

PAPER

Clinical Translation of PEGylated Liposomal Drug Delivery Systems in Oncology: Progress, Barriers, and Future Prospects

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ABSTRACT

The development of PEGylated liposome drug delivery systems as a method to deliver nanomedicine in oncology, is a means to improve the pharmacokinetic and therapeutic properties of cytotoxic agents. Liposomes are flexible phospholipid bilayers capable of encapsulating hydrophilic and/or hydrophobic drugs; thereby providing protection to unstable drugs and control over how drugs distribute within the body. Once the surface of liposomes is modified using poly (ethylene glycol) (peg), 'stealth' liposomes are formed that demonstrate steric stabilization and inhibit the opsonization of the liposome. As a result of being less susceptible to removal from the circulation by the reticuloendothelial system (RES) due to sterically stabilized surfaces, stealth liposomes will remain in circulation longer than traditional liposomes. Additionally, stealth liposomes will increase the duration in which they remain in proximity to solid tumors through enhanced permeation and retention (EPR) effects. Pegylated liposomal doxorubicin (doxorubicin HCl liposome) and nanoliposomal irinotecan have been the first two liposomal formulations to receive FDA approval. Each formulation provided improvements to the therapeutic index and reduced dose-limiting toxicities relative to their non-pegylated counterparts. However, many obstacles exist that must be addressed prior to widespread adoption of pegylated liposomes. These obstacles include uniform delivery of the liposomes to tumors, ability of liposomes to penetrate into tumor tissue, the "peg dilemma", and the possibility of eliciting an immune response leading to accelerated blood clearance (ABC) or anti-peg antibodies. Researchers continue to develop novel stealth polymers, PEG-based architectures that respond to stimulus, theragnostic liposomes, and combine liposomes with immunotherapy as methods to achieve more reliable delivery of liposomes and better clinical outcomes for patients. Although pegylated liposomes represent one of the most efficient platforms available for the delivery of chemotherapeutic agents, it is critical to take into consideration both the design of the liposome and the individual characteristics of each patient to obtain optimal results in treating cancers. This revised synthesis explicitly reframes EPR-based deposition as one component of a broader, heterogeneous tumor-transport process; highlights the quantitative limitation that generally less than 2% of the injected nanoparticle dose reaches tumors; incorporates anti-PEG antibody prevalence and patient-stratification considerations; adds manufacturing or CMC reproducibility barriers; and proposes testable

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translational hypotheses for imaging-guided patient selection and rational immunotherapy combinations.

KEYWORDS

poly (ethylene glycol) (PEG)ylated liposomes, nanomedicine, drug delivery systems, oncology, stealth liposomes

1 INTRODUCTION

Liposomes are self-assembled phospholipid bilayer vesicles that can encapsulate hydrophilic therapeutic agents within an aqueous core and incorporate lipophilic or amphipathic agents within, or in association with, the lipid bilayer. By virtue of this structural versatility, encapsulated labile drugs can be protected against degradation by enzymes or chemicals, the biodistribution can be controlled by a lipid-based carrier as well as the release kinetics can be tailored based on the bilayer composition and vesicle size. In cancer therapy, these advantages enable longer systemic availability, reduced off target delivery of free drug and an increased likelihood of delivery into tumors compared with traditional formulations [1–4].

PEGylation is defined as the surface modification of liposomes with the stable incorporation or chemically bonding of poly (ethylene glycol) (PEG), usually through the use of PEG-lipid conjugates, which provide anchorage in the lipid bilayer while providing hydrated polymer chains in the aqueous phase. The resultant “PEG-corona” affects several interfacial characteristics such as colloidal stability, protein adsorption behavior and cellular recognition, and thus enables prolonged circulation [5–9].

At the molecular level, PEG chain conformation is governed by polymer length and surface grafting density, producing conformations that transition from “mushroom” to “brush” regimes. These regimes generate steric–hydration repulsion that reduces short-range interactions with proteins and cell membranes, which is foundational to the reduced opsonization and extended systemic half-life characteristic of sterically stabilized (“stealth”) liposomes [10–12]. Figure 1 depicts a spherical liposomal nanocarrier composed of a phospholipid bilayer enclosing an aqueous core containing encapsulated drug molecules.

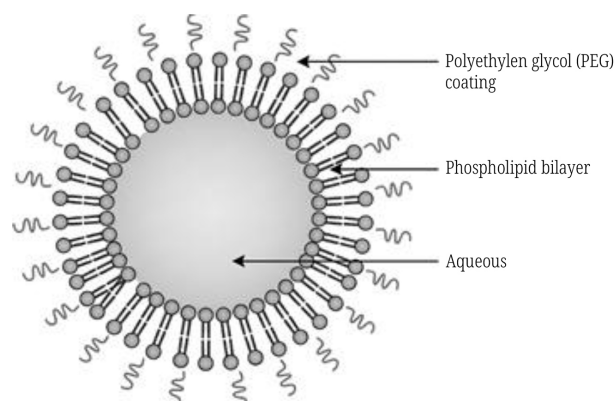


Fig. 1. Three-dimensional schematic representation of a PEGylated liposome

Following hydration and vesicle formation, PEG–lipids preferentially present PEG chains on the outer leaflet, creating a dense, water-rich shell that exerts repulsive

steric and hydration forces. This steric stabilization reduces aggregation and fusion and decreases adsorption of opsonins (e.g., immunoglobulins, complement components, apolipoproteins) that promote recognition by the mononuclear phagocyte system (MPS). By attenuating opsonization, PEGylation reduces uptake by Kupffer cells and splenic macrophages, thereby extending systemic circulation and increasing the probability of repeated tumor-pass exposure [8–12].

