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Review

Engineered Liposomes in Interventional Theranostics of Solid Tumors

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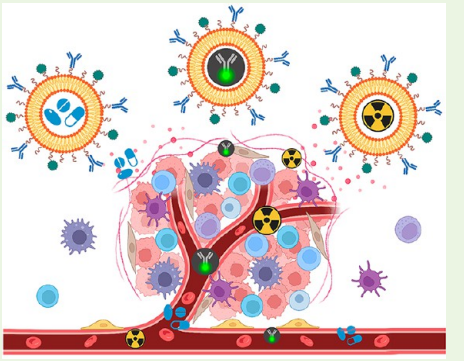
ABSTRACT: Engineered liposomal nanoparticles have unique characteristics as cargo carriers in cancer care and therapeutics. Liposomal theranostics have shown significant progress in preclinical and clinical cancer models in the past few years. Liposomal hybrid systems have not only been approved by the FDA but have also reached the market level. Nanosized liposomes are clinically proven systems for delivering multiple therapeutic as well as imaging agents to the target sites in (i) cancer theranostics of solid tumors, (ii) image-guided therapeutics, and (iii) combination therapeutic applications. The choice of diagnostics and therapeutics can intervene in the theranostics property of the engineered system. However, integrating imaging and therapeutics probes within lipid self-assembly "liposome" may compromise their overall theranostics performance. On the other hand, liposomal systems suffer from their fragile nature, site-selective tumor targeting, specific biodistribution and premature leakage of loaded cargo molecules before reaching the target site. Various engineering approaches, viz., grafting, conjugation, encapsulations, etc., have been investigated to overcome the aforementioned issues. It has been studied that surface-engineered liposomes demonstrate better tumor selectivity and improved therapeutic activity and retention in cells/or solid tumors. It should be noted that several other parameters like reproducibility, stability, smooth circulation, toxicity of vital organs, patient compliance, etc. must be addressed before using liposomal theranostics agents in solid tumors or clinical models. Herein, we have reviewed the importance and challenges of liposomal medicines in targeted cancer theranostics with their preclinical and clinical progress and a translational overview.



KEYWORDS: *liposomes, interventional theranostic, solid tumors, nanoimaging*

INTRODUCTION

Many nanoparticles have shown tremendous progress in cancer imaging, therapeutics, or theranostics for solid tumor applications. Better biocompatibility and stability, circulation, specificity for tumor targeting and biodistribution, unique physicochemical properties, high surface area for high payloads, better stability, and multifunctionality are major advantages of nanoparticles over conventional diagnostics and therapeutics agents or molecules. Multiple doses of conventional diagnostics and therapeutics agents (chemo and radiation doses) are standard in hospitals today for cancer diagnosis and treatment despite several side effects.[1] More importantly, traditional imaging agents (iodinated and gadolium contrast) and therapeutic agents (several anticancer drugs) suffer from poor blood circulation, nonspecific biodistribution, and vital organ toxicities (mainly for heart and liver). However, they are hidden parameters in the literature. To overcome these significant health-related issues of small molecules contrast agents and chemotherapeutic drugs, nanoparticles-based cancer diagnostics and therapeutics approaches have been proposed as an alternative for conventional cancer therapies due to their specific physicochemical properties, site-selective binding to cancer cells/tumors, prolonged circulation time, etc.[2] These nanoparticles based imaging and therapeutic agents have shown their promising impact on tumor diagnosis and treatment in preclinical models. But they have tried individually and reported the major degradation concern.[3,4] In the last few decades, integrated nanoimaging and therapeutic platforms named nanotheranostics have gained massive attention in cancer theranostics research, which has grown exponentially.[2] Theranostics systems (integrated imaging and therapeutic agents) are essential for localized diagnostics and treatment at the minimum required dose. Importantly, theranostics helps in (i) making a distinction between pathologic and normal

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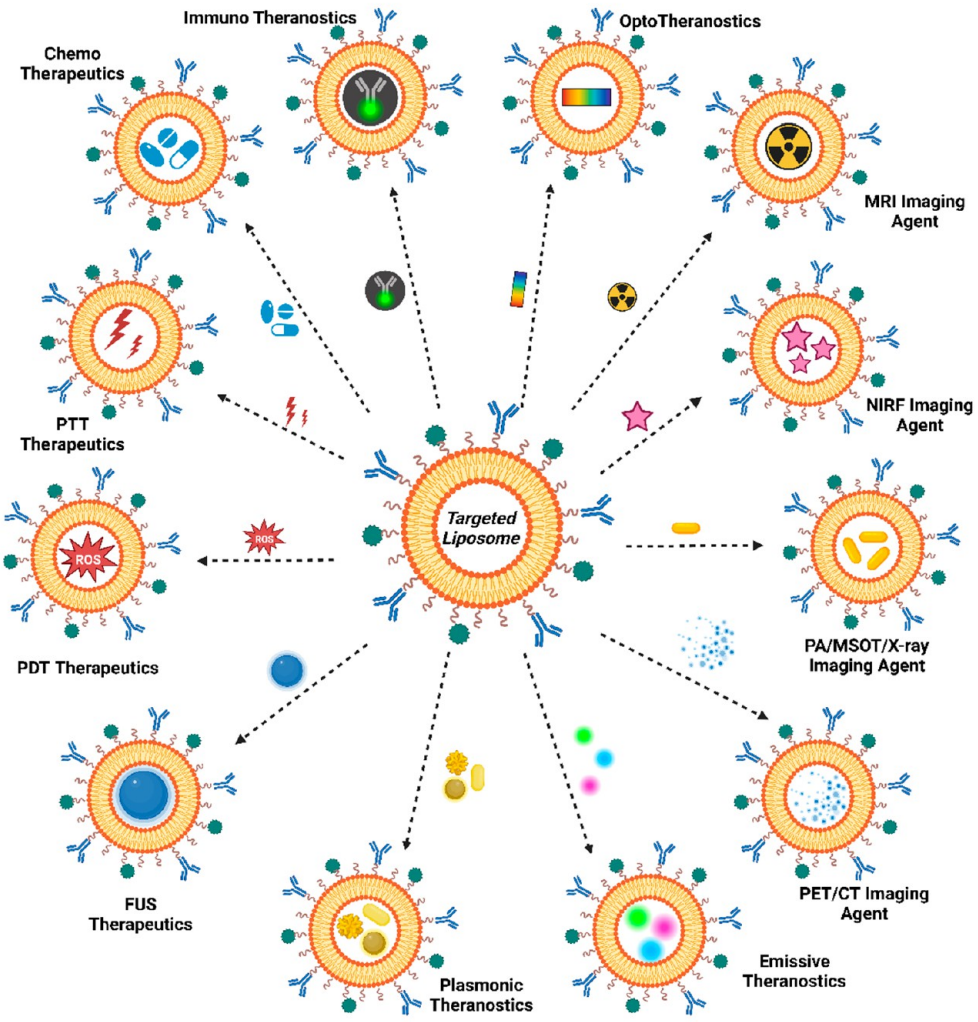

Figure 1. Schematic showing the imaging and therapeutic interventions in the targeted liposomal system. Self-assembly and bioengineering of liposomal systems with different imaging or therapeutic probes can intervene with cancer diagnostics and therapeutic modalities.

tissues, (ii) targeted diagnosis and disease prognosis, and (iii) evaluating therapeutic progress. In the last few decades, nanoparticle-based theranostics research has gained attention for imaging and targeted therapy of cancer.[5] It should be noted that both probes or agents, viz., diagnostics and therapeutics, have been injected separately, which causes several side effects. Hence, integrated imaging and therapeutics probes in a single system theranostics at the nanoscale without losing an individual's performance are always in demand.[5] However, this is one of the critical tasks to address for developing safe nanotheranostics agents for cancer care and treatment. More importantly, nanoparticulate systems tend to have prolonged blood circulation and localized targeting compared to free diagnostics and therapeutic agents. To our knowledge, various engineered nanoparticle therapeutics agents have been proposed and tested in multiple clinical applications. About 25 nanoformulations are approved and almost 100 are in clinical trials now.[6] Moreover, surface engineering approaches have been proposed and tested to improve circulation, showing that surface-engineered nanocarriers have many benefits over free medicines.[7] Further, nanocarriers possess numerous advantages, such as improved solubility, enhanced cell penetration, aiding drug accumulation inside cells, reduced drug clearance, and codelivery of drugs. More precisely, significant problems, such as poor absorption, solubility, bioavailability, rapid degradation rate, a short shelf life, and inadequate therapeutic efficacy, are connected to the therapeutic administration of free medicines.[8] The therapeutic efficacy of bioactive chemicals is increasing. At the same time, their adverse effects are decreased by incorporating them into reasonably biocompatible inert carriers or delivery systems with ligand-guided site-specific administration. As a result, the constraints of pharmacokinetics, drug absorption, and side effects are all eliminated by nanoformulation.[9] Nanoparticles face various problems of biodegradation, toxicity, rapid clearance, reproducibility, scalability, reliability, specific targeting, biodistribution, and regularity of approvals. Nanoparticles are proposed for surface functionalization with specific tumor targeting agents, which improve the site-selective delivery of small imaging or therapeutic agents.[9−11] Given their ease of membrane crossing and prolonged circulation duration in the reticuloendothelial system, they have enormous promise as theranostics agents against various disorders.[9] Surface-engineered nanoparticle systems can be changed with multiple chemical moieties, including polyethylene glycol, maleimide, poly amino acids, carbohydrates, and fatty acids, to bypass the reticuloendothelial system. However, there are always significant issues with achieving high tumor accumulation of

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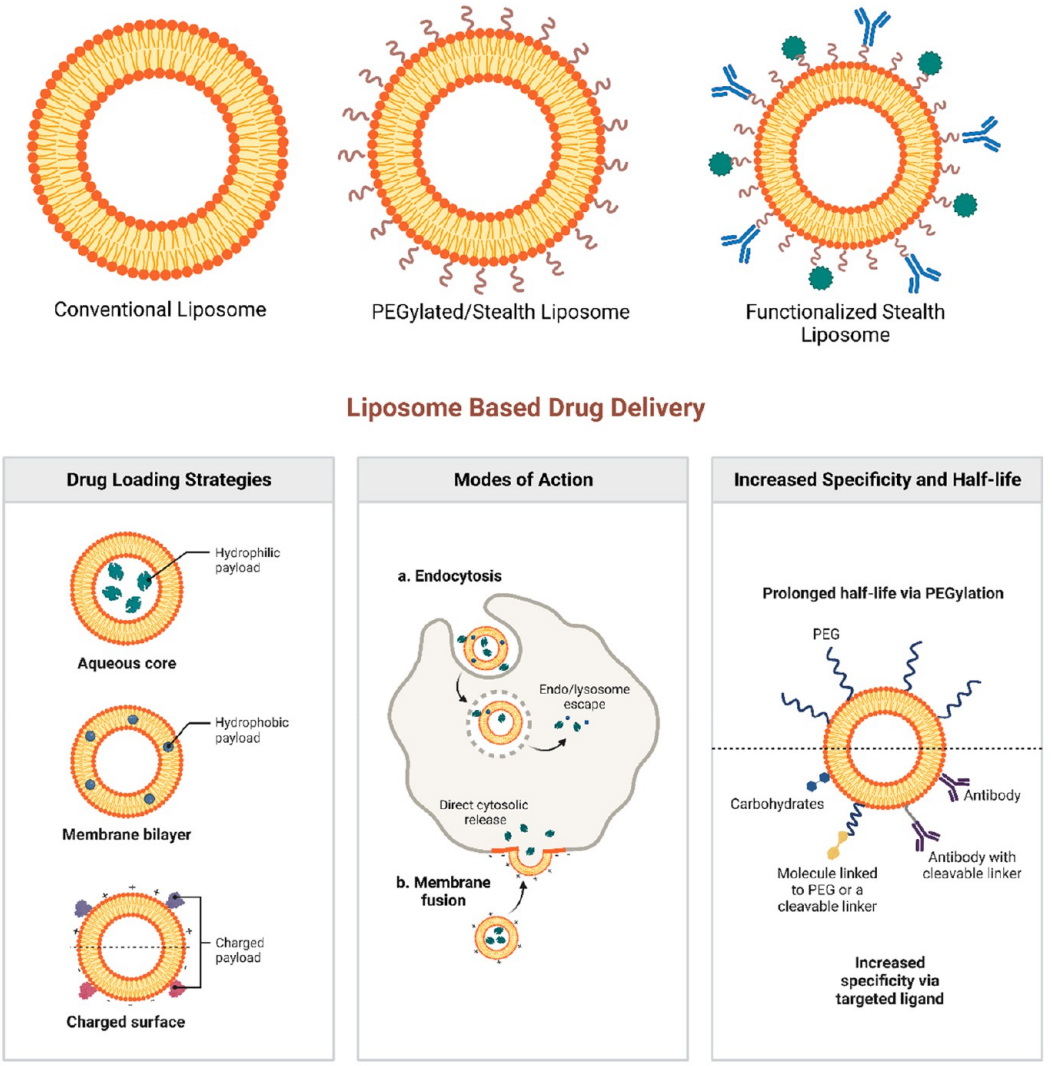

