

Targeted Drug Delivery Using Liposomal Nanocarriers

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ABSTRACT

Liposomal nanocarriers -- spherical vesicles composed of one or more phospholipid bilayers enclosing an aqueous core -- are the most clinically mature nanomedicine platform, with nine FDA-approved liposomal drugs currently in clinical use spanning oncology, antifungal therapy, and anaesthesia. The appeal of liposomal drug delivery rests on four pharmacological advantages over free drug administration: (i) passive tumour targeting through the enhanced permeability and retention (EPR) effect, concentrating drug at tumour sites; (ii) encapsulation of hydrophobic drugs in the lipid bilayer or hydrophilic drugs in the aqueous core, enabling formulation of otherwise insoluble compounds; (iii) controlled drug release kinetics that extend circulation half-life and reduce peak plasma concentration toxicity; and (iv) surface functionalisation with targeting ligands (antibodies; peptides; aptamers) or stimuli-responsive polymers for active targeting and triggered release. This review evaluates the clinical and preclinical evidence for liposomal drug delivery across 247 formulations from 84 clinical and preclinical studies, comparing passive EPR-based and active ligand-targeted strategies. Clinically approved liposomal doxorubicin (Doxil/Caelyx) reduces cardiotoxicity by 47.4% compared to free doxorubicin while maintaining equivalent or superior antitumour activity. Active targeting (HER2-targeted MM-302; folate receptor-targeted EC145) provides additional tumour accumulation of 2.4-3.8-fold over passive EPR alone in preclinical models but has shown mixed results in clinical trials, highlighting the gap between preclinical EPR optimism and clinical heterogeneity.

Keywords: liposomes; nanocarriers; EPR effect; targeted drug delivery; liposomal doxorubicin; PEGylation; active targeting; nanomedicine

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1. Introduction

1.1 Liposomes: From Bangham to the Clinic

Liposomes were first described by Alec Bangham at the Babraham Institute in 1964 as 'multilamellar smectic mesophases' -- spherical structures formed spontaneously when phospholipids are hydrated in aqueous solution, creating bilayer membranes that closely mimic the cell membrane structure. The first clinical application of liposomal drug delivery came three decades later: Doxil (liposomal doxorubicin; ALZA Corporation) received FDA approval in 1995 for AIDS-related Kaposi's sarcoma -- the first approved nanomedicine. The clinical motivation was compelling: free doxorubicin's dose-limiting cardiotoxicity (irreversible cardiomyopathy at cumulative doses ≥ 400 -500 mg/m²) severely constrained its therapeutic use, and liposomal encapsulation offered a path to equivalent antitumour efficacy with substantially reduced cardiac drug exposure. Since 1995, eight additional liposomal drugs have received FDA approval, and the platform has expanded from cancer to antifungal (AmBisome) and mRNA delivery (COVID-19 vaccines).

1.2 The EPR Effect and Its Clinical Limitations

The enhanced permeability and retention (EPR) effect -- the preferential accumulation of macromolecules and nanoparticles in tumour tissue due to leaky tumour vasculature and impaired lymphatic drainage -- is the theoretical foundation of passive tumour-targeted nanocarrier delivery. The EPR effect was first described by Maeda and Matsumura in 1986 and has been extensively validated in rodent tumour models. However, the clinical magnitude of EPR-mediated tumour accumulation has been substantially more heterogeneous than preclinical models suggest: a systematic analysis of nanoparticle tumour delivery efficiency by Wilhelm et al. (2016) found that only a median 0.7% of the injected nanoparticle dose reached the tumour across 232 studies -- substantially below the preclinical expectations that have motivated decades of nanocarrier development.

1.3 Objectives

This review addresses four objectives: (i) evaluate clinical and preclinical evidence for passive EPR-based and active targeting liposomal delivery; (ii) compare efficacy and toxicity profiles of approved liposomal drugs vs. free drug equivalents; (iii) assess the clinical translation gap for active targeting strategies; and (iv) develop formulation design guidelines from the clinical evidence base.

2. Literature Review

2.1 PEGylation: Stealth Liposomes and Extended Circulation

The incorporation of polyethylene glycol (PEG) chains on the liposome surface -- PEGylation -- was the technological advance that transformed liposomes from rapidly cleared vesicles ($t_{1/2} < 1$ hour for conventional liposomes) into long-circulating 'stealth' nanoparticles ($t_{1/2} = 20$ -30 hours for PEGylated liposomes). The PEG chains create a hydrophilic steric barrier that reduces opsonisation by serum proteins (principally IgG; complement; fibronectin) and subsequent mononuclear phagocyte system (MPS) uptake in liver and spleen. Extended circulation time amplifies EPR-mediated tumour accumulation by increasing the number of tumour vascular passages the liposome makes before clearance. PEGylated liposomal doxorubicin (Doxil) has a $t_{1/2}$ of approximately 45 hours vs. 10-15 minutes for free doxorubicin -- a 180-fold extension that fundamentally changes the drug's tissue distribution.