PEGylation generally reduces systemic clearance and increases overall exposure (AUC), often restricting early biodistribution largely to the vascular compartment compared with free drug. These PK effects increase the integrated likelihood of tumor extravasation over time and can alter dose-limiting toxicities by reducing peak concentrations of unencapsulated drug in sensitive tissues. Importantly, prolonged circulation improves opportunity for deposition, whereas intraliposomal retention and release kinetics govern whether deposited drug becomes bioavailable at clinically meaningful levels within tumor tissue [13–16]. Figure 2 displays the pharmacokinetics of PEGylated liposomes and non-PEGylated liposomes.

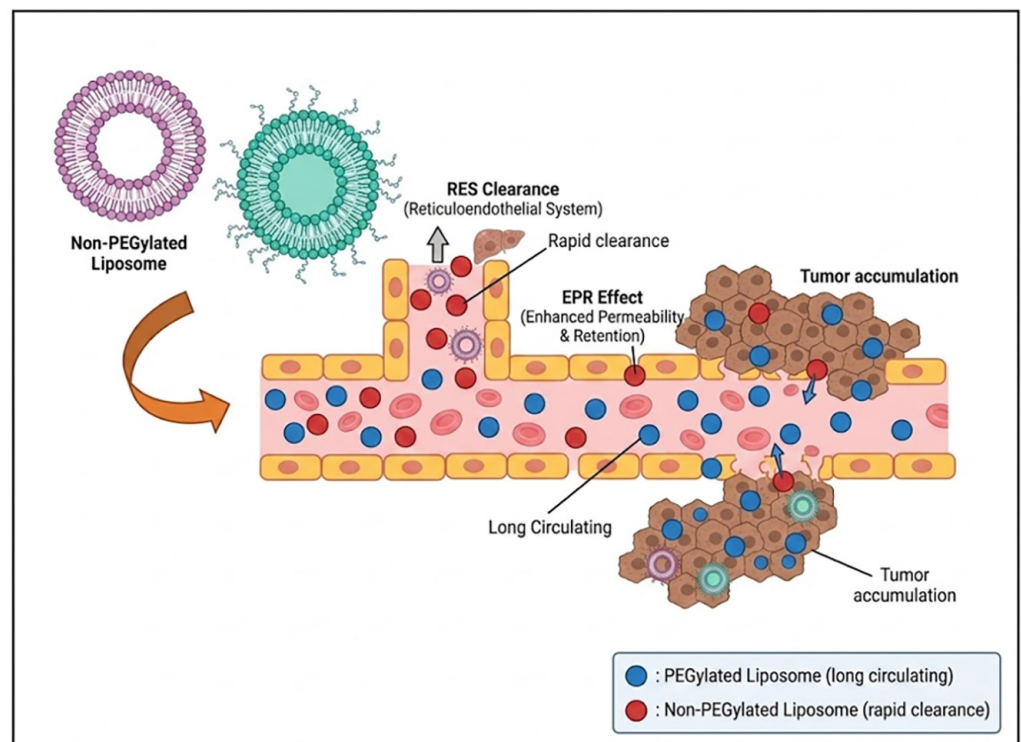


Fig. 2. Schematic illustrate of the pharmacokinetics and tumor delivery of PEGylated versus non-PEGylated liposomes

A clinically informative demonstration of these principles is radiolabeled PEGylated liposome imaging in patients, which showed prolonged blood residence and measurable tumor localization in solid malignancies, providing direct translational evidence that long-circulating liposomes can accumulate in human tumors under clinical conditions [17].

Pegylated liposomal doxorubicin (PLD; Doxil[®]/Caelyx[®]) remains the prototypical “stealth liposome” anticancer nanomedicine, and it provides a benchmark for understanding how PEGylation integrates with loading chemistry and release behavior to determine clinical performance. Its profile reflects (i) stable intraliposomal drug loading and retention, classically enabled by transmembrane gradient loading;

(ii) prolonged systemic circulation due to PEG-mediated steric stabilization; and (iii) reduced early distribution of free doxorubicin to normal tissues, yielding a toxicity profile that differs materially from conventional doxorubicin [15, 16, 18].

Regulatory product information characterizes PLD as a PEGylated liposomal formulation with pharmacology distinct from non-liposomal doxorubicin, including altered distribution and exposure patterns consistent with carrier-controlled delivery. These regulatory descriptions reinforce that clinical behavior emerges from the combined effects of PEGylation, membrane composition, loading strategy, and release kinetics rather than from any single design feature in isolation [19, 20].

1.1 Key advantages in oncology

The enhanced permeability and retention (EPR) effect describes preferential accumulation of macromolecules and nanoparticles in tumors due to hyperpermeable tumor vasculature and impaired lymphatic drainage, which together can increase retention of appropriately sized, long-circulating carriers. PEGylation supports EPR-based deposition primarily by extending blood residence time, thereby increasing cumulative exposure of tumor microvasculature to circulating liposomes and raising the likelihood of extravasation in permissive regions [21–23].

However, mechanistic work increasingly indicates that tumor entry is not exclusively “passive.” Endothelial transport routes (including transcytosis), macrophage-assisted transport, and heterogeneity of vessel phenotypes can substantially modulate nanoparticle flux into and within tumors. These insights have motivated broader conceptual frameworks (e.g., active transport and retention, ATR) that complement classical EPR and provide mechanistic explanations for variability in delivery outcomes across tumors and patients [24–28].

