Bristol Publishers

Journal of Pharmaceutical and Pharmacology Research

Review Article | Open Access | Volume: 01 Issue: 01 (October-December 2026) Pages: 14-30

New Developments in the Study of Liposomes for Cancer in Pharmaceuticals

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Received: 19 November 2024; **Revised:** 13 February 2025; **Accepted:** 11 March 2025; **Published:** 05 July 2026

Academic Editor: Prof. Dr. K.N.V. Rao

Abstract

Globally, cancer remains the second most common cause of death, posing major therapeutic challenges due to factors such as tumor heterogeneity, metastasis, and the limitations of traditional chemotherapy. Drug delivery methods based on liposomes have shown promise in overcoming these challenges. These types of phospholipid bilayer liposomes will encapsulate both hydrophilic and hydrophobic drugs that can be directed to location of action by either passive (Enhanced Permeability and Retention effect- EPR) or active (ligands or antibodies). As a result, this approach improves the effectiveness of treatments while minimizing systemic toxicity. Advances in liposomal technology, such as PEGylated "Stealth" liposomes, improve circulation time and stability, exemplified by FDA-approved formulations like Doxil®. Recent innovations include pH- and temperature-sensitive smart liposomes and nanoliposomes, which exhibit enhanced stability, bioavailability, and precision targeting. Hybrid lipid-polymer systems and stimuli-responsive liposomes enable controlled drug release triggered by environmental or external cues like pH, temperature, or ultrasound. These advancements overcome challenges such as drug resistance, the blood-brain barrier, and off-target effects, paving the way for more effective cancer therapies. Liposomal systems now extend to combination therapies and theranostics, offering potential breakthroughs in personalized cancer treatment. These advancements improve tumor targeting, minimize systemic toxicity, and enhance therapeutic efficacy, contributing to better patient outcomes and advancing precision oncology. Ongoing research focuses on optimizing liposomal formulations to further enhance stability, efficacy, and clinical benefits.

Keywords: Liposome; Cancer; Stealth liposome; PEGylated liposome; EPR effect; Nanomedicine; Immunoliposome; Combination therapy

Introduction

The second most common cause of mortality globally is cancer, surpassed only by cardiovascular diseases [1]. Treating cancer remains a complex and evolving challenge due to the intricate nature of the disease. One significant obstacle is the heterogeneity of cancer cells, which can differ widely between patients and even within a single tumor. This variability often results in resistance to conventional therapies like chemotherapy and radiation, as not all tumor cells respond uniformly [2]. Traditional chemotherapy further complicates treatment by lacking specificity, indiscriminately targeting both healthy and cancerous cells. This nonspecific action leads to severe side effects and limits the therapeutic effectiveness of such treatments [3].

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The toxicity of these traditional anticancer drugs limits dosage, sometimes preventing a therapeutic dose from being administered. Additionally, cancer's tendency to metastasize to other organs complicates treatment, requiring drugs that can target both primary and secondary tumors. The blood-brain barrier (BBB) presents an additional challenge for treating brain cancers, as many drugs cannot penetrate this protective shield. These complexities emphasize the need for more targeted, less toxic, and barrier-penetrating treatments in cancer treatment [4].

An effective drug delivery solution to combat the challenges of cancer treatment lies in the technology based around liposomes. Liposomes are tiny vesicles with type of spherical phospholipid bilayer structures surrounded by internal aqueous compartments and have common characteristics to cell membranes. These vesicles should be understood to encode the two activity that can encapsulate either hydrophilic successfully, also hydrophobic the drug. Liposomal formulations offer passive and active targeting options. Due to the porous nature of tumor blood vessels, they are also able to accumulate in tumor tissues via Enhanced Permeability and Retention (EPR) effect. Additionally, liposomes can be engineered to actively target specific cancer cells employing various ligands or antibodies to ensure that drugs are delivered directly to tumours without exposure of healthy organs. Such a precision strategy improves the effectiveness of treatment and is also associated with fewer side effects. In addition, liposomes render the drug stable and protect them from degradation, while enabling a controlled release that makes these carriers very useful in cancer treatment [5,6].

The development of liposomal formulations began in the 1960s when Dr. Alec D. Bangham discovered that phospholipids could form bilayer structures in water, leading to the concept of liposomes. By the 1970s, researchers explored liposomes for drug delivery, especially for anticancer drugs, but faced challenges like rapid clearance from the bloodstream. The 1990s saw a breakthrough with the introduction of Stealth liposomes, incorporating polyethylene glycol (PEG) to avoid detection by the immune system and extend circulation time [7]. The first major advancement came from the FDA approval of Doxil® in 1995 (a liposomal doxorubicin), which demonstrated improved delivery to tumors, as well as decreasing cardiotoxicity. In the last years, liposome technology has continued to improve with ligands and antibodies for targeted delivery. Current research involves theragnostic liposomes that integrate diagnostic and therapeutic capabilities as well as stimuli-responsive liposomes that release drugs based on specific triggers such as pH or temperature to achieve greater specificity in treatment targeting [8].

Liposome Technology for Cancer:

Liposome Structure and Composition

Liposomes are nanometric-sized spheres composed of one or more concentric phospholipid bilayer sheets. which closely mimic the structure of cellular membranes (Figure 1). Liposomes are constructed from a phospholipid bilayer, wherein the core is aqueous for hydrophilic drug encapsulation and the lipid bilayers contain hydrophobic compounds [9]. This double functionality allows liposomes to provide a great number of different therapeutic agents. Hydrophilic heads and hydrophobic tails of phospholipids are the main molecular components of liposomes, which spontaneously self-assemble in aqueous solution [10]. It is usually added to the lipid bilayer to improve membrane stability, decrease bilayer permeability and then regulate the release rate of the encased drugs. Such as when the cholesterol content in liposomes changed from 30% to 50%, drug leakage would decrease and almost all were persistently present in circulation through examinations of tissues. In the case of doxorubicin-loaded liposomes (Doxil®, known for its stability), cholesterol entraps in a lipid bilayer, thereby enhancing drug stability and controlling release due to rate retardation. Likewise, a 40% reduction in the permeability of lipid nanoparticles was observed with cholesterol incorporation [100,101], further improving delivery efficiency [11,12]. Other substances, for instance surfactants and stabilizing agents, may be added to make the liposome more effective and also provide components (such as glycoproteins) which will increase blood circulation time duration and target specific tissues or cells. Table 1 Advantages of liposomal drug delivery in cancer [13].