Figure 2. Schematic representation showing lipid self-assembled liposome and their surface engineering such as conventional liposome, PEGylated liposome, and stealth liposome. Different payloads (hydrophilic, hydrophobic, and charged cargos) in liposomal nanoparticles with their action for cell uptake and half-life have been represented here. These graphics are reproduced with permission from ref 83. Copyright 2015 Frontiers Media S. A.

administrated nanoparticles due to several biological barriers.[12] Many nanoparticles demonstrate necessary imaging and therapeutic responses *in vitro* and *in vivo*, but most pilot-level preclinical and clinical models failed.[13,14] Various research groups have developed stimuli-responsive nanotechnology-based transport systems for the simultaneous delivery of diagnostics and therapeutic drugs due to the ongoing advancements in nanotechnology. Now, we are at the stage of better understanding these nanotechnology-based solutions at a cellular level.[15,16] Theranostics is one such strategy that combines diagnostics and therapeutics at the nanoscale without compromising an individual's performance. It enables simultaneous target identification, drug dissemination tracking, and therapeutic performance evaluation to produce personalized medicine.[17−19] The selectivity and the possibility of accumulation in the tumors have enabled organic and inorganic nanotheranostics to be extensively developed. So far, various nanoplatforms have been proposed for cancer theranostics applications. Among them, liposomal nanotheranostics have been successfully established for *in vitro* studies and tested for multimode-targeted *in vivo* bioimaging and near-infrared (NIR) light-mediated cancer therapeutic applications. The choice of diagnostics and therapeutics can intervene in the theranostics property of the engineered liposomal system. On the other hand, imaging and therapeutic interventions in liposomal systems have been achieved and tested in *in vitro* and *in vivo* models as shown in Figure 1. Such intervention has been achieved in other nanotheranostics systems, viz., mSilica, polymeric, plasmonic shell, etc. based hybrid systems. Among all nanotheranostics used, liposomes offer several advantages, viz., heterogeneity, better biocompatibility, easy penetration within solid tumor matrix, a degree of protection from degradative processes, better carriers for large molecules, as well as targeted potentiality of these carriers.[20,21] Additionally, they offer site specificity and allow for surface modifications, such as targeting ligands like hyaluronic acid and aptamers, further improving their target-specific competencies.[22,23] Liposomes have found their usage not only in cancer theranostics applications but also for age-related diseases, inflammatory diseases, phototriggered local anesthesia, etc.[24,25] On the basis of various engineering and medical issues, liposomal cancer theranostics are in the developing phase and

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still in its nascent stage[26,27] though they have been proposed for clinical trials. For example, anticancer drug doxorubicin (DOX) loaded liposomal nanoparticles have been extensively studied for targeted therapeutics in solid tumor reduction applications. This was mainly attributed to the fact that DOX could form adducts with the DNA because of its presence in the liposome.[28]

Further, engineered liposomal hybrid nanoparticles have entered preclinical and clinical studies for biomedical applications such as virus and cancer vaccines, cancer chemotherapeutics, drug/gene delivery, targeted bioimaging, and light-mediated photodynamic and photothermal ablation therapy of cancer. *In vitro* and *in vivo* examinations indicate their biocompatibility, safety, diagnostics, and therapeutic efficacy for further clinical trials. The article addresses liposomal nanotheranostics agents' significant advantages and translational aspects for targeted cancer imaging and therapeutics applications.[29−33]

LIPOSOMES

Liposomes have been recognized as versatile cargo delivery systems and proposed as an early version of nanomedicine applications.[34,35] Today, in various pharmaceutical industries, small-size lipid particles have shown their tremendous response in terms of potential vehicles for delivering different types of therapeutic cargo. Lipid-based formulations, viz., COVID-19 mRNA vaccines, are in the spotlight today because they are essential in efficiently protecting and carrying mRNA to the target cells.[36,37] However, before this formulation, various liposomal structures had been designed and tested for biomedical applications. Liposomal engineering, one of the early first-generation nanomedicines, has successfully transitioned from conception to commercialization. Since their discovery in almost the mid-1900, numerous technological developments have been made to increase their usefulness, including liposome processing and targeting.[9] In the past 25 years, liposomal structures have received tremendous attention as potential medication and gene delivery systems for cancer treatment. More interestingly, 1990 brought better hope in medical applications with the term "lipid nanoparticle" when the era of nanotechnology was established. Since then, nanoscale lipid formulation named liposomes has been proposed for biomedical applications, which has been considered the earliest generation of nanosized lipid particles as shown in Figure 2.[38] Liposomes are tiny phospholipid bubbles or lipid vesicles with a single molecular lipid bilayer inside and a regular arrangement of amphiphiles in the border layers. The primary structural element of the liposome is a phospholipid (phosphatidylcholines, phosphatidylserines, phosphatidylethanolamines, phosphatidylglycerols, etc.); when combined with an aqueous solution, the phospholipid forms a spherical structure. The structural stability of liposomes is aided by another crucial component viz., cholesterol, which also improves the solubility of medications during blood circulation. Cholesterol may generate vesicles with diameters ranging from 0.025 to 2.5 μm during the creation of liposomes but has several limitations for cancer nanomedicine applications.[35,36] Liposomal hybrid systems can entrap both hydrophilic and hydrophobic cargo molecules, where hydrophilic small cargo molecules can be encapsulated in the aqueous interior core of liposomes. In contrast, hydrophobic cargos can be embedded in the hydrocarbon chain assembly of the liposome's lipid bilayer. The ability to carry multiple cargo molecules makes liposomes a versatile cargo delivery system. The preparation method changes the overall structures of liposomal systems that are either unilamellar [small unilamellar vesicles (SUV with 20−100 nm size), large unilamellar vesicles (LUV with 100−1000 nm size)] or multilamellar vesicles (MLV with >500 nm in size). The composition, size and form, preparation techniques, benefits, and certain restrictions related to liposomes are presented in Table 1.[39,40] The discovered liposome drug carriers can be targeted either passively or actively by the cells and taken up. In the case of passive targeting, the drug-loaded liposome is absorbed by the cell membrane through molecular diffusion.[41] Circulation half-life and cargo loading capacity or encapsulation efficiency depend on their size, a primary parameter for escaping phagocyte uptake.[42] ≤100 nm size of liposomes has been considered for pharmaceutical purposes and biomedical applications. Lipid-based nanosize carrier systems start with different generations. Nanoscale solid lipid particles, nanostructured lipid carriers, and nucleic acid-encapsulated ionic lipid particles demonstrate better therapeutic responses due to their unique physicochemical properties. The earliest nanomedicines of the first generation, liposomes, have jumped from idea to market.

Liposomal drug composition has been the subject of numerous investigations for various biological purposes.[43−45] In one of the investigations, glutamic acid is successfully used as a cross-linker to conjugate polyethylene glycol (PEG) to the lipid monomer.[46] Postadministration of PEGylated formulations/drugs results in the production of antibodies which bind to PEG selectively showing reduced treatment efficacy.[47−50] For example, PEGylated proteins and peptides demonstrate anti-PEG antibody responses by the T-cell pathway. Over the past years, PEG has been reported to be nonimmunogenic, but there are some evidence claiming that PEG might be more immunogenic than the reported ones.[39,51,52] There are also evidence for existence of anti-PEG antibodies in healthy bodies which are exposed to PEG additives heavily. In the case of PEGylated therapeutics, 25−42% anti-PEG antibodies have been reported in healthy blood donors which may trigger immunogenic responses.[53−56] It is reported that PEG conjugation increases the hydrophilicity and functional response against the biological barrier which also protect nanocarriers from serum protein adsorption.[17,47] Functionalization of liposomes with PEG has been reported with reduced adsorption of protein opsonins on liposomal surfaces and improved clearance of the liposomal system from phagocytic cells in the liver and spleen during blood circulation.[17,57,58] Various PEGylated and non-PEGylated liposomes have received clinical relevance and approval.[59] For example, Doxil (doxorubicin loaded PEGylated liposomes) is one such approved for breast and ovarian cancer.[49,60] PEGylation improves overall circulation, specific bio distribution, biocompatibility, therapeutics efficacy and response, and better tumor retention ability of liposomal hybrid nanotheranostics.[61,62] However, the immunogenicity concern associated with PEGylated liposomes, particularly the accelerated blood clearance (ABC) phenomenon, is an important consideration in developing and clinical applications of PEGylated liposomal formulations.[63] ABC is characterized by a rapid clearance of subsequent doses of PEGylated liposomes from the bloodstream upon repeated administration, leading to decreased therapeutic efficacy.[53] In the year 2000, accelerated blood clearance (ABC) phenomenon was conceptualized when

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Table 1. Nanosized Lipid Carriers

lipid nanoparticles	components	shape/size	preparation method	major advantages	limitations	refs
liposomes	phosphatidylserine, 1,2-dipalmitoyl-sn-glycero-3-phospho-choline monohydrate, and phosphatidylcholine	spherical/ ≤1 μm	solvent dispersion/evaporation and thin-film hydration/deter-gent method	controlled drug release	low stability, high-cost ap-proach, low production, and scale-up process	12
niosomes	alkyl amide and ethers/fatty acid and alcohols/cholesterol	spherical/ ≤ 1 μm	thin-film hydration/sonication/re-verse phase solvent evaporation/ microfluidization	sustained drug release, better stability, and nonimmunogenic	low shelf life, high cost for production, and time-taking process	84
solid lipid nanoparticles	phospholipid (90 NG)/glycerol tristearate and monostearate/cetyl palmitate	spherical/ 50−1000 μm	solvent emulsification and double emulsion/spray drying/sonica-tion	better stability, multiple cargo capacity, and better compati-bility and scalability	low drugs encapsulation effi-ciency	12,17
nanohybrid lipid carriers	stearic acid/cetyl palmitate/tristearin/cholesterol/precirol ATO5/CompritolR888/ DynasanR116/SoftisanR154/GelotR64/Emulcire 61/paraffin oil/oleic acid/squa-lene/triglycerides/isopropyl myristate/vitamin E	tens of nm to 3 μm	cold and hot homogenization/ microemulsion	better solubility, storage ability, half-life, bioavailability, and permeability	low stability and high oper-ating temperatures	9,11
lipid polymer hybrid sys-tems	PLGA/PCL/poly-L-arginine/PEI/DSPE/PLA/PLA/cholesterol/DSPE lipid-lecithin/ DSPE/DLPC/DMPE	spherical/ ~60−280 nm	solvent evaporation/nanoprecipi-tation/emulsification	better stability and half-life and sustained drug release	expensive and complicated scale up	18

PEGylated liposomes were injected in small and large animals (rats and rhesus monkeys).[64] These PEGylated liposomes showed improved clearance after a week of administration.[53] So far, this concept has not yet been implicated in clinical use especially for repeated administration of doxorubicin loaded PEGylated liposomal formulations but may be implicated for other types of PEGylated products in the future.[53] Repeated injections of PEGylated liposomes can induce the production of anti-PEG antibodies, including both immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies.[65,66] These antibodies recognize and bind to the PEG moiety on the liposome surface, triggering an immune response and resulting in faster clearance of PEGylated liposomes from circulation.[53] Absence of surface receptors on macrophage for Fc fragment of IgM antibodies reduce the phagocytosis of postinjected liposomal nanohybrids.[53,54] It was reported that preinjection of empty liposomes (uncoated) resulted in reduced infusion reactions against PEGylated drugs and prevented anti-PEG antibody responses too.[53] However, drug loaded PEGylated liposomes do not induce anti-PEG antibody responses as compared to empty liposomes.[53] For example, doxorubicin loaded PEGylated liposomes prevents the production of anti-PEG IgM and prolongs its half-life after injecting a second dose which was validated in beagle dogs.[53,67] Similarly, mitoxantrone or oxaliplatin-loaded PEGylated liposomes do not show anti-PEG IgM antibody responses.[53] However, low dose administration of liposome may cause anti-PEG antibody responses in small animal models.[53] Complement (C) activation related pseudo allergy (CARPA) hypersensitivity was another immune response of PEGylated liposomes.[53,68] This immune response can compromise the prolonged circulation and drug delivery properties associated with PEGylated liposomes.