Therefore, the passive targeting paradigm has been revised from a deterministic EPR model to a probabilistic and mechanism-dependent model. In this interpretation, PEGylated liposomes benefit from prolonged circulation, but tumor entry and retention depend on endothelial transcytosis, macrophage-mediated transport, vascular phenotype, tumor exit pathways, and patient-specific microenvironmental barriers. This avoids overstating EPR as the sole mechanism and aligns the review with recent evidence supporting active transport and retention mechanisms [24–28].

In PEGylated liposomes, “passive targeting” is most rigorously interpreted as statistical targeting: prolonged circulation increases the probability of deposition events, while high intraliposomal retention sustains local exposure after deposition even when the fraction of injected dose reaching tumor is modest. Accordingly, therapeutic benefit may arise not only from the amount delivered but also from the exposure time course and the balance between stability during circulation and release within tumor tissue [29–32].

Quantitatively, nanoparticle tumor delivery remains a major translational bottleneck. Meta-analytic evaluations indicate that only a small fraction of the injected nanoparticle dose reaches solid tumors, commonly below 2% of the injected dose. For PEGylated liposomes, this limitation means that improved circulation does not automatically translate into therapeutically sufficient intratumoral exposure. Clinical benefit therefore depends on the combined effects of dose, circulation time, tumor accumulation, intratumoral distribution, release kinetics, and the pharmacodynamic potency of the liberated payload [29–32].

Clinical and translational observations further show that tumor accumulation is measurable yet heterogeneous and that intratumoral distribution is frequently

perivascular unless penetration constraints are mitigated. Therefore, the clinically relevant endpoint is effective exposure across meaningful tumor compartments rather than mere detection of carrier presence within tumor tissue [17, 33–35].

A principal advantage of PEGylated liposomes is improvement of therapeutic index through reduced systemic exposure to free drug during circulation. Encapsulation limits peak free-drug concentrations in plasma and normal tissues, thereby mitigating dose-limiting toxicities associated with conventional formulations while preserving antitumor activity in settings where tumor deposition and release are adequate [36, 37].

In PLD specifically, clinical experience illustrates that toxicity is not eliminated but redistributed: cardiotoxicity risk is reduced compared with conventional doxorubicin, whereas exposure-pattern toxicities such as palmar–plantar erythrodysesthesia and mucositis can become more prominent, reflecting prolonged circulation and altered tissue distribution. This underscores that therapeutic-index gains depend jointly on circulation time and release kinetics that determine where and when active drug becomes bioavailable [36–38].

PEGylated liposomes provide two stabilizing layers: the lipid bilayer sequesters payloads from direct enzymatic or chemical attack, and the PEG corona reduces destabilizing protein–membrane interactions and aggregation, maintaining colloidal integrity in vivo. These effects improve formulation robustness during circulation and support predictable systemic exposure [38].

For gradient-loaded anthracyclines and other amphipathic weak bases, intraliposomal precipitation/complexation further suppresses premature leakage, enabling long-circulating “drug reservoirs” that preferentially release drug in tissue compartments rather than in blood. This stabilizing architecture is particularly consequential for narrow-therapeutic-index cytotoxics, where modest changes in free-drug exposure can substantially affect tolerability and feasible dosing [18]. Figure 3 presents the key advantages of PEGylated liposomal formulations in oncology.

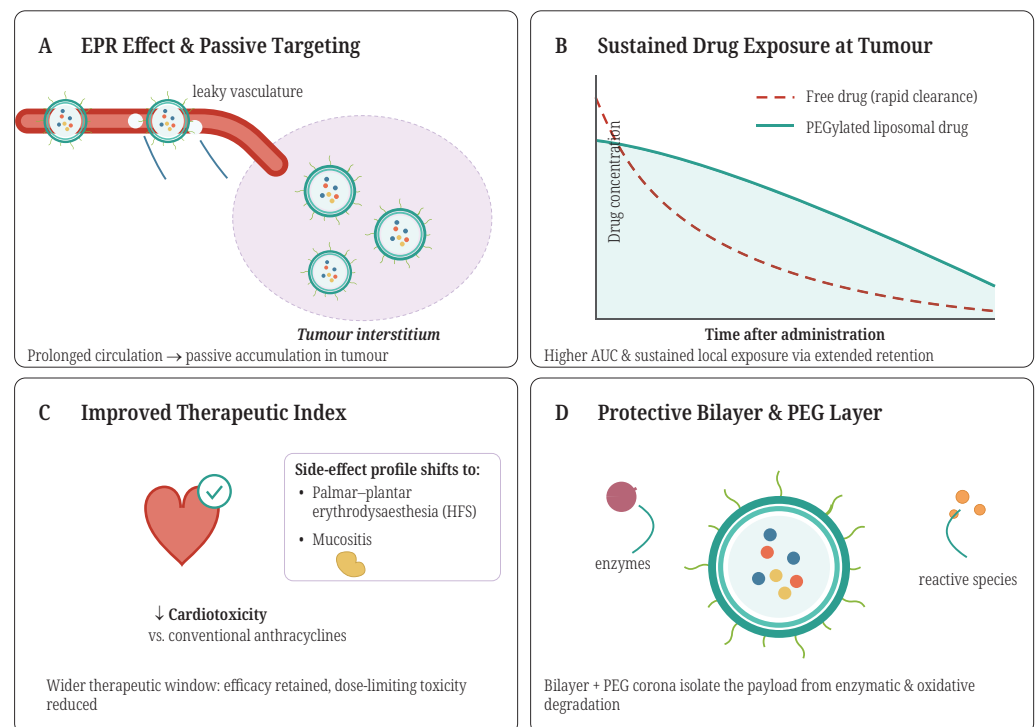


Fig. 3. Key advantages of PEGylated liposomal formulations in oncology

1.2 Clinical successes and approved formulations

A number of malignancies are being studied using PLD, and there is considerable evidence demonstrating that it has significant clinical activity and a differentiated safety profile due to its liposomal formulation and PEG-mediated pharmacokinetic modification. The regulatory label for PLD clearly establishes its PEGylated liposomal nature and that it is pharmacologically distinct from traditional doxorubicin [19, 20, 39].

