-cell lung cancer

Small-cell lung cancer (SCLC) represents 20% of total lung cancer cases and exhibits a more rapid spread compared to non-small cell lung cancer. Consequently, treating it proves challenging. The strong association between tobacco carcinogen exposure and SCLC is well-established. A significant proportion of patients receive a diagnosis at an advanced, metastatic stage, while only one-third present with earlier-stage cancer that may respond to potentially curative diagnostic treatment (21).

1.6 Breast Cancer

Breast cancer (BC) stands as the predominant form of cancer among women globally and persists as the second highest contributor to cancer-related deaths among them. In 2020, it surpassed lung cancer to become the most frequently diagnosed cancer worldwide.

The worldwide tally of breast cancer cases has exceeded 2.2 million lung cancer diagnoses, making it the foremost cancer globally. Breast cancer, a prevalent form of the disease, poses a significant health hazard to women. It carries a notable mortality rate, contributing to 6.9% of

all cancer-related deaths worldwide and holding the top position in women's global cancer mortality rates (7).

The nuclear transcription factor Estrogen Receptor Alpha (ERα), encoded by the ESR1 gene, typically activated by estrogen, is present in over 70% of diagnosed cases of breast cancer. A burgeoning body of evidence elucidating the structure and transcriptional activation mechanisms of ERα has firmly established the pivotal role of estrogen-ER signaling in the onset, progression, and metastasis of breast cancer (22).

1.7 Cancer treatment and chemotherapy

Current cancer treatment primarily involves surgical procedures, chemotherapy, radiation therapy, or a combination of these modalities (23). Conventional methods of targeting cancer cells involve disrupting DNA synthesis and mitosis, effectively eliminating rapidly proliferating cancer cells. However, due to their non-specific nature, these mechanisms often harm normal, healthy cells, leading to significant unintended side effects such as nausea, loss of appetite, bone marrow suppression, alopecia, and hypersensitivity reactions. Furthermore, the substantial and severe side effects induced by chemotherapeutic agents are primary contributors to the elevated mortality rates observed in cancer patients.

Moreover, a majority of chemotherapeutic medications lack optimal physicochemical properties for formulation and exhibit limited penetration into tumors *in vivo*. Consequently, elevated doses are often required, resulting in heightened toxicity and the development of multidrug resistance (24).

Various classes of chemotherapeutic agents include alkylating agents, antimetabolites, antimicrotubule agents, antitumor antibiotics, and topoisomerase inhibitors, among others. Alkylating agents function by binding to DNA, disrupting DNA replication, and impeding subsequent cell division.

Examples of alkylating agents encompass nitrogen mustards (such as cyclophosphamide, ifosfamide, chlorambucil, melphalan), tetrazines (including dacarbazine and mitozolamide), and nitrosoureas (such as carmustine and lomustine). Organoplatinum complexes like cisplatin, carboplatin, and oxaliplatin operate by crosslinking with DNA strands, thereby inhibiting DNA, RNA, and protein synthesis (25).

Antimetabolites constitute another category of anti-cancer agents that share structural similarities with endogenously available vitamins, nucleosides, or amino acids. They function by inhibiting enzymes or incorporating into DNA, thereby hindering the synthesis of DNA, RNA, or proteins in cancer cells. Examples of antimetabolites include folate antagonists (such as methotrexate and pemetrexed), purine analogues (including cladribine, fludarabine, pentostatin, and mercaptopurine), pyrimidine analogues (such as fluorouracil, capecitabine, and gemcitabine), and hydroxyurea.

Taxanes (including paclitaxel, docetaxel, and cabazitaxel) and vinca alkaloids (such as vincristine, vinblastine, and vinorelbine) target the spindle apparatus during cell division. Topoisomerases are enzymes that act on the three-dimensional structure of DNA by cleaving, unwinding, and rejoining DNA strands during replication. Examples of topoisomerase inhibitors include camptothecin analogues (like irinotecan and topotecan), anthracyclines (including doxorubicin, daunorubicin, epirubicin, and idarubicin), and epipodophyllotoxins (such as etoposide and tenoposide).

