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Liposomal Paclitaxel In Breast Cancer Therapy: Advances, Clinical Insights, and Future Perspectives

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Breast cancer remains a leading cause of cancer-related morbidity and mortality worldwide, and conventional chemotherapy with paclitaxel (PTX) is limited by poor solubility, systemic toxicity, and suboptimal tumor targeting. Liposomal formulations of PTX have emerged as promising nanocarrier-based strategies to overcome these challenges by enhancing drug solubility, improving pharmacokinetics, reducing adverse effects, and enabling targeted delivery. This review provides a comprehensive overview of PTX liposomal formulations, including conventional, PEGylated, targeted, and stimuli-responsive liposomes, with emphasis on their composition, preparation methods, and optimization parameters. Clinical and preclinical studies, including recent advances (2024–2025), highlight improved cellular uptake, antitumor efficacy, and safety profiles compared to conventional PTX. The review also discusses approved formulations (e.g., Lipusu®), ongoing clinical trials, and combination therapy approaches. Finally, challenges related to scale-up, stability, regulatory approval, and clinical translation are addressed, alongside emerging trends such as smart liposomes, hybrid nanocarriers, and personalized therapy. Collectively, liposomal PTX represents a promising platform for enhancing therapeutic outcomes in breast cancer, with ongoing research paving the way for next-generation, safer, and more effective chemotherapy strategies.

Keywords: Breast cancer; paclitaxel; liposomes; targeted therapy; antitumor efficacy; nanomedicine.

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List of Abbreviations

5-FU — 5-fluorouracil
AI — Artificial intelligence
CAFs — Cancer-associated fibroblast
DOX — Doxorubicin
DTX — Docetaxel
EPR — Enhanced permeability and retention
MDR — Multidrug resistance
ML — Machine learning
PD — Pharmacodynamics
PK — Pharmacokinetics
PTX — Paclitaxel
Que — Quercetin
Res — Resveratrol
REV — Reverse-phase evaporation
TME — Tumor microenvironment
TNBC — Triple-negative breast cancer

1. Introduction

Breast cancer is one of the most common malignancies that plagues women across the world and is one of the most common causes of cancer-related deaths.[1] It has developed from the epithelial cells that line the ducts or lobules of the breast and has been divided into several subtypes, including hormone receptor-positive, HER2-positive, and triple-negative breast cancer (TNBC), which have distinct molecular properties and responses to therapy. Genetic, hormonal, and lifestyle factors, such as obesity, alcohol, and long-term exposure to estrogens, are some of the factors that have continued to increase the burden of breast cancer all over the world. The combination of mammography and molecular diagnostics has led to better survival rates in breast cancer, but metastatic breast cancer remains a major challenge to treatment because of resistance to drugs, heterogeneity across tumors, and the systemic toxicity of chemotherapeutic agents.[2–4]

The major therapeutic modalities of breast cancer include conventional chemotherapy, radiotherapy, surgery, and targeted therapy. The most common of them is chemotherapy with agents such as paclitaxel (PTX), docetaxel (DTX), and doxorubicin (DOX), which remain central, particularly in advanced and metastatic disease.[5] Although effective, poor aqueous solubility, non-selective biodistribution, dose-dependent toxicity, and multidrug resistance (MDR) limit the use of PTX therapy.[6] The traditional PTX formulation requires the solubilization of additional solubilizers, such as Cremophor EL, which can cause severe hypersensitivity reactions and may be neurotoxic, with variable pharmacokinetics.[6] Moreover, rapid systemic clearance and minimal tumor accumulation reduce treatment efficacy. Therefore, there are some acute requirements for the development of sophisticated drug delivery methods that can maximize the therapeutic index of PTX and reduce adverse effects.[7]

Nanocarriers, which are liposomes, polymeric nanoparticles, micelles, dendrimers, and others, have become hopeful approaches to overcome the shortcomings of traditional chemotherapy.[8] These nanosystems improve the solubility, stability, and bioavailability of poorly soluble drugs such as PTX, as well as passive or active targeting of tumor tissues (Fig. 1).[9] In particular, liposomes offer a biocompatible platform that is flexible enough to accommodate both hydrophilic and hydrophobic agents. Their capacity to extend the circulation period, protect medications against rapid degradation, and take advantage of the enhanced permeability and retention (EPR) effect renders them most appropriate for cancer therapy. It can also be targeted to specific cells by surface modification with targeting ligands, including antibodies, peptides, or folic acid, to result in selective concentration in cancer cells and reduce systemic toxicity and therapeutic efficacy.[10,11] In this way, the liposomal formulations of PTX are a potential breakthrough in attaining safer and more effective treatment results in the management of breast cancer.

2. PTX: An Overview

2.1. *Chemical structure and mechanism of action*

PTX is a naturally derived diterpenoid compound originally isolated from the bark of the Pacific yew tree (*Taxus brevifolia*). It possesses a complex taxane ring system with an ester side chain at the C-13 position, which is essential for its antineoplastic activity.[12] The chemical formula of PTX is $C_{47}H_{51}NO_{14}$, and it is characterized by poor aqueous solubility and high lipophilicity.[13]

PTX acts as a cytotoxic agent primarily by binding to the β-subunit of tubulin, inducing microtubule polymerization and stabilizing them, thereby inhibiting depolymerization. The stabilization interferes with the normal dynamic reorganization

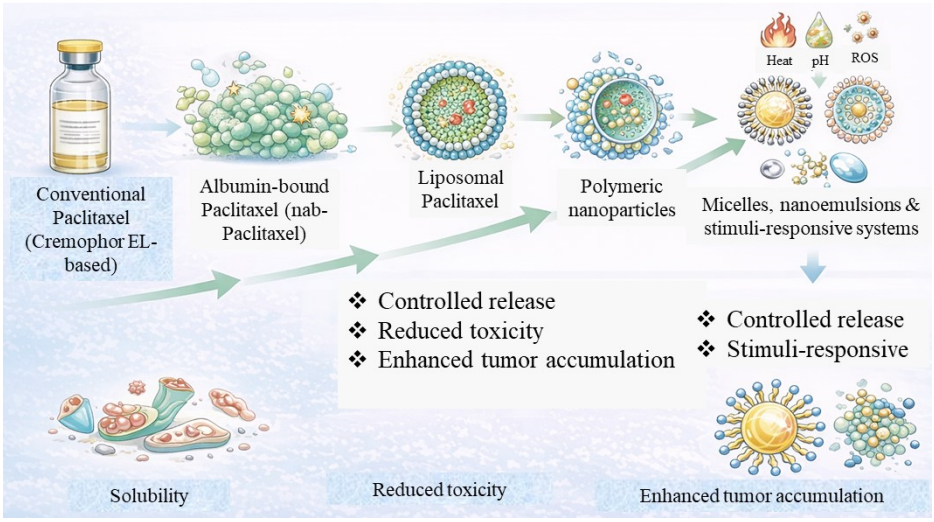

Fig. 1. Evolution of PTX formulations and delivery systems, illustrating the transition from conventional Cremophor EL-based PTX to albumin-bound, liposomal, polymeric nanoparticle, and advanced micellar/nanoemulsion and stimuli-responsive platforms, highlighting improvements in solubility, reduced toxicity, enhanced tumor accumulation, and controlled or stimulus-triggered drug release.

of the microtubule network required to form a mitotic spindle. This causes arrest of the cell cycle at the G 2/M stage, which causes apoptosis in fast-dividing cancer cells (Fig. 2).[14] In addition to induction of mitotic arrest, PTX also induces cell death by activating pro-apoptotic signaling and blocking anti-apoptotic proteins (Bcl-2) and disrupting angiogenic and metastasis-related intracellular signaling signals.[15]

2.2. *Breast cancer clinical uses*

PTX is commonly used in the treatment of breast cancer. It is utilized in the treatment of breast cancer as either first-line or adjuvant therapy in early-stage breast cancer, advanced breast cancer, and in metastatic breast cancer as a single agent or in combination with other cytotoxic agents, including DOX, cisplatin, and targeted agents such

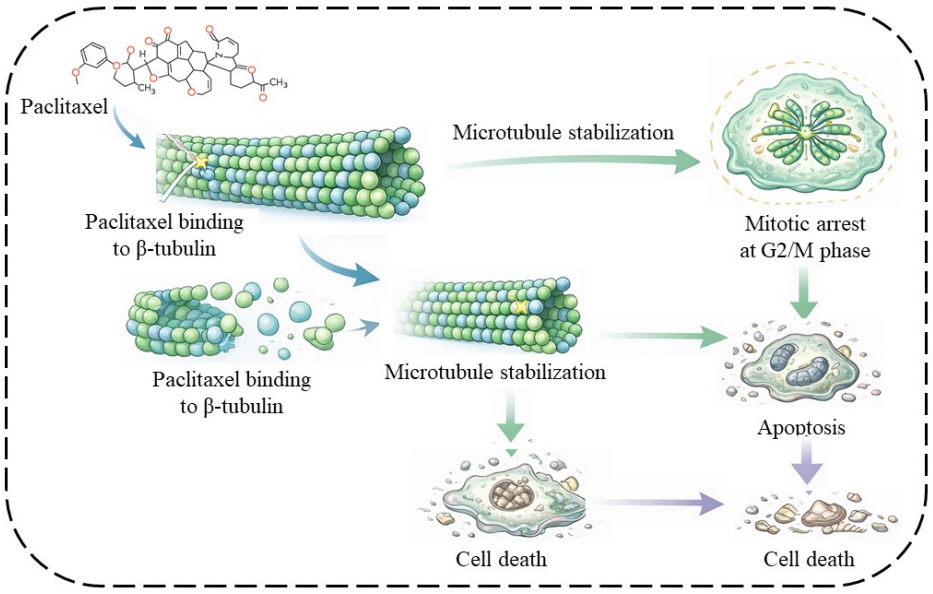

Fig. 2. Depiction of the mechanism of action of PTX in cancer therapy. PTX binds to β-tubulin subunits of microtubules, stabilizing them and inhibiting their depolymerization. This disrupts spindle dynamics, resulting in mitotic arrest in the G2/M phase, activation of the apoptotic pathway, and cell death in cancer cells.