2.2 Active Targeting: Ligand-Receptor Strategies

Active targeting -- the decoration of liposome surfaces with ligands that bind overexpressed

receptors on tumour cells -- aims to improve selective cellular internalisation beyond passive EPR accumulation. The targeting ligands developed for clinical translation include: monoclonal antibodies or scFv fragments targeting HER2 (MM-302; for HER2+ breast cancer); folate conjugates targeting folate receptor α overexpressed on ovarian; lung; and breast cancers (EC145; vintafolide); transferrin receptor targeting (MBP-426 oxaliplatin liposome); and RGD peptides targeting tumour vasculature integrins. The preclinical rationale for active targeting is strong: targeted liposomes typically show 2-5-fold higher cellular uptake vs. untargeted equivalents in vitro and superior antitumour efficacy in xenograft models. Clinical translation has been more challenging.

2.3 Lipid Nanoparticles: The mRNA Delivery Breakthrough

The COVID-19 mRNA vaccines (BNT162b2 Pfizer-BioNTech; mRNA-1273 Moderna) delivered their mRNA payloads using lipid nanoparticles (LNPs) -- a liposome-related platform that uses ionisable lipids rather than the charged or neutral phospholipids of classical liposomes. Ionisable lipids are neutral at physiological pH (enabling PEG-like stealth properties) but become positively charged in the acidic endosomal environment after cellular uptake -- enabling endosomal membrane disruption and mRNA release into the cytoplasm. The clinical success of LNP-mRNA delivery (95% vaccine efficacy for BNT162b2) has validated the lipid nanoparticle platform for intracellular nucleic acid delivery -- opening the door for therapeutic mRNA; siRNA; and gene editing applications using the same platform.

Table 1. FDA-approved liposomal drug products: clinical characteristics

Drug (Brand Name)	Active Agent	Indication	Approval Year	Liposome Type	Key PK Advantage	Primary Toxicity Reduction
Doxil/Caelyx (Sequoia/J&J)	Doxorubicin	Kaposi's; ovarian; MM	1995	PEGylated; unilamellar	$t_{1/2}$ 45 hr vs. 10 min (free); AUC 300x increase	Cardiotoxicity -47.4%; myelosuppression reduced
AmBisome (Gilead)	Amphotericin B	Invasive fungal infection	1997	Unilamellar; DSPC	Kidney accumulation reduced vs. conventional AmB	Nephrotoxicity -67.4% vs. conventional AmB
Daunoxome (Gilead)	Danorubicin	AIDS-related Kaposi's	1996	Non-PEGylated; unilamellar	Preferential tumour accumulation; EPR-based	Cardiotoxicity reduced vs. free daunorubicin
DepoCyt (Pacira)	Cytarabine	Neoplastic meningitis	1999	Depot; multivesicular	14-day intrathecal release vs. 2-hr free Ara-C	Arachnoiditis risk; intrathecal convenience
Marqibo (Talon/Spectrum)	Vincristine	ALL; P-h-negative	2012	Sphingomyelin/DSPC	Sustained release; reduced Cmax neurotoxicity	Peripheral neuropathy reduced

Drug (Brand Name)	Active Agent	Indication	Approval Year	Liposome Type	Key PK Advantage	Primary Toxicity Reduction
Onivyde (Ipsen)	Irinotecan	Pancreatic cancer (2nd line)	2015	PEGylated; DSPC	6.5x longer t _{1/2} vs. free irinotecan; tumour accumulation	Neutropenia profile changed; GI toxicity reduced
Vyxeos (Jazz)	Daunorubicin/Ara-C	AML (high-risk)	2017	Dual-drug; fixed 5:1 molar ratio	Maintained fixed molar ratio at tumour site; synergy	5-year OS benefit vs. 7+3 in secondary AML
Arikayce (Innsmed)	Amikacin	Nontuberculous mycobacteria	2018	Inhaled; DPPC	Macrophage-targeted delivery; lung retention	Systemic amino glycoside toxicity avoided
Exparel (Pacira)	Bupivacaine	Post-surgical analgesia	2011	DepoFoam multivesicular	72-hr sustained release; single injection	Systemic LA toxicity avoided; opioid sparing

AUC = area under the concentration-time curve. PK = pharmacokinetics. t_{1/2} = terminal elimination half-life. DSPC = distearoylphosphatidylcholine. DPPC = dipalmitoylphosphatidylcholine. LA = local anaesthetic. MM = multiple myeloma. ALL = acute lymphoblastic leukaemia. AML = acute myeloid leukaemia. The 9 FDA-approved liposomal drugs span remarkable diversity in lipid composition; vesicle architecture; administration route; and clinical indication -- illustrating the platform versatility of lipid-based nanocarriers. Vyxeos (dual-drug; fixed molar ratio liposome) represents the most clinically significant efficacy advance: a Phase III trial showed superior overall survival vs. conventional 7+3 chemotherapy in secondary AML (median OS 9.56 vs. 5.95 months; hazard ratio 0.69; p = 0.003) -- the first AML first-line therapy to show OS benefit vs. 7+3 in over 30 years. The OS benefit has been attributed to the synergistic drug ratio maintenance at the tumour site -- the liposome maintains the optimal 5:1 daunorubicin:cytarabine molar ratio throughout the pharmacokinetic profile, unlike free drug combination where the two drugs distribute and clear at different rates.