Cytotoxic agents predominantly impact cells that undergo rapid cell division, lacking precise targeting of cancer cells in the G0 phase of the cell cycle. Additionally, they exhibit limited efficacy in addressing aspects of cancer progression such as metastasis and tissue invasion. These agents demonstrate similar toxicity towards both cancer and normal cells. In addition to cytotoxic drugs, treatment options for cancer include monoclonal antibodies (mAbs) like trastuzumab and bevacizumab, as well as hormonal agents such as tamoxifen, letrozole, and

bicalutamide. These alternatives act specifically on receptors, modulating their function in the treatment of cancer.

Despite notable progress, lung and breast cancer continues to pose significant challenges in terms of incidence and mortality rates. Many existing chemotherapy treatments are accompanied by severe side effects, and a considerable number of patients develop resistance to new drugs. Consequently, there is an urgent need to develop more potent medications and investigate novel therapeutic targets for lung and breast cancer. Natural products emerge as a vital reservoir for drug discovery, particularly in anti-tumor therapies, often showcasing superior biological activity, specificity, efficacy, and safety profiles for cancer treatment. Thus, the exploration of new therapeutic agents derived from natural sources represents a critical avenue of research for both preventing and treating cancer.

1.8 Limitations of conventional cancer chemotherapy

Although the conventional chemotherapy regimens are being followed in treatment of various cancers either alone or in combination with other treatment strategies, they pose several limitations as enumerated below.

Poor aqueous solubility: Most of the chemotherapeutic agents are poorly soluble in water and require solvents in formulation of their dosage forms. These agents suffer meager bioavailability and requires frequent and high dosing to reach the therapeutic concentrations. To formulate intravenous dosage forms, the active agents must be capable of going into solution in aqueous media for them to stay in the system. For example, Paclitaxel (PTX), a highly successful chemotherapeutic agent suffers very low solubility of less than 0.5 mg/L. Cremophor EL (castor oil derivative) and dehydrated alcohol had to be used in its formulation which causes toxicities of heart, kidney, brain, systemic toxicity, and peripheral neuropathy along with hypersensitivity reactions. On the other hand, surfactants when employed in the

formulation to solubilize the drug, may lead to precipitation of drug *in vivo*, due to their high critical micelle concentration in physiological fluids.

Non-specific toxicities: Conventional anticancer agents when administered by intravenous route, pass through the blood circulation and reaches cancer cells as well as normal cells. Such drugs which are currently in use often show unwanted toxicities due to their broad distribution in the body resulting in serious adverse effects such as bone marrow depression, systemic toxicity, hair loss, anaemia, weight loss, nausea, diarrhoea, infertility, cardiovascular toxicity, vomiting etc. to name a few. Also, cells have slowly attained drug resistance to these single agent therapies. The absence of selectivity in mechanism of action is a conspicuous drawback of conventional chemotherapy. Most number of anti-cancer drugs does not act on intracellular mechanisms exclusive to cancer cells but on pathways common in both normal and cancerous cells.

Uptake by reticuloendothelial system (RES): Macrophages and monocytes present in the reticular connective tissue (liver, spleen) compose the reticuloendothelial system and are responsible for rapid clearance of hydrophobic agents from the blood circulation. They remove the cell debris, foreign substances, and pathogens from the circulation by phagocytosis. As most of the cancer chemotherapeutics are hydrophobic in nature, they get engulfed by the RES which results in rapid clearance of drug from the blood stream. Therefore, high concentrations of drug need to be administered to reach the therapeutic concentrations at the target site. Hydrophobicity of the delivery system along with its particle size properties and surface charge influences the uptake by RES.

To overcome this problem, the delivery system can be coated by polyethyleneglycol (PEG) which is a synthetic, water-soluble polymer. PEG improves the hydrophilicity of the system and masks it from being identified by macrophages. It inhibits opsonization and protein adsorption of the delivery system which improves its circulation time. This technique has been

effectively used to minimize the rapid clearance of drug delivery systems and improving the overall half-life of the drug.

Multidrug resistance (MDR): One of the major problems associated with conventional chemotherapy is the cells acquiring resistance to drugs. Resistance could occur by cellular or non-cellular mechanisms. The cellular mechanism involves a plasma membrane receptor protein P-glycoprotein (P-gp) which repels the drugs out of the cell obstructing intracellular accumulation of therapeutic agents. P-gp is the major efflux protein in the body belonging to the family of ATP binding cassette (ABC) transporters.