Several alternatives have been explored in the surface engineering of liposomes to mitigate the immunogenicity and ABC phenomenon.[53] Hydrophilic polymers such as hyperbranched polymers (e.g., POEGMA) and synthetic polymers [e.g., poly(glycerols), poly(oxazolines), and poly(hydroxypropyl methacrylate)] have been investigated.[69] These polymers exhibit advantageous properties such as reduced antigenicity, improved stealth behavior, and oxidative or thermal stress resistance. Zwitterionic polymers like poly(carboxybetaine) and poly(sulfobetaine) have shown intense hydration and resistance to nonspecific protein fouling, making them attractive alternatives to PEG.[70,71] Additionally, glycosaminoglycans (GAGs) and polysialic acid (PSA) have demonstrated increased circulation times for nanoparticle conjugates.[72] Despite promising results, further research is needed to fully understand the immunogenic profiles and address limitations associated with these alternative polymers for functionalizing liposomes. On the other hand, active targeting is accomplished by coating the carriers' surfaces with ligands such as folic acids, peptides, antibodies, hyaluronan, small proteins, etc.[73−76] The new liposomal vesicle's stability, bioavailability, and increased toxicity to malignant cells are all credited to it. Likewise, RGD-modified liposomes significantly increase the bioavailability of curcumin in MCF-7 cells.[77] Another team of researchers came up with an intriguing idea: anticancer medications were directed toward brain tumors via the GLUT receptor, which upholds the brain's energy requirements. Glucose is coupled with liposomal vesicles to cross the blood-brain barrier using polyethylene glycol.[78] Cancerous cells have successfully used a highly active glycolysis

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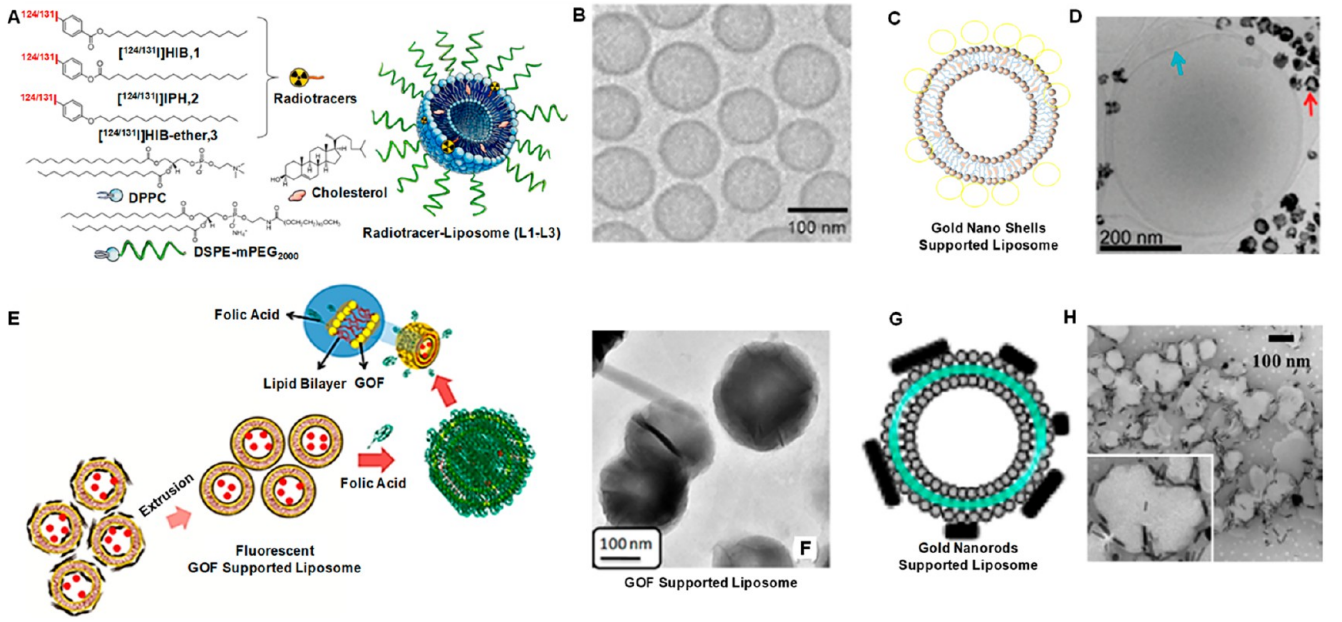

Figure 3. Surface-engineered liposomal theranostics hybrid systems. Engineered liposomes for tumor-targeted radionuclide therapy. (A) Required components for engineering liposome-based radiotracers and (B) TEM image of engineered liposomes. Panels A and B are reproduced from ref 110. Copyright 2021 American Chemical Society. (C) Graphic showing gold nanoshell supported liposomal hybrids system and (D) TEM image of gold nanoshell-liposomal hybrids. Panels C and D are reproduced from ref 111. Copyright 2008 American Chemical Society. (E) Schematic representation of folic acid attached emissive graphene oxide supported liposomal hybrids theranostics systems, and (F) TEM image of graphene oxide flakes supported liposomes. Panels E and F are reproduced from ref 31. Copyright 2019 American Chemical Society. (G) Graphic of gold nanorods decorated liposomal theranostics systems and (H) TEM image of gold nanorods supported liposomal nanoparticles. Panels G and H are reproduced from ref 98. Copyright 2012 American Chemical Society.

pathway. The aforementioned metabolic pathway aids in increasing the cell's warmth and lowering its pH.

In response to this occurrence, temperature-sensitive liposomal vesicles for actively targeting anticancer medicines have been created. siRNA is a commonly used pharmacological medication for cancer treatment.[79] Due to the increased production of P-glycoprotein, the root of drug resistance, active siRNA targeting is complex. As previously stated, a polyethylene glycol-conjugated liposomal vesicle has been successfully developed to target the siRNA and actively solve the problem.[80] Similarly, connecting the overexpressed estrone receptor with the PEGylated DOX-liposome has been successfully formulated for site-selective targeting.[81] Melanoma cells that overexpress the survival protein become resistant to dacarbazine and eugenol. Therefore, the safe engineering of hyaluronic acid decorated lipid carriers have been proposed for transporting the dacarbazine and eugenol-resistant melanoma cells to battle resistance to these anticancer treatments. In a few independent trials, dye-tagged 1,2-dipalmitoyl-*sn*-glycero-3-phosphodiglycerol-modified polyethylene glycol-based liposomal delivery systems have been developed to examine the cellular interactions of these systems in solid tumors.[82] Further, these thermosensitive liposomal systems have demonstrated effective liposome-cell interactions with better drug accumulation and cell toxicity.[10] To make nanomedicines for theranostics, preparation and delivery methodologies of liposomes for improved drug accumulation in cells must be thoroughly assessed.[11]

Intervention in lipid-based assemblies help in multimode imaging and therapeutics of cancer, especially solid tumors with respect to decorated diagnostics or therapeutics probes at nanoscale. Moreover, various other strategies, viz., small molecules conjugations, graphene oxide flakes wrapping, gold nanoparticles support, etc., for liposomal surface engineering have been applied, and designed nanotheranostics systems demonstrated successive theranostics performance in various cancer models (Figure 1−Figure 3). Liposomes mainly comprise sonication, extrusion, and microfluidic jet-blowing approaches from the thin film hydrated lipid suspensions followed by mechanical agitation or tedious freezing-thawing processes, hot ethanol injection, and water/oil emulsion protocols.[85,86] Methods such as sonication, extrusion, homogenization, and microfluidics have been tested for lipid nanoparticle preparation and their particle size control. Still, microscopic validation, elemental analysis, dynamic light scattering analysis, and surface charge zeta potential are promising enough to ensure their morphology, compositions, size distribution, and stability, respectively.[86−90]

SURFACE ENGINEERED LIPOSOMES

Lipid self-assembled nanoscale particles are soft and fragile in nature; hence, there is a concern about their easy degradation in physiological conditions before delivering loaded cargo molecules (imaging and therapeutics or both) to the target site. On the other hand, unmodified liposomal nanotheranostics or nanomedicines show nonspecific biodistribution and poor tumor uptake (in solid tumors) though the solid tumor has good vascular network and blood perfusion. To improve the stealthy, stability, specific biodistribution, and targeting ability of liposomal systems, various liposomal nanostructures have been engineered with a surface covering agent viz., small molecules, nanosheets wrapping, support of plasmonic nanoparticles, and surface-functionalized targeting ligands that recognize and bind to specific receptors on the target cells as

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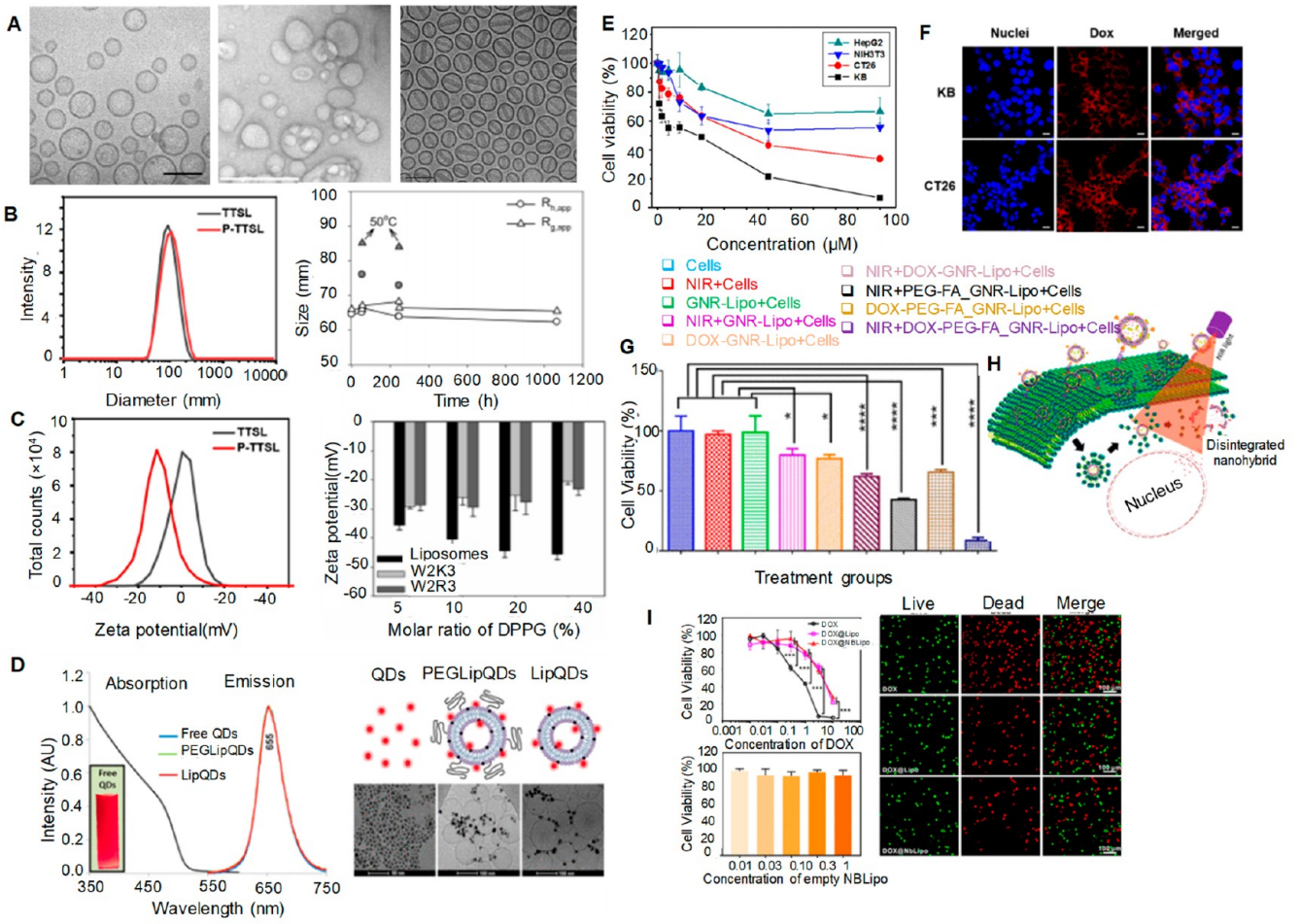

Figure 4. Characteristics of liposomal nanotheranostics. (A) Cryo-Transmission electron microscopic (Cryo-TEM) images of liposome, surface-engineered liposome, and drug-loaded liposomal particles at 100 and 200 nm scale bar. Panel A: Reproduced with permission from ref 87. Copyright 2003 Elsevier. Reproduced with permission from ref 88. Copyright 2014 PLoS One. (B) Dynamic light scattering particle size and time-dependent size distribution of liposomal nanostructures and (C) surface charge measurements of liposomes and surface-engineered liposomal nanoparticles. Panel B is reproduced from ref 89. Copyright 2019 American Chemical Society. Panel C is reproduced from ref 90. Copyright 2014 American Chemical Society. (D) Absorption and emission spectra, and Cryo-TEM of quantum dots (QDs) decorated liposomes in aqueous media; the inset is a photograph of a colloidal suspension of QDs at λ_{ex} = 300 nm with red emission. Panel D is reproduced from ref 112. Copyright 2019 American Chemical Society. (E) Concentration dependent *in vitro* cell compatibility followed by MTT assay and (F) *in vitro* cell images. Panels E and F are reproduced from ref 113. Copyright 2016 American Chemical Society. (G) *In vitro* cancer theranostics performance of surface engineered and drug-loaded liposomal theranostics systems in terms of % cell viability measurements followed by MTT assay and (H) schematic representation of gold nanorods decorated liposomal theranostics for near-infrared light mediated cancer cell ablation. Panels G and H are reproduced from ref 29. Copyright 2018 American Chemical Society. (I) Concentration-dependent biocompatibility of designed liposomal hybrid systems and cancer therapeutics performance of drug doxorubicin loaded liposomal system at various concentrations along with live−dead cell imaging. Panel I is reproduced from ref 96. Copyright 2020 American Chemical Society.