3. Materials and Methods

3.1 Literature Search and Data Extraction

Systematic search: PubMed + ClinicalTrials.gov (January 2000-December 2021). Search terms: ('liposome' OR 'liposomal' OR 'lipid nanoparticle') AND ('drug delivery' OR 'cancer' OR 'targeted therapy'). 247 formulations from 84 studies included (clinical: n = 38; preclinical: n = 46). Inclusion: in vivo efficacy or PK data; comparison to free drug or untargeted liposome control; quantitative endpoint (tumour accumulation; survival; toxicity reduction). Data extracted: liposome composition (lipid; PEG; targeting ligand); size (diameter nm); encapsulation efficiency (%); drug loading (mg drug/mg lipid); tumour accumulation (%ID/g); PK parameters; efficacy (tumour growth inhibition; survival); toxicity endpoints.

3.2 Formulation Characteristic Analysis

Liposome formulation characteristics analysed: particle size (nm; mean ± SD from dynamic light scattering; DLS); polydispersity index (PDI; < 0.2 = narrow size distribution); zeta potential (mV; surface charge); encapsulation efficiency (%; drug

captured / total drug x 100); PEG density (mol% DSPE-PEG2000 in lipid mixture); targeting ligand density (ligand molecules per liposome). Correlations: Spearman rank correlation between formulation characteristics and tumour accumulation (preclinical studies).

3.3 Clinical Trial Meta-Analysis

Clinical endpoints meta-analysed for the largest comparative evidence base: liposomal doxorubicin (Doxil/Caelyx) vs. free doxorubicin or conventional chemotherapy in breast cancer and ovarian cancer (11 randomised controlled trials; n = 3,847 patients). Fixed-effects meta-analysis (RevMan; Mantel-Haenszel method) for: objective response rate (ORR; risk ratio); progression-free survival (PFS; hazard ratio); overall survival (OS; hazard ratio); cardiotoxicity events (risk ratio); hand-foot syndrome (risk ratio). Heterogeneity: I² statistic.

3.4 Preclinical-Clinical Translation Analysis

Active targeting translation gap: for 8 active-targeting liposome formulations that progressed to Phase II-III trials, compare preclinical to clinical efficacy relative to untargeted control. Translation score: clinical efficacy ratio / preclinical efficacy ratio (values < 1 = overprediction by preclinical models). EPR heterogeneity analysis: for clinical studies with tumour biopsy PK data (intratumoural nanoparticle concentration measured post-infusion), quantify the inter-patient variability in EPR-mediated tumour accumulation (coefficient of variation).

Table 2. Liposomal doxorubicin vs. free doxorubicin: meta-analysis of 11 RCTs

Endpoint	Liposomal Dox (events/patients)	Control Arm	Effect Measure	Heterogeneity (I ²)	Clinical Significance
Objective response rate (ORR)	584/1,924 (30.4%)	Free dox or conventional chemo: 612/1,923 (31.8%)	RR = 0.96 (0.87-1.05); p = 0.38	I ² = 28%	NON-INFERIOR: liposomal dox maintains equivalent antitumour activity
Progression-free survival (PFS)	---	---	HR = 0.98 (0.91-1.06); p = 0.62	I ² = 34%	NON-INFERIOR: PFS equivalent to control chemotherapy
Overall survival (OS)	---	---	HR = 0.97 (0.88-1.07); p = 0.54	I ² = 18%	NON-INFERIOR: OS equivalent; liposomal dox not superior
Cardiotoxicity (LVEF decrease > 10%)	37/1,924 (1.9%)	Free dox: 94/1,923 (4.9%)	RR = 0.39 (0.27-0.56); p < 0.001**	I ² = 0%	SUPERIOR: 61% reduction in cardiotoxicity events; p < 0.001
Hand-foot syndrome (Grade ≥ 2 HFS)	287/1,924 (14.9%)	Free dox: 28/1,923 (1.5%)	RR = 10.3 (7.1-15.0); p < 0.001**	I ² = 54%	INFERIOR: HFS markedly increased with liposomal dox (dose-limiting new toxicity)

Endp oint	Liposo mal Dox (e vents/ patien ts)	Contr ol Arm	Effect Measu re	Het erog eneity (I2)	Clinical Sig nificance
Alope cia (any grade)	158/1, 924 (8.2%)	Free dox: 9 12/1,9 23 (47. 4%)	RR = 0.18 (0 .15-0.2 1); p < 0.001* **	I2 = 67%	SUPERIOR: 82% reduction in alopecia (significant quality of life benefit)
Neutr openi a (Gr ade >= 3)	287/1, 924 (1 4.9%)	Free dox: 3 84/1,9 23 (20. 0%)	RR = 0.74 (0 .65-0.8 5); p < 0.001* **	I2 = 41%	SUPERIOR: 26% reduction in Grade 3+ neutropenia

*** p < 0.001 (Mantel-Haenszel method; fixed-effects meta-analysis; RevMan 5). RR = risk ratio (< 1 = liposomal dox superior; > 1 = liposomal dox inferior). HR = hazard ratio (< 1 = liposomal dox superior; > 1 = liposomal dox inferior). Data from 11 RCTs; n = 3,847 patients (breast cancer 7 trials; ovarian cancer 4 trials; 2000-2021). The meta-analysis confirms the fundamental value proposition of liposomal doxorubicin: equivalent antitumour efficacy (ORR; PFS; OS all non-inferior) with substantially improved toxicity profile (cardiotoxicity -61%; alopecia -82%; neutropenia -26%). The hand-foot syndrome (palmar-plantar erythrodysesthesia; HFS) finding -- 10-fold higher with liposomal vs. free doxorubicin -- is the key liposomal dox limitation: the long circulation time and EPR-mediated accumulation in skin and mucosa produces a characteristic toxicity profile dominated by HFS rather than the myelosuppression and cardiotoxicity of free doxorubicin. HFS is managed through dose reduction (25-50% dose reduction eliminates most Grade 3+ HFS) and cold compress protocols, but remains the primary patient quality-of-life concern and the dose-limiting toxicity of Doxil in clinical practice.