as trastuzumab.[16] The drug is very effective in enhancing the overall survival rates and the disease-free progression rates, especially in cases of patients with HER2-positive and TNBC.[17]

PTX is used in clinical practice in multi-cycle regimens, usually on a weekly or every-three-week schedule, and as an intravenous drug. Its system of selective attack on dividing cells makes it a part and parcel of multiple combination chemotherapy regimens (e.g., AC-T: DOX + cyclophosphamide, then PTX). Nevertheless, clinical response is usually poor due to systemic toxicity and low tumor selectivity, even though it has therapeutic advantages.[18]

2.3. *Limitations to conventional formulations of PTX*

Poor water solubility (<0.01 mg/mL) is the greatest weakness of traditional PTX formulations, and it necessitated the addition of solubilizing agents (Cremophor EL, polyoxyethylated castor oil, or ethanol) to the commercially available Taxol product. These excipients, nevertheless, are linked to severe hypersensitivity, neurotoxicity, nephrotoxicity, and changes in drug pharmacokinetics.[19] Corticosteroids and antihistamines are usually used to premedicate the patient to minimize such adverse effects, which complicates matters even more.[20]

Also, traditional PTX is not specific in its biodistribution, leading to low concentrations in the tumor and significant exposure of normal tissues to the drug. Its clinical efficacy is further jeopardized by rapid systemic clearance, dose-limiting toxicity, and the emergence of MDR, all of which are mediated by p-glycoprotein efflux pumps. Such constraints have necessitated the formulation of new delivery systems, especially liposomal and nanoparticle delivery systems, to promote solubility, toxicity reduction, and targeted delivery of PTX to breast cancer tissues.[21]

3. Liposomal Drug Delivery System Requirement

3.1. *Benefits of liposomes versus traditional systems*

The traditional methods of drug delivery usually have several limitations, such as low solubility, limited circulation time, non-selective biodistribution, and intense systemic toxicity. To eliminate these shortcomings, liposomes provide a novel platform for delivering therapeutic agents in biocompatible lipid bilayers. Their structural flexibility allows the inclusion of hydrophilic and lipophilic drugs, which is very appropriate in agents like PTX.[22]

Liposomes help increase the solubility, stability, and bioavailability of drugs, resulting in better therapeutic effects. They prevent enzymatic degradation of encapsulated drugs, as well as reduce the premature elimination of encapsulated drugs in the systemic circulation.[23] Liposomes can be engineered to release drugs sustainably and controllably through modulation of particle size, surface charge, and lipid composition.[24] Moreover, liposomal encapsulation limits drug exposure to normal tissues, thus eliminating dose-limiting toxicities typical of conventional chemotherapy. Altogether, liposomes can be used to promote the anticancer drug therapeutic index, leading to an improved patient compliance and safety profile.[25]

Zhang *et al.* developed hydrophobic cell-penetrating peptide (CPP)-modified liposomes, termed PFV-Lip-PTX, to enhance PTX delivery and antitumor efficacy in breast cancer. The study aimed to broaden the use of hydrophobic CPPs as a strategy to improve cancer treatment. Physicochemical characterization revealed spheroid-like vesicles approximately 120 nm in diameter with a negative surface charge and a narrow size distribution. *In vitro* release studies demonstrated controlled, sustained release of PTX. At the same time, cellular uptake experiments in MCF-7 cells demonstrated higher internalization efficiency of PFV-Lip-PTX than that of non-modified liposomes. PFV modification enhanced PTX cytotoxicity via hydrophobic interactions between PFV-Lip and cancer cell lipid membranes. *In vivo*, PFV-Lip-PTX exhibited efficient tumor targeting and accumulation in MCF-7 xenograft models, leading to enhanced antitumor efficacy relative to non-modified liposomes. Importantly, PFV-Lip-PTX showed low systemic toxicity, as evidenced by minimal body weight changes and absence of histological alterations in major organs.[26]

3.2. *Types of liposomes*

Liposomes can be differentiated according to the composition, size, lamellarity, and surface modification strategies that affect biological behavior and pharmacokinetics (Table 1).[27]

Table 1. Comparative analysis of PTX nanoformulation platforms.

Nanocarrier platform	Key composition	Advantages	Limitations	Novel advancements	Ref.
Conventional liposomes	Phospholipids + cholesterol	Biocompatible, scalable, and improved solubility	Premature leakage, limited stability	PEG-free stealth lipids, ionizable lipids, and microfluidic fabrication	38
Stealth liposomes	PEGylated lipids	Prolonged circulation and reduced RES uptake	Anti-PEG immunogenicity	Polysacrosine, Pox, zwitterionic coatings	39
Polymeric nanoparticles (PLGA, PEG-PLA)	Biodegradable polymers	Sustained release, high stability	Lower loading of PTX versuslipid carriers	Hybrid lipid-polymer core-shell systems	40
Solid-lipid nanoparticles	Solid lipids + surfactants	High drug solubility, low cost	Risk of drug expulsion during storage	Matrix-lipid blending + stabilizers	41
Nanocrystals	Pure drug crystals	Very high loading, no carrier toxicity	Burst release, aggregation	Surface-functionalized nanocrystals (biomimetic coatings)	42
Exosomes/biomimetic vesicles	Cell-derived vesicles	High biocompatibility, immune-evasive	Expensive, difficult to standardize	Engineered exosomes (ligand-grafted, hybrid exosomes)	43

3.2.1. *Conventional liposomes*

These are the oldest types of liposomes, mainly composed of natural phospholipids and cholesterol. Although they offer an easy way of encapsulating drugs, they are eliminated promptly by the mononuclear phagocyte system (MPS), thus healthcare professionals are limited in their circulation duration.[28]

3.2.2. *PEGylated liposomes (stealth liposomes)*

PEG glycol is attached to the liposomal surface to form a hydrophilic coating that minimizes opsonization and macrophage recognition. This change increases blood flow, enabling passive targeting of tumor tissues via the EPR effect. A typical example of such technology is doxil (PEGylated liposomal DOX).[29]

3.2.3. *Targeted liposomes*

Active targeting is achieved by conjugating ligands to the liposomal surface, such as antibodies, peptides, aptamers, folic acid, or transferrin. These ligands interact with overexpressed receptors on tumor cells, including HER2 or folate receptors, which are then endocytosed. Directed liposomes increase the intracellular delivery of drugs and reduce off-target toxicity.[30]

3.2.4. *Stimuli-responsive liposomes*

These are complex formulations designed to deliver the encapsulated drug in response to stimuli such as pH, temperature, redox potential, or enzymes present in the tumor microenvironment. They provide site and controlled drug release to provide superior therapy precision.[31] Despite their remarkable results in preclinical research, stimulus-responsive liposomes face major obstacles to clinical translation. Inconsistency between *in vivo* stimuli and controlled laboratory conditions is one of the major challenges. The tumor pH, temperature gradients, and enzyme levels vary widely between patients and within the same tumor, making it challenging to achieve predictable trigger-based drug release. The example is that pH variations between tumor and normal tissues generally are not high enough to trigger pH-responsive systems across the board, whereas thermosensitive liposomes demand highly specific external heating conditions that cannot be clinically standardized.[32–34]

Formulation complexity and batch reproducibility are other severe constraints. Stimulus-responsive liposomes often involve specialized lipids, cleavable linkers, or sensitive compositions that are difficult to scale up under GMP conditions while maintaining stability and consistent trigger sensitivity. It is possible that small changes in lipid ratios or linker chemistry during large-scale production can cause a large change in release behavior. Moreover, it makes it difficult to evaluate the drug's therapeutic performance, as the reliability of external or internal stimuli determines this. All these reasons contribute to the explanation of why several bright

liposomal systems demonstrate good laboratory results and minimal advancements to advanced clinical results.[35–37]

3.3. *Mechanism of liposomal drug delivery*

Liposomal drug delivery mechanism constitutes a complex interaction of passive, active, and more recently recognized stimuli-responsive targeting pathways that, in combination, increase the accuracy and therapeutic efficacy of anticancer drugs (Fig. 3).[44]