shown in Figure 2−Figure 4 and Table 2. Surface functionalization of liposomes with different approaches play a crucial role in overcoming the current limitations of liposomal nanocarrier systems to treat solid tumors with efficient targeting and therapeutic response. For various advantages, soft liposomes have been decorated or supported with different covering agents such as fluorescent quantum dots, graphene sheets, gold nanoshells/nanorods, polymeric frameworks, cell-derived biological nanovesicles, etc. These supported systems not only improve the stability, overall theranostics performance, and circulation of liposomal platforms but also prevent the premature leakage or release of loaded therapeutics or imaging agents. Recently, it has been noticed that soft liposomal hybrid systems demonstrate better stability after surface engineering with (i) gold nanorods that provide mechanical strength for both the inner and outer surfaces of lipid-based assemblies. This self-assembled gold nanorods liposomes hybrids platform performs anticancer therapeutics with deep tissue localization in living animals followed by optoacoustic tomography imaging. In another case, small gold nanoparticles have been engineered on the exterior surface of liposomal soft vesicles which have been tested for *in vivo* tumor ablation applications under NIR light irradiation as highlighted in Figure 3. In a recent study that reported liposome gold nanoparticles, the liposomal surface indicates irregular decoration of gold nanoparticles on a liposomal surface that may not prevent the predegradation of soft liposomal vesicles before reaching the target site. However, this formulation has the advantage of phototriggered successive tumor reduction with short irradiation of NIR due to better photothermal response.

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Table 2. Liposome Surface Functionalization Methods and Their Applications

surface functionalization method	summary	relevant applications
PEGylation	PEGylation is the attachment of polyethylene glycol (PEG) chains to the liposome surface, providing stealth properties, prolonged circulation time, and reduced immunogenicity	enhanced tumor accumulation, improved stability, and reduced side effects
targeted ligand conjugation	ligands such as antibodies, peptides, or small molecules are attached to the liposome surface to specifically target tumor cells	selective delivery, improved cellular uptake, and enhanced therapeutic efficacy
pH-sensitive surface modification	liposomes are functionalized with pH-responsive moieties, allowing them to undergo changes in surface charge or structure in response to the acidic tumor microenvironment	triggered drug release and improved therapeutic index
magnetic nanoparticle coating	magnetic nanoparticles are coated onto the liposome surface, enabling magnetic targeting and imaging through external magnetic fields	magnetic guidance, imaging, and hyperthermia therapy
photosensitizer incorporation	photosensitizers are incorporated into the liposome bilayer, enabling light activation for photodynamic therapy (PDT)	tumor-localized photoactivation and targeted cell death
imaging agent conjugation	liposomes are functionalized with imaging agents (e.g., fluorescent dyes, radioisotopes) to enable real-time imaging and tracking of liposomes *in vivo*	noninvasive imaging, biodistribution studies, and theranostic approaches
stimuli-responsive modifications	liposomes are engineered with stimuli-responsive components (e.g., temperature, light, and enzymes) that trigger changes in their structure or cargo release upon exposure to specific stimuli	on-demand drug release and personalized therapy approaches

Table 3. Formulated Liposomes and Their Clinical Status[93,94]

liposomal formulation	size (nm)	application	clinical status	ref
Myocet (Cephalon)	190	cancer therapy	approved	119
Depocyt	120	neoplastic meningitis	approved	120
DaunoXome (Galen)	100−180	cancer therapy	approved	121
Mepact (IDM Pharma SAS)	about 100	cancer therapy	approved	122
Lipo-Dox (Taiwan Liposome)	−	cancer therapy	approved	123
Lipoplatin	100−120	solid tumor therapy	phase III	124
Lipoplatin (Regulon)	100	cancer therapy	phase II	125
CFTR gene liposome	−	cancer therapy	phase II	
lip glucan-time	100−200	solid tumor therapy	under trials	126
Marqibo	−	acute lymphoblastic leukemia	under trials	127
Lipotecan	100−110	colorectal carcinoma	under trials	128
liposomal alendronate	150−200	coronary artery stenosis	under trials	129
Versamune PDS0101	100−120	cancer therapy	approved	130
Daunoxome (Gilead): classical (phosphatidyl choline + cholesterol)	30−70	cancer therapy	approved	131
Stealth Liposomes Doxil/Caelyx (Johnson & Johnson)	100	Kaposi's sarcoma	approved	132
Doxil (Janssen products)	100	cancer therapy	phase II	133
ThermoDox (Celsion Corp)	100	cancer therapy	approved	134
ThermoDox (Celsion)	−	cancer therapy	phase II	134
biomimetic liposomes Stimuvax (Oncothyreon/Merck)	−	nonsmall cell lung cancer	phase III	135
ATI-1123 Docetaxel	60−80	solid tumors	phase I	136
Stimuvax	−	nonsmall cell lung cancer	phase III	137
Liprostin	−	restenosis after angioplasty	phase III	39
liposomal annamycin	−	refractory acute myeloid leukemia	phase II	138
SPI-077 (soybean phosphatidylcholine and cholesterol)	−	lung and head and neck cancer	phase II	139
mPEG covalently bound phospholipids (S-CKD602)	−	cancer therapy	phase II	140
Endotag-I (DOTAP: DOPC: Paclitaxel)	−	cancer therapy	phase II	141
LEP-ETU (Paclitaxel DOPC, cholesterol, and cardiolipin)	−	cancer therapy	phase II	142
LE-SN38 (DOPC, cholesterol and cardiolipin)	−	advanced colorectal cancer	phase II	143
LEM-ETU (mitoxantrone DOPC, cholesterol, and cardiolipin)	−	cancer therapy	phase I	144
INX-0076 (topotecan, cholesterol and sphingomyelin)	−	solid tumors	phase I	39
INX-0125 (vinorelbine tartrate cholesterol and sphingomyelin)	−	solid tumors	phase I	145
2B3-101 (DOX glutathione PEGylated liposomes)	−	solid tumors	phase I	146

On the other hand, graphene oxide flakes have been proposed for the complete wrapping of liposomal vesicles that provide better stealth nature and stability of soft vesicles in biological conditions and targeted tumor imaging, but this nanoengineered hybrid system demonstrates its easy degradation in a cancer-mimicked environment in both conditions (inherent tumor environment and external photothermal conditions). Further, the above-engineered hybrid systems exhibit significant photothermal response within short NIR exposure that ablate solid tumors selectively and significantly. To our knowledge, some surface-engineered stealth liposomes have been reported with passive targeting in solid tumors which is explained based on their naturally leaky vasculature and defective lymphatic drainage.[25,91,92] Namely, Myocet,

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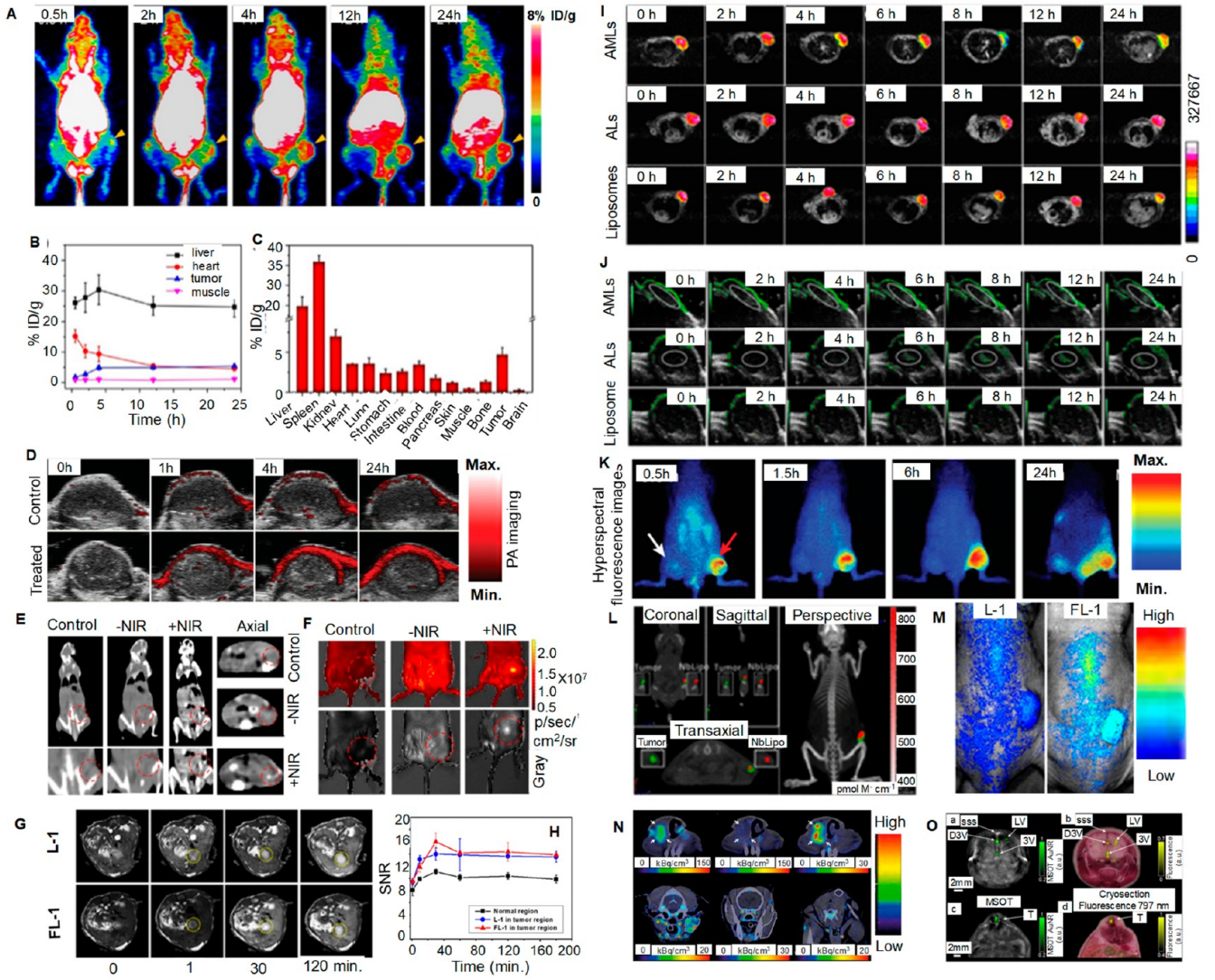

Figure 5. Engineered liposomal hybrid platforms in imaging multimodality for tumor diagnosis. (A) Time-dependent PET images of 4T1 tumor-bearing mice after injecting $^{64}Cu^{2+}$ tagged AQ4N-hCe6 engineered liposomes where yellow arrows give the direction for the tumor area, (B) quantification of injected AQ4N-^{64}Cu-hCe6-liposome from the liver, heart, tumor, and muscle of 4T1 tumor-bearing mice at different time points of postinjection, and (C) biodistribution of injected AQ4N-^{64}Cu-hCe6-liposome 4T1 tumor bearing mice. Panels A–C are reproduced from ref 114. Copyright 2017 American Chemical Society. (D) Time-dependent photoacoustic imaging of a prostate tumor after intravenous injection of surface-engineered liposomes. Panel D is reproduced from ref 115. Copyright 2021 American Chemical Society. (E) X-ray computed tomography images and (F) near-infrared fluorescence (NIRF) imaging of a solid 4T1 tumor in female balb/c mice using a gold nanoparticle-decorated emissive liposomal nanoimaging agent. Panels E and F are reproduced with permission from ref 25. Copyright 2020 Springer Nature. (G, H) T1MR images for the early diagnosis of metastatic liver cancer (yellow circles are for the metastatic tumor area) and signal-to-noise ratio (SNR) comparison of normal and metastatic regions in the liver treated using engineered liposomal hybrids system, viz., Gd^{3+}-texaphyrin conjugated DOX-liposomes. Panels G and H are reproduced from ref 112. Copyright 2019 American Chemical Society. (I) *In vivo* T2MR imaging and (J) US dual imaging for tumors diagnosis at various times (2, 4, 6, 8, 12, and 24 h) using liposomal nanoimaging agents. Panels I and J are reproduced from ref 116. Copyright 2017 American Chemical Society. (K) Time-dependent hyper spectral fluorescence imaging of tumor bearing mice after injecting liposomal nanoparticles. Panel K is reproduced from ref 115. Copyright 2021 American Chemical Society. (L) Liposomal nanocontrast for 4T1 tumor targeting observed by the IVIS-CT multimodal imaging system. Tumor imaging is after 24 h postinjection. The green signals are for tumor location, and the red fluorescence signals are for injected DiD-labeled liposomes. Panel L is reproduced from ref 96. Copyright 2020 American Chemical Society. (M) *In vivo* fluorescence scans for solid tumor imaging in a xenograft model using liposomes. Panel M is reproduced from ref 112. Copyright 2019 American Chemical Society. (N) PET/CT scans for tumor imaging in large animals, viz., dogs, using ^{64}Cu tagged liposomes. Panel N is reproduced from ref 117. Copyright 2015 American Chemical Society. (O) MSOT and fluorescence imaging using gold nanorods (GNR) supported liposome as imaging agents administrated in [a, b] brain and [c, d] tumor tissue (HT29 xenograft). Superior sagittal sinus (SSS); lateral ventricle (LV); third ventricle (3 V), green signals from the nanorods, grayscale background for an optoacoustic signal at 900 nm and yellow fluorescence is detected from NIR-797. Panel O is reproduced from ref 98. Copyright 2012 American Chemical Society.