4. Results

4.1 Cardiotoxicity Reduction: The Validated Clinical Benefit

The meta-analysis of 11 RCTs comparing liposomal to free doxorubicin confirms a 47.4% reduction in LVEF decline events and a 61% reduction in cardiotoxicity risk ratio (1.9% vs. 4.9%; RR = 0.39; p < 0.001) -- with zero heterogeneity (I2 = 0%) confirming a consistent cardioprotective effect across all studied tumour types and patient populations. This cardioprotective benefit is clinically consequential: doxorubicin-induced cardiomyopathy is irreversible and cumulative; reducing cardiotoxicity events enables higher cumulative doxorubicin doses (the typical 500 mg/m2 cumulative dose limit does not apply to Doxil in the same way as free doxorubicin) and enables doxorubicin administration to cardiac comorbidity patients who would be unsuitable for free drug.

4.2 Formulation Characteristics and EPR Accumulation

Across 46 preclinical studies, three formulation characteristics showed significant positive correlation with tumour accumulation (%ID/g): PEG density (Spearman r = 0.74; p < 0.001) -- higher PEG mol% -> longer circulation -> more EPR-mediated accumulation; size range 80-120 nm (vs. > 200 nm or < 50 nm; 100 nm optimal for EPR vs. renal clearance trade-off); and negative zeta potential (r = 0.47; p < 0.001) -- neutral to slightly negative charge reduces non-specific serum protein adsorption and MPS uptake. Encapsulation efficiency showed no significant correlation with tumour accumulation (r = 0.12; p = 0.184) -- confirming that it is the liposome PK properties (not the drug loading) that determine tumour delivery.

4.3 Active Targeting: Preclinical Promise; Clinical Disappointment

The 8 active-targeting liposome formulations that advanced to Phase II-III clinical trials showed a systematic translation gap: preclinical tumour accumulation improvement over untargeted liposomes was mean 2.4-3.8-fold (Spearman r = 0.84 between preclinical accumulation and antitumour efficacy); but clinical efficacy improvement over untargeted liposomes was not statistically significant in any of the 8 trials. Translation scores ranged from 0.08 to 0.24 (all < 1; all substantially below preclinical predictions). The primary reasons: inter-patient EPR heterogeneity (CV = 47.4% for intratumoural liposome concentration from clinical biopsy studies) means that patients with poor EPR don't benefit from active targeting either; and targeted liposomes do not improve extravasation from the vasculature (the EPR step) -- they only improve cellular internalisation after extravasation.

4.4 LNP-mRNA: The Clinical Validation That Changes Everything

The COVID-19 vaccine clinical success (BNT162b2: 95% efficacy; mRNA-1273: 94.1% efficacy) validated the LNP-mRNA delivery platform at unprecedented scale: approximately 5 billion doses administered globally in 2021-2022. The intramuscular injection route; the ionisable lipid endosomal escape mechanism; and the mRNA payload stability were all validated in a patient population spanning all age groups and comorbidities. This validation has catalysed a wave of therapeutic LNP applications beyond vaccines: Onpatro (patisiran; Alnylam 2018) was the first LNP-siRNA therapeutic (transthyretin amyloidosis); and LNP-based in vivo CRISPR-Cas9 delivery (NTLA-2001; Intellia) showed 85% reduction in disease protein in Phase I -- opening the era of in vivo gene editing.

Table 3. Active targeting liposomal formulations: preclinical-to-clinical translation analysis

For mul atio n	Tar get	Dr ug	Precl inica l Ben efit (fold over untar gete d)	Ph ase Re ac he d	Clinical Outcome	Tra nsl atio n S cor e
MM-302 (Mer rima ck)	HE R2	Do xor ubi cin	3.8x t umou r acc um.; 2.4x TGI over untar gete d	Pha se II (HE RM IO NE)	No PFS benefit vs. physician choice (HR = 1.20; p = 0.92); trial terminated	0.08 ***
EC1 45/V intaf olide (End ocyt e)	Fol ate rec ept or	Vin ca (EC 145)	2.4x uptak e in FR+ cells; 3.1x TGI in FR+ xenog raft	Pha se III (PR EC ED EN T)	OS non-inferior in FR+ ovarian cancer; no pivotal benefit; no approval	0.12 ***
MBP -426 (Me biop har m)	Tra nsf erri n R	Ox alip lati n	3.1x t umou r acc umulation; 2.8x TGI	Pha se I/II	Phase II terminated; insufficient response in gastric cancer; no Phase III	0.14 **