P-gp transporter protein is overexpressed in cancer cells and diminishes the entry and retention of drug in the cells. This phenomenon is majorly observed when drug diffuses passively into the cell through the lipid cell membrane. After entering into cell, anticancer agents, using the energy of ATP hydrolysis forms transmembrane channels by binding to P-gp and expel these molecules out of the cell. Such actives which are susceptible to P-gp efflux are called P-gp substrates. Doxorubicin, docetaxel, paclitaxel, actinomycin D, etoposide are a few of the anti-cancer agents which are P-gp substrates. Oral bioavailbility of such molecules is seriously comprimised. The co-administration of inhibitors of P-gp was investigated to improve the delivery to cancer cells. For example, a delivery system encapsulating elacridar, a P-gp inhibitor along with paclitaxel in a micellar system was reported to overcome the multidrug resistance problem of paclitaxel.

Multidrug-resistance-associated protein 1 (MRP1) and Breast cancer resistance protein (BCRP) are two other major drug transporters after P-glycoprotein. MRP1 was reported to be overexpressed in cancers of lung, colon and different forms of leukaemia. Further, high first-pass metabolism of taxoids by P-450 enzymes also contribute to their poor oral bioavailability. Also, poor vascularization of tumor regions reduces the drug access to the entire cancer tissue leading to poor therapeutic efficacy in cancer cells. Some of the basic drugs get ionized in the

acidic environment of the tumour which prevents their movement across the cell membrane. High interstitial pressure and low microvascular pressure may also hold back the extravasation of drug molecules. Apart from the drug efflux pumps, cancer cells also show drug resistance by decreasing the uptake of drug, increasing the drug metabolism, altering the drug targets, impair apoptotic pathways and cell cycle checkpoints.

As a solution to overcome the pitfalls of conventional chemotherapy, pursuit for effective therapeutic approaches warrants more attention, and understanding. The aim of the ideal cancer chemotherapy is to deliver the right amount of drug at a controlled rate and for adequate period of time to the site of action (tumor) to achieve the desired therapeutic outcome, causing no harm to the normal cells at the same time. To accomplish this objective, the delivery systems should be designed so as to hold required amount of therapeutic agent and eliminate the setbacks associated with bioavailability, biodistribution, clearance, non-specific toxicities, and drug resistance. The delivery system should be able to remain in the blood circulation for prolonged time, with tumor specificity, retention in tumor, and tunable drug release, providing the maximum pharmacological efficacy of the drug.

1.9 Exploring the potential of natural products-based nanomedicine for cancer therapy

1.9.1 Nano drug delivery

Nanotechnology is a rapidly growing field that has gained great attention in drug delivery and cancer theranostics (26). Nanomedicines in cancer aim to improve the pharmacokinetics and distribution of anti-cancer drugs, increasing their specificity to cancer cells. Various nanotechnology-based products have already established their benefits clinically and made their way into the market. Further, there are more than 60 active clinical trials going on involving nano drugs most of which are for anti-cancer drug delivery. The years from 2013 to 2015 had witnessed the highest number of nanoformulation-based applications of clinical trials

to USFDA, suggesting the high potential of nanotechnology-based products in the treatment of various diseases (27).

The nanometer size (1-1000 nm) and, the high surface-to-volume ratio of these materials give them a unique biological advantage which allows them to carry, bind, and absorb a variety of drugs, genetic material, peptides, and, diagnostic agents with great efficacy (28). Nanoformulations can enhance the solubility of poorly soluble drugs and improve the bioavailability of poorly absorbed drugs.

Nanocarriers investigated in cancer chemotherapy can be classified majorly into targeted and non-targeted delivery systems. Liposomes, polymeric nanoparticles, nanocrystals, dendrimers, polymeric micelles, albumin-bound nanoparticles, metal nanoparticles, and polymer-drug conjugates are some of the widely explored nano systems for the delivery of chemotherapeutic agents (29).