Doxil, and Caelyx are the forms of DOX-loaded liposomes used for cancer therapies.[93,94]

Further, for site-selective targeting, liposomes have been grafted with targeting ligands (peptides, aptamers, folic acid, antibody fragments, small molecules, etc.) using surface

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engineering techniques viz., click chemistry, bioconjugate chemistry, self-assembly, noncovalent binding, etc.[87,89] For example, conjugation with antibodies has been proposed for developing targeted immunoliposomes (actively targeted liposomes) where the efficiency of IgM ligand-attached liposomes was 100 times higher than that of unmodified liposomes. These lipid structures can deliver loaded bioimaging agents and an anticancer payload from their internal aqueous cavity or entrenched positions within the hydrophobic phospholipid bilayer. Liposomal-based nanohybrids have been studied for targeted nanoimaging and therapeutics applications, and recently they have been documented for image-guided nanotheranostics in tumor models.[25,95] Various liposomal formulations have been tested for multiple cancers with promising therapeutic efficacy.[11] Some have been tested for *in vivo* and clinical trials, as highlighted in Table 3.[39,89−94,96−98]

Cancer cells are overexpressed with specific receptors like the folate receptor and transferrin receptor, which easily bind to their corresponding ligands attached to the liposomal surface. For example, folic acid ligand-attached liposomes can strongly bind to the folate receptors expressed in cancer cells that differentiate the specificity of tumor cells from non-cancerous cells. It should be noted that various macrophages are also overexpressed with folate receptors, characteristic of inflammatory diseases. Therefore, folic acid-based targeting can also be applied to deliver anti-inflammatory therapeutics. Further, liposomal systems have also been engineered with other targeting molecules named transferrin which can firmly attach to specific receptors, such as transferrin receptors overexpressed proliferating cancer cells. The liposomes were surface-conjugated with RGD peptide and NGR. The cell line study found that liposomes combined with the RGD peptide demonstrated greater cocultures uptake than single cultures. This is possibly due to the interaction between the RGD peptide and M cells, which eventually engulf the liposomes through receptor-mediated endocytosis. This demonstrated the superiority of surface-functionalized liposomes as compared to regular liposomes.[99] In another study for cancer, surface-functionalized liposomes for HER2-positive breast tumor cells were prepared and loaded with the anticancer drug DOX.

The liposomes were prepared using the thin film hydration method. The study established that the peptide attached to the liposomal surface at 1−2% results in nonspecific binding and uptake by HER2-negative cells MCF-7. This is evident from the indication of nonselective binding and uptake at a peptide density of 2%. Additionally, the *in vitro* study that employed different lengths of peptide linkers showed a significant increase in cellular uptake with a shorter linker length (EG8). Still, no cellular uptake was observed with a longer linker length (EG18) at a 1% peptide density. The peptides' efficient display prevented any obstruction from lipids or polyethylene glycol (PEG), improving their binding to receptors. This highlighted that peptide lengths attached to the liposomes could have an overall effect and bind to tumor cells.[100] In another study, for effective delivery of the nutraceutical camptothecins, the surface functionalization of the liposomes to make them pH-sensitive has been carried out by sialic acid using thin film hydration and an active loading technique. There are other targeting ligands, such as the epidermal growth factor receptor (EGFR, a tyrosine kinase receptor), which are overexpressed in various solid tumors (breast cancer, colorectal cancer, and lung cancer), and the granulocyte-macrophage colony-stimulating factor (GM-CSF) ligand for the GM-CSF receptor, anti-CD20 antibody (Rituximab, Ibritumo).[101−109] Liposomal-based nanoformulations have been recognized as the most clinically acceptable systems for various therapeutic applications, including drugs, genes, vaccines, and imaging agents.

LIPOSOMES IN CANCER IMAGING AND THERAPEUTICS

The self-assembly and amphiphilic nature of liposomes make them versatile for encapsulating or entrapping multiple cargo agents, viz., hydrophilic, hydrophobic, or amphiphilic imaging probes. So far, various liposomal hybrid systems have been tested for multimode imaging named as photoacoustic, ultrasound, T1/T2MR imaging, X-ray computed tomography, NIR imaging, positron emission tomography (PET)-computed tomography, multispectral optoacoustic tomography (MSOT), and fluorescence imaging applications for cancer cells and solid tumors as shown in Figure 5. However, enhanced tumor entry/penetration and high tumor retention are still the main focus of research. In brief, immunoliposomes have been used to transport contrast agents for magnetic resonance imaging (MRI) and deliver radionuclides for targeted radionuclide therapy[147] diagnostic radionuclide imaging.[148−150] Liposomal contrast agents have been created for MRI by enclosing contrast metals chelated into a soluble chelator through a passive encapsulation process.[151,152] One option to modify chelating groups is to attach a hydrophobic group like phosphatidylethanolamine or stearic acid and then fix them onto the liposomal surface either during or after liposome preparation.[153,154] Alternatively, polychelating amphiphilic polymers can increase metal contrast agents (like Gd) loaded onto liposomes.[155] Attaching multiple chelating groups to the main chain of specific polymers makes it possible to load them with several metal atoms.[156−158] These metal-chelated polymers can then be integrated into the lipid bilayer of a liposome using the lipid residue at one end. The benefit of this method is that a single lipid anchor can transport a polymer molecule with multiple chelating groups, which enhances the amount of metal contrast agent delivered per carrier on the surface of the liposome.[159] In this regard, Park et al. prepared liposomes containing hyaluronic acid and ceramide, including DOX and Magnevist, a type of contrast agent utilized for MRI. The nanohybrid liposome was designed to take advantage of the lipid and HACE components,[160,161] and its effectiveness in cancer therapy and the diagnosis was demonstrated. The formulation of the nanoliposomes involved a liposome preparation method that had been modified. The cellular uptake of DOX from the nanohybrid liposome was more significant than conventional liposome due to receptor-mediated endocytosis through interactions with the HA-CD44 receptor. It was also discovered that the release of DOX from the nanohybrid liposome increased under acidic pH conditions. The nanohybrid liposome's *in vivo* targeting was verified by MR imaging, as evidenced by the contrast-enhancing effect in the tumor region. Compared to the DOX solution and conventional liposome groups, the clearance *in vivo* of DOX was reduced in the nanohybrid liposome, suggesting a longer circulation time in the bloodstream.

In another study, Saito and co-workers formulated liposomes colabeled with gadolinium (Gd) and a fluorescent indicator, 1,1-dioctadecyl-3,3,3,3 tetramethylindocarbocyanine-5,5-disulfonic acid [DiI-DS; formally DiIC18(3)-DS].

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The study provided real-time analysis of developing liposomes through convection-enhanced delivery (CED). The research also shows that gadodiamide might be added to liposomes containing anticancer medication or given concurrently with liposomes containing gadodiamide. This made it possible to measure the drug's distribution in tissues directly during CED. After infusion of the formulation to rats, a distinct signal was observed through MRI for Lip/Gd/-DS (20 μL), which could be tracked after 2 h.

On the other hand, an engineered liposomal system named Lip/DiI-DS, which was not formulated along with the Gd contrast agent, did not exhibit any MRI signal for localized imaging. Following the infusion of Lip/Gd/DiI-DS via CED, Lip/Gd/DiI-DS nearly enveloped the entire tumor mass. These suggested liposomes were excellent carriers for nano-assisted delivery and imaging at the localized tumor site.[162−164] In another study, Feng and co-workers developed cisplatin prodrug liposomes for biomedical image-guided cancer therapy. The liposomes were prepared by conjugating them with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine. The DiR-Pt (IV)-liposome was a highly effective tool for conducting *in vivo* bimodal imaging using NIR fluorescence and photoacoustic techniques. This is due to its intrinsic cis-Pt (IV) prodrug doping, efficient conversion of light to heat, and exceptional ability to target tumors.

Furthermore, 5 h postinjection, the level of the DiR-Pt (IV)-liposome was found to be increased. The results show that the formulation could be effectively used as a probe for photoacoustic imaging to reveal the accumulation of drugs at the tumor site.[165] In a study, Du et al. investigated the liposomes containing a dual-targeted drug delivery for radio frequency and imaging for tumor detection and therapy. Initially, a hybrid liposomal system consisting of C_{60}-Fe_3O_4-PEG2000 was created by attaching iron oxide nanoparticles to the surface of fullerene (C_{60}) and then followed by PEGylation. This hybrid platform was engineered with thermosensitive liposomes (consisting of dipalmitoylphosphatidylcholine, DPPC, and DSPE-PEG2000-folate) and integrated with docetaxel. The objective was to utilize biological and physical (magnetic) targeting for fullerene-triggered drug release. The MRI investigations showed that liposomes were an effective tool for both photothermal ablation of the tumor and acting as a contrast agent. Also, remarkable koinonia multifunctional liposomes could transduce radiofrequency energy into thermal energy, enhancing the release of pharmaceutical agents from formulated thermosensitive liposomal systems at *in vitro* and *in vivo* levels.[166]

A new image-guided therapeutic liposomal agent composed of Gd^{3+} texaphyrin core conjugated to the anticancer drug DOX[167,168] has been studied for its promising role in the imaging and treating of metastatic liver cancer. Liver-specific contrast agents such as Gd chelates can provide enhanced lesion-to-liver contrasts that facilitate early diagnosis of the liver tumor with better accuracy and characteristics. Further, for molecular imaging where we get diagnostics information at the cellular level, the combination of magnetic resonance imaging and fluorescence imaging offers good spatial resolution and high sensitivity for tumor detection. Interestingly, it should be noted that glutathione, an upregulated molecule in cancer mimicking the environment in cancer cells or solid tumors, cleaved the Gd3+ texaphyrin-DOX conjugation rapidly. Further, the designed conjugate was encapsulated within folic acid targeting ligand-attached liposomes for enhanced tumor targeting which causes selective uptake and release of anticancer drug DOX into the folate receptors overexpressed cancer cells. Upon separation, free DOX releases, producing the desired antitumor effect. Further, it was also observed that the liposomes demonstrated enhanced magnetic resonance imaging under conditions of T_1 contrast, thereby enabling the imaging of metastatic cancer progression.[113] In a study by Béalle et al., ultramagnetic liposomes could load various magnetic nanoscale particles for MRI contrast imaging and site-selective targeting. The magnetic properties of these hybrid systems are conversed by the developed ultramagnetic liposomes that make them an ideal platform for stimuli-responsive targeted therapeutics in a controlled manner. Initially, the alkaline coprecipitation method has been proposed for synthesizing the magnetic nanoparticles composed with maghemite and then decorated with citrate ligands and dispersed in water to form lipid assemblies, viz., liposomes. Magnetic nanoparticles encapsulated liposomal hybrid platforms demonstrate enhanced penetration into the tumor cells.