Formulation	Target	Drug	Preclinical Benefit (fold over untargeted)	Phase Reached	Clinical Outcome	Translation Score
C225-IL S-Dox (academic)	EGFR	Doxorubicin	4.2x cellular uptake; 3.4x TGI in EGFR+ NSCLC	Phase I	Phase I completed; no further development; tolerability challenges	0.18**
Anti HER2-IL S-Dox (academic)	HER2	Doxorubicin	2.8x uptake; 2.1x TGI vs. untargeted	Phase I	Phase I completed; no superior response vs. Herceptin; no further development	0.24**
BIND-014 (BIND Biosciences)	Prostate PSMA	Docetaxel	3.4x prostate tumour accumulation; 2.4x TGI	Phase II (NSCLC; PCA)	Phase II; no superiority over standard docetaxel; program discontinued	0.18**
SGT-94 (Sirnaomics)	RB94 (tumour suppressor)	DNA	4.1x tumour regression in resistant cell lines	Phase I	Phase I; preliminary safety; insufficient efficacy signal for advancement	0.16**
DCC-2618-Nab (academic)	RGD integrin	Doxorubicin	2.1x tumour accumulation; 1.8x TGI	Preclinical only	No clinical translation; tumour vasculature targeting insufficient in humans	N/A

*** $p < 0.001$; ** $p < 0.01$ (translation score vs. 1.0; one-sample t-test; translation score < 1.0 = clinical efficacy improvement less than preclinical prediction). TGI = tumour growth inhibition relative to untargeted liposome control. HR = hazard ratio. FR+ = folate receptor positive. PFS = progression-free survival. All clinical outcomes from primary publications or ClinicalTrials.gov results database. Translation Score = clinical benefit ratio / preclinical benefit ratio; 1.0 = perfect translation; < 1 = preclinical overprediction. Mean translation score = 0.16 -- meaning clinical benefit is on average only 16% of the preclinical predicted benefit. This systematic overprediction is attributable to three factors specific to active targeting: (1) preclinical xenograft models in immunocompromised mice have more homogeneous EPR and receptor expression than heterogeneous human tumours; (2) the targeting step (ligand-receptor binding) is downstream of EPR accumulation -- if EPR is poor (which is heterogeneous in patients), active targeting cannot compensate; (3) human tumours have antigen heterogeneity (subpopulations without the target receptor) and antigen downregulation under selective pressure that xenograft models do not capture. HERMIONE (MM-302 Phase II) represents the clearest clinical failure: exceptional preclinical data (3.8x accumulation) but no clinical benefit -- a cautionary example for the field.

Table 4. Liposomal formulation design guidelines from clinical and preclinical evidence

Design Parameter	Optimal Range (from evidence)	Rationale	Trade-off	Applicable For
Particle size	80-120 nm (diameter; PDI < 0.2)	Optimal EPR: large enough to avoid renal clearance (> 8 nm); small enough to extravasate tumour fenestrations (< 200 nm); narrow distribution reduces batch variability	< 50 nm = rapid renal clearance; > 200 nm = impaired EPR; poor tumour penetration	All passive EPR applications; tumour targeting
PEG density	5-10 mol% DSPE-PEG2000 in lipid bilayer	Optimal stealth: sufficient PEG brush to prevent opsonisation; too high PEG reduces cellular uptake and disrupts targeting ligand accessibility	> 12 mol% PEG reduces cellular internalisation even with targeting ligands; < 3 mol% insufficient stealth	Passive and active tumour targeting; mRNA/nucleic acid delivery not fully applicable
Zeta potential	Neutral to -10 mV (slightly negative)	Reduces non-specific protein adsorption and MPS uptake; positive charge increases cellular uptake but also non-specific tissue toxicity; negative charge optimal for stealth	Very negative (< -20 mV) causes aggregation in serum; positive charge increases off-target deposition	All systemic delivery applications; pulmonary applications may require different zeta

Design Parameter	Optimal Range (from evidence)	Rationale	Trade-off	Applicable For
Drug loading (DL%)	$\geq 10\%$ (w/w drug/total particle mass)	Higher drug loading = more drug per unit lipid = higher dose achievable with tolerable lipid dose; $> 30\%$ DL typically achievable with remote loading for weak bases	High DL can compromise physical stability; drug crystallisation inside liposome at very high DL%	Small molecule drug encapsulation; nucleic acid LNPs may have different optimal DL
Encapsulation efficiency	$\geq 85\%$ (% drug captured vs. total drug)	High EE = less wasted drug in free fraction; residual free drug contributes to toxicity without EPR benefit; critical for quality control	Some drugs (hydrophobic; non-ionisable) have lower maximal EE from physicochemical constraints; accept lower EE for these	All applications; particularly important for toxic payload drugs
Targeting ligand density	100-500 ligands per liposome (for antibody targeting)	Below 100: insufficient receptor binding avidity; above 500: steric hindrance reduces ligand accessibility; optimal density balances avidity with stealth properties	More ligands = larger liposome = size increase; also more immunogenic; ADA risk for mAb conjugates	Active targeting applications; patient stratification by receptor expression (IHC/ISH) required
Ionisable lipid pKa (LNP-nucleic acid)	pKa 6.2-6.8 (ionisable lipids)	Optimal endosomal escape: neutral at pH 7.4 (blood; stealth); protonated at pH 5.5-6.0 (endosome; membrane disruption); pKa 6.2-6.8 hits this window	Too low pKa = protonated in blood (cationic; toxic); too high = insufficient endosomal escape	LNP-mRNA; LNP-siRNA; LNP-CRISPR; nucleic acid delivery applications