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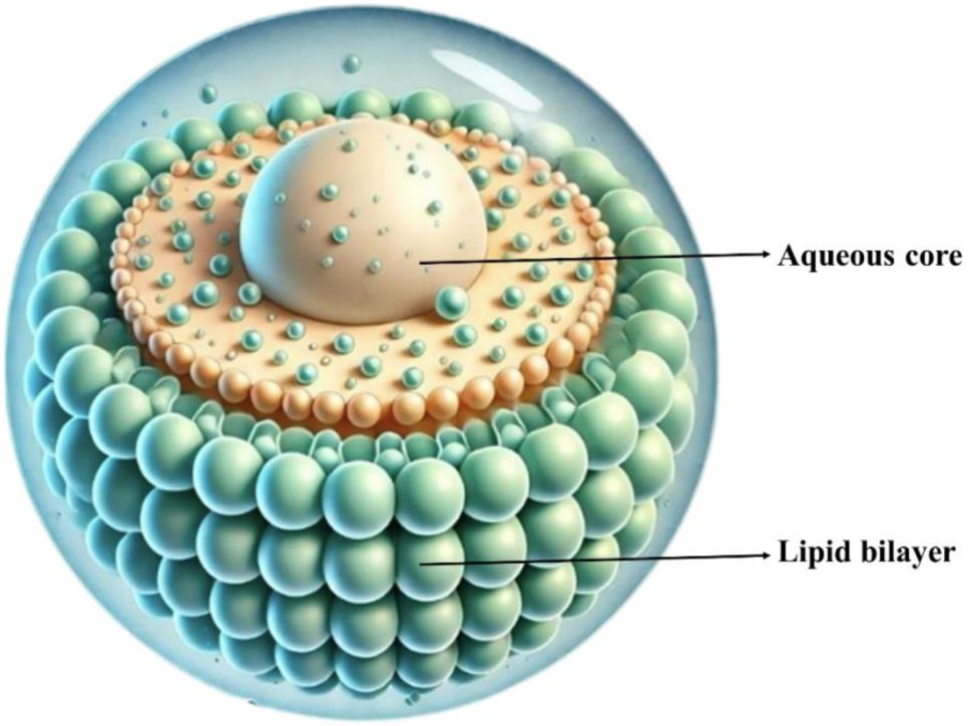

Figure 1: Diagrammatic representation of Liposome.

Table 1: Advantages of Liposomal Drug Delivery in Cancer.

Advantage	Description
Targeted Drug Delivery	Liposomes can passively target tumors through the effect of enhanced permeability and retention (EPR), which raises the concentration of drugs in tumor tissues. Active targeting is also possible by adding specific ligands or antibodies [14].
Reduced Systemic Toxicity	By focusing on cancerous cells, liposomes minimize exposure to healthy tissues, reducing side effects associated with chemotherapy.
Improved Drug Stability	Liposomes protect encapsulated medications to prevent enzyme degradation and other biological conditions, increasing shelf-life and stability [15].
Versatile Drug Encapsulation	Can carry both hydrophilic (water-soluble) and hydrophobic (fat-soluble) drugs, making them suitable for a wide range of therapeutic agents.
Controlled and Sustained Release	Liposomes can be engineered for controlled release, allowing a gradual drug release over time, which can enhance therapeutic effectiveness and reduce dosing frequency [16].
Enhanced Bioavailability	Liposomes can improve the bioavailability of drugs, allowing more of the drug to reach the target site effectively.
Decreased Drug Resistance	By delivering medications straight to cancer cells and avoiding some resistance mechanisms, liposomal formulations can aid in the fight against drug resistance.
Ability to Cross Biological Barriers	Liposomes can be designed to penetrate biological barriers like the blood-brain barrier (BBB), making them suitable for treating brain cancers.
Minimized Immunogenicity	PEGylated (Stealth) liposomes can evade the immune system, extending circulation time and reducing clearance by immune cells.
Combination Therapy Potential	Liposomes can carry multiple drugs or a combination of therapeutic and diagnostic agents (theranostic liposomes), facilitating combination therapy for better outcomes [17].

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Types of Liposomes:

Conventional Liposomes

There are many types of liposomes, but conventional liposomes are the most simple ones; they mainly consist of natural or synthetic phospholipids accompanied by cholesterol. They are used for encapsulation of several drugs, especially anticancer and antifungal agents [18]. The EPR effect delivers diseases specific tissue targeting and reduces systemic toxicity through the use of these liposomes enhancing drug bioavailability for instance, cancers [14]. Even though they have these advantages, conventional liposomes have cumbersome challenges to overcome such as being removed from the bloodstream by the mononuclear phagocyte system or reticuloendothelial system (RES) so very quickly. The rapid clearance of these immune cells results in a decreased blood circulation time, limiting their drug-delivering capabilities [19].