Further, based on the results of magnetic resonance imaging, it has been noticed that the stimuli active liposomes could be magnetically vectorized to the solid tumors *in vivo*, beginning their targeting ability. Also, the heating response of these ultramagnetic liposomes enables their utility in hyperthermia therapeutics to kill or destroy the tumor cells. The thermosensitive properties of the developed magnetic liposomes can be used for triggered drug release, making it a fascinating theranostics tool in imaging and treating cancer.[169]

Multifunctional liposomal theranostic systems are functionalized with numerous imaging agents,[32,170] each capable of providing specific information at a cellular level. This enables the monitoring of disease treatment and evaluation of drug pharmacokinetics.[76] In a study by Li et al., multifunctional liposomal theranostics vesicles were developed by incorporating NIR fluorescent tracer called IRDye-DSPE onto remanufactured liposomes. Further, the anticancer drug DOX was effectively entrapped into the multifunctional liposomal structures. The liposomes were subsequently labeled with ^{99m}Tc or ^{64}Cu for imaging through single-photon emission computed tomography (SPECT) and positron emission tomography (PET). These imaging results demonstrate the high levels of distribution of the liposomes after their intratumoral administration in squamous cell carcinoma of the head and neck. Hence, the engineered multifunctional liposomes fulfill all requirements of a potential theranostics system. The engineered functional hybrid liposomal system enables real-time monitoring of both systemic and microdistribution of the injected therapeutic agents, allowing a personalized drug administration scheme with a precise treatment response prediction.[171] Loaded MRI contrast agents in the aqueous core of thermosensitive liposomes with the combination of anticancer drugs provide a promising potential for thermo-triggered drug delivery under external stimulus, viz., magnetic resonance image guidance.[172−175] These temperature-sensitive functional liposomal systems enable the triggered release of MRI contrast agents at the melting phase transition temperature (T_m) which also causes a distinct change in the MRI signal. This helps visualize and quantify drug release patterns at the site of the lesion. On the basis of this concept, Smet et al. have explored hyperthermia-mediated triggered delivery of anticancer drug DOX from thermal-sensitive liposomal hybrid systems. The study involved the

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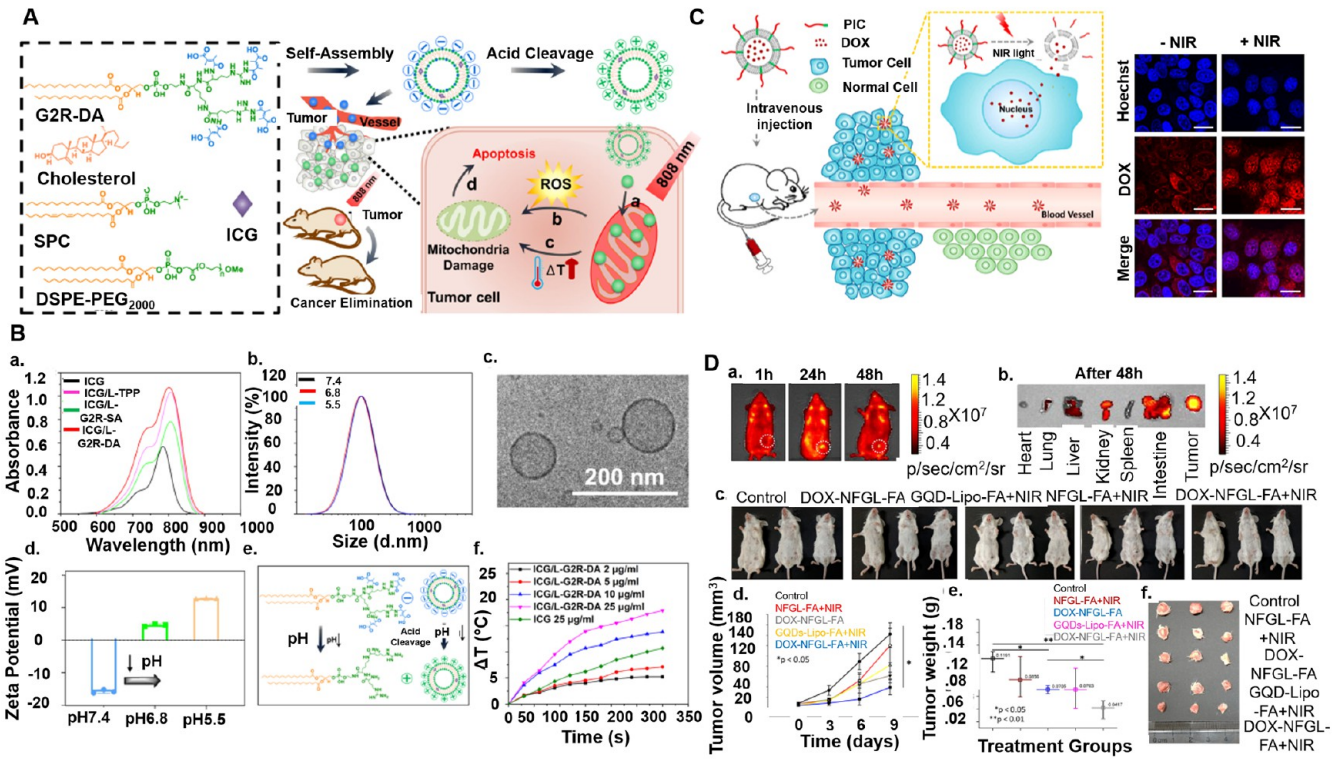

Figure 6. (A) Basic components and schematic representation of self-assembled liposomal system for targeted cancer therapy and (B) physicochemical characterization of engineered liposomes. (a) UV/vis spectra, (b) particle size distribution, (c) electron microscopic image (scale bar is 200 nm), (d) surface charge zeta potential, (e) degradation of engineered liposomal hybrids in the cancer-mimicked environment, and (f) time-dependent light to heat response of engineered liposomal system at various concentrations (2−25 μg/mL). Panels A and B are reproduced with permission from ref 92. Copyright 2021 Springer Nature. (C) Schematic diagram of PEG-IR780-C13 (PIC) and DOX tagged liposomal structure for synergetic photo thermal-chemotherapy under NIR light exposure followed by intravenous injection and their cancer cell imaging before and after NIR light treatment. Panel C is reproduced from ref 118. Copyright 2017 American Chemical Society. (D) Emissive liposomal nanotheranostics for (a) targeted solid tumor (4T1 breast tumor) imaging at various time points (1, 24, and 48 h), (b) *ex vivo* imaging of major organs and tumor after 48 h, (c) photographs of treated animals during therapeutic courses, (d, e) tumor volume and weight measurements for tumor reduction performance in tumor bearing female mice, and (f) photograph of collected tumors after different therapeutics courses. Panel D is reproduced with permission from ref 25. Copyright 2020 Springer Nature.

application of local hyperthermia for 30 min in solid tumor-bearing animal models through high-intensity focused ultrasound (HIFU). The release of the contrast agent Gd was monitored using T_1 mapping of the tumor tissue.

A good correlation between ΔR_1 and the uptake of DOX and Gd has been found in the tumor, confirming the *in vivo* DOX release from stimuli-responsive liposomes.[176] Emissive graphene oxide flakes supported liposomal hybrid systems have been tested for phototriggered tissue visualization and tumor regression which has been studied by Prasad et al. In this formulation, the liposomal cavity was filled by the anticancer drug DOX and the exterior surface of liposomes was covered by photo thermally active red emissive graphene oxide flakes which induce the photothermal response under NIR light irradiation. This formulation has been proposed and tested for solid tumor ablation due to the potential effect of released anticancer drugs and produced photothermal heat in the solid tumor microenvironment. Further, the developed nanohybrid system "wrapped a flimsy liposomal matrix with multifunctional graphene oxide" demonstrates its biodegradability, safety, and targeted theranostics ability.

In the reported study, the graphene oxide flakes fortified liposome, functionalized with folic acid, caused deep intracellular localization, 4T1 breast tumor diagnosis, and prolonged binding capacity resulting in practical tumor regression. The developed system caused no harm to the surrounding healthy tissues, overcoming one of the critical concerns in cancer nanomedicine as shown in Figure 6.[31] This could be a clinically acceptable nanotheranostics for further preclinical and clinical studies. In another study, nitric oxide precursors have been encapsulated in phosphatidylcholine liposomes to design an image-guided therapeutic platform for the controlled release of nitric oxide with simultaneous fluorescent imaging.[177] Ostrowski et al. encapsulated a nitric oxide precursor in the liposomes and demonstrated a steady and controlled release of nitric oxide upon photolysis, whereas the unencapsulated complex demonstrated burst release in oxygenated solutions. Importantly, the quantum yield for nitric oxide release from liposomal formulations was five times higher than the unencapsulated precursors. Hence, the amount of nitric oxide released upon photolysis enhances the therapeutic response of liposomal hybrid systems. The fluorescence (low-intensity blue light) accompanying the nitric oxide release enables the development of theranostics systems for simultaneous tracking and imaging nitric oxide release.[178] During these studies, there are chances of radical species generations which may cause oxidative damage in surrounding healthy tissues in live animals. Hence, this could limit the theranostics applicability of liposomal hybrid systems in further preclinical and clinical tumor models.

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It should be noted that the liposomal formulations for contrast-enhanced magnetic resonance imaging of molecular targets are widely explored.[179−181] Mulder et al. have developed liposomes that were prepared by using the popular lipid film hydration method. These liposomes were then PEGylated, and paramagnetic and fluorescent immunoliposomes were synthesized to permit the expression of molecular markers on endothelial cells. Researchers have utilized human umbilical vein endothelial cells (HUVEC) as a study model to evaluate the specificity of molecular markers. A pro-inflammatory cytokine was used to treat these cells to enhance the expression of the adhesion molecule E-selectin/CD62E, TNFR (tumor necrosis factor R). Later, HUVEC cells expressed E-selectin were treated with PEGylated paramagnetic fluorescently labeled liposomes with anti-E-selectin monoclonal antibodies as targeting ligands. MRI and fluorescence microscopy were utilized to observe the association of the liposomal MR contrast agent with stimulated HUVEC cells demonstrating that the liposomes were explicitly bound to the treated cells. This report indicates that the engineered system could be a promising diagnostic platform for investigating pathological processes *in vivo* with MRI.[182] In another study by Kamaly, thin film hydration-prepared liposomes composed of paramagnetic and fluorescent lipids exhibit bimodal characteristics for their multifunctionality. Furthermore, PEG-lipid amphiphiles were used to prepare liposomal systems for prolonged circulation. Remarkably, folic acid targeting ligand-attached bimodal paramagnetic and fluorescent liposomes demonstrate improved accumulation in the folate receptor-expressing solid tumor model. The study was reported with IGROV-1 cells to initiate tumors in Balb/c mice lacking immune function, and the mice were administered the folate-targeted liposomes through intravenous injection. It has been observed that the folic acid-modified and unmodified liposomal systems demonstrate significant differences in tumor targeting and accumulation, indicating the importance of folic acid as a targeting ligand. Additionally, folic acid-attached liposomal theranostics systems demonstrate 4-fold tumor accumulation and T1 signal intensity within 2 h of postinjection compared to unmodified liposomal hybrid systems. These liposomes without a folic acid coating show tumor accumulation results after 24 h of injection. Moreover, the fluorescence pattern of folic acid-attached liposomes seems to be more confined compared to the diffused and dispersed fluorescence observed in passively accumulated liposomal formulations. These findings indicate that folic acid targeting is a successful approach for real-time MRI of solid tumors diagnosis and also sheds light on the dynamics of nanoparticle targeting, both targeted and nontargeted, to solid tumors.[183] Researchers have proposed the existence of a "binding site barrier" that could affect the binding of immunoliposomes targeted toward localized tumor areas. Carter et al. described liposome formulations of porphyrin phospholipid assemblies and designed them to be permeabilized by NIR light. Photothermally active monomers derived from clinically approved components that could absorb light has been studied in detail by molecular dynamics simulations and exhibit improved stability of the porphyrin bilayers. Apart from this, the effectiveness of molecularly engineered liposomes (DOX-PoP-liposomes) was administered through the bloodstream (dose of 10 mg per kg of body weight) and evaluated for anticancer therapy in nude mice with KB cancer cell line-derived tumors. Fifteen minutes of the intravenous injection, these tumor-bearing mice were irradiated with a 658 nm laser light at 200 mW power of light for 12.5 min (150 J per square centimeter) and mice left unexposed were considered as controls. After 24 h of light treatment, the organs were harvested, and the distribution of DOX was analyzed. Laser treatment enhanced the accumulation of DOX in irradiated tumors by three times compared to nonirradiated ones, while there were no significant differences in other vital organs. Interestingly, there was a notable increase in DOX deposition/or accumulation in the skin covering the tumor and in the muscle near the tumor in other laser-irradiated tissues. The precise mechanism is still unknown and being researched. These results emphasize the importance of precise light delivery to cancer, not to nearby healthy organs. The light-based stimuli-responsive therapeutics concept has been noted as a safe modality for solid tumor treatment and triggered the delivery of anticancer agents from loaded nanoparticles. In preclinical studies, *in vivo* injection of PoP-liposomes into the tumor followed by light irradiation demonstrated the photo-triggered release of the loaded therapeutics agents, for example, the anticancer drug, but the biodistribution observations were challenging. These PoP-liposomes were created using porphyrin-lipid monomers, which showed remarkable stability and prevention in preleakage of loaded cargo molecules even under high temperatures. Moreover, these therapeutically engineered liposomes could effectively release their contents upon exposure to clinically relevant levels of NIR light. For this clinically acceptable therapeutic modality, the amount of porphyrin integration, laser irradiation time, and power could be adjusted to control the release of loaded cargo.[184]