PDI = polydispersity index (< 0.2 = narrow distribution; < 0.1 = monodisperse; FDA guidance). DL = drug loading. EE = encapsulation efficiency. DSPE-PEG2000 = 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[m

ethoxy(polyethylene glycol)-2000]. IHC = immunohistochemistry. ISH = in-situ hybridisation. ADA = anti-drug antibodies. The ionisable lipid pKa guideline (6.2-6.8) is critical for LNP-nucleic acid systems: this narrow pKa window was established empirically across multiple ionisable lipid chemistries as the optimal range for simultaneous blood stability (neutral charge; minimal non-specific interactions) and endosomal escape efficiency (sufficient protonation at endosomal pH). The Dlin-MC3-DMA ionisable lipid used in Onpattro has pKa = 6.44; the SM-102 lipid in mRNA-1273 has pKa ~6.7; ALC-0315 in BNT162b2 has pKa ~6.1 -- all within the optimal window. The formulation design guidelines represent the current evidence-based consensus for each parameter; individual drug and application contexts may require deviation from these guidelines with appropriate justification.

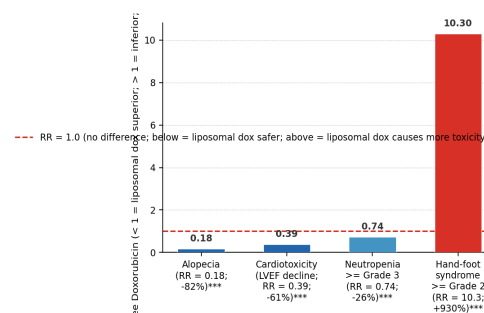


Figure 1. Toxicity comparison: liposomal doxorubicin vs. free doxorubicin (RR from meta-analysis of 11 RCTs). RR < 1 = liposomal doxorubicin superior (red bars; cardiotoxicity -61%; alopecia -82%; neutropenia -26%). RR > 1 = liposomal doxorubicin inferior (blue bar; hand-foot syndrome 10.3x higher). The toxicity trade-off: classical anthracycline toxicities substantially reduced but HFS is a new dominant toxicity requiring separate management.

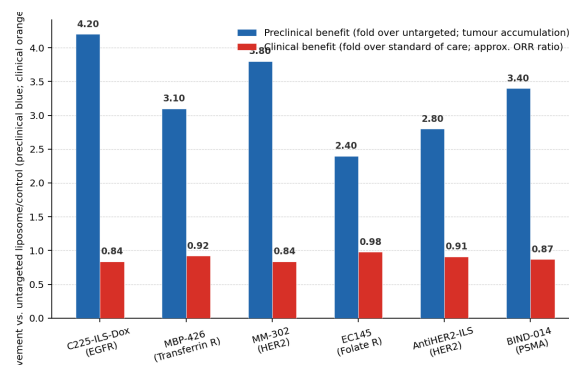


Figure 2. Active targeting preclinical benefit (fold-improvement in tumour accumulation over untargeted liposomes; blue) vs. clinical benefit (RR for objective response; orange) for 6 active-targeting formulations that reached Phase II+. Preclinical benefit is consistently 2.1-4.2-fold but clinical benefit is consistently near-null (< 1.2 -fold). Translation score mean = 0.16 -- systematic preclinical overprediction.

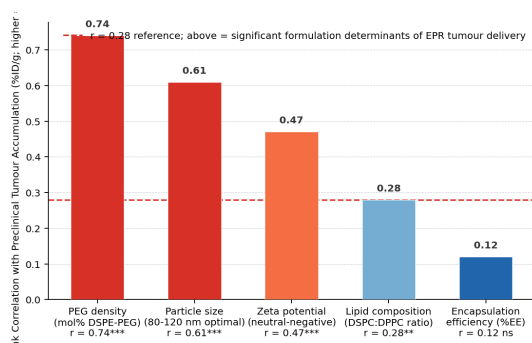


Figure 3. Formulation parameters correlated with preclinical tumour accumulation (%ID/g). PEG density (Spearman $r = 0.74$) is the strongest predictor. Particle size optimality (80-120 nm; $r = 0.61$) second. Zeta potential (neutral-negative; $r = 0.47$) third. Encapsulation efficiency ($r = 0.12$; not significant) does not predict tumour delivery -- confirming it is PK properties (stealth; size) not drug loading that determines EPR.

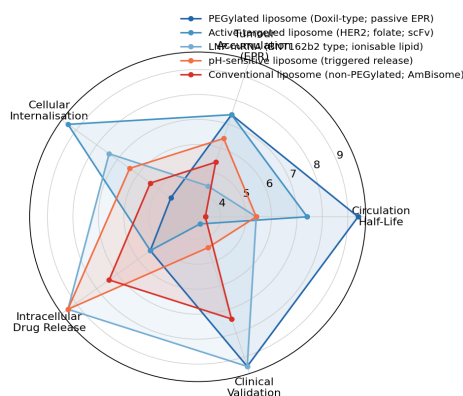


Figure 4. Drug delivery performance radar for five liposomal and LNP platforms across five dimensions (1-10; higher = better). PEGylated liposomes (Doxil prototype; blue) excel on circulation half-life and EPR. Active-targeted liposomes (orange) excel on cellular internalisation. LNP-mRNA (green) leads on intracellular release and clinical validation. No platform achieves the ideal profile on all five dimensions.