The drug is either entrapped in the delivery system or covalently conjugated to the system. Entrapment of drugs in the nano systems prevents the degradation of drugs in the biological system and also by-passes first-pass metabolism leading to enhanced bioavailability (30). As the solubility of the drug improves when encapsulated, it prevents the precipitation of the drug *in vivo*. The first clinical trial of nanoformulations for chemotherapeutic drug delivery dates back to the mid-1980s, and in 1995, the first nanosystem liposomes incorporating doxorubicin hit the clinic. Consequently, various nanoparticulate systems for the delivery of anti-cancer agents have been investigated owing to the benefits they offer such as enhancing solubility and stability of hydrophobic drugs, decreasing non-specific uptake, prolonging circulation time, evading undesirable off-target effects, improving cellular association, and effective targeting of tumours.

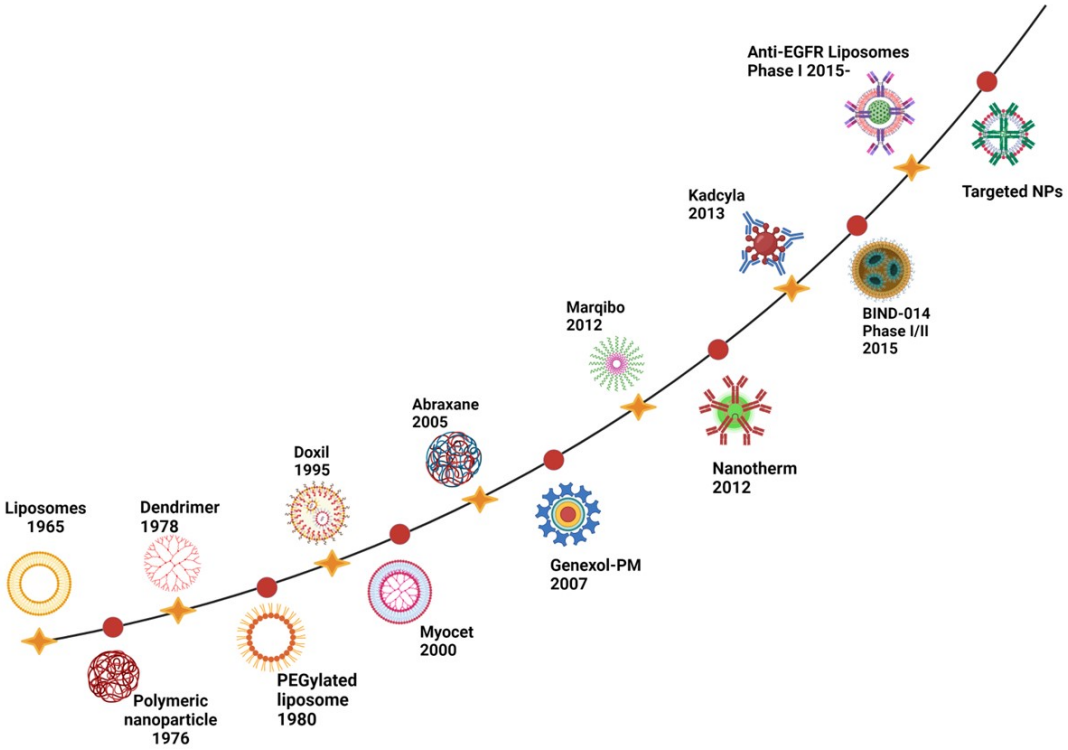

Figure 4 Types of nano systems investigated for cancer drug delivery

1.9.2 Advantages of nanocarrier systems

Nanocarriers improve the therapeutic index of drugs encapsulated in comparison to their conventional delivery systems. They increase the drug efficacy by maintaining the steady state therapeutic concentrations by controlling the drug release over a period of time. Nanocarriers are capable of evading phagocytosis by macrophages by surface modification with hydrophilic polymers such as polyethyleneglycol. The surface of nanocarriers can be tuned to attach a variety of ligands which can actively target the receptors specific to the cell. Due to the nano size of the system, they can accumulate in the tumour tissue passively by enhanced permeation and retention (EPR) effect.

Polymers used for formulation of nanocarriers such as poloxamers act as P-gp inhibitors and thereby reverse the multidrug resistance which improves the intracellular drug concentration.