The randomized trials in patients with AIDS-related Kaposi's sarcoma demonstrated PLD to be an effective treatment with acceptable tolerance compared to the many multi-drug cytotoxic regimens that were used historically, which supports the broader clinical use of this drug in a population that is particularly vulnerable to systemic toxicity [39].

The randomized phase III study of PLD versus topotecan in patients with recurrent epithelial ovarian cancer provided support for PLD as a standard treatment, and subsequent analyses indicate that PLD continues to provide a durable benefit to certain clinically relevant subgroups of patients, which will help determine when and how to use PLD in different disease states and treatment histories [40–42].

PLD was shown in the phase III studies to be equivalent to conventional doxorubicin in terms of efficacy, but to be associated with significantly less cardiac toxicity, which demonstrates the rational basis for the development of PEGylated liposomal anthracyclines in situations where cumulative anthracycline exposure is a critical clinical concern [43].

The combination of PLD and bortezomib in patients with multiple myeloma resulted in superior time-to-progression compared to bortezomib monotherapy, demonstrating that PLD can serve as a viable anthracycline in combination regimens that offer both clinically meaningful efficacy and a modified toxicity profile [44].

Onivyde (nanoliposomal irinotecan) is approved for metastatic pancreatic cancer after prior therapy with gemcitabine-containing chemotherapy, based on phase III evidence (NAPOLI-1) showing that nal-IRI plus 5-FU/leucovorin results in longer survival than the comparator therapy [45, 46].

As far as formulation goes, the labeling for Onivyde indicates that methoxy-PEG2000-DSPE is one of the excipients included in the formulation, which shows that the formulation includes a PEGylated lipid component as part of the membrane structure of the liposomes, in keeping with the intention to alter the circulation and biodistribution of irinotecan. Studies involving analysis of the formulation of Onivyde and reverse-engineering of the formulation also show that nal-IRI performance is dependent on a variety of interrelated factors related to lipid composition, stability, retention and release, all of which influence how much of irinotecan and its active metabolite are delivered to tumors vs. normal tissues [45, 47].

Marqibo is a liposomal formulation of vincristine made with sphingomyelin and cholesterol designed to increase the dose intensity of vincristine compared to the conventional formulation and illustrates another possible approach to stabilizing liposomes that is different from the traditional method of PEGylation [48, 49].

Marqibo was first approved by the FDA in 2009; however, the approval was revoked in 2022 as the company failed to meet requirements for follow-up evidence, and it illustrates that simply developing a liposomal version of a drug is insufficient to guarantee long-term clinical acceptance and that fulfillment of post-marketing evidentiary commitments is necessary for successful translation of nanomedicines into practice [50].

Although numerous studies have been performed, relatively few PEGylated liposomal anticancer drugs have received and maintained FDA approval, whereas a number of non-PEGylated liposomal formulations have gained entry into clinical testing.

This reflects the existence of a number of viable paths to develop liposomal formulations (e.g., lipid composition, particle size, loading methods, and release designs), and each path may be best suited to a particular payload or disease context [51–53].

An instructive example of successful liposomal engineering without PEGylation is CPX-351 (liposomal daunorubicin/cytarabine), which demonstrates that liposomes can preserve pharmacologically advantageous drug ratios and improve outcomes in selected hematologic malignancies, reinforcing that PEGylation is a powerful strategy but not a universal prerequisite for clinical success [54]. Table 1 summarizes the clinical successes and approved liposomal formulations.

Table 1. Clinical successes and regulatory status of selected liposomal anticancer formulations

Formulation	Liposomal Design/ PEGylation Status	Approved Indications & Key Clinical Evidence	Clinical Impact and Translational Significance
Doxil®/Caelyx® (Pegylated liposomal doxorubicin)	PEGylated liposome (DSPE-PEG2000); long-circulating sterically stabilized formulation	Approved for AIDS-related Kaposi's sarcoma, recurrent epithelial ovarian cancer, metastatic breast cancer, and multiple myeloma (in combination). Randomized trials demonstrated efficacy comparable or superior to standard therapies, including superiority to topotecan in ovarian cancer and improved time-to-progression with bortezomib in myeloma.	Established clinical proof-of-concept for PEGylated liposomes. Demonstrates improved therapeutic index via reduced cardiotoxicity and altered biodistribution, validating PEG-mediated PK modulation as a viable oncology strategy.
Onivyde® (Nanoliposomal irinotecan; nal-IRI)	PEGylated liposomal formulation containing methoxy-PEG2000-DSPE	Approved (with 5-FU/leucovorin) for metastatic pancreatic cancer after gemcitabine failure. Phase III NAPOLI-1 trial showed significant overall survival benefit versus control regimens.	Illustrates PEGylated liposomes beyond anthracyclines. Clinical performance depends on integrated lipid composition, stability, retention, and release behavior, supporting rational liposomal design for difficult-to-treat solid tumors.
Marqibo® (Vincristine sulfate liposome injection)	Non-PEGylated liposome (sphingomyelin/ cholesterol)	Initially approved for relapsed/refractory acute lymphoblastic leukemia to enable higher vincristine dose intensity; FDA approval withdrawn in 2022 due to failure to complete confirmatory studies.	Highlights that liposomal reformulation can modify PK but does not guarantee durable clinical success. Underscores the importance of confirmatory evidence and post-marketing obligations in nanomedicine translation.
CPX-351	Non-PEGylated dual-drug liposome with fixed synergistic drug ratio	Approved for high-risk and secondary acute myeloid leukemia based on improved survival versus standard 7 + 3 therapy in phase III trials.	Demonstrates that PEGylation is not universally required for clinical success. Validates liposomes as platforms for ratio-controlled combination therapy, expanding the translational landscape beyond long-circulating PEGylated systems.