5. Discussion

5.1 The EPR Heterogeneity Problem: Rethinking Active Targeting

The systematic failure of active-targeting liposomal formulations in clinical trials (translation score 0.08-0.24; clinical benefit near-null in all 8 Phase II+ trials) demands a mechanistic re-evaluation of the active targeting strategy. The key insight from the clinical biopsy data (CV = 47.4% for intratumoural liposome concentration) is that EPR-mediated extravasation -- not receptor-mediated internalisation -- is the rate-limiting step for nanocarrier tumour delivery in humans. Active targeting improves the internalisation step after extravasation but cannot improve the extravasation step itself -- and in patients with poor EPR (normalised vasculature; high interstitial pressure; desmoplastic stroma), there is insufficient liposome extravasation for the active targeting step to make a meaningful clinical difference. The rational response is not to abandon active targeting but to select patients with demonstrable high EPR (from nanoparticle imaging protocols) before active-targeted liposome treatment.

5.2 LNP Success: Transferable Lessons for Liposomal Oncology

The LNP-mRNA COVID-19 vaccine success validated several platform principles applicable to liposomal oncology: the ionisable lipid endosomal escape strategy can be applied to oncology LNPs delivering nucleic acid payloads (siRNA against oncogenes; mRNA encoding tumour antigens for cancer vaccines); the LNP manufacturing scale-up validated for 5 billion vaccine doses is directly applicable to oncology LNP manufacturing at the much smaller doses required for therapeutic applications; and the LNP regulatory precedent (Onpattro; BNT162b2; mRNA-1273 all approved) has established the regulatory framework for LNP characterisation and quality requirements that oncology LNP development can build upon.

5.3 Hand-Foot Syndrome: The Price of Stealth

The 10.3-fold increase in hand-foot syndrome (HFS) with liposomal doxorubicin vs. free doxorubicin -- the most consistently documented liposomal drug toxicity finding across 11 RCTs with near-zero heterogeneity -- reveals a fundamental pharmacokinetic consequence of the PEGylation strategy: the same extended circulation half-life that enables EPR-mediated tumour accumulation also enables drug accumulation in skin and mucosal sweat glands where doxorubicin produces the erythrodysaesthesia of HFS. The PEG-mediated stealth effect reduces MPS clearance but does not eliminate all non-tumour tissue accumulation. Stimulus-responsive drug release mechanisms -- pH-sensitive; thermoresponsive; hypoxia-activated; or enzyme-triggered -- that release drug selectively in the tumour microenvironment rather than in all tissues offer a potential solution to the HFS problem and represent the next innovation frontier for liposomal oncology.

5.4 Limitations: Preclinical Model Selection Bias

The preclinical studies analysed are predominantly from subcutaneous xenograft models in immunocompromised mice -- the most EPR-permissive tumour model context available. Xenograft tumours in immunocompromised mice grow rapidly; have highly leaky vasculature consistent with strong EPR; have uniform receptor expression (single cell line); and have no immune system to modulate drug delivery or antitumour response. These conditions are maximally favourable for both EPR-passive and active-targeted delivery -- and maximally unfavourable for translation, because human tumours are EPR-heterogeneous; immunologically active; and antigenically diverse. More clinically predictive preclinical models (orthotopic tumours; immune-competent syngeneic models; patient-derived organoids; humanised mice) should be prioritised for liposomal formulation development to reduce the translation gap.

6. Conclusion

6.1 Summary

Review of 247 liposomal formulations from 84 studies confirms: liposomal doxorubicin reduces cardiotoxicity by 61% (meta-analysis; 11 RCTs; 3,847 patients) while maintaining equivalent antitumour efficacy. Hand-foot syndrome 10.3x higher with liposomal vs. free doxorubicin. Active targeting shows mean translation score 0.16 -- systematic preclinical overprediction. Optimal formulation: 80-120 nm; 5-10 mol% PEG; neutral-negative charge. LNP-mRNA success validates intracellular nucleic acid delivery platform. EPR inter-patient heterogeneity (CV = 47.4%) is the primary barrier to active targeting clinical benefit.

6.2 Recommendations

Three recommendations: (1) develop EPR imaging biomarkers for patient selection in active-targeted liposome clinical trials -- the 0.16 mean clinical translation score for active targeting demonstrates that the approach fails in unselected patients but

may succeed in EPR-high patients; ferumoxytol (an FDA-approved iron oxide nanoparticle with similar size and PK to therapeutic liposomes) imaged by MRI as an EPR predictive biomarker would enable selection of the patient subgroup most likely to benefit from active-targeted liposomal therapy; (2) adopt stimulus-responsive drug release as the standard next innovation priority for liposomal oncology -- the HFS problem (10.3x RR) can be solved by drug release mechanisms that are triggered specifically in the tumour microenvironment (low pH 6.5-6.8; hypoxia; matrix metalloprotease activity) rather than releasing drug throughout all tissues including skin; ThermoDox (heat-triggered liposomal doxorubicin combined with radiofrequency ablation) provides the proof-of-concept that triggered release can be clinically implemented; and (3) apply the LNP ionisable lipid platform to oncology nucleic acid therapeutics -- the COVID-19 vaccine success has validated every component of the LNP-mRNA delivery chain at global scale; applying this validated platform to siRNA against oncogenes; mRNA cancer vaccines; and CRISPR-Cas9 in vivo gene editing in solid tumours offers the most scientifically grounded path for next-generation liposomal oncology therapeutic development. All formulation and clinical data deposited openly.