Nano formulation Abraxane, which is an albumin-bound particle of paclitaxel, has been approved for the treatment of various cancers. It was found to be well tolerated compared to

the conventional paclitaxel formulation, which contains cremophor EL as the solubilizer. This has facilitated the administration of Abraxane at the desired quantities to achieve maximum benefits. Opaxio, a polyglutamic acid-conjugated paclitaxel nano drug, is under investigation. Also, a polymeric micelle-based formulation of paclitaxel (Genexol-PM) has been approved for the treatment of metastatic breast cancer and has shown a better therapeutic profile compared to conventional formulations. Nanocarriers possess the prowess to be delivered actively or passively to the target site by various approaches. Most of the approved nanoformulations are passively targeted and accumulate in the cancer tissue by a phenomenon called enhanced penetration and retention (EPR) effect.

1.9.3 Targeted drug delivery

Targeted drug delivery of nanocarriers enables the selective and efficient localization of therapeutic molecules in the target site while restricting access to adjacent normal tissues. This helps in reducing the non-specific toxicities, maximizing the therapeutic index, and improving the biodistribution of the drug, which is a major aspect in the triumph of anti-cancer drug delivery. Cancer targeting by nanoscale systems can be accomplished based on the considerations of leaky vasculature of tumors, angiogenesis, and ligand-based approach for over-expressed receptors in cancer, EPR effect, and intracellular targeting (31). Particle size, potential, hydrophilic-lipophilic properties, and chemical attachment of ligands are a few of the factors that should be looked into for effective targeting of nano-drug delivery systems.

1.9.3.1 Protein-based nanoparticles

In recent years, protein-based nanoparticles have gained remarkable interest due to their inimitable functionalities and possible applications in the field of nanomedicine and nanotechnology. A vast variety of nanoparticulate systems have been developed based on proteins such as albumin (e.g. human serum albumin, bovine serum albumin, and ovalbumin), gelatin, apotransferrin, lactoferrin, fibrinogen, ferritin, heat shock protein (Hsp), viral

nanoparticles such as cowpea chlorotic mottle virus (CCMV), cowpea mosaic virus (CPMV), lectin, collagen, milk proteins (e.g. casein, whey proteins), silk proteins, elastin, zein, gliadin, soy proteins, etc (32-35). Biomacromolecule protein overcomes various limitations of conventional therapy, including poor solubility of the drug, storage stability, poor bioavailability, and therapeutic efficacy of the drug. These protein-based systems have inherent properties of preferential uptake in tumor and inflamed tissue, biodegradability, lack of toxicity, and immunogenicity. The amphiphilic nature of the proteins allows them to interact with both the therapeutic moieties and the surrounding solvent system. The presence of functionally charged groups, including carboxylic and amino groups, offers protein numerous possibilities for surface modification and interaction with various nanoparticles and therapeutic molecules. These properties make protein an ideal delivery system.

Table 1 Food and Drug Administration and European Medicines Agency approved therapeutic nanoparticles

Nanostructure	Production	Nanoparticle formulation	Drug	Indication(s)	Confirmed
Protein-drug conjugates	Kadcyla®	Maytansine derivative DM1	Trastuzumab	HER2+breast cancer	FDA 2013
Protein-drug conjugates	Abraxane®	Albumin	Paclitaxel	Metastatic breast cancer, Non-small lung cancer, pancreatic cancer,	FDA 2012 Europe 2005, FDA 2013 Europe 2008

Protein-drug conjugates	Krystexxa®	PEGylated uricase	Pegloticase	Gout disease	FDA 2010 Europe 2013
Protein-drug conjugates	Plegridy®	PEGylated interferon-1a	Interferon-1a	Multiple sclerosis	FDA 2014 Europe 2014
Protein-drug conjugates	Adynovate®	PEGylated factor VIII	Factor VIII	Hemophilia	FDA 2015
Protein-drug conjugates	Rebinyn®	Glycopegylated coagulation factor IX	Factor IX	Hemophilia	FDA 2015