1.3 Challenges and limitations of conventional PEGylated liposomes

PEGylation extends circulation time but can attenuate biological interactions required for maximal therapeutic effect at the tumor–cell interface, particularly when efficacy depends on efficient cellular internalization, intracellular trafficking, or endosomal escape (as in nucleic-acid delivery). This “PEG dilemma” arises because the same steric barrier that reduces opsonization can also reduce membrane association and receptor engagement unless ligand presentation is engineered to overcome PEG shielding [55].

In actively targeted systems (e.g., antibody-, peptide-, or small-molecule ligand conjugates), PEG may function either as a beneficial spacer that projects ligands into the biological milieu or as an obscuring layer that decreases productive binding,

depending on PEG length, ligand density, and architectural placement. In order to achieve maximum benefit from PEGylated liposome-based drug delivery, it is necessary to balance the stealth characteristics of PEGylated liposomes against those factors that determine how they are taken up into cells, transported through tissues, and delivered to target sites of action—all of which are involved in determining the pharmacodynamic effects of drugs administered in this manner [56, 57].

The repeated administration of PEGylated liposomes can lead to an increase in the rate at which they are cleared from the circulation, a phenomenon known as the accelerated blood clearance (ABC) reaction. The ABC reaction occurs when the first administration of PEGylated liposomes induces an immune response mediated by IgM antibodies that leads to their removal from the circulation at a faster rate than would occur otherwise. Upon subsequent administration, the reduced concentration of these antibodies results in decreased exposure of tumor sites to the drug and the potential for a diminished therapeutic effect [58, 59].

Studies have shown that anti-PEG antibodies, including both pre-existing antibodies to PEG and antibodies formed as a result of exposure to PEGylated liposomes, play a role in reducing the efficacy of drugs delivered using this method, increasing the variability of drug concentrations among individuals, and altering the pharmacokinetic profiles of drugs delivered in this way. In addition to the clinical implications associated with the formation of anti-PEG antibodies, such as reduced efficacy, increased variability among individuals, and altered pharmacokinetic profiles, these immune responses present a significant challenge to delivering drugs chronically or repeatedly via PEGylated liposomes and therefore require careful consideration during both the formulation design process and the clinical dosing strategy process [60–62].

Pre-existing anti-PEG antibodies should also be treated as a patient-level covariate rather than only as a post-treatment phenomenon. Published reviews report that anti-PEG antibody positivity may be detected in a substantial minority of individuals, frequently cited in the approximate 20–40% range depending on assay platform, antibody class, threshold definition, and population sampled. This variability has direct implications for pharmacokinetics, infusion reactions, complement activation, ABC, and repeat-dose efficacy. Accordingly, future clinical protocols should consider baseline anti-PEG IgG/IgM assessment, complement-activation risk evaluation, prior PEG exposure history, infusion-reaction history, and early PK monitoring as candidate stratification tools [61–65].

Activation of the complement system may also contribute to infusion reactions and acute hypersensitivity-like reactions, such as those that occur as part of complement activation-related pseudoallergy (CARPA), which can be influenced by the surface chemistry of the nanoparticles, the infusion parameters used to administer the nanoparticles, and the individual's predisposition to developing allergic reactions. Because of the risk of such adverse reactions and because managing them is essential to ensuring the safety and tolerance of administering nanoparticles to humans, there are several constraints related to CARPA that will need to be managed during the clinical development and use of PEGylated liposomes [63–65].

Strategies for administering PEGylated liposomes have been evaluated as one means of potentially mitigating some of the negative consequences of the ABC reaction; for example, the slow infusion of PEGylated liposomes has been shown in pre-clinical studies to decrease the rate at which they are removed from the circulation by decreasing the rate of antibody-mediated clearance and demonstrating that the regimen design used to deliver PEGylated liposomes can influence the interaction of the nanoparticles with the host immune system [66].

One of the main limitations of the passive targeting approach to delivering drugs is that the delivery of nanoparticles to cancerous tissue is unpredictable and

heterogeneous across different types of cancers, different stages of cancer, different sites of metastasis, and different individuals. There is also heterogeneity in the delivery of nanoparticles within a single tumor as a result of differences in the vascular architecture of the tumor, the perfusion of the tumor, and the composition of the tumor microenvironment, all of which make it difficult to predict the extent to which nanoparticles will penetrate the tumor and be retained within the tumor [33, 67].

More recent analyses have highlighted the fact that the delivery of nanoparticles to tumors involves many complex mechanisms that vary in space and include different types of vessels that supply oxygen to the tumor and active transport routes through which particles can enter and leave the tumor, and that the combined effects of tumor entry and exit routes may significantly affect net retention. As a result of the complexity of these mechanisms, the heterogeneity of the delivery of nanoparticles to tumors creates challenges for predicting the effectiveness of doses of drugs administered using nanoparticles and for understanding why clinical outcomes have been inconsistent and thus motivates the use of biomarkers to identify patients who are likely to experience favorable delivery of drugs via nanoparticles [31, 68].