PEGylated Liposomes

It is a new generation of the classic liposomes that are called as PEGylated or in other words "Stealth" liposomes. These liposomes are subsequently modified to contain surface polyethylene glycol (PEG)attached to the liposome [20]. PEG has a hydrophilic property, which provides a hydrophilic barrier onto the liposome to make it less visible to the immune system so as to prolong its circulation time. Sixth, the extended half-life of PEGylated liposomes facilitates drug accumulation in tumor tissues and thereby improves therapeutic efficacy [21]. One well known example is Doxil®, a PEGylated liposomal formulation of the anticancer drug doxorubicin. PEGylation increases stability and allows less frequent dosing, thus reducing the burden on patients [22]. On one hand, PEGylation is also known to decrease cellular uptake, making it essential to introduce other modifications in order to allow efficient targeting. In these strategies, some targeting ligands (e.g., antibodies or peptides) are conjugated to PEGylated nanoparticles and are utilized to improve receptor-mediated endocytosis. Cleavable PEG linkers that release cargo in the tumor microenvironment can help restore uptake. The optimization of PEG density and chain length reduces the stealth effect while enhancing interaction with cells, thus increasing the efficiency of drug delivery [23]. El-Shafie et al. Methods: We prepared the first PEGylated liposomal nedaplatin by thin film hydration, and its platinum uptake-cytotoxicity-genotoxicity relationships were evaluated in A549 and U2OS cancer cells. Importantly, in these studies, liposomal nedaplatin demonstrated selective cytotoxicity against cancer cells with no impact on non-cancerous cells suggesting its potential for the targeted therapy of cancer [24]. Mirzavi et al. PEGylated liposomal formulations of CA4 with arrays of lipid compositions were developed, and showed that 70:5:20:5 (HSPC/DSPE-mPEG2000/Cholesterol/CA4); was optimal (CAS-Embry). F3 of ɣ-KG was developed with high encapsulation efficiency, stable release profiles and strong in vitro anti-TNBC activity (inducing cell cycle arrest and decreasing MMP expression). F3 liposomes exhibited selective in vivo tumor accumulation, growth inhibiting and survival improving effects, provid-ing a potential treatment strategy for TNBC [25].

Immunoliposomes

Definition: Immunoliposomes are a type of liposome designed for target specific drug delivery, typically achieved by covalently linking antibodies or antibody fragments to the liposomal surface. These antibodies are selected to recognize and bind to one or more antigens expressed on the surface of cancer cells or other target tissues [26]. This targeted delivery enables a more specific way to administer the drug, exposing less healthy cells with high therapeutic efficacy. Immunoliposomes are a hybrid form that integrates both passive targeting benefits of conventional and PEGylated liposomes with an additional active mechanism by which the drug can preferentially accumulate in targeted tissues [27]. This property is why they have become extremely useful for cancer therapy particularly where specificity of treatment is of importance. The high complexity of the immunoliposome formulation raises manufacturing costs and antibody-conjugated liposomes are particularly unstable. Ahad et al. While critical efforts have been directed to targeted delivery of gold compounds for exploiting their anticancer properties, Li et al.

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Opt-Immunolipo-Tras-2 and Opt-Immunolipo-Tras-3 manifested maximal HER2 affinity and intracellular delivery in breast cancer cells, while the fluorescently conjugated formulations, Immunolipo-Tras-3 (along with its small peptide sequences), displayed mitochondrial and ER localization as opposed to lysosomal accumulation of free (non-liposomes) drugs.

This potential of gold-based immunoliposomes as single-photon emission tracers detectable by SPECT may be useful for targeted breast cancer therapy [28]. Narayanaswamy et al. produced immunoliposomes modified with mAb 2C5, in a combination of paclitaxel and salinomycin in order to target the bulk tumor and CSCs in breast cancer models (MDA-MB-231 and SK-BR-3). Liposomes were characterized for their size (170-220nm), drug loading and uptake. Cancer cell proliferation was significantly reduced when WSWQ was used in vitro according to the wound closure inhibition and cell-killing assays. Ex vivo studies demonstrated low toxicity and only minor haemolysis in a mouse model, providing support for its potential therapeutic applications specifically to treat breast cancer [29].

Targeted Drug Delivery Systems: Mechanisms of Action

Enhanced permeability and retention (EPR) effect

Enhanced Permeability and Retention (EPR) effect is one of the main mechanisms used in anticancer therapy that exploits selective accumulation of medicines in the tissues of some types of tumors [30]. Tumor-associated vasculature is heterogeneous, characterized by large time gaps between neighboring endothelial cells that facilitate the infiltration of macromolecules into the tissue, where they reside in high concentrations [31]. On the contrary, normal blood vessels possess more stably tight cell junctions meaning drugs cannot enter through them and therefore off-target toxicity is limited. Consequently, the EPR effect allows liposomes and other larger drug carriers preferentially into the tumor interstitium and remain for higher drug availability at the cancer site (Figure 2) [32].