3.3.1. *Passive targeting through EPR effect*

The EPR effect, which is a compartmental mechanism of liposome accumulation in tumor tissues, is traditionally activated by the pathological features of tumor vasculature, such as irregular endothelial junctions, impaired basement membranes, and the lack of lymphatic drainage. All these properties make nanosized liposomes (usually 80–200 nm) extravasate and selectively concentrate in tumor interstitial spaces.[45] Recent reports indicate that the intensity of the EPR effect depends on tumor type and patient group, and there are active efforts to increase passive accumulation using vascular modulation, EPR enhancers (e.g., nitric oxide donors), and tumor-priming techniques.[46] Despite the fact that the EPR effect offers a theoretical basis to passive targeting, there is strong evidence in the clinical observations that this effect is very heterogeneous in human tumors as opposed to animal models. Breast tumors in humans tend to have dense stromal structure, high interstitial fluid pressure, poor blood perfusion and fibrotic extracellular matrices that combine to restrict uniform liposomal extravasation. Now this patient-to-patient variation is identified as a key cause of the poor clinical behavior of most nanomedicine formulations that are based only on passive targeting.[47,48] To address this shortcoming, tumor-priming approaches have been suggested to temporarily alter the tumor microenvironment and establish permeability apertures to facilitate enhanced nanoparticle penetration. Strategies, including low-dose PTX or DOX pretreatment, radiation therapy, hyperthermia, nitric oxide donors, collagenase, or hyaluronidase, have been shown to decrease the stromal density, normalize abnormal vasculature and decrease interstitial pressure. These treatments greatly enhance intratumoral distribution and retention of liposomes. Hence, tumor priming is gaining recognition as a crucial complementary approach to addressing EPR heterogeneity in clinical nanotherapy in breast cancer.[48–50]

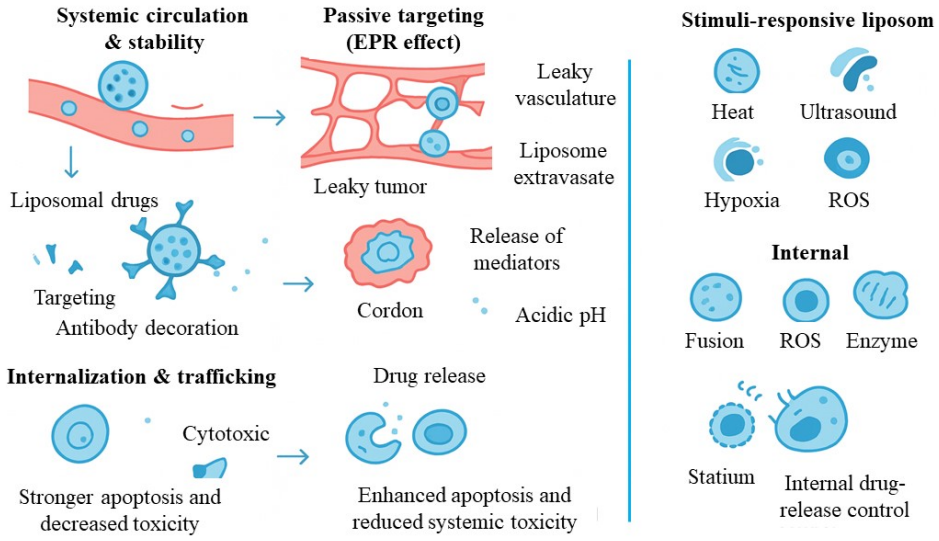

Fig. 3. Illustration of systemic circulation and presence of liposomal drugs, passive targeting through the EPR effect, cellular targeting, intracellular trafficking, and eventual extracellular release of the drug in the tumor tissue. It also draws attention to external (heat, ultrasound, hypoxia, ROS) and internal (fusion, enzymes, ROS) stimuli, leading to controlled drug release and improved apoptosis, with reduced systemic toxicity.

3.3.2. *Active targeting via surface functionalization*

Active targeting through the functionality of surfaces promotes the specificity of liposomal drug delivery by circumventing the constraints and heterogeneity of the EPR effect. In this method, surface ligands are incorporated onto the surface of liposomes, which include antibodies, peptides, aptamers, hyaluronic acid, or other small molecules that selectively bind overexpressed receptors on cancer cells. This specific receptor-ligand binding enhances endocytosis via these receptors, and makes the internalization of liposomes into malignant cells easy and specific.[51] Recent developments have enhanced this strategy by coming up with dual-ligand liposomes that have the ability to interact with more than one receptor at a time, hence, overcoming receptor variability in tumors.[52] Moreover, biomimetic coatings that have been obtained based on cancer-cell membranes or exosomes are being increasingly utilized in order to improve immune evasion and homotypic targeting. The other critical innovation is the incorporation of click-chemistry-based conjugation methods that also enhance the stability of the ligand and capture correct ligand orientation on the surface of the liposomes, which in the long-term leads to an increase in targeting fidelity and therapeutic efficacy.[53]

3.3.3. *Intracellular drug release and endosomal escape*

After internalization, the liposomes are transported into the endosomal and lysosomal system, and acidic pH, enzyme activity, and redox gradient cause destabilization of the lipid bilayers, releasing the encapsulated drug into the cytosol.[54] Modern liposomal preparations combine pH-sensitive lipids that destabilize under acidic conditions with ionizable or fusogenic lipids to promote membrane fusion and ensure rapid cytosolic release, as well as endosomolytic polymers tailored to enhance endosomal escape, especially for fragile cargo (nucleic acids and peptides). This better intracellular release not only increases the local concentration of drugs in tumor cells but also circumvents efflux pumps such as P-glycoprotein (P-gp), thus overcoming the problem of MDR via efflux and greatly increasing the treatment efficacy.[35] This occurs because free PTX primarily enters cells via passive diffusion across the plasma membrane, where it is rapidly recognized and actively effluxed by membrane-bound P-glycoprotein (P-gp) transporters. In contrast, liposomal PTX is taken up via energy-dependent endocytic pathways, including clathrin- and caveolae-mediated endocytosis. This process enables the intact liposome to avoid a direct interaction with P-gp pumps at the cell membrane. After endosomal trafficking, destabilization of liposomal membranes in acidic intracellular compartments causes cytosolic release of PTX from the plasma membrane, thereby preventing immediate drug efflux. This physical distance between P-gp recognition sites leads to the extended intracellular retention of drugs and recovery of cytotoxic effects on tumor cells with resistance.[55,56]

3.3.4. *Membrane composition and fusion capability*

The lipid composition plays a critical role in determining the pharmacokinetic and pharmacodynamic performance of liposomes, directly influencing their stability, circulation time, and interaction with cellular membranes.[57] Components such as cholesterol, PEGylated lipids, and lipid blends with defined phase transition temperatures can be strategically incorporated to enhance membrane rigidity, prolong systemic retention, or improve fusion with target cells.[58] Recent advancements in liposomal design have introduced high-transition-temperature phospholipids to achieve superior *in-vivo* stability, as well as lipid raft-mimicking structures enriched with sphingolipids to strengthen membrane interactions and facilitate cellular uptake.[59] Additionally, thermosensitive liposomes have gained prominence for their ability to release encapsulated drugs in response to mild hyperthermia (~42°C), offering precise spatiotemporal control through externally applied heat triggers and enabling more targeted and effective therapeutic interventions.[60]

3.3.5. *Stimuli-responsive liposomal systems*

Liposomal systems of the next generation have internal (pH, redox potential, enzymes, hypoxia) and external (temperature, ultrasound, magnetic field, near-infrared light) signaling molecules to control and release drugs on demand. These intelligent liposomes have the ability to improve the

drug bioavailability at the tumor site, accompanied by a reduction in systemic toxicity.[61]

Liposomal PTX preparations take advantage of these mechanistic benefits to attain greater therapeutic efficacy than traditional PTX preparations solubilized with Cremophor EL. These formulations are expected to minimize systemic toxicity and hypersensitivity effects, enhance target cell retention, and increase drug retention at the site of action by encasing PTX inside liposomes.[62] The increased levels of intracellular PTX stimulate more robust apoptotic effects, and the next generation of evidence might be synergistic activation of immunotherapies by affecting the tumor microenvironment. Recent developments in liposomal PTX development have involved PEG-free stealths, increased drug-loading efficiencies and hybrid lipid-polymer nanostructures, and they are all associated with enhanced safety, stability, and the overall therapeutic efficacy.[63]

4. Methods of Composition and Preparation

PTX Liposomal PTX formulations are aimed at increasing the solubility, stability, and the tumor-targeted delivery of the drug. These generally include phospholipid (PC, PG or DSPC) and cholesterol, which, when combined to make a stable bilayer, help regulate the rigidity of the membrane and reduce the leakage of drugs. PEG can be used as a surface modifier to enhance the circulation time and minimize opsonization and ever further increase therapeutic performance.[64.65] Different methodologies are used to process liposomal PTX, which affect important formulation properties including particle size, lamellarity, drug loading efficiency and stability.[66]

Thin-film hydration method is considered one of the most popular methods in which lipids and PTX are dissolved together in an organic solvent and then dried to create a uniform thin lipid film. With hydration of aqueous buffer, multilamellar vesicles are produced and can be further downsized with sonication or extrusion. Although a traditional method, some of the recent advances, like regulated rotary evaporation, optimizing hydration environment, and co-solvents, have improved the loading of PTX and the uniformity of the vesicles.[67,68]

Reverse-phase evaporation method (REV) makes it possible to achieve higher encapsulation efficiency by forming a stable water-in-oil emulsion, which is then slowly dried out under low pressure. The method results in large and internal aqueous cores of liposomes and enhanced phosphotransporter tubes.[69] The recent contributions include the utilization of greener solvents, minimization of the amount of the residual solvent, and the realization of continuous-flow REV to improve the scalability and reproducibility.[70]