Even when PEGylated liposomes extravasate, deep tumor penetration may be restricted by dense ECM networks, elevated interstitial fluid pressure, and solid stress that compress vessels, reduce convection, and retain large carriers near perivascular regions. These constraints are particularly prominent in desmoplastic tumors and contribute to no uniform intratumoral exposure that can permit resistant niches to persist [35, 69].

Accordingly, improving penetration frequently requires either localized/triggered release to liberate diffusible drug beyond the perivascular space or microenvironment-modulating adjunct strategies (e.g., vascular normalization, stromal remodeling) that reduce transport resistance. In this context, PEGylation can be necessary for systemic persistence but insufficient for uniform intratumoral exposure unless integrated with release engineering and/or micro-environmental intervention [32, 68]. Table 2 summarizes the key challenges and limitations of conventional PEGylated liposomes.

A critical determinant of heterogeneous tumor exposure is the quantitative relationship between liposome physicochemical properties and transport through tumor tissue. Smaller particles, especially those below approximately 50–100 nm, generally diffuse and penetrate more deeply than larger carriers, whereas particles above approximately 200 nm are more likely to remain near perivascular regions. Shape, deformability, surface charge, PEG grafting density, PEG chain length, and alternative hydrophilic coatings further determine extravasation, extracellular matrix transport, cellular contact, and uptake. Thus, the PEG dilemma is not only a circulation-versus-cellular-uptake trade-off, but also a penetration-versus-stealth optimization problem [70–75]. Table 2 presents the physicochemical design parameters that influence the extravasation, tumor penetration, intratumoral distribution, and cellular uptake of PEGylated liposomes.

Table 2. Physicochemical design parameters governing extravasation, penetration, intratumoral distribution, and cellular uptake of PEGylated liposomes

Design Parameter	Expected Transport Effect	Therapeutic Implication
Hydrodynamic diameter	Particles below about 50–100 nm tend to penetrate more deeply; larger particles, especially above about 200 nm, are more often retained near vessels.	Optimizing size may improve intratumoral distribution, but very small carriers may lose payload retention or circulation advantages.
Shape and deformability	More deformable or non-spherical carriers may navigate pores and ECM differently from rigid spherical particles.	Mechanical design can complement biochemical stealth strategies for transport through dense tumors.

(Continued)

Table 2. Physicochemical design parameters governing extravasation, penetration, intratumoral distribution, and cellular uptake of PEGylated liposomes (*Continued*)

Design Parameter	Expected Transport Effect	Therapeutic Implication
PEG density and PEG chain length	Higher PEG density/longer chains reduce opsonization but can hinder ECM transport, membrane association, receptor binding, and uptake.	Requires balancing circulation half-life against cellular internalization and deep penetration.
Surface charge and terminal groups	Near-neutral hydrated surfaces reduce nonspecific protein adsorption; cationic surfaces may improve cell interaction but increase toxicity/clearance risk.	Surface chemistry should be tuned to the payload, dosing schedule, and tumor microenvironment.
Release kinetics	Stable carriers may reach tumor but retain payload too strongly; triggered or local release can expand exposure beyond perivascular zones.	Effective therapy depends on bioavailable drug, not merely carrier detection in tumor tissue.
Alternative hydrophilic coatings	Poly(2-oxazoline), zwitterionic coatings, or modified PEG architectures may alter hydration, immune recognition, and interstitial transport.	Next-generation coatings should be evaluated by penetration, immunogenicity, and manufacturability, not circulation time alone.

Table 3 provides a comprehensive overview of the key challenges and limitations associated with conventional PEGylated liposomes in tumor drug delivery. Despite their ability to prolong circulation time and enhance passive tumor accumulation through the EPR effect, conventional PEGylated liposomes face several obstacles, including limited tumor penetration, heterogeneous intratumoral distribution, reduced cellular uptake due to the PEG shielding effect, ABC upon repeated administration, and variability in therapeutic efficacy across different tumor types. These limitations can significantly affect drug delivery efficiency and overall treatment outcomes.

Table 3. Challenges and limitations of conventional PEGylated liposomes in tumor drug delivery

Challenge Category	Underlying Mechanism	Impact on Therapeutic Performance
PEG dilemma: prolonged circulation vs. biological interaction	The PEG corona provides steric shielding that reduces opsonization and prolongs circulation but simultaneously impedes membrane association, receptor engagement, cellular internalization, intracellular trafficking, and endosomal escape. The effect is particularly detrimental for payloads requiring active cellular uptake, such as nucleic acids.	Reduced tumor cell uptake and intracellular drug bioavailability; diminished pharmacodynamic response despite adequate tumor accumulation; limited efficacy of actively targeted PEGylated systems without optimized ligand presentation.
Accelerated Blood Clearance (ABC) and anti-PEG immunity	Repeated exposure to PEGylated liposomes can induce IgM-mediated immune responses and anti-PEG antibody production, leading to accelerated clearance of subsequent doses. Complement activation may also occur, contributing to hypersensitivity reactions (CARPA).	Decreased systemic exposure upon repeat dosing; increased inter-patient variability; reduced tumor accumulation over time; safety concerns including infusion-related reactions; challenges for chronic or multi-cycle treatment regimens.
Heterogeneity of the EPR effect	Tumor delivery depends on heterogeneous vascular permeability, perfusion, endothelial transport pathways, and microenvironmental features that vary across tumor types, disease stages, anatomical sites, and individual patients. Both tumor entry and exit pathways influence net nanoparticle retention.	Unpredictable tumor accumulation and dose–response relationships; inconsistent clinical outcomes; limited generalizability of passive targeting; need for patient stratification and delivery biomarkers.
Limited intratumoral penetration (ECM, IFP, solid stress)	Dense extracellular matrix, elevated interstitial fluid pressure, and mechanical solid stress compress blood vessels, reduce convection, and restrict nanoparticle transport, retaining PEGylated liposomes near perivascular regions—particularly in desmoplastic tumors.	Non-uniform intratumoral drug distribution; persistence of poorly perfused, drug-resistant tumor niches; reduced overall therapeutic efficacy despite measurable tumor deposition; requirement for release engineering or microenvironment-modulating adjunct strategies.