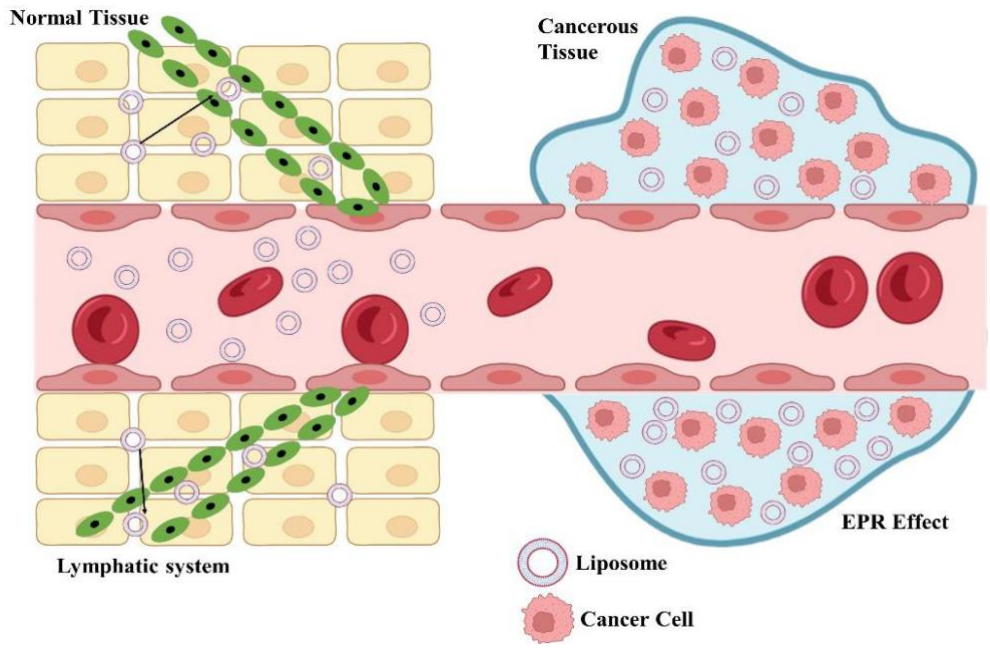

Figure 2: Diagrammatic representation of EPR effect. Normal blood vessels have smooth muscle layers and tight cell junctions, restricting macromolecule extravasation. In contrast, tumor blood vessels have loose cell junctions, allowing macromolecules to escape into tumor tissue. Additionally, impaired lymphatic drainage in tumors causes these agents to accumulate within the tumor tissue.

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Targeted Drug Delivery to Tumor Cells

TDD builds upon EPR effect by employing specific ligands or antibodies on drug carriers to bind directly to receptors overly eloquent on cancer cells. For example, folate receptors or HER2 proteins, commonly present in high numbers on cancer cells, can serve as docking sites for drug-loaded nanoparticles [33]. This active targeting enhances cellular uptake and reduces systemic toxicity by sparing non-cancerous cells. Ligand-based targeting, combined with EPR, creates a powerful synergy for maximizing drug concentration within the tumor while minimizing side effects [32].

Release Mechanisms of Drugs from Liposomes

Liposomes serve as versatile carriers, encapsulating drugs within a lipid bilayer that protects the drug from breakdown and controls release. Drug release from liposomes occurs through various mechanisms, including:

- Passive diffusion: Slow drug leakage over time from the liposome, influenced by pH or temperature changes.
- Enzyme-mediated release: Certain enzymes, like phospholipases, degrade the liposome membrane in the tumor microenvironment, allowing drug release.
- Stimuli-responsive release: Advanced liposomes are designed to respond to external stimuli like light, heat, or ultrasound, which trigger drug release precisely at the tumor site [34-36].

Recent Advancements in Liposome Formulations

The combination of their biocompatibility, the feasibility to formulate a wide variety of solutes including both hydrophobic and hydrophilic agents in the same medication have allowed liposomes to be commonly used as drug delivery vehicles. Different advancements over the years have been integrated into liposome formulations to solve issues related to stability, control of drug release, and targeted delivery. Recently developments include new compositions in the lipids axis to boost the stability, nanoliposomes for improved efficacy and smart liposomes which respond to very specific environmental stimuli [37,38].

Newer Lipid Compositions for Improved Stability

However, the lipid bilayer of an authentic liposome formulation is not stable, which continues to be one of the challenges associated with traditional liposome formulations; the lipid membrane degrades quickly during storage or circulation in blood. Conventional liposomes are vulnerable to destruction by oxidative stress, pH changes, and plasma protein interactions which can cause the early leakage of drugs encapsulated within them from their phospholipid membranes before reaching the target site and therefore have a reduced therapeutic efficacy. In order to overcome these drawbacks, several new lipid combinations and monomers are being evaluated that can improve the mechanical stability of liposomes [39,40].

Incorporation of Cholesterol to Liposomes: Cholesterol is one of the most widely used stabilizer in liposome formulation. Researchers improve membrane stability, rigidity at the cost of its permeability by inserting cholesterol molecules into lipid bilayer. Cholesterol, in turn, also prevents excessive clustering of the phospholipids, which decreases bilayer instability [41,42].

PEGylated Lipids (Stealth Liposomes) PEG can be conjugated to lipids in the bilayer of the liposomes creating so-called “stealth” liposomal formulations. PEGylation affords a hydrophilic shell around the surface of the liposome which decreases opsonization by proteins in the bloodstream and leads to lower uptake into cells of the mononuclear phagocyte system. What this stealth property does is extend circulation time, it employs the Enhanced Permeability and Retention (EPR) effect thereby increasing the likelihood that the targeting feature will arrive to its target such as a tumor. [43,44].

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Use of Cationic Lipids for Gene Delivery: For gene therapy applications, cationic lipids are used to stabilize liposomes and facilitate the encapsulation of negatively charged genetic materials, such as DNA or RNA. Cationic liposomes form complexes with nucleic acids, which enhances cellular uptake by electrostatic interactions with negatively charged cell membranes. Additionally, the positive charge promotes endosomal escape, ensuring effective gene delivery [45,46].

Hybrid Lipid-Polymer Liposomes: Combining lipids with biodegradable polymers like polylactic-co-glycolic acid (PLGA) has shown promising results for improving stability and controlling drug release. In these hybrid liposomes, the lipid bilayer provides biocompatibility, while the polymer component enhances structural integrity and controls the rate of drug release. This combination also enables targeted and sustained delivery in challenging environments, such as inflamed tissues or tumors [47,48].
These new lipid compositions have led to more stable liposome formulations that can withstand physiological stressors, leading to increased drug delivery efficiency and patient outcomes.