The ethanol or ether injection technique is a rapid injection of lipid-organic phase into an aqueous medium, resulting in spontaneous liposome formation as a result of solvent replacement.[71] Current versions of the method incorporate microjet injection systems, temperature-controlled mixing, and solvent-free versions that enhance the uniformity of vesicle size and reduce the problem of PTX precipitation.[72]

Microfluidics can be used to produce uniform-sized liposomes in a precise, scalable way.[73] Extrusion is also used to further refine vesicle size and lamellarity, and modern automated extrusion, high-pressure homogenizers, and hybrid systems are now capable of efficient large-scale manufacturing that is GMP compliant.[74] Although such modes of preparations are beneficial at the laboratory level, they pose a number of challenges when applied at the industrial level of production. The methods of thin-film hydration and REV are batch-type processes that have problems of reproducibility, solvent remnants, and the inability to reproduce the same vesicle size at scale-up. The injection techniques have restrictions associated with solvent manipulation and the rapid mixing at increased volumes. Microfluidics offers great control over the properties of liposomes but has a low volumetric throughput, limiting large-scale production. To eliminate this, adaptation in industry is bound to numbering-up strategies, where several microfluidic chips work concurrently under continuous-flow circumstances. Moreover, high-pressure homogenization, automated extrusion systems, and process analytical technology (PAT) tools are also becoming more combined to allow continuous, GMP-compliant production, without loss of batch-to-batch consistency. These developments show that the process of preparing liposomes in the lab is increasingly being adjusted to the needs of industrial manufacturers.[75–77]

4.1. *Optimization (Parameters: Particle size, charge, encapsulation efficiency)*

The key to safe, stable, and effective physicochemical properties of lipid-based nanoparticles, including particle size, surface charge (zeta potential), and encapsulation efficiency (EE), is important in the generation of liposomal PTX formulations. All attributes are related to the composition of the formulation and the production process; thus, a systematic development plan (QbD/DoE) should be suggested.[78]

4.1.1. *Particle size and size distribution*

Particle size and size distribution are critical in the liposomal PTX performance as they determine the circulation time, tumor penetration (EPR effect), and REC clearance, and general biodistribution. The optimum size of 80–150 nm (maximum allowable size of 200 nm) and a PDI of less than 0.20 are used to provide the stability of uniform and regulation-compliant formulations.[79] The dimensions are regulated by way of making the preparations (microfluidics, solvent-injection, thin-film hydration with extrusion/sonication, and high-pressure homogenization) and the process parameters (flow rates, injection speed, temperature, membrane pore size) and the composition of lipids (saturation level, cholesterol content).[80] Such post-processing approaches as extrusion or homogenization are used to further refine size and PDI. The size/PDI characterization is done by DLS, the number-based distribution is done by NTA and the morphology and lamellarity are done using cryo-TEM, with the values reported as mean ± SD to make it clear.[81]

4.1.2. *Zeta potential (Surface charge)*

A major measure of liposomal stability, protein association, circulation duration, and cellular absorption is zeta potential. The charge of neutral to mildly negative (−10 to −30 mV) is normally desired as it reduces aggregation and nonspecific interactions but allows extended circulation time.[82] Cationic liposomes can enhance cellular internalization yet can also promote serum protein adsorption, complement activation and systemic toxicity hence are not used unless the advantages of increased intracellular delivery offset their disadvantages.[28] Recent developments are on the surface charge tailoring to enhance stability and tumor targeting.[83] PEG-free stealth surfaces with zwitterionic lipids or polysarcosine are also employed in order to circumvent PEG-related immunogenicity and low opsonisation.[84] Charge-switch liposomes, which are neutral in bloodstream, but are cationic in low tumor pH, improve tumor-cell contact with minimal toxicity to the system.[85] Receptor-mediated targeting can also be reinforced by controlled ligand-density strategies that do not enhance opsonisation.[86] Zeta potential is normally measured in electrophoretic light scattering in buffer, serum, to consider protein corona-induced variation and is more realistic of *in vivo* behavior.[87]

4.1.3. *Encapsulation efficiency (EE) and drug loading*

The efficiency of the delivery of liposomal PTX is dependent on the efficiency of encapsulation (EE) and drug loading because the ability limits the dose and release dynamics and stability of the formulation.[88] In the case of hydrophobic PTX, EE should be aimed at above 80% and drug loading at 3–15% w/w. High EE is obtained through optimization of the lipid composition, the ratio of drugs to lipid, and by temporary solubilizers or sophisticated techniques, namely, lipid-drug conjugates, cyclodextrin complexes, or hybrid lipid-polymer scaffolds.[89] EE is measured through HPLC/UPLC in the presence of separated free drug, whereas stability and drug state are measured with the use of DSC, XRD, and cryo-TEM, and leakage is measured during storage and during exposure to serum.[90]

4.1.4. *Stability, storage, and scalability*

The formulations of clinically viable liposomal PTX require stability, storage, and scalability as critical considerations.[91] Serum stability is assessed by incubating liposomes in biological media and recording size, PDI, zeta potential, and drug leakage. Since the formation of protein corona can dramatically change biodistribution and cellular uptake in more advanced studies, the composition of the corona may be measured by proteomics.[92] To achieve long-term storage, lyophilization using appropriate cryo/lyoprotectants, e.g., trehalose or sucrose, serves to function as an integrity preserving factor to vesicles; however, validation of reconstitution behavior and drug retention is

necessary.[93] Continuous operations, including microfluidics, continuous reverse-phase evaporation, or tangential-flow filtration (TFF), are used to achieve scalable manufacturing, whereby the critical parameters of the process (CPPs) and critical quality attributes (CQAs) are identified and managed to maintain batch-to-batch consistency and be in line with the GMP standards.[94]

4.1.5. *Analytical and biological characterization*

A thorough evaluation of liposomal PTX requires both analytical and biological characterization. Physicochemical testing typically includes measuring particle size and distribution using DLS and NTA, examining vesicle morphology with cryo-TEM, determining surface charge, and quantifying encapsulation efficiency through validated HPLC methods.[95] Additional tests, such as lipid content assays and structural analyses using DSC, XRD, or SAXS help assess bilayer organization and stability. *In-vitro* studies focus on drug-release behavior under sink conditions, stability in serum, hemolytic potential, complement activation, and cellular uptake using techniques like flow cytometry or confocal imaging.[96] Cytotoxicity assays and P-gp interaction studies are included when MDR is relevant. For *in vivo* assessment, preclinical models are used to study pharmacokinetics, organ and tumor distribution, therapeutic performance, and safety to build a complete profile of the formulation's behavior and effectiveness.[97]

4.1.6. *Qbd/DoE and process control*

Quality by Design (QbD) is used to understand how formulation components and processing conditions shape the key quality attributes of a liposomal PTX system, such as particle size, PDI, surface charge, encapsulation efficiency, and drug-release behavior.[98] A typical Design of Experiments (DoE) workflow begins with a screening phase—often using fractional factorial designs—to pinpoint influential variables like lipid composition, cholesterol proportion, drug-to-lipid ratio, hydration temperature, or flow parameters.[99] Once the major factors are identified, optimization is carried out using response surface designs, such as Box-Behnken or central composite designs, to refine operating ranges and establish an acceptable design space. These studies generate predictive equations that link process inputs to product performance and help define a clear control strategy.[100] For manufacturing, process-analytical tools such as in-line DLS, NIR, or UV sensors can be integrated to track critical properties in real time. Modern microfluidic systems also simplify scale-up by allowing parallel expansion of the same optimized conditions while maintaining consistent product quality.[101]

4.2. *In vitro and In vivo assessment*

Physicochemical properties, including size distribution, zeta potential, polydispersity index (PDI), and encapsulation efficacy, are evaluated *in vivo* for liposomal PTX formulations. The sustained-release behavior is determined by conducting drug-release studies in physiological and tumor-mimicking environments.[109] The antiproliferative activity of the preparation, compared with traditional PTX preparations, is tested using cytotoxicity assays (e.g., MCF-7 and MDA-MB-231). Also, fluorescence labeling of cells assists in determining the internalization efficiency of liposomes.[110]

Table 2. Physicochemical attributes influencing PTX loading and release.