1.9.3.1.1 Albumin nanoparticles

During the last few decades, albumin-based nanoparticles have been extensively explored for their clinical applications because of their low cost, high availability, easy purification, better drug loading capacity, and implication in medicine, but the major breakthrough came with the development of FDA-approved paclitaxel–albumin nanoparticles (Abraxane, an example of nanometre albumin-bound technology (nabTM) for the treatment of metastatic breast cancer and advanced non-small-cell lung cancer. Since then, several albumin-based drugs or imaging agents have been marketed, and numerous other such products have been under preclinical and clinical trials for various biomedical applications, including oncology, rheumatoid arthritis (RA), diabetes, hemophilia, and hepatitis C. That augmented the interest in the use of albumin (mainly HSA and BSA) as a nanocarrier for biomedical applications. Albumin is the most copious plasma protein exclusively synthesized by specialized liver hepatocytes having a

molecular weight of 66.5 kDa, which constitute more than half of the human plasma protein (average plasma concentration of 40 mg/mL) and remain stable over a wide range of pH from 4–9, and also remain thermally stable when heated at 60 ºC for up to 10 h without any deleterious effects. These plasma proteins constitute a critical component in numerous biological processes, such as maintaining colloidal osmotic pressure, solubilizing long-chain fatty acids, balancing plasma pH, and delivering nutrients to cells.

Moreover, the inimitable hydrophobic and hydrophilic ligands binding property of albumin impart enhanced solubility and half-life for albumin-conjugated therapeutic molecules in plasma and thus help in improving the pharmacokinetic properties of therapeutic moieties in the biological environment (36).

Previous reports have shown that ALB is more abundant around tumors compared to normal tissues, as cancerous cells utilize ALB as a source of nutrition and energy within the tumor microenvironment. Previous studies have reported that ALB preferentially interacts and binds with albondin, which is a membrane-associated gp60 protein expressed on endothelial cell surfaces. This interaction facilitates the active internalization and transportation of ALB. Albondin primarily engages with caveolin-1, an intracellular membrane protein, and assists in the folding of the membrane, which creates small vesicles called caveolae that play a role in transcytosis. Caveolae exhibit the ability to transport ALB or ALB-related structures to the extravascular compartment (37). Thus, small anticancer compounds conjugated or entrapped within ALB-NPs are simultaneously transported to the tumor environment.

The present research indicates that the utilization of ALB-NPs as a carrier for small anticancer compounds enables their targeted delivery to the tumor environment. The conjugation or entrapment of these compounds within ALB-NPs facilitates their simultaneous transportation to the tumor site. Furthermore, the research findings demonstrate that ALB exhibits interactions with and binding to SPARC (secreted acidic protein rich in cysteine), which is over-expressed

in various types of cancer cells. The interaction between ALB and SPARC leads to the accumulation of conjugated ALB or ALB-entrapped anticancer drugs within the cancer milieu and cells (38).

A large number of therapeutic agents, including drugs, proteins, peptides, and so on, suffer severe drawbacks due to their rapid clearance from the body via the liver and kidney. This may be overcome by exploiting albumin's long half-life and its inherent property to accumulate at tumor sites and inflamed tissues.

1.10 Exploring the potential of natural products based semi-synthetic derivatives for cancer therapy

1.10.1 Structural modification of natural products

Natural products have served as a rich reservoir of compounds for pharmaceutical exploration. Several cancer-fighting medications utilized in medical practices today are derived from natural product frameworks, including vinblastine, vincristine, paclitaxel, as well as semi-synthetic options like Etoposide and Teniposide (39).

The cancer-fighting capabilities of natural products (NPrs) have garnered significant interest from both the industrial and academic sectors. Numerous screening initiatives have yielded valuable lead compounds and potential frameworks for drug development, as evidenced by the proliferation of NPr-derived drugs in clinical trials or already on the market. Collections of compounds modeled after or influenced by NPr structures are anticipated to be abundant in bioactivity, influencing various biological mechanisms.

Terpenoids stand out as the most prevalent and broadly dispersed secondary metabolites found in plants. This expansive class of natural products encompasses roughly 25,000 distinct chemical structures, showcasing a wide array of pharmaceutical properties (40).