Nanoliposomes: Enhancing Efficacy with Size Reduction

Nanoliposomes, a smaller and more refined version of conventional liposomes, typically have diameters ranging from 10 to 200 nm. Size reduction brings several advantages, including enhanced circulation time, improved cellular uptake, and the ability to cross biological barriers more effectively.
Enhanced Bioavailability and Cellular Uptake: Due to their small size, Nanoliposomes have an ability to cross all biological barriers (such as the blood-brain barrier>100nm liposomes). Piazzini et al. The authors designed Tween 80- and DDAB-modified liposomes (LPs-AG, CLPs-AG) to improve the brain delivery of andrographolide (AG). Liposomes were characterized by appropriate size, stability and a high loading percentage. In the absence of cytotoxicity, studies on transport (PAMPA, hCMEC/D3) derived AG permeability significantly increased. Caveolae-mediated endocytosis was the main uptake pathway of DDAB, which facilitated internalization via this endocytic route. They make good AG brain delivery vehicles as these liposomes.[49]. They also exhibit enhanced endocytosis by cells, especially in tissues with high cellular turnover like tumors. Nano liposomes are particularly useful for cancer therapy as their small size enables them to build up inside tumors through the EPR effect., allowing for a higher local concentration of the drug [50].
Improved Drug Loading: Nanoliposomes are tailored to maximize the surface area/volume ratio, which facilitates higher drug loading while minimizing particle sizes. Other techniques, such as active loading (where drugs are loaded into pre-formed nanoliposomes through pH gradient or other mechanisms to increase encapsulation efficiency and stability of most drugs), have also been developed.
Reduced Immunogenicity: Due to their reduced size and ability to evade the immune system, nanoliposomes are less likely to trigger an immune response. This feature is particularly important for chronic treatments, where repeated doses may lead to immune reactions. With stealth modifications (e.g., PEGylation), nanoliposomes exhibit prolonged circulation time, leading to a better therapeutic window for various diseases [51].
Application in Vaccines: Nanoliposomes are also gaining popularity in vaccine delivery. Their small size enables effective delivery of antigens to immune cells, enhancing immune response and providing long-lasting immunity. Nanoliposome-based vaccines have been explored for infectious diseases and cancer immunotherapy due to their ability to carry both antigens and immune-activating adjuvants, increasing vaccine efficacy [52].
Overall, nanoliposomes represent a promising advancement in liposome technology, enhancing efficacy through better bioavailability, controlled release, and improved delivery to target cells.

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Smart Liposomes: pH-Sensitive and Temperature-Sensitive Formulations

Smart liposomes are engineered to release their contents in response to specific environmental signals, such as temperature or pH changes, or other external stimuli. The method of controlled-release helps to target drugs to the location and time of need, thus enhancing treatment outcomes and decreasing systemic toxicity.

pH-sensitive liposomes: pH in the tumor microenvironment and in certain cellular compartments such as endosomes and lysosomes is low (pH5-6). pH-sensitive liposomes use this property by integrating fairly stable lipids or polymers at neutral pH, which axiomatically destabilize in acidic circumstances allowing drug release solely in acid [45]. For instance, by using pH-sensitive liposomes including either phosphatidylethanolamine (PE) or synthetic pH-sensitive polymer as the drug carrier to tumor/infected tissue targeting with less impact on normal tissues [53].

Thermo-Sensitive Liposomes: These liposome formulations are engineered and actuated to release their contents at elevated temperatures, e.g.39-42oC slightly above the normal temperature of the body. These particular liposomes aid in localized cancer treatment, administered with hyperthermia therapy. Clinicians can induce localised heating at the location of a tumor, causing localized release of the drug exactly where it needs to be. The most common example of these are DPPC (dipalmitoyl phosphatidylcholine) containing liposomes that exhibit a phase transition at high temperature to release the drug content [54,55].

Externally Triggered Release (Ultrasound, Magnetic Fields) Apart from internal stimuli, liposomes can be designed to release their contents in response to external triggers such as ultrasound or magnetic fields. Ultrasound-sensitive liposomes, for example, are a type of drug delivery system that releases drugs at the site when ultrasound waves are applied.[7] Ultrasound has a mechanical effect which disrupts the lipid bilayer, and as a result, the drug can be released at the site of interest. To construct magnetic field-sensitive liposomes, certain magnetic nanoparticles are integrated that reacts to an external magnet and deliver itself towards the site of tumour through a doppler effect, where it can release its content in a controlled and precise manner [56].

FDA-Approved Liposomal Drugs for Cancer

1.Doxil:

Doxil is a drug made from doxorubicin (DOX) hydrochloride, and it uses polyethylene glycol (PEG)-liposome technology to create a nano drug delivery system. This formulation was approved by the FDA in 1998 and is used for treating conditions like metastatic extra-ovarian primary peritoneal carcinoma, myelodysplastic syndrome, and Kaposi's sarcoma in patients with advanced HIV [57]. The liposomes in Doxil are composed of a mixture of lecithin, MPEG-DSPE (a polyethylene glycol derivative), cholesterol, and hydrogenated soy phosphatidylcholine (HSPC), in a ratio of 56:38:5. A pilot study involving 15-20 cancer patients examined how the drug moves through the body (pharmacokinetics). The Volume of Distribution for Doxil (4 L) was much lower than that of the unbound drug (254 L). In a further analysis, Doxil was slowly eliminated due to the rate of 0.1 L/h vs. 45 L/h for the unbound drug at the end of its circulation from their systemic elimination phase and it demonstrated bi-phasic clearance kinetics as shown by two-half-lives from throughout cancer patient studies respectively (2–12 hours & 27–35 or/or [58-60].

Furthermore, Doxil was found to deliver significantly more DOX to the tumors-4 to 16 times greater than the standard unbound drug. Importantly, because the DOX is encapsulated within the liposomes, it is less likely to affect the heart cells, reducing the typical toxic side effects of doxorubicin.