1.4 Manufacturing and CMC scalability barriers

Translation of PEGylated liposomes also depends on chemistry, manufacturing, and controls (CMC) robustness. Batch-to-batch variability in particle-size distribution, polydispersity, PEG-lipid molar ratio, PEG surface density, lamellarity, encapsulation efficiency, drug-to-lipid ratio, residual solvent, sterility, and in vitro release can change pharmacokinetics, biodistribution, immune recognition, and safety. Therefore, clinical translation requires predefined critical quality attributes, orthogonal analytical characterization, validated release assays, stability-indicating methods, and comparability testing after scale-up or process transfer [76–80].

The reviewer-suggested nanomaterial studies have been incorporated as broader characterization examples to reinforce this CMC principle. Although Au/ZnO and CNF@Au systems are not PEGylated liposomal oncology products, they illustrate how synthesis route, core-shell architecture, surface chemistry, and nanoparticle characterization can influence biological and functional performance; this broader nanomaterial lesson supports the need for rigorous material-attribute control in liposomal translation. As shown in Table 4, the translational success of PEGylated liposomes depends not only on their design characteristics but also on the robustness of manufacturing and CMC processes. Factors such as lipid purity, formulation composition, process scalability, particle size control, encapsulation efficiency, sterility assurance, and long-term stability play pivotal roles in maintaining reproducible product performance from laboratory development to commercial production. Understanding and controlling these variables are essential for minimizing variability, ensuring regulatory acceptance, and achieving reliable clinical outcomes.

Table 4. Manufacturing and CMC variables that influence translational reproducibility of PEGylated liposomes

CMC Variable	Critical Quality Attribute	Potential Translational Consequence	Control Strategy
Size distribution/PDI	Mean diameter, PDI, fraction of oversized particles	Altered clearance, tumor penetration, perivascular retention, and batch comparability	Validated extrusion or microfluidic process; DLS/NTA/cryogenic imaging; release specifications
PEG density and PEG-lipid stability	Molar ratio, surface density, PEG chain length, PEG shedding	Changed opsonization, ABC/CARPA risk, cellular uptake, and penetration	Quantitative lipid analysis; accelerated stability; complement and anti-PEG risk assays
Drug loading and retention	Encapsulation efficiency, drug-to-lipid ratio, leakage, release kinetics	Reduced efficacy or increased off-target toxicity if free drug or leakage varies	Validated loading process; in vitro release and serum stability assays
Lipid composition and lamellarity	Membrane rigidity, cholesterol ratio, phase behavior	Changes in circulation, release, and payload bioavailability	Orthogonal physicochemical characterization and comparability testing
Scale-up and storage	Sterility, residual solvent, endotoxin, aggregation, shelf-life	Regulatory delays, safety risks, and inconsistent clinical performance	GMP controls, stability-indicating assays, sterility/endotoxin testing, cold-chain validation

1.5 Future perspectives and emerging directions

Given increasing recognition of anti-PEG immunity and ABC-related variability, next-generation stealth strategies aim to retain hydration-based circulation extension while reducing immunogenicity and complement activation. Alternative hydrophilic polymers (e.g., poly(2-oxazoline), zwitterionic coatings) have been proposed and investigated as potential substitutes that can provide strong hydration shells with distinct immune-recognition profiles [81–84].

In parallel, modified PEG chemistries and terminal-group engineering seek to reduce immunogenic epitopes while preserving stability, and regimen-level approaches (including infusion-rate modulation) remain pragmatic tools to mitigate antibody-mediated clearance when platform switching is not feasible. Increasingly, stealth coatings are evaluated using multidimensional criteria immune compatibility, complement activation propensity, manufacturing robustness, and regulatory viability rather than circulation time alone [85–87].

In the short term, the use of imaging biomarkers is likely to be an effective method of determining whether individual tumors will allow accumulation of PEGylated liposomes. This can be achieved by directly labeling liposomes with radioisotopes or by using nanoparticles to act as surrogate tracers. The ability to observe delivery would enable patient stratification into two groups, those who can accumulate nanoparticles and those who cannot. Patient selection could then be refined to ensure that only those capable of accumulating the treatment are exposed to it. Furthermore, the level of dose required to achieve a therapeutic effect would be better determined if one knew how much was being delivered, allowing the most effective use of the adjunct delivery enhancement methods available in tumors with poor inherent permeability to nanoparticles.

To make the future-perspectives section operational, the revised manuscript now frames key directions as testable hypotheses rather than generic opportunities. These hypotheses connect patient selection, tumor delivery, PEG immunity, combination therapy, and manufacturing control to measurable endpoints (refer to Table 5).