The monoterpene framework is inherently versatile and widely recognized as a bioactive scaffold. It serves as a structural basis for numerous biologically active compounds,

demonstrating efficacy against various human illnesses. A prime illustration is chloroquine, a key drug in malaria treatment. Given its significance as a pharmacophoric scaffold, integrating the quinoline structure presents a solid foundation for devising and creating more targeted anticancer medications.

Monoterpenoids, characterized by their structural diversity, are prevalent throughout the plant world. They have been noted for their manifold biological effects and hold considerable importance in contemporary efforts to discover new drugs.

A semi-synthetic approach is employed to create analogs by utilizing the functional groups present in natural products. The modification of biologically active compounds, known as leads, plays a crucial role in drug development for both pharmacological and agricultural purposes. While the structural modification method enhances structural diversity, it has successfully produced improved versions of existing lead natural product molecules compared to their original forms. Derivatization of lead structures serves various objectives, including gaining insights into structure-activity relationships, boosting intrinsic potency, enabling oral applicability, enhancing bioavailability, mitigating unwanted side effects, and overcoming resistance mechanisms in the case of antibiotics (41).

The customization of scaffolds derived from numerous plants by researchers has led to the creation of highly potent drugs. Programs focused on optimizing these leads, known as pharmacomodulation, have yielded significant outcomes by enhancing the drugs' activity and pharmacokinetic properties (42).

Structural units featuring nitrogen-based heterocycles are of considerable interest in diverse scientific fields, including anticancer, antinociceptive, antipyretic, antimicrobial, antiepileptic, antituberculosis, antiviral, anticoagulant, and antiplatelet applications. Several synthesized compounds within these domains have gained approval from the FDA (43, 44). In this context, triazoles have proven to be highly effective against various types of tumor cells (45-47). Hybrid

compounds that incorporate 1,2,3-1H-triazole linked with various natural products have demonstrated encouraging *in vitro* anticancer outcomes (48-50).

The 1,2,3-triazole group serves as an essential pharmacophore with diverse pharmacological effects. Employing Click chemistry enables the straightforward synthesis of 1,2,3-triazoles, which has proven effective in enhancing the pharmacokinetic profiles of target drugs.

In recent times, the utilization of Click chemistry with natural compounds has garnered significant attention. Various molecules under investigation encompass alkaloids, coumarins, saponins, steroids, and triterpenes like betulinic acid. Triazoles and their variations hold substantial significance in medicinal chemistry, facilitating the creation of numerous heterocyclic compounds with diverse biological effects, including antiviral, antibacterial, antifungal, anti-tuberculosis, anticonvulsant, antidepressant, anti-inflammatory, and anticancer properties.

Within the domain of drug development, there's a growing interest in altering the structure of natural products to either boost their effectiveness or unveil new properties. Among these methodologies, the utilization of click chemistry to incorporate 1,2,3-triazoles into drug compounds stands out as a noteworthy tactic (51).

1.10.2 Molecular hybridization approach and its role in enhancing the therapeutic effect of natural products

Natural products have consistently served as a unique reservoir of potential lead compounds for medicinal chemists, offering a diverse array of promising drug candidates for the treatment of various diseases and disorders in clinical practice (52).

The strategy of molecular hybridization is a proven and efficient approach in drug development. It involves combining two or more pharmacophores, with or without a linker, to create a hybrid molecule that inherits the pharmacological properties of the parent

pharmacophores. This process aims to achieve a hybrid with enhanced potency, decreased toxicity, and reduced side effects. Past pharmacological findings indicate that monoterpenes such as thymol and carvacol can serve as distinctive pharmacophores suitable for combination in the development of new anticancer agents (53).

The 1,2,3-triazole serves as a significant bioisostere for ester, carboxylic acid, amide, and various heterocycles. It engages with a range of proteins, enzymes, and receptors through diverse hydrophobic and hydrophilic interactions (54). Additionally, the 1,2,3-triazole acts as an effective linker with a wide array of pharmacological properties. Successfully employed in combining terpenes with different pharmacophores, the 1,2,3-triazole demonstrates inhibitory properties and favorable molecular interactions with receptors like EGFR, Erα etc. This justifies the optimal pairing of terpenes and 1,2,3-triazole pharmacophores.