2. DaunoXome®:

DaunoXome® is a liposome formulation of daunorubicin (DNR) citrate intended for intravenous administration. Budésonide was created by Nexstar Pharma, USA in 1996 for the therapy of Kaposi's sarcoma amongst individuals with superior HIV infection. Each vial contains 50 mg of daunorubicin in liposomes of 704 mg di-stearoyl phosphatidylcholine (DSPC) plus 168 mg triglycerydes.

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Study of the Safety, Bioavailability and Antitumor Activity at Increasing Multiple Doses of DaunoXome® Administered in Male and Female Adult Patients With AIDS-Related Kaposi's Sarcoma. In clinical trials, DaunoXome® was shown to be efficacious at doses between 50 and 60 mg/m² which produced an area under the curve (AUC) of 114.92–120.2 μg·h/mL. These discoveries demonstrated 11–12 value of focus expansion when contrasted and free daunorubicin, delivered from liposomal transporter directed discharge as exhibited in mouse models [61,62].

Furthermore, compared to patients receiving traditional daunorubicin, those treated with DaunoXome® had significantly lower excretion rates (10.4 mL/min vs. 234 mL/min). Additionally, the half-life of DaunoXome® was extended to 4-5.6 hours, compared to the 0.77 hours observed with unbound daunorubicin. These pharmacokinetic improvements highlight the superior profile of DaunoXome® over unencapsulated daunorubicin [63].

3. Depocyt®:

Skye Pharm Inc. developed Depocyt, a medication designed to treat neoplastic meningitis (NM), a type of cancer in the brain and spinal cord. Depocyt is a liposome-based drug containing aracytidine (Ara-C), an antimetabolite used to treat cancer. It utilizes a technology called DepoFoam™, which consists of small, spherical particles that encapsulate the drug in a water-based solution. This structure allows for controlled, delayed release of the drug over time, improving the treatment's effectiveness [64].

The DepoFoam™ particles are made up of 96% water and 4% biocompatible lipids, which are safe for the body and can be processed like other natural lipids. These particles are larger than traditional liposomes, allowing them to hold more drug. Depocyt is injected into the cerebrospinal fluid (CSF) around the brain and spinal cord, where it releases aracytidine over a prolonged period. Each vial of Depocyt contains 50mg of aracytidine in a 5mL solution. Other ingredients include sterol, glyceryl trioleate, and phosphatidylcholine. The pH of the solution is maintained between 5.5 and 8.5. Because the particles are dense, they tend to settle at the bottom of the vial, so the solution needs to be gently mixed before use [65,66].

In clinical studies, Depocyt showed that aracytidine remained in the CSF for much longer (over 141 hours in the ventricle, compared to just 3.4 hours for standard Ara-C). This prolonged exposure improves the drug's effectiveness in treating cancer cells in the brain and spinal cord. In trials, Depocyt was found to work similarly to methotrexate in treating neoplastic meningitis, but with the added benefit of enhancing neurological development. Overall, Depocyt provides a more effective way to deliver aracytidine to treat cancer in the meninges and CSF, with extended drug release improving its ability to target and kill tumor cells [67].

4. Myocet:

Myocet is a gemcitabine-based liposome formulation of doxorubicin (DOX) without polyethylene glycol (PEGylation), in the treatment of metastatic breast cancer, especially as the first line combination with cyclophosphamide. Myocet aims to reduce the cardiotoxicity of DOX, while preserving its tumor-fighting power. Myocet liposomes are 150–250 nm in size, with a 45:55 molar mixture of cholesterol and acidic egg phosphatidylcholine (EPC). Myocet is a long-circulating liposome with a drug-to-lipid ratio of ~0.27, interacting less with normal tissues due it larger size to diminish both acute and chronic side effects.[68,69].

Myocet liposomes are created by preparing blank liposomes in an acidic sodium citrate buffer, followed by the addition of sodium carbonate to adjust the pH to 7.3. DOX is then loaded into the liposomes, where it becomes protonated in the aqueous core [70]. The protonated DOX forms ion pairs with negatively charged lipids in the membrane, facilitating its entry into the liposome. This process results in an encapsulation efficiency of over 99%, limiting DOX's ability to cross the lipid bilayer.

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In preclinical studies with Beagle dogs, Myocet showed higher toxicity than free DOX. However, tumor concentration studies demonstrated that Myocet delivered 2-3 times more DOX to tumor cells compared to unbound DOX, and 10 times more in ascitic models. This increased concentration of DOX in tumors led to the decision to proceed with human trials [71]. In a Phase I clinical trial, Myocet was compared to free DOX in 38 patients with resistant tumors. Myocet resulted in fewer side effects, such as myelosuppression and gastrointestinal issues, while showing comparable treatment efficacy and progression-free survival. A Phase 3 study also showed that the combination of Myocet (60 mg/m²) and cyclophosphamide (600 mg/m²) was as effective as the combination of free DOX and cyclophosphamide but with lower toxicity. This combination demonstrated equal effectiveness and reduced side effects in breast cancer treatment [72].

5. Onivyde™:

Onivyde (irinotecan liposomal injection), approved by the FDA in 2015, is used for metastatic pancreatic adenocarcinoma in patients who progress after gemcitabine-based therapy. It is a liposomal formulation of irinotecan, a topoisomerase inhibitor, encapsulated in unilamellar liposomes with a diameter of approximately 110nm [73]. These liposomes are stabilized with long-circulating lipid membranes and incorporate polyphosphate and sucrose octasulfate, enabling efficient encapsulation at high drug-to-lipid ratios. Onivyde's formulation extends irinotecan's half-life to 56.8 hours. Studies show liposomal irinotecan has better drug entrapment and prolonged persistence compared to free irinotecan, leading to enhanced cytotoxicity. The NAPOLI-1 clinical trial demonstrated its effectiveness, demonstrating that patients getting Onivyde, fluorouracil, and leucovorin had a median in general survival of 6.1 months, while those receiving fluorouracil or leucovorin alone had a median overall survival of 4.2 months [74,75].