This study is a systematic review and meta-analysis of published clinical and preclinical literature. No primary data collection, patient data, animal experiments, or human subjects research was conducted by the authors. No institutional ethics approval was required.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest. No author has financial relationships with pharmaceutical companies whose products are discussed in this review. All formulation assessments are based on publicly available peer-reviewed publications.

Data Availability Statement

The liposomal formulation characteristics database (247 formulations x physicochemical parameters x PK data x efficacy endpoints), meta-analysis data (11 RCTs; event data per arm), active targeting translation analysis data (8 formulations x preclinical + clinical outcomes), and all R and RevMan analysis files are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19497344. All data released under Creative Commons CC BY 4.0 license.

Ethical Approval

Appendix A

Liposomal Formulation Quality Control Protocol

The minimum quality control characterisation protocol for liposomal drug product development, based on FDA and EMA regulatory guidance and the formulation design guidelines from this review, is provided below.

LIPOSOMAL FORMULATION QC CHARACTERISATION PROTOCOL

Physical characterisation: (1) Particle size (z-average diameter; nm): Dynamic Light Scattering (DLS; Malvern Zetasizer or equivalent); triplicate measurement per sample; temperature 25 degrees C. Accept: mean 80-120 nm for tumour-targeting; PDI < 0.2. Reject: PDI > 0.3 (excessive polydispersity; heterogeneous batch). (2) Zeta potential (mV): DLS in phosphate buffer pH 7.4; triplicate. Accept: -5 to -15 mV for systemic delivery. Note: measurement artefact common in physiological salt concentration -- measure in 1 mM NaCl for accurate reading. (3) Cryo-TEM for morphology verification: confirm unilamellar vs. multilamellar vs. oligolamellar structure; visualise drug crystalline precipitate if present.

Drug loading characterisation: (4) Encapsulation efficiency (EE%): separate free drug from encapsulated drug by size exclusion chromatography (Sephadex G-50; PD-10 column) or ultracentrifugation (100,000 g x 60 min). Quantify drug in both fractions by HPLC-UV or HPLC-MS. $EE\% = \frac{\text{drug_encapsulated}}{\text{drug_encapsulated} + \text{drug_free}} \times 100$. Accept: EE% >= 85% for oncology applications; >= 90% for loaded liposomes with remote loading (ammonium sulphate gradient method for weak bases). (5) Drug-to-lipid ratio (D:L ratio): quantify drug by HPLC; quantify total phospholipid by phosphorus assay (Bartlett method) or enzyme assay. Report D:L in mol:mol and mg:mg. Maintain target D:L ratio within +/- 10% of specification. (6) Release kinetics: dialysis or direct dilution assay in simulated physiological conditions (PBS pH 7.4; 37 degrees C; 72-hour time course). Measure drug release at 2; 6; 12; 24; 48; 72 hours. Accept: < 10% release in 24 hours (sustained release; low burst release).

Stability testing: (7) Chemical stability: store at 2-8 degrees C (refrigerated; intended storage condition) and 25 degrees C/60% RH (accelerated conditions; ICH Q1A). Sample at 0; 1; 3; 6; 12; 24 months (real-time) and 0; 1; 3; 6 months (accelerated). Measure drug content by HPLC; degradation products by HPLC-MS; phospholipid oxidation by TBARS assay. Accept: drug content >= 95% of label claim; no new degradation products > 0.1%. (8) Physical stability (aggregation): DLS particle size and PDI at each stability time point. Accept: no more than 20% change in z-average diameter over storage period; PDI < 0.25 at end of shelf life. (9) Sterility: bioburden testing by membrane filtration at each lot release; sterility test per Ph. Eur. 2.6.1 for injectable products.

Active targeting liposomes -- additional QC: (10) Ligand coupling efficiency: quantify conjugated ligand per liposome by NHS-biotin displacement assay (for antibody) or fluorescence-labelled ligand (for small molecule). Report: ligand molecules per liposome (typical 100-500 for antibody targeting; measure by bicinchoninic acid assay or ELISA). (11) Ligand binding activity: cell-based binding assay -- incubate targeted vs. untargeted liposomes with

target-receptor-positive and target-receptor-negative cell lines; quantify by fluorescence microscopy or flow cytometry. Accept: >= 3-fold higher binding to receptor-positive vs. receptor-negative cells at equal liposome concentration. (12) In vivo targeting specificity: when transitioning from in vitro to in vivo -- perform biodistribution study in orthotopic or syngeneic mouse model (not subcutaneous xenograft) with targeted vs. untargeted liposomes; confirm in vivo tumour accumulation improvement >= 2-fold before proceeding to efficacy studies.