Law et al. have recently studied a liposomal formulation named Iohexol (an iodinated clinical contrast) with encapsulated liposomes for a nose-to-brain delivery followed by chemical exchange saturation transfer (CEST) at a 3T field strength. It has been observed that these liposomal systems could easily penetrate mucus. In brief, the loading of Iohexol is in a liposomal system (Ioh-Lipo), a CT contrast agent featuring amide protons that exchange at 4.3 ppm on the Z-spectrum at an *in vitro* level that results in contrasts of 35.4% at 4.3 ppm, 1.8% at −3.4 ppm, and 20.6% at 1.2 ppm, respectively. Confirmation of successful Ioh-Lipo delivery has been noticed in brain regions with elevated cerebrospinal fluid levels including various regions, viz., external plexiform layer (EPL), left and right olfactory bulb (LROB), and olfactory limbus (OL), etc. For *in vivo* examinations, the contrast values of engineered PEGylated Ioh-Lipo peaked at 0.5 h (4.3 ppm) and 1 h (−3.4 ppm) in the olfactory bulb. This contrast distribution phenomenon could be attributed to the propensity of lipophilic molecules to utilize the intracellular olfactory and trigeminal pathways, whereas solutes and water molecules are more likely to utilize transcellular pathways. Hence, the unique contrast property and mucus penetrating ability of designed Ioh-Lipo systems may provide promising drug delivery opportunities by bypassing the blood brain barrier and tracking the regional distribution of therapeutic drugs and liposomes in the brain. Moreover, determining regional distribution associated with the delivery pathways could yield appreciated information for engineering effective cargo or drug delivery systems for chemo-theranostics.[185] Liu and Quan et al. have engineered and tested a liposomal hybrid system named as PEGylated 3 nm γ-Fe_2O_3 nanoparticles embedded lipid self-assembly (Lp-IO). These designed liposomes induce ferroptosis in cancer cells by promoting the generation of hydroxyl

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radicals (OH) from hydrogen peroxide (H_2O_2). In the engineered liposomes (Lp-IO), the decorated amphiphilic PEG moieties enhance the permeability of the lipid membrane to H_2O_2 and OH which improve lipid peroxidation and result in efficient ferroptosis of targeted cancer cells. This above-discussed liposomal hybrid system has been tested for solid tumor (4T1 breast tumor) treatment in female Balb/c mice. The tumor reduction is due to *in vivo* antineoplastic effects which is associated with ferroptosis. In this therapeutic course of Lp (20 mg/kg), IO-PEG (2.5 mg Fe/kg), and Lp-IO at low (L, 1 mg Fe/kg) and high (H, 2.5 mg Fe/kg) doses is given via intravenous administration every day and done for 1 week. From a solid tumor therapeutic course, it has been analyzed that IO-PEG did not reduce tumor growth significantly compared to the biocompatible Lp. Further, researchers have examined the r1 and r2 relaxivities of IO-PEG and Lp-IO to perform *in vivo* MR imaging at a high magnetic field 7.0T. These results showed that IO-PEG and Lp-IO had r1 relaxivities of 0.71 and 0.21 mM^{-1} s^{-1} and r2 relaxivities of 30.7 and 62.7 mM^{-1} s^{-1}, respectively. The higher r2 value of Lp-IO which is twice that of IO-PEG can be explained by the magnetic dipole interaction (MDI) of aggregated IO-PEG nanoparticles present in the bilayer of engineered Lp-IO system. Obtained results demonstrate that combining ferroptosis and chemotherapy produces synergistic antineoplastic effects while reducing toxicity. These results present an efficient strategy for initiating lipid peroxidation in ferroptosis and a novel approach for drug delivery in combination therapies for cancer.[186]

In another study, superparamagnetic iron oxide nanoparticles encapsulated liposomal hybrids systems were proposed for targeted imaging of cancer cells/tumors.[187] In this report, Zn-doped Fe_3O_4 nanoparticles were prepared using a solvothermal methodology. Purified nanoparticles were decorated with liposomes and conjugated to a tumor-penetrating peptide (RGERPPR) and have been tested for the MR imaging properties. Designed $Zn_{0.4}Fe_{2.6}O_4$-PEG nanoparticles were assessed using T1-mode MR imaging revealing that the MR signal intensity of the nanoparticles was corroborated with the concentration of Fe. These observations indicate that the nanoparticles are positive contrast agents for T1-weighted MR imaging applications. The MRI images in both groups (phosphate buffer saline, PBS and $Zn_{0.4}Fe_{2.6}O_4$-PEG- RGERPPR) displayed similar MR features. However, the group treated with RGERPPR nanoparticles (NPs) for 20 min showed a mere 7.29% signal increase as compared to the control group (treated with PBS only). Overall, engineered $Zn_{0.4}Fe_{2.6}O_4$-PEG- RGERPPR NPs display a high potential for MR imaging and can be considered a valuable option for tumor diagnosis. However, significant studies are yet to be demonstrated to make this nanoparticle system clinically useful and it is crucial to conduct additional research on its targeted imaging capabilities, toxicity, and biodistribution.[188]

Next, various other liposomal nanomedicines/theranostics systems have been designed for targeted imaging and therapeutics of various tumors especially for solid tumor applications. Cheng et al. has synthesized a liposomal hybrid system which is self-assembly of an aza-BODIPY lipid building block named as BODIPYsome. The BODIPY lipid is aza-boron dipyrromethenes (aza-BODIPYs), an NIR fluorophore with high fluorescence and is better in the photostability ability which is the ideal system for cancer cell/tumor imaging. It should be noted that the engineered liposomes were highly stable with NIR J-aggregation. Importantly, cholesterol was introduced during the preparation of such self-assembled platforms which improve the colloidal stability and the J-dimer within the vascular structure. This stability and provided fluorescence and absorption properties lead to high quenching efficiency and significant extinction coefficients of imaging probes in the engineered hybrid system. Remarkably, the intact BODIPYsome exhibited exceptional photoacoustic activity in phantoms, while its disrupted nanostructure displayed outstanding NIR fluorescence properties *in vitro* which is a novel concept of multimode imaging and image-guided therapeutics. The engineered BODIPYsome demonstrated favorable results as an optical imaging agent for photoacoustic and fluorescence imaging in an *in vivo* orthotopic prostate tumor in a preclinical mouse model. The study concluded that the novel design of the BODIPYsome and its dye-lipid building block demonstrated the potential of a versatile nanoparticle design as a promising biophotonic imaging agent at nanoscale.[189] In another study, a novel imaging technique named multispectral optoacoustic tomography (MSOT) was used to track the drive and distribution of indocyanine green (ICG, FDA approved molecules) loaded liposomes within the heterogeneous solid tumor microenvironment. The MSOT imaging modality allows for noninvasive, real-time monitoring of the distribution and movement of the ICG tagged liposomes in live animals providing valuable insights into the behavior of the imaging agent for specific biodistribution and targeted tumor imaging. To understand solid tumor imaging and deep tissues visualization, the ICG tagged/of encapsulated liposomes and ICG were injected into CD-1 albino mice which were compared with plasmonic gold nanorods named as AuNRs or GNRs. The post injected animals were subjected to photoacoustic imaging where ICG-encapsulated liposomes demonstrate about 1.3-fold improvement in the MSOT signal intensity with compared to free ICG and 3.2 folds compared to gold nanorods. Further, the reported study found that using PEGylated ICG-loaded liposomes result in prolonged circulation times in the bloodstream which also improve the accumulation of the imaging agent within the solid tumor microenvironment. The ICG molecules absorbed specific wavelengths of light and emitted light in the NIR region which penetrated deeply into biological tissues, making it an ideal imaging agent for noninvasive imaging. Overall, the use of MSOT imaging allowed for the real time tracking of the ICG tagged/encapsulated liposomes within the tumor microenvironment, demonstrating the potential impact of this imaging modality for monitoring the distribution and effectiveness of liposomal theranostics systems. Moreover, the study's results showed the unique importance of MSOT imaging for optimizing cancer treatments by optimizing real time monitoring and adjustment of nanoparticles blood circulation strategies. This technology can help researchers to understand the dynamics of nanoparticles-based imaging and therapeutics delivery systems and optimize treatment strategies to enhance the efficacy of cancer treatments.[190]

In the literature, the efficacy of ^{99m}Tc-radiolabeled antisense oligonucleotides (ASONs) decorated liposomes has been tested for targeting mouse double-minute 2 (MDM2) mRNA (mRNA) for *in vivo* imaging of MDM2 expression. The MDM2 oncogene is overexpressed in many human cancers and is considered a potential target for cancer therapy. Radionuclide targeting of MDM2 expression can provide valuable diagnostic information in malignant tumors. ASON

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and a mismatch oligonucleotide (ASONM) targeting MDM2 mRNA were synthesized and radiolabeled with ^{99m}Tc using hydrazinonicotinamide (HYNIC) as a bifunctional chelator. The radiolabeled probe was characterized *in vitro*, and MCF-7 cells (a human breast adenocarcinoma cell line) were incubated with liposome-coated ^{99m}Tc-HYNIC-ASON/ASONM at different concentrations for 24 h. The MDM2 mRNA and protein level were assayed using reverse-transcriptase polymerase chain reaction and Western blotting. The liposome-coated ^{99m}Tc-HYNIC-ASON/ASONM biodistribution was investigated in MCF-7 bearing nude mice models. The labeling efficiencies of ^{99m}Tc-HYNIC-ASON and ^{99m}Tc-HYNIC-ASONM were 57.2% ± 2.98% and 56.3% ± 3.01%, respectively, with radiochemical purity above 95%. The radiolabeled ASON retained its ability to hybridize with the sense oligonucleotide. The antisense probe had a more significant effect on the MDM2 mRNA and protein levels than the mismatch probe. The tumor radioactivity uptake of the antisense probe was significantly higher than that of the mismatch probe ($P < 0.01$). The excretion of both probes was primarily through the liver and kidneys. The accumulation of liposome-coated ^{99m}Tc-labeled ASONs in breast cancer tissue was specific. Using liposome-coated ^{99m}Tc-HYNIC ASON for antisense imaging may be a promising method for visualizing the MDM2 expression in human breast cancer. Tumors were visible in images acquired between 1 to 10 h after injection of the antisense probe, while tumors were not visible after injection of the mismatch probe at any time.[191] On the other hand, in a study performed by Garcia Ribeiro et al., the potential of magneto-liposomes (MLs) as magnetic resonance imaging contrast agents and delivery vehicles have been engineered and evaluated for imaging and therapeutic applications of cancer. MLs were targeted toward the $\alpha v\beta 3$ integrin overexpressed on tumor neovascularization and different tumor cell types, including glioma and ovarian cancer. MLs functionalized with a Texas Red fluorophore (anionic MLs) and cRGD; cRGD-MLs targeting the $\alpha v\beta 3$ integrin were produced. The biodistribution of MLs was evaluated in Swiss nude mice subcutaneously injected with 107 human ovarian cancer SKOV-3 cells. Results showed that cRGD-MLs had a higher uptake in SKOV-3 xenografts than control anionic MLs, as visualized by MRI and fluorescence imaging (FLI). The most increased ML uptake was observed after 4 h using MRI and confirmed by *ex vivo* electron paramagnetic resonance spectroscopy (EPR) and FLI. The results showed that cRGD-MLs could effectively target the SKOV-3 xenograft in Swiss nude mice and can be visualized using both MRI and FLI, indicating their potential as theranostics.[192] Another study used dual targeted paramagnetic liposomes with two angiogenesis-targeting ligands, the $\alpha V\beta 3$ integrin-specific RGD and the neuropilin-1 (NRP-1) receptor-specific ATWLPPR (A7R), which were prepared. These liposomes were nanoparticle-sized and effectively encapsulated gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) as a paramagnetic MRI contrast agent. The T1 relaxivity of the liposome formulations was lower than pure Gd-DTPA, but there was no statistically significant difference. *In vitro* cellular uptake and the competitive inhibition assay showed that the dual targeted liposomes had a higher binding affinity to HUVECs and A549 cells than pure Gd-DTPA, nontargeted, and single-targeted liposomes, which was mediated by the binding of RGD/$\alpha v\beta 3$-integrin and A7R/NRP1. *In vivo*, MR imaging of mice-bearing A549 cells showed that the dual targeted liposomes had the highest signal enhancement rate (SER) value with a significant difference at all experimental time points, which was an about 3-fold increase compared to pure Gd-DTPA and nontargeted liposomes and 1.5-fold of single-targeted liposomes at 2 h postinjection. The SER gradually decreased but only by 40% of the peak value in 6 h. The dual-targeted liposomes were likely to exert a synergistic effect and enhance the specificity of delivering Gd-DTPA to the tumor site. Therefore, the dual-$\alpha v\beta 3$-integrin-NRP1-targeting paramagnetic liposome with RGD-ATWLPPR heterodimeric peptide could be potent for molecular imaging of tumors.[193]

Apart from the above-discussed reports, it should also be noted that the surface-modified liposomes with the ability to monitor within the body, specifically in the gastrointestinal (GI) tract, were prepared after oral administration using a real-time *in vivo* imaging system (IVIS). Liposomes were labeled with ICG, a NIR dye, and modified with chitosan (CS) or glycol CS (GCS). The dynamic behavior of the liposomes was observed in rats using IVIS, a noninvasive imaging technique. The IVIS results were validated by comparing them to quantitative measurements of ICG fluorescence intensity in tissue homogenates. Nanosized small unilamellar vesicles in the GI tract were retained longer than in microsized multilamellar vesicles. Additionally, surface-modified liposomes showed longer retention in the GI tract than unmodified liposomes in fasted rats. Furthermore, surface modification with CS or GCS effectively prevented the excretion of liposomes from the GI tract and prolonged their retention in fed rats.[194] Overall, liposomes hold great potential as cancer imaging agents due to their ability to encapsulate various imaging contrast agents and target cancer cells.[195−198] Liposome surface modifications can further enhance their specificity and retention in the tumor site, improving diagnostic accuracy.[199−201] Various imaging modalities, such as MRI, CT, and PET, etc., can be utilized to visualize and monitor the distribution and accumulation of liposomes in the body and within the tumor environment.[202−204] With continued advances in liposome technology, these nanosized therapeutic systems will likely play an increasingly important role in cancer imaging and diagnosis in the future. However, further research is needed to optimize the design and synthesis of liposomes and to evaluate their safety and efficacy in clinical settings.