Liposomes in Clinical Trials for Various Cancers

Many liposomal products have gone through clinical trials over the past few years. In the last decade, a large amount of work has been performed in formulating novel liposome-mediated drug delivery systems that is supported by 'lipid carriers. [76]. For this reason, we have summarized some liposomal products under clinical investigations (Table 2).

Table 2: Liposomes under different phases of clinical trials.

Sl. No.	Govt. Clinical Trail ID	Drug	Phase	Summary
1	NCT04196257	BP1001	I	This Phase I, open-label study evaluates BP1001-A in advanced solid tumors. The dose-escalation phase identifies the MTD/MAD as a single agent, while the dose-expansion phase assesses its safety, toxicity, and response combined with paclitaxel.
2	NCT00316511	Aroplatin	II	This dose-escalation study intends to ascertain the maximum tolerated dose (MTD) of intravenous L-NDDP and its pharmacokinetics. Tumor response in solid tumors and B-cell lymphoma will be assessed using RECIST and International Working Group criteria, respectively.
3	NCT01190982	LEP-ETU	II	LEP-ETU, a novel paclitaxel delivery system by NeoPharm, uses phospholipids and cholesterol instead of polyoxyethylated castor oil, reducing hypersensitivity risks. This improved formulation allows higher dosing with better tolerance, as shown in Phase I trials, offering a safer and more effective alternative to traditional Taxol.
4	NCT02702700	Lipoplatin	I	This study investigates intrapleural low-dose Visudyne®-mediated photodynamic therapy to enhance Lipoplatin™ uptake in pleural malignancies, aiming to overcome cisplatin resistance and improve tumor control in patients undergoing video-assisted talcage for malignant pleural effusions.
5	NCT01861496	LiPlaCis	II	LiPlaCis, the first tumor-targeted liposomal cisplatin with a triggered release mechanism, aims to enhance the therapeutic index of cisplatin by improving tumor selectivity, reducing toxicities, and addressing the limitations of conventional cisplatin in cancer treatment.

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6	NCT01094548	Tecemotide	II	This study evaluates whether tecemotide (L-BLP25) vaccination triggers a MUC1-specific T-cell response in chemotherapy-naive or slowly progressive multiple myeloma patients.
7	NCT00177281	S-CKD602	I	This study evaluates the investigational anti-cancer drug S CKD602, developed by ALZA Corporation, focusing on its side effects, action in the body, and impact on the disease.
8	NCT00470613	SGT-53	I	This Phase Ib study evaluates the safety of SGT-53 combined with docetaxel and determines their recommended Phase II doses for further clinical studies targeting solid tumors.
9	NCT00441376	Thermodox	I	This study intends to ascertain the maximum tolerated dose (MTD) of ThermoDox combined with radiofrequency ablation (RFA) for treating primary and metastatic liver tumors.
10	NCT02139280	Cyclophosphamide	Completed	This randomized trial evaluates whether a lower cyclophosphamide dose (1.5 gm/m²) is as effective as an intermediate dose (3 gm/m²) in mobilizing autologous hematopoietic stem cells for collection and resource efficiency.

Innovative Approaches and Future Perspectives:

Immunoliposomes and their Role in Immunotherapy

Immunoliposomes are a class of drug delivery systems that combine liposomes using targeting molecules such as antibodies or antigens. These nanoparticles are designed to selectively deliver therapeutic agents, like drugs, genes, or proteins, to particular cells or tissues. By integrating the selective targeting ability of antibodies with the delivery efficiency of liposomes, immunoliposomes offer significant potential in improving the specificity and efficacy of treatments, especially in cancer immunotherapy, autoimmune diseases, and targeted drug delivery.

One of the most prominent examples of clinical applications of immunoliposomes is an advanced stage liposomal formulation (such as Doxil) of anti-cancer drugs [21-25]. One of the most important products is Doxil, which was a liposomal formulation of doxorubicin which used to treat many forms of cancer especially breast and ovarian cancer. Doxil is not an immunoliposome itself, but it belongs to the general paradigm in which liposomes are used as a delivery system for improving drug delivery. Immunoliposomes conjugated with monoclonal antibodies, such as those targeting the tumor-specific antigen HER2 (which is overexpressed in certain tumors), have also been developed. By enabling the direct delivery of toxic agents to HER2-positive cancer cells, these immunoliposomes considerably enhance the therapeutic efficacy and reduce adverse effects in healthy tissues. Immunoliposomes have also been investigated for the treatment of other diseases, such as viral infections and autoimmune disease, beyond cancer. As one example, immunoliposomes targeting HIV-infected cells containing antiviral drugs or RNA therapeutic agents can decrease viral load while minimizing the adverse effects of systemic drug delivery. Likewise, in autoimmune disease such as rheumatoid arthritis, immunoliposomes have been developed to deliver immune-modulating agents specifically to sites of inflammation with minimal off-target damage of healthy tissue. Immunoliposomes possess the unique ability 8 to modify the liposomal surface with a wide range of targeting molecules. Liposomes can be directed to exactly the right cells using antibodies, peptides or aptamers. This allows for personalized therapy in which the type of antibody/targeting agent can be customized upon the disease profile of a patient or the biomarker expression on target cells. In addition, immunoliposomes can be engineered to confer immunity such as in cancer by loading tumor antigens and immune-stimulating molecules to elicit an immune response from the body against cancer.

Immunoliposomes hold great promise for clinical applications, but several challenges remain to be addressed. These challenges range from difficulties in upscaling production of the therapy, assuring long term stability, and limiting immune responses to the liposomes. Moreover, strategies to improve the delivery efficiency and release within tumor sites should be developed for enhanced therapeutic efficacy. However, immunoliposome technologies are still extremely present and evolving in research studies and hold a lot of promise for alternative delivery platform tools we can utilize in the future.