Attribute	Influence on PTX	Formulation considerations	Ref.
Lipid phase transition temperature (Tm)	Higher Tm improves retention	Use DSPC/DPPC for rigid bilayers	102
Cholesterol ratio	Controls membrane fluidity	25–40% optimal for reducing leakage	103
Particle size	Affects PK, EPR effect, and release	80–150 nm ideal for tumor targeting	104
Surface charge (Zeta potential)	Stability, opsonization, uptake	Slightly negative (−10 to−30 mV) preferred	105
Drug-lipid ratio	Determines EE and stability	Overloading causes crystallization and expulsion	106
Bilayer rigidity	Modulates diffusion/release rate	Rigid bilayers = slower release	107
Hydrophobicity of PTX analogues	Solubility and loading efficiency	PTX-lipid conjugates for controlled release	108

In Vivo testing entails testing pharmacokinetics, biodistribution, and therapeutic efficacy in animal models of breast cancer. Tumor growth inhibition, the ratio of tumor growth to the organ drug concentration, and the survival rate are the parameters analyzed to assess therapeutic performance. The liposomal PTX usually demonstrates longer circulation, increased tumor localization, and decreased systemic toxicity of the conventional solution of PTX.[111]

4.3. *Pharmacokinetic and pharmacodynamic enquiries*

PTX liposomal encapsulation has a big impact on the pharmacodynamics (PD) and pharmacokinetics (PK) behavior. PEGylated or stealth liposomes have a longer plasma half-life, lower clearance, and higher area under the curve (AUC) than free PTX. The liposome-controlled release is associated with stable therapeutic plasma concentrations with long-term duration, which reduces the dosing frequency.[112,39]

The increased development of PTX in tumor tissue results in increased drug concentration and antitumor effects in these tissues and reduced drug exposure in healthy tissues pharmacodynamically.[113] MDR is also avoided by the liposomal system as it minimizes the recognition of the PTX by the P-glycoprotein efflux pumps. As a result, liposomal PTX preparations have a high therapeutic efficacy and reduced toxicity at the systemic level, and thus, this characteristic makes them good alternatives to traditional chemotherapy.[114]

4.4. *Targeted liposomal PTX in breast cancer*

4.4.1. *Ligand-based targeting*

Ligand-based targeting is a strategy to enhance the specificity of liposomal PTX delivery to tumor cells while sparing normal tissues. In this approach, ligands that recognize overexpressed receptors on breast cancer cells are conjugated to the liposomal surface (Table 3).[115]

Folate-Targeted Liposomes: Many breast cancer cells overexpress folate receptors. Conjugation of folic acid to liposomes facilitates receptor-mediated endocytosis, improving intracellular delivery and therapeutic efficacy.[116] The study by Liziane *et al.* aimed at the creation and testing of PTX-loaded folate-coated long-circulating and pH-sensitive liposomes (SpHL-folate-PTX) as a therapy against breast cancer. The objective of the study was to overcome the low solubility of PTX and the side effects associated with traditional formulations. Cellular uptake, cytotoxicity, apoptosis, and cell cycle effects were determined in the MDA-MB-231 breast cancer cell line, whereas the *in vivo* antitumor efficacy was assessed in BALB/c nude mice bearing MDA-MB-231 tumors. Findings showed increased cellular delivery of PTX by SpHL-folate-PTX, resulting in improved cytotoxicity and activation of apoptotic pathways compared with free PTX and folate-coated SpHL-PTX. Flow cytometry has shown a high concentration of cells in the G0/G1 phase after treatment with SpHL-folate-PTX. A significant decrease in tumor growth was observed *in vivo, as evidenced by reduced radiopharmaceutical uptake in scintigraphic imaging of the SpHL-folate-PTX group, indicating* increased therapeutic efficacy. Histomorphometric studies revealed more necrosis and inflammation in tumors that were treated with SpHL-folate-PTX. The immunohistochemical tests also showed reduced proliferative cells and an increase in apoptotic cells relative to controls. In general, the article by Liziane *et al.* supports the idea that SpHL-folate-PTX is an effective antitumor agent, which can deliver greater effectiveness and lower toxicity to the systemic level in breast cancer models, whilst it may also enable lower systemic toxicity.[117]

Antibody-Targeted Liposomes: Monoclonal antibodies such as trastuzumab (against HER2) are used to target HER2-positive breast cancer. This ensures selective uptake in tumor cells and reduces off-target effects.[118]

In 2024, Kumar *et al.* developed PTX-anti-ABCB1 siRNA-based multifunctional cationic liposomes targeting HER2, conjugated with trastuzumab on the liposome surface to enable synergistic treatment of HER2-positive breast cancer. Optimized trastuzumab-decorated liposomes had a size of 229 ± 4 nm, a zeta potential of 43.46 ± 0.61 mV, and a PDI of 0.31 ± 0.01, which is a measure of homogeneity and stability. Their morphology was identified as spherical, as confirmed by SEM. Trastuzumab-conjugated liposomes exhibited superior cellular uptake (2.79x) and drug retention compared to free PTX and non-targeted liposomes and reduced IC50 values in *in vivo* studies in BT-474 cells. These liposomes have

Table 3. Summary of key research studies on PTX-loaded nanocarriers.

Authors	Nanocarrier type	Model used	Key findings	Ref.
Zhang *et al.*	Hydrophobic CPP-modified liposomes (PFC-Lipo-PTX)	MCF-7 cells, MCF-7 xenograft mice	~120 nm stable liposomes; enhanced cellular uptake; improved cytotoxicity; strong tumor accumulation; superior antitumor effect with low systemic toxicity.	26
Liziane *et al.*	Folate-coated long-circulating pH-sensitive liposomes (SpHL-folate-PTX)	MDA-MB-231 cells, BALB/c nude mice	Higher PTX uptake; increased cytotoxicity & apoptosis; G0/G1 arrest; significant tumor inhibition; enhanced radiopharmaceutical localization; increased necrosis with low toxicity.	117
Monteiro *et al.*	Radiolabeled folate-targeted PTX liposomes	Breast cancer tissue models	High folate-receptor binding and tumor-selective liposomal accumulation, confirming targeted delivery suitability.	120
Chen *et al.*	Redox-sensitive PTX + anti-survivin siRNA liposomes (PTX/siRNA/SS-L)	4T1 cells, 4T1 tumor-bearing mice	High cellular uptake; efficient endosomal escape; surviving downregulation; reduced migration; strong tumor inhibition & metastasis reduction; low systemic toxicity	121
Cetin *et al.*	nab-PTX + liposomal cisplatin combination	MCF-7, MDA-MB-231 cells	Synergistic anticancer activity; decreased viability; increased apoptosis (AI↑); reduced proliferation (LI↓); potential for combination nanotherapy.	126
Chen *et al.*	Mitochondria-targeted KLA-modified 5-FU/PTX liposomes	MDA-MB-231 cells, xenograft mice	Enhanced mitochondrial delivery; dual-drug synergy; strong tumor growth inhibition; limited systemic toxicity.	127
Kumar *et al.*	Trastuzumab-conjugated cationic PTX + ABCB1-siRNA liposomes	HER2+ breast cancer cells, mice	~229 nm, +43 mV liposomes; enhanced uptake; sustained release; potent targeted therapy; reduced ABCB1-mediated resistance.	119
Meng *et al.*	PEGylated liposomes co-loaded with resveratrol + PTX (Res/PTX-Lipo)	MCF-7/Adr drug-resistant cells, mice	~50 nm, >50% EE; strong synergistic cytotoxicity; improved tumor retention; Res reduced efflux (MRP1↓); major inhibition of MDR tumor growth.	130
Duan *et al.*	MMP-triggered dual-targeting micelle-in-liposome (RPM@NLQ)	CAF-rich breast cancer models	Sequential Que + PTX delivery; ECM remodeling (Wnt16↓); deeper intratumoral penetration; high tumor accumulation; significantly enhanced antitumor efficacy.	137

demonstrated high tumor uptake (2.67x higher) in xenograft nude mouse models and increased tumor growth inhibition (3.66x reduction in tumor volume compared to control). A Western blot analysis proved the significant down-regulation of HER2, ABCB1, and BCL2 proteins, which supported a synergistic anticancer effect. The article emphasized that targeted liposomes can enhance PTX, siRNA, and trastuzumab therapies to increase therapeutic effect, serum stability, and specificity and reduce off-target drug toxicity. Before clinical translation, Kumar *et al.* recommended additional scale-up, long-term stability, and toxicity studies.[119]

4.4.2. *Stimuli-responsive liposomes*

pH-Sensitive Liposomes: The development of pH-sensitive liposomes involves the release of PTX in acidic conditions specific to tumors (pH 5-6) and endosomes. Ionizable or imidazole-based lipids, PEG, which can be cleaved by acid, or charge-switching polymers that are stable in blood but dissociate at low pH to release a drug and accumulate in the tumor, have been developed using modern designs to enable the delivery of drugs to their therapeutic targets. More recent developments include improved lipid compositions to achieve stability, enhanced loading using a hybrid lipid-polymer system, and improved endosomal-escape properties, which have made pH-responsive liposomes more accurate and efficient for tumor-targeted therapy.

In 2018, Monteiro *et al.* developed folate-coated, long-circulating, and pH-sensitive liposomes (SpHL-folate-PTX) in which PTX was encapsulated and targeted to folate receptor-positive

breast cancer cells. Both folate-coated and non-coated liposomes were radiolabeled with technetium-99 m (99Tc), and the radiochemical purity, stability, and pharmacokinetics of the radiolabeled liposomes were assessed to determine their *in vivo* behavior. Formulations were found to have high radiochemical purity (>98%) and high *in vivo stability (>90%), demonstrating their suitability for* biological research. Biodistribution and scintigraphic imaging experiments in healthy and breast tumor-bearing mice showed that the 99Tc-SpHL-folate-DTPA-PTX formulation had a longer circulation half-life (541.8 min) and higher tumor uptake than both non-functionalized liposomes and free 99Tc-PTX. The folate-coated liposomes attained a much more important tumor-muscular percentage 4 h after injection, and maintained a tumor for up to 8 h, which revealed successful folate receptor-mediated targeting of breast cancer tissues. These findings vindicated the claim by Monteiro *et al.* that they managed to develop a radiolabelled folate-targeted liposomal PTX system that could be selectively concentrated in folate receptors of breast cancer tumors, thus proving useful as an effective nanocarrier of a specific drug to the tumor.[120]

Redox-Sensitive Liposomes: Tumor cells often have higher intracellular glutathione (GSH) levels. Disulfide linkages in liposomes can be cleaved under reductive conditions, thereby releasing PTX selectively within cancer cells.