Table 5. Testable translational hypotheses and measurable endpoints for next-generation PEGylated liposomal oncology systems

Testable Hypothesis	Study Design/Measurable Endpoint	Translational Value
Imaging-guided patient selection improves response to PEGylated liposomes.	Use radiolabeled or theranostic liposomes/surrogate tracers before treatment; correlate tumor uptake and spatial distribution with response, PFS, toxicity, and PK.	Identifies patients likely to benefit and avoids exposing non-accumulators to ineffective therapy.
Baseline anti-PEG antibody status predicts altered PK, ABC risk, or infusion reactions.	Measure anti-PEG IgG/IgM and complement markers before treatment and after cycle 1; associate with clearance, AUC, reactions, and repeat-dose exposure.	Enables patient stratification, infusion-risk mitigation, and alternative stealth-coating selection.
Rational combinations with immunotherapy outperform empiric combinations.	Combine PLD or liposomal immune agonists with checkpoint blockade only when schedule, ICD markers, immune infiltration, and tumor drug exposure are prospectively measured.	Aligns cytotoxic release with immune priming while minimizing systemic inflammation.
Optimized PEG architecture improves penetration without sacrificing circulation.	Compare PEG chain length/density, cleavable PEG, and alternative coatings using tumor penetration, cellular uptake, PK, complement activation, and efficacy endpoints.	Addresses the PEG dilemma through measurable design criteria.
Tighter CMC specifications improve clinical reproducibility.	Relate size/PDI, PEG density, drug loading, release, and stability to PK variability and clinical outcomes across batches.	Connects manufacturing quality to therapeutic reliability and regulatory confidence.

In the longer term, there is potential to develop additional mechanisms to improve delivery of PEGylated liposomes into “cold” tumors and therefore expand the range of tumor types to which this form of cancer treatment is applicable. These may include the identification of specific endothelial cell types that are involved in the process of delivering PEGylated liposomes into tumors and the development of methods to stimulate these cells to facilitate delivery. Alternatively, the development of methods to stimulate the vasculature surrounding tumors to become more permeable to

nanoparticles could be used to enhance delivery. Such targeted and mechanism-based interventions could help to broaden the applicability of PEGylated liposome treatments to a wider range of tumor types than has been previously possible [87–89].

The potential to use imaging to assess the effectiveness of delivery of theranostic PEGylated liposomes to tumors is substantial. As discussed above, the use of imaging biomarkers would enable the assessment of whether or not the treatment has accumulated in sufficient quantities to be therapeutic. In addition, imaging would provide a way to determine how well the treatment had penetrated into the tumor, providing valuable information about the efficiency of delivery. Additionally, the presence of an imaging agent in the PEGylated liposomes would enable researchers to correlate the delivery of the therapeutic agent with the biological responses to the treatment, such as changes in tumor size or alterations in metabolic activity. The ability to measure the effectiveness of delivery of theranostic PEGylated liposomes would allow for the refinement of dosing and scheduling and would provide a powerful tool for the evaluation of new treatments [80–84].

PEGylated liposomes can complement immunotherapy through at least two mechanisms. First, certain chemotherapeutics (including anthracyclines) can induce immunogenic cell death, promoting antigen release and immune priming that may synergize with checkpoint blockade and other immune-directed interventions.

Second, liposomes can deliver innate immune agonists (e.g., STING agonists) or immunomodulatory in a manner that concentrates exposure within tumors or tumor-draining compartments while limiting systemic distribution, thereby improving the therapeutic window of potent immunostimulatory agents. The overarching strategic objective is to control where and when immune activation occurs relative to tumor cell killing while minimizing off-target inflammation [90–93]. Figure 4 illustrates key advancement strategies, including the development of next-generation stealth coatings to overcome anti-PEG immunity, cleavable and stimuli-responsive PEG architectures for tumor-selective activation, co-delivery and rational combination therapies, theranostics liposomes integrating imaging and treatment, personalized oncology enabled by imaging biomarkers for patient stratification, and the integration of PEGylated liposomes with immunotherapy to enhance antitumor immune responses.

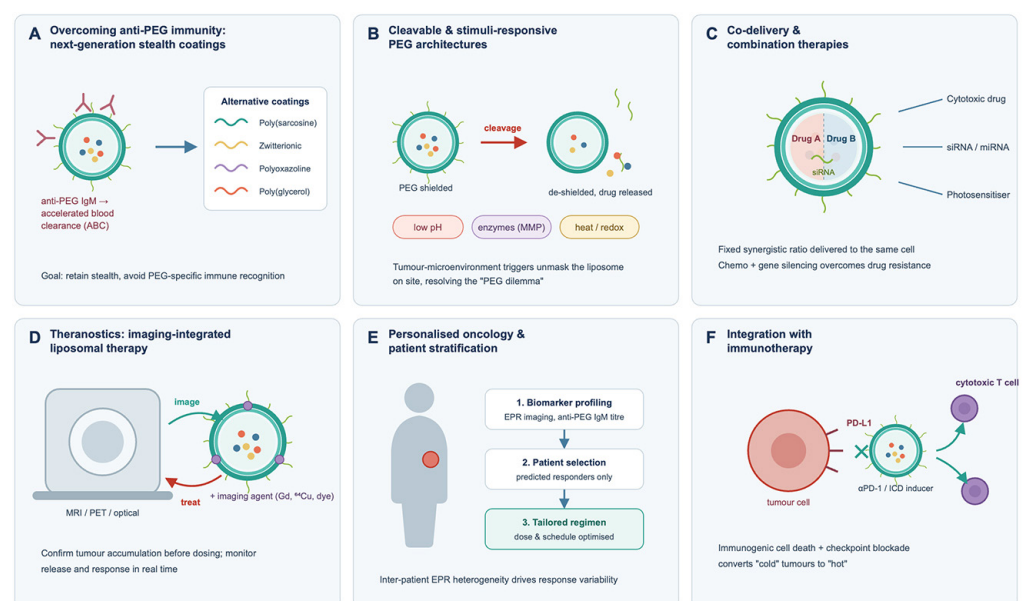


Fig. 4. Future perspectives and emerging directions in PEGylated liposomal drug delivery for cancer therapy

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