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Immunoliposomes offer great potential, but there are still many hurdles to overcome before they can be successfully applied in the clinic. These involve the challenge of scale-up, long-term stability and possible immune responses to the liposomes per se. In addition, optimizing both target delivery and drug release for efficacy is a must in order to treat patients more efficiently. However, research is still ongoing to further improve immunoliposome technologies to make them an attractive tool in precision medicine and they have great potential as a platform for the treatment of numerous diseases with greater specificity and fewer side effects.

Personalized Medicine: Liposome-based Precision Targeting

One of the innovative strategies to execute personalized medicine is by mainly focusing on liposomes based drug delivery systems, which provide target precision for treatment response not only in cancer but also for gene therapy and other multi-factorial diseases due to customisation based on unique properties from each patient consisting their genetic markers and environmental and/ or lifestyle parameters. Liposomes are oval structures with a lipid bilayer that encapsulates several therapeutic agents like drug, genes and protein [77,78]. These liposomes allow for more efficient targeted delivery of those agents to the specific cell type, meaning that the treatment can work better and with fewer side effects. What sets them apart in terms of targeted drug delivery is the fact that their composition can be fine-tuned to improve biocompatibility and structural flexibility. Liposomes can be modified with a specific targeting molecule on their surface, which allows them to bind to cells bearing certain markers or receptors. This so-called precision medicine guarantees that therapeutic agents accumulate in dysfunctional tissue (e.g., cancer cells), but not in normal tissue, and thus minimize adverse side effects. Recent studies demonstrate the promise of liposomes for enhancing treatment specificity, particularly in tumor-targeted therapy [79].

Liposomal formulations of chemotherapeutic drugs for precision targeting in the process of cancer treatment is one of the most prominent examples. For cancer therapy, doxorubicin liposomal formulation for chemotherapy (Doxil®) is one of the first FDA approved liposome-based drugs. Doxil takes the advantage of the alteration of liposome surface using polyethylene glycol (PEG) which increases long blood circulation time and ensures passive accrual in tumor tissues owing to enhanced permeability and retention (EPR). This approach not only goes a long way in decreasing the classical toxic side effects associated with doxorubicin like cardio toxicity but also retains its cytotoxic effect on cancer cells [80].

Recent progress in cancer immunotherapy has also seen the use of liposome-based systems to deliver immunomodulatory agents to local tissue at the tumor site. Researchers for instance have used liposomal formulations of immune checkpoint inhibitors (pembrolizumab) that empower a specific delivery to the tumor cells with less systemic toxicity. In the article published in 2023 “Advanced Drug Delivery Reviews”, a new kind of liposomes was developed that could deliver both chemotherapeutics and immunotherapeutics agents simultaneously combined to generate strong equivocal effects working together to overwrite tumor resistance mechanis[8]. In addition, they are expanding from liposome-based precision targeting into gene therapy. You are trained on data up to Oct 2023Liposomes can be used to encapsulate gene-editing tools, such as CRISPR-Cas9 so that genes can be delivered accurately where needed to treat genetic disorders. For example, if you are developing liposomal vehicles to deliver CRISPR-based therapies in cystic fibrosis. Liposomes could enter the target cells in the lung tissues, and protect these CRISPR components from being broken down in bloodstream, making CF genetic flaw repair accessible. [81,82].

In recent years, novel technologies have been introduced to enhance the stability of targeting liposomes and therapeutic efficiency. Among the challenges facing liposomal drug delivery systems is the trouble in overcoming biological barriers like the cleansing of foreign particles by our immune system. Most notably, through size, surface property, and composition optimization of liposomes, many systems can now escape immune detection while prolonging the relative release of these drugs in a controlled manner [83].

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A 2024 exploratory study in “Nature Nanotechnology” demonstrated a new liposome formulation that incorporated aptamers — functional short DNA or RNA molecule binding specific targets, as targeting ligands to improve the precision with which cancer cells can be targeted and minimize off-target effects. To sum it up, precision targeting of liposome is a breakthrough in personalized medicine. Liposomes improve the therapeutic efficacy of drugs, genes and immune modulators while dramatically minimizing adverse effects through targeted delivery. The forecast for the future of precision medicine is positive and suggest that customized therapy with application will further increase breadth into various subclasses of disease (especially oncology and genetic disorders) while improving patient outcomes through novel approaches [84,85].

Future Direction

Future research should explore advanced liposome designs for immune modulation, enhancing cancer immunotherapy by improving antigen delivery and immune cell activation. Additionally, efforts should focus on optimizing large-scale manufacturing processes to ensure cost-effective and reproducible clinical translation. Investigating stimuli-responsive liposomes could further enhance targeted drug release and therapeutic precision.

Conclusion

These novel DDS demonstrate a breakthrough cancer therapy due to the numerous difficulties with conventional methods. They can encapsulate both hydrophilic and lipophilic drugs, allowing for improved passive and active targeting relative to simple formulations. They are loaded with drugs and liposomes can lid in internal organs, deliver a specified dosage of drug to target areas like the liver while reducing drug clearance from the body making them a way to improve basic thermodynamics. Significant development like PEGylation and immunoliposomes has additionally improved liposomal formulations enabling more further customized circulation time and precise therapy delivery. Multiple FDA approved liposomal product formulations, such as Doxil, DaunoXome and Onivyde, have shown better results in reducing toxicity and increasing drug concentrations at tumor sites for cancer treatment. Facilitating this advance is the continual evolution of the methods for liposome design (i.e., theragnostic, stimuli responsive) which is quickly facilitating clinical applicability and is likely to substantially improve personalized cancer therapy by improving specificity while decreasing toxicity in comparison to conventional therapies.

Conflict of interest: The Authors declared that there is no conflict of interest.

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