The article by Chen *et al.* resolved the dilemma of cancer resistance to PTX treatment due to survivin, which is an anti-apoptotic protein overexpressed in breast cancer. To overcome this, they developed a redox-sensitive liposomal nanosystem (PTX/siRNA/SS-L) to co-deliver PTX and anti-survivin siRNA. It was formulated with a cationic oligopeptide lipid (LHSSG 2 C 1 4) with disulfide linkages (to be redox-sensitive), soybean phosphatidylcholine (SPC), and cholesterol. This design enabled effective encapsulation and rapid, redox-sensitive delivery of both therapeutic agents within the reducing tumor microenvironment. The PTX/siRNA/SS-L system demonstrated higher cellular uptake, endolysosomal escape, more effective survivin downregulation, and improved cytotoxic and apoptotic effects in 4T1 breast cancer cells in *in vivo* studies compared with control groups. This formulation also showed reduced wound healing and migration, suggesting it could prevent metastasis. When used in *tumor-bearing (4T1) mice, PTX/siRNA/SS-L showed reduced systemic toxicity and synergistic inhibition of tumor growth and lung metastasis compared with* single-agent or non-redox-sensitive formulations. Taken together, Chen *et al.* demonstrated that redox-sensitive cationic oligopeptide liposomes can offer a promising co-delivery system for PTX and siRNA, thereby bypassing drug resistance and effectively inhibiting breast cancer growth and spread. These smart delivery systems enhance therapeutic efficacy, minimize systemic toxicity, and enable controlled, site-specific drug release.[121]

4.4.3. *Combination therapy*

Combination therapy using liposomal PTX with other anticancer agents is an effective strategy to enhance antitumor efficacy and overcome drug resistance.

Chemotherapy Combinations: PTX can be co-encapsulated with agents like cisplatin and 5-fluorouracil to achieve synergistic cytotoxic effects and minimize tumor regrowth.[122]

A major strength of co-encapsulating drugs in a single liposomal nanocarrier is that it is possible to sustain a pre-determined drug ratio between drugs in systemic circulation and at the tumor site. The traditional combination chemotherapy therapy drugs are given individually and therefore have a different pharmacokinetic profile, and hence there is a variation in plasma concentration, and the optimum synergistic ratio is not achieved until the drug reaches tumor tissues. Conversely, liposomal co-delivery allows both drugs to stay physically linked in the same carrier and thus be biodistributed, cellularly absorbed, and intracellularly released in a synchronized manner. This synchronized delivery maintains the synergetic ratio during the circulation and tumor uptake, thus maximizing synergy of the therapy and minimizing the risk of antagonistic effect.[123–125]

A study by Cetin *et al.* evaluated the *in vivo* cytotoxicity of nab-PTX combined with liposomal cisplatin against MCF-7 and MDA-MB-231 breast cancer cell lines. Using cell viability assays, xCELLigence RTCA (Real-Time Cell Analysis), mitotic index (MI), apoptotic index (AI), and labeling index (LI), the study applied various drug combinations: A1L25, A1L5, A10L5 for MDA-MB-231 and A1L5, A1L10, A5L1 for MCF-7 over 24-72 h.

Results showed a significant reduction in cell viability and cell index values in both cell lines, with MI and LI increasing at 24 h and decreasing at 72 h, while AI significantly increased, indicating apoptosis induction. The study concluded that nab-PTX combined with liposomal cisplatin exhibits promising anticancer activity across different breast cancer subtypes, suggesting potential for combination nanotherapy.[126] Furthermore, Chen *et al.* developed KLA-modified liposomes co-loaded with 5-fluorouracil (5-FU) and PTX, termed KLA-5-FU/PTX Lps, to enhance therapeutic efficacy against TNBC. The liposomes were prepared using the thin-film dispersion method and designed to achieve mitochondria-targeted drug delivery through surface modification with the KLA peptide, a known mitochondria-penetrating sequence. *In vivo* studies using MDA-MB-231 human breast cancer cells revealed that KLA-5-FU/PTX Lps exhibited enhanced cytotoxicity, efficient mitochondrial accumulation, and induction of mitochondria-mediated apoptosis compared to unmodified or single-drug-loaded liposomes. *In vivo* evaluation in MDA-MB-231 tumor-bearing mice confirmed that these dual-drug-loaded, KLA-functionalized liposomes exhibited superior tumor-targeting capability, significant tumor growth inhibition, and minimal systemic toxicity.[127]
Targeted Therapy Combinations: Co-delivery with targeted agents (e.g., trastuzumab for HER2-positive tumors) allows simultaneous inhibition of proliferative signaling and induction of apoptosis.[128] A study by Kumar *et al.* developed trastuzumab-conjugated cationic liposomes co-loaded with PTX and ABCB1-siRNA for HER2-positive breast cancer therapy. The nanoparticles (~229 nm, +43 mV) showed spherical morphology, enhanced cellular uptake, and sustained drug release. *In vitro* and *in vivo* studies demonstrated superior tumor targeting, higher anticancer efficacy, and reduced toxicity compared to non-targeted or free PTX, highlighting their potential for targeted and synergistic HER2-positive cancer treatment.[119]
Natural Compounds or Phytoconstituents: Liposomal co-delivery of PTX with compounds like Resveratrol and quercetin can enhance chemosensitivity, reduce oxidative stress, and provide additional therapeutic benefits.[129]

A study by Meng *et al.* developed a PEGylated liposomal co-delivery system encapsulating resveratrol (Res) and PTX for the treatment of MDR tumors. The liposomal formulation, approximately 50 nm in diameter, achieved encapsulation efficiencies above 50% for both drugs and exhibited sustained release behavior. This study, reported as the first attempt to co-encapsulate Res and PTX in a single PEGylated liposome, aimed to enhance drug synergy and overcome resistance in drug-resistant MCF-7/Adr breast cancer cells. The results demonstrated that liposomal co-delivery of Res and PTX significantly enhanced cytotoxicity against drug-resistant cells *in vivo and improved the bioavailability and tumor retention of both agents*. In animal models, systemic administration of the composite liposome effectively inhibited tumor growth without notable systemic toxicity ($p < 0.01$). Neither PTX- nor Res-loaded liposomes alone achieved comparable efficacy, highlighting the synergistic action between the two agents. Mechanistically, Res served as a natural chemosensitizer, reducing drug resistance by modulating mitochondrial apoptosis pathways and downregulating MRP1 expression, while PTX acted as the primary cytotoxic drug. PEGylation of the liposome prolonged circulation time, improved tumor accumulation via the EPR effect, and minimized clearance by the liver and spleen.[130] Despite the extensive use of PEGylation in order to increase the systemic circulation time of liposomes by inhibiting opsonization and uptake by the RES, repeated treatments with PEGylated liposomes have been linked with the Accelerated Blood Clearance (ABC) effect. This is caused by the production of anti-PEG IgM antibodies after the initial dose that recognize PEG on the subsequent doses and lead to the rapid activation of complement, and hepatic clearance. This causes a decrease in therapeutic efficacy when PEGylated nanocarriers are used again and again clinically, causing a major limitation in chronic chemotherapy protocols like PTX therapy of breast cancer.[131,132] Recent studies have been on the way to overcoming this drawback by using alternative methods of surface engineering. The addition of zwitterionic lipids (which include phosphorylcholine, sulfobetaine, and carboxybetaine groups) which mimic cell membrane phospholipids and confer stealth effects, but do not trigger immunogenicity are a promising approach. The zwitterionic coatings exhibit low protein adsorption

and decreased antibody recognition in comparison with PEG, thus reducing the ABC effect. Also being investigated are cleavable PEG linkers, PEG analogs (e.g., polyglycerol, polysarcosine), and biomimetic surface functionalities based on erythrocyte or leukocyte membrane coats to keep in circulation without immune sensitization. Next-generation stealth strategies will be used in the future to substitute the traditional PEGylation in the liposomal PTX formulations to enhance clinical performance with repeated dosing.[133–136]

Beyond conventional PEGylation strategies, recent studies have focused on developing multifunctional liposomal systems that integrate targeting, stimulus responsiveness, and tumor microenvironment modulation to enhance therapeutic efficacy. A study on quercetin-based combination by Duan *et al.* developed a matrix metalloproteinase (MMP)-triggered dual-targeting hybrid micelle-in-liposome system (RPM@NLQ) designed for sequential delivery of quercetin (Que) and PTX to remodel the fibrotic tumor microenvironment (TME) and enhance chemotherapy efficacy. In this system, PTX-loaded RGD-modified micelles (RPM) and antifibrotic quercetin were co-encapsulated within MMP-sensitive liposomes, which were further functionalized with the NGR peptide for targeted delivery. Upon intravenous administration, RPM@NLQ selectively accumulated at the tumor site via NGR-mediated targeting. The overexpression of MMPs in the TME triggered the release of both Que and RPM. Quercetin acted locally to suppress Wnt16 expression in cancer-associated fibroblasts (CAFs), thereby reducing fibrosis and stromal barrier density and enhancing intratumoral penetration of RPM. The released RPM then effectively targeted and eradicated tumor cells. This cascade-targeted delivery system exhibited prolonged circulation, enhanced tumor accumulation, improved penetration, and superior antitumor activity both *in vivo* and *in vivo*. Overall, Duan *et al.* demonstrated that sequential delivery strategies combining TME remodeling and chemotherapy could provide a promising therapeutic approach for CAF-rich tumors, including breast cancer.[137] Combination liposomal formulations improve tumor-targeted drug delivery, maximize cytotoxic synergy, and reduce systemic side effects, offering a promising approach for personalized breast cancer treatment.[138]

5. Systems Pharmacology & AI-Driven Optimization of PTX Nanocarriers

Data-driven, systems-level optimization of precision design of PTX nanocarriers is now beyond single-variable engineering.[139] The integration of mechanistic physiological paradigms (systems pharmacology) with machine learning (ML) and digital twin simulations can be used to predict interactions among formulation, process, and patient heterogeneity to determine biodistribution, tumor uptake, intracellular release, and therapeutic outcome, enabling rational, faster, and safer translation.[140]

5.1. *ML models for formulation: Predicting encapsulation, leakage, and stability*

Supervised ML models (random forests, gradient boosting, neural network, and physics-informed algorithm) are currently employed to predict relevant PTX-liposome characteristics such as encapsulation efficiency, leakage, particle size, and stability, depending on input data such as lipid composition, drug: lipid ratio, cholesterol level, solvent system, and process parameters.[141] A curated dataset of multiple preparation methods can be built, formulations transformed into molecules, and a process description, a cross-validated model trained, and SHAP or feature-ranked analysis can be used to understand which factors contribute to poor EE or instability. Recent investigations indicate that such ML tools can reduce effort in formulation-screening over 60% and are able to give accuracies in predictions ($R^2 > 0.8$), with focused experimentation, rather than extensive trial and error experiences.[142]

5.2. *PK-PD digital twins for PTX-liposome performance and dosing personalization*

A digital twin is a patient model, a computer-based model, which connects physiology (perfusion, interstitial pressure), nanoparticle behavior (opsonization, transport, cellular uptake), and PTX pharmacodynamics to predict the treatment outcomes. It may be utilized to predict tumor exposure and toxicity with virtual what-if tests of liposome size, surface chemistry or dosing schedule

after calibration with PK/PD data and imaging data. This is a new methodology, which has been recently used in PTX systems, which optimizes faster, doesn't require trial-and-error experimentation, and enables smarter and responsive trial design by determining the best regimen and probable responders before clinical testing.[143,144]

5.3. *Multi-omics integration for patient-stratified PTX delivery*

The heterogeneity of tumors is a crucial determinant of liposomal PTX performance. The combination of proteomic and transcriptomic profiling facilitates the detection of molecular signatures that can be directly used to design nanocarriers and select patients. For example, tumors that have increased expression of matrix metalloproteinases (e.g., MMP-9) can be considered good targets for enzyme-responsive liposomes with cleavable linkers. Ligand-targeted formulations with an optimized ligand density can be used in patients with a high degree of folate receptor or transferrin receptor expression. On the other hand, anti-PEG antibodies detected in plasma proteomic analysis indicate that PEG-free stealth coatings, such as zwitterionic lipids or polysarcosine lipids, should be used to prevent rapid clearance. Similarly, transcriptomic indicators of an acidic tumor microenvironment or hypoxia can be used to justify the use of pH-responsive or stimulus-sensitive liposomes. Therefore, multi-omics profiling offers a logical rationale to associate particular PTX nanocarrier designs with biologically defined subgroups of patients to achieve personalized nanomedicine.[145–147]

5.4. *AI-guided surface engineering: ligand density, PEG alternatives and stimulus thresholds*

The optimization of liposome surface engineering (to predict optimal ligand density for uptake and to reduce opsonization), the identification of suitable PEGyl substitutes for polysarcosine (POx) (e.g., zwitterionic lipids) using protein-adsorption models, and the control of ionizable lipid pK or cleavable-PEG linkers to ensure charge switching at tumor-relevant pH are being improved with AI and ML tools. These methods simplify design, reduce experimental workload, and increase targeting accuracy.[148] A representative example of AI-assisted liposome optimization was reported, where a supervised machine learning workflow was trained using experimental datasets of targeted liposomes varying in ligand density, PEG content, and lipid ratios to predict cellular uptake efficiency. The model revealed a nonlinear relationship between ligand density and receptor-mediated endocytosis, demonstrating that excessive ligand grafting reduced targeting due to steric hindrance and increased opsonization, whereas an optimal intermediate density maximized uptake. The same model also identified optimal phospholipid–cholesterol compositions that balanced membrane rigidity and drug retention. Experimental validation confirmed the AI predictions with significantly fewer trial formulations, highlighting how data-driven modeling can rationally guide liposome surface engineering rather than relying on empirical trial-and-error approaches. Such case studies demonstrate the practical utility of AI in optimizing PTX liposome design for enhanced targeting performance.[149–151]

5.5. *Ongoing clinical trials*

Real-life and preclinical studies of liposomal PTX (e.g., Lipusu) in breast cancer - A retrospective real-life study of 647 advanced breast cancer patients in China taking PTX liposome (Lipusu) as salvage therapy found PFS and ORR results.[152] See Table 4 for a summary of liposomal PTX formulations in breast cancer. Other indications (e.g., lung cancer) are also undergoing trials of Lipusu: one randomized Phase 3 trial in locally advanced/metastatic squamous cell carcinoma of the lungs compared Lipusu + cisplatin to gemcitabine + cisplatin.[153] Guidelines and continued use in practice indicate that there is still clinical interest, whereas a specific large randomized trial of liposomal PTX in breast cancer may be still lower (or less published). The comparative efficacy and safety profiles were compared.[154] Regarding Lipusu: The liposomal formulation has been reported to be equally or slightly more effective than conventional PTX (Taxol) with Cremophor EL/ethanol solubilizer, but with a better safety profile (especially less hypersensitivity reactions by virtue of abandoning Cremophor EL).[155] The excipient Cremophor EL is said to cause hypersensitivity and complement activation; removal of Cremophor in Lipusu is said to create a greater

Table 4. Summary of liposomal (or liposome-based) PTX formulations in breast cancer - approval status, study population, key findings.

Formulation	Type	Indication (Breast Cancer)	Key Study or Status	Comments
PTX liposome (Lipusu®)	Liposomal PTX, Cremophor-free	Breast cancer (approved in China)	Phase I/II trials; real-world study in advanced breast cancer (647 patients) showed ORR ~46.7% (1st line), median PFS ~5.5 mo.	Approved in China in 2003 for breast, ovarian, and NSCLC.
Liposomal PTX (generic investigational)	Liposome-encapsulated PTX in early trials	Breast cancer	Real-world & neoadjuvant comparisons: a study showed liposomal PTX in NAT had tpCR ~34.4% versus ~19.4% for DTX and ~13.3% for PTX.	Shows promise in axillary node clearance and lower AEs.
PEGylated liposomal DOX(PLD) +PTX	Though not PTX-liposome alone, it uses a liposomal carrier plus PTX	Metastatic breast cancer	Phase II: PLD +PTX showed a 48% response in previously treated MBC.	Included here as an example of a liposome combination with PTX.
Investigational liposomal PTX in the neoadjuvant setting	Liposomal carrier of PTX	Neoadjuvant breast cancer	Study April 2014–2020 (647 pts) comparing PTX, docetaxel, liposomal PTX: liposomal group had significantly better apCR (63.5% versus 24.6% & 34.8%).	Good data for liposomal form in early/locally advanced setting.

safety margin. In real-world breast cancer data, the retrospective study determined a significant use of PTX liposome in first-line, second-line, and further in advanced breast cancer, but the ORR and PFS data should be put into perspective (patient population, salvage lines).[156] In a head-to-head real-life compare and contrast (advanced breast cancer) of PTX liposome versus nab-PTX. The research found that the DCR (disease control rate - nab -nab-PTX versus PTX liposome) was higher compared to PTX liposome ($p = 0.023$), but PFS was longer with PTX liposomes than with nab-PTX ($p = 0.0276$). Regarding the number of adverse events, PTX liposome was more prone to all grades of AEs and neutropenia than nab-PTX; neurotoxicity was more frequent with nab-PTX.[157] In Lipusu, in animals, volume of distribution (Vd) and clearance (CL) were greater than with free PTX; however, higher tissue retention (particularly in liver, spleen, lymph nodes) indicated changed biodistribution that was favorable in therapy of tumors/lymph nodes. Benefits in safety: Fewer cases of hypersensitivity (no Cremophor EL), and (possibly) a lowered need for steroid premedication.[158] Several clinical trials have investigated the safety and efficacy of liposomal PTX in breast cancer (Table 5). Although Tables 4 and 5 summarize formulations and registered trials, a comparison of clinical study design, endpoints, and outcomes is essential to understand the translational performance of liposomal PTX. Therefore, a comparative analysis of major reported clinical studies is presented in Table 6.

A clearer clinical distinction can be drawn when comparing Lipusu with Abraxane, as both are Cremophor-free nanocarrier formulations of PTX

Table 5. Summary of clinical trials using liposomal PTX in breast cancer.

NCT Number	Phase	Formulation Description	Indication	Ref.
NCT02142790	Phase 4	PTX liposome injection (PTX Liposome) is administered weekly versus every 3 weeks.	Metastatic breast cancer	159
NCT06632574	Phase 1	PTX cationic liposomes via arterial infusion.	Advanced solid tumors (includes possible breast cancer)	160
NCT06481553	—	PTX liposomes combined with an anti-HER2 monoclonal antibody.	HER2-positive locally advanced/ metastatic breast cancer	161

Table 6. Clinical Translation Status and Key Challenges of Nanomedicine Platforms Relevant to Liposomal PTX.

Platform/ category	Clinical stage/ success	Breast cancer relevance	Translation challenges/ key failure reasons	Supporting evidence	Ref.
Liposomal PTX (Lipusu®)	Marketed in China; real-world studies	Approved for breast cancer (China)	Limited global development beyond China; variable efficacy across populations	Liposome clinical landscape review showing limited approvals of nanomedicines despite many preclinical reports	162,163
PEGylated liposomes (general)	Phase I/II studies	Included in early nanomedicine pipelines	ABC phenomenon and immunogenicity upon repeated dosing; poor predictability of benefit in humans	Review on nanomedicine clinical translation challenges including immune clearance issues	164
Active-targeted liposomes (ligand modified)	Preclinical / early phase	Models tested in HER2, TNBC	Complex design increases regulatory/CMC hurdles; preclinical models overpredict clinical benefit	Nano translation review noting difficulty predicting clinical outcome from preclinical models	163,165
Stimuli-responsive nanocarriers	Preclinical	TNBC models	Inconsistent stimuli, *in vivo* versus lab, scalability & stability issues	Translational challenges in drug delivery nanoparticles include a lack of proper mechanistic understanding and clinical predictivity	166
Combination co-delivery liposomes	Preclinical	MDR models	Maintaining optimal synergistic ratio *in vivo*; regulatory complexity	General review on clinical translation hurdles for nanoparticles, including complex *in vivo* behavior	164
Nanomedicines in cancer overall	Multiple stages	Includes breast cancer trials	High attrition from phase II to III due to poor efficacy rather than toxicity	Survey shows ~48% success in phase II dropping to ~14% in phase III for cancer nanomedicines	167,168

but differ fundamentally in drug release kinetics and toxicity profiles. Lipusu®, being a liposomal encapsulation, provides sustained drug release and lower peak plasma concentrations, which translates into reduced peripheral neurotoxicity during repeated chemotherapy cycles. In contrast, Abraxane®, an albumin-bound nanoparticle formulation, facilitates rapid dissociation of PTX after administration, leading to higher free drug exposure and a comparatively higher incidence of grade ≥3 peripheral neuropathy (reported in the range of 10–17% in breast cancer trials). While both formulations effectively eliminate Cremophor EL-associated hypersensitivity reactions and reduce the need for steroid premedication, Lipusu® demonstrates an additional safety advantage due to its controlled release behavior. Conversely, Abraxane® often shows faster tumor response attributed to gp60-mediated endothelial transcytosis and SPARC-assisted tumor accumulation. Clinical observations therefore suggest that Lipusu® may be preferable in patients requiring prolonged therapy with better tolerability, whereas Abraxane® may be selected when rapid tumor shrinkage is desired despite a higher neuropathy risk. This comparison highlights the therapeutic relevance of liposomal PTX in improving safety without compromising efficacy in breast cancer management.[152,155,169]

Although the preclinical results of most liposomal PTX platforms are promising, the transfer of these systems to clinical practice has been largely unsuccessful. A number of the formulations that have shown very good tumor targeting and efficacy in animal models have not gone on to clinical stages because of variability of the EPR effect in human tumors, unpredictable immunogenicity, instability during large-scale production and no reproducible therapeutic benefit over existing formulations. Complex surface changes in certain instances contributed to instability in formulation and regulatory challenges but did not offer proportional clinical value. Moreover, the biology of tumors in animal models and in patients with humans tended to be different, which subsequently led to lower accumulation of tumors and inaccurate

therapeutic responses. Such considerations indicate that though advanced liposomal designs are scientifically appealing, the success of their clinical translation demands the balancing of the formulation complexity, stability, scalability, and the heterogeneity of tumors in the real world.[164,170]

5.6. *Toxicity and safety issues*

5.6.1. *Reduced systemic toxicity*

A major benefit that liposomal PTX has over traditional preparations is the decrease in systemic toxicity. Conventional preparations of PTX involve the Cremophor EL as a solubilizer, which is linked to severe hypersensitivity reactions, neurotoxicity, and abnormal pharmacokinetics.[171] Liposomal encapsulation eliminates the need for Cremophor, thereby reducing the adverse effects of solvents. It has been clinically reported that liposomal PTX has good tolerability and reduced peripheral neuropathy, cardiac toxicity, and alopecia than traditional PTX. Besides, controlled release of liposomes into the bloodstream and tumor targeting lowers the peak plasma concentration, thereby exposing healthy tissue to the fewest liposomes.[172]

5.6.2. *Hematological and hypersensitivity reactions*

The toxicity in the hematology during chemotherapy is still of concern. The liposomal PTX preparations are associated with a reduced grade 3-4 neutropenia incidence and reduced grade 3-4 neutropenia with low myelosuppression. Selective delivery of drugs to the tumor also decreases thrombocytopenia and anemia.[173]

The present liposomal formulations have greatly decreased or removed hypersensitivity reactions that are prevalent with Cremophor-based PTX. The majority of patients do not need premedication with either corticosteroids or antihistamines, which enhances safety and compliance with patients. Infusion-related reactions can still take place, but are usually mild and treatable and are rare.[174]

5.6.3. *Long-term safety aspects*

Clinical trials and real-life studies indicate that liposomal PTX is generally well tolerated in more than one cycle with no cumulative toxicity that was not expected. The chronic side effects like neurotoxicity, cardiotoxicity, and hepatotoxicity, are not as strong as compared to conventional PTX. Also, liposomal preparations lower the exposure of the body at large, and this may decrease the chances of secondary malignancy and organ destruction.[175].

Pharmacovigilance should, however, be continued especially during combination therapy, or with repeated long-term therapy, to detect unusual but severe toxicity, such as infusion reactions, hypersensitivity, or delayed hematological impacts.[176]

5.7. *Challenges and future prospects*

5.7.1. *Scale-up and stability problems*

Although there have been encouraging preclinical and clinical outcomes, liposomal PTX on a large scale is not easily produced. The reproducibility of manufacture, the control of the size of particles, as well as the efficacy of encapsulation of drugs, must remain constant to use in the clinic.[177] Liposomes are thermodynamically unstable and, over time, may either aggregate, fuse, or leak out, which may influence the drug release profile and therapeutic efficacy. The stability of long-term storage and the prevention of the oxidation or hydrolysis of the lipids, and keeping the compound sterile are key elements in the development of commercial formulations.[178] Shelf-life has been improved by developments in freeze-drying (lyophilization) and stabilizing excipients, although cost-efficient and scalable approaches to this challenge have not been developed yet.[179]

5.7.2. *Clinical and regulatory translation problems*

Regulatory requirements have made it difficult to translate liposomal PTX bench-to-bedside. The variation in lipid composition, size, surface modification, and encapsulation methodologies may affect pharmacokinetics and safety, necessitating a rigorous preclinical and clinical testing process. Regulatory organizations require rigorous quality control, batch-to-batch reproducibility, and stability information, which may increase development cycles.[180]

Comparative trials with traditional PTX, nab-PTX, and other nanoformulations should be used clinically to justify the advantages of the therapy and cost-effectiveness. In addition, heterogeneous

populations of breast cancer require strata of patients and the identification of biomarkers to maximize benefit in these patients.[181]

5.7.3. *Novelties*

The future of liposomal PTX is in complex, target-based, and individualistic nanomedicine strategies:

Smart Liposomes: Stimulus-responsive liposomes, releasing drugs in response to pH, temperature, redox potential, or enzymes of the tumor microenvironment, improve site-specific delivery and reduce systemic toxicity.

Hybrid Nanocarriers: Liposomes may be improved by the addition of polymers, micelles, or inorganic nanoparticles to enhance stability and drug loading and multi-functionality, allowing combination therapies and theranostics.

Individualized Therapy: Optimization of efficacy and minimization of adverse effects can be achieved by personalization of liposomal preparations depending on the tumor receptor expression, genetic profile, and the patient's pharmacokinetics. Ligand-based targeting can be integrated (e.g., antibodies or peptides) to deliver the package to a particular subtype of breast cancer (e.g., High-level expression of HER2 or TNBC).

6. Conclusion

The introduction of liposomal PTX is a breakthrough in the treatment of breast cancer since it overcomes the numerous drawbacks of traditional PTX preparations. Liposome-based encapsulation is associated with increased tumor-targeted delivery, decreased systemic toxicity, and increased therapeutic outcome. Ligand-based targeting, stimulus responding systems, and combination therapy are additional advances to the possibilities of liposomal formulations to target cancer with precision. Clinical safety and efficacy of approved formulations, including Lipusu, have been proven, and many investigational liposomal PTX formulations are in clinical trials to improve pharmacokinetics, adverse effects, and antitumor activity. The next generation of nanomedicine will be based on progress in smart liposomes, hybrid nanocarriers, and personalized therapy despite the difficulties of scale-up, stability, and regulatory approval. In general, liposomal PTX can be considered an effective platform to enhance the outcomes in patients with breast cancer, and the future perspectives of its use in the clinical setting and its therapeutic properties are likely to be extended through future research.

Data Availability

No new data was generated as this is a review article.

Statement of Usage of Artificial Intelligence

No AI was used in this article.

Conflict of Interests

None to declare.

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