LIPOSOMES IN SOLID TUMOR THERANOSTICS

As highlighted above uncoated/parent liposomal systems may be limited for solid tumor theranostics applications due to their fragile nature; therefore, self-assembly of phospholipids, phospholipid-like copolymers, and other stabilizing agents have been used to prepare liposomal carriers for delivering diagnostic and therapeutic moieties with good biocompatibility and extra functions.[205] Among all amphiphilic block polymers, the FDA has approved PEG-distearyol phosphatidylethanolamide (DSPE) for use in a clinical setting.[206] This is due to the reduced uptake of liposomes by the reticuloendothelial system.[207] Furthermore, liposomes with PEG modifications have been shown to activate immune responses following multiple administrations.[208] It also induces accelerated blood clearance, decreasing the drug's half-life.[209] Further, liposomes-containing surfaces functionalized with radioisotopes (^{64}Cu, ^{67}Ga, ^{111}In, ^{99m}Tc, and ^{18}F) for PET/SPECT scanning in the detection/treatment of cancers have been synthesized. Theranostic liposomes containing DSPE-PEG2000-DOTA-

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Lu, indocyanine green (ICG), were synthesized in this regard. Results demonstrated that the ICG-DPDCs successfully accumulated at the tumor site and aided in prolonging circulation time. It was also inferred that, when compared to ICG, ICG-DPDCs showed excellent photostability that helped in the PTT of cancer.[210] Lozano et al. prepared monoclonal antibody-targeted PEGylated liposomes encapsulating ICG and DOX for preclinical study. The anti-MUC1 humanized monoclonal antibody (MoAb) hCTM01 is used in this study to create targeted PEGylated liposome-ICG as a tumor-specific theranostics system.[211]

In accordance with liposomal ICG monitoring in the tumor, targeted and nontargeted liposome-ICG formulations are selectively accumulated in the cancer models under investigation. Early time points showed rapid accumulation of targeted liposomes, primarily at the periphery of the tumor volume, indicating binding to accessible MUC-1 receptors. At later periods, nontargeted PEGylated liposomes got concentrated at the tumors' center compared to targeted PEGylated liposomes.[211,62] To increase bioavailability and cause the photothermal release of pharmaceuticals in response to NIR light radiation,[212−214] Li et al. integrated IR825 in the lipid membrane of DOX-loaded stealth liposomes. This is one of the clinically acceptable approaches and systems because NIR light has deep penetration and nonabsorption with healthy tissues. The findings showed that, compared to blank liposomes, more than 80% of DOX was released in 30 min under near-infrared light exposure, along with a 6-fold increase in cell mortality over that same period. It has been demonstrated that improved chemo-photothermal therapy can be produced using liposomal-based formulations that tolerate very hydrophobic IR825 and DOX. A new cyclic nanopeptide with iRGD-modified liposomal nanoplatforms that comprise ICG was described by Lee et al. Due to their excellent tumor targeting and strong penetrating capabilities, iRGD-modified ICG-loaded liposomes could specifically penetrate solid tumors by targeting neuropilin-1 dose-dependently.[215] Prasad et al. developed biodegradable photosensitive nanohybrids for photosensitive tumor diagnosis and growth inhibition. A biodegradable and photothermal active red emissive carbon dots were decorated to the liposomal surface. In this report, supported or decorated carbon dots perform imaging properties and produce photothermal heat under NIR irradiation that helps in targeted solid tumor theranostics. It has been shown that a nonmetallic nanohybrid can degrade breast tumors by photothermal and oxidative processes. The liposomes developed showed successful aqueous solubility, biocompatibility, and enhanced cellular uptake facilitated by enhanced photoluminescence. It was noted that there was enhanced tumor ablation using photoactivated localized photodynamic-photothermal therapy when there was significant tumor regression.[30] It is worth mentioning that carbon dots also cause the generation of reactive oxygen species (ROS) that destroy the cancer cells in the form of photodynamic cancer therapy, but these produced ROS are uncontrolled and affect the surrounding healthy tissues. Hence, this is a major limitation of near NIR-mediated imaging and therapeutics when photothermally active organic dye loaded nanoparticles are used for theranostics applications. To overcome this issue, similar research has been developed on liposomal-based ROS scavengers which is a theranostics platforms. In this report, engineered liposomes are contained with gold nanoparticles (contrast imaging as well as ROS scavenging probes) and red emissive graphene quantum dots (NIR imaging agent). The engineered liposomal hybrid theranostics system has been functionalized with folic acid targeting ligands and anticancer drug DOX which are applicable not only for targeted theranostics but also for combination therapeutics for solid tumor ablation applications as shown in Figure 6. Because of their high contrast and emissive properties, encapsulated agents demonstrated imaging bimodality for *in vivo* tumor diagnosis. Surface functionalization showed that injected nanotheranostics aided the distribution of the liposomal vesicles.[25] Various importance such as smooth circulation, multimode imaging and therapeutics, biocompatibility, degradation, better thermal response, etc. of such design has been reported which are essential parameters for setting up clinically relevant cancer theranostics medicines/platforms. In another study, Jeon and co-workers created theranostics dual-layered nanoparticles by adding a liposomal layer to the Au-coated layer (AL) to form liposomal (LAL). This extra layer improved the liposomes ability to target tumors compared to gold nanoparticles alone. Furthermore, these liposomes were further enhanced by adding radiolabeling for imaging *in vivo* and a PEG group to increase stability and passive targeting ability. Their study also indicated that LAL is more stable than AL *in vitro* and *in vivo* and has a similar photothermal effect. Noninvasive imaging with 64Cu-LAL was also possible. LAL displayed a prolonged circulation time and efficient tumor targeting through passive targeting (16.4%ID g^{-1}) in a mouse orthotopic breast cancer model. *In vivo* fluorescence imaging showed that LAL had 2.9 times greater tumor targeting ability than AL. Finally, the *in vivo* tumor growth inhibition rate of LAL-mediated photothermal therapy (PTT) was 3.9 times greater than that of AL-mediated PTT (79.4% vs 20.4%, respectively). The study underlined that LAL could be used as a promising photothermal agent.[216]

Liposomal probes with specific targeting ligands are extensively explored as imaging and delivery vehicles for *in vivo* diagnosis. In a study by Wang et al., a dual-peptide targeting liposomal probe named BTLS was constructed that could synergistically bind to two different sites of prominin-1, a cancer stem cell marker. The investigation acquires the appropriate spatial structure upon insertion into the hollow pocket of prominin-1, showing strong binding affinity in both cellular and *in vivo* levels. The nanoprobe enhances the specific recognition by tumors and improves the *in vivo* delivery efficiency. Thus, it can be concluded from the study that the design of density-optimized peptide-targeted liposomes could be a potential approach to maximize the targeting effects, enabling significant improvements in cancer theranostics.[217] Radiolabeled liposomes also serve as theranostics agents, i.e., diagnostic and therapeutic agents, for various malignancies.[218,219] In a study by Hansen et al., $[Cu\text{-}64]^+$ loaded PEGylated liposomes were developed to study the effect of enhanced permeation. The high uptake of liposomes in carcinomas and increased retention in several sarcomas was evident from the results of high-resolution PET/CT imaging. This establishes the crucial role of nanocarrier-based radiotracers as theranostics imaging agents that have the potential to guide the possible interventions required for several malignancies in future clinical practice.[117] The liposomes encapsulated within anethole dithiolethione, hydrogen sulfide prodrug, and superparamagnetic nanoparticles can be converted into microsized H_2S bubbles intratumorally. The liposomes accumulate preferentially in the tumor tissue due to

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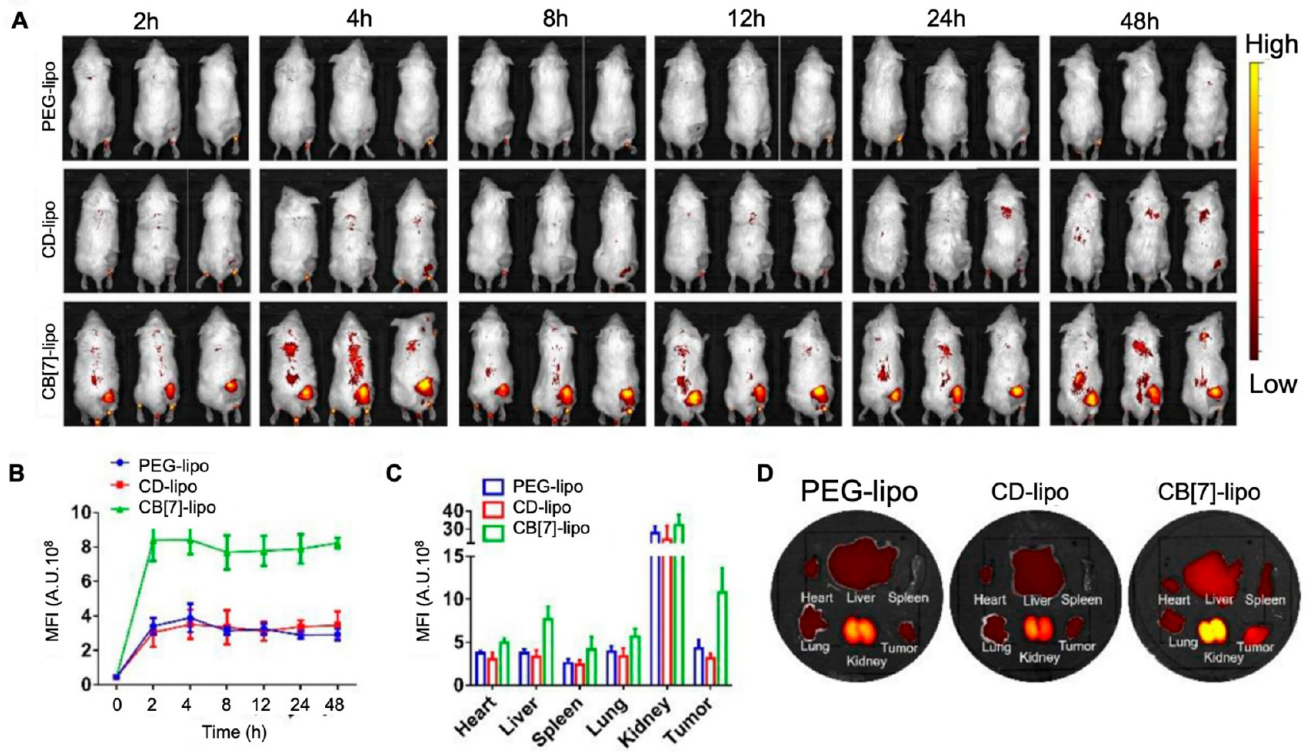

Figure 7. Cy5.5 tagged CB [7]-liposomes, PEGylated liposomes, and CD attached liposomal hybrid system for tumor imaging and their biodistribution in tumor bearing mice after intravenous administration. (A) Whole body near-infrared fluorescence (NIRF) imaging of tumor-bearing mice using intravenously injected dye tagged surface engineered liposomal hybrids. (B,C) Time-dependent mean Cy5.5 fluorescence intensities from the tumor area and organs after intravenous injection of engineered liposomal nanoparticles, and (D) *ex vivo* NIRF images of major organs (heart, liver, spleen, kidneys, and lungs) and tumor after 48 h of post injection. Panels A−D are reproduced with permission from ref 221. Copyright Theranostics Ivyspring International Publisher.

spatiotemporal navigation through an external magnetic field. Apart from radiolabeled liposomes, magnetic nanoliposomes have been extensively explored for their ability to maximize tumor-cell-specific uptake of the therapeutic agents. Liu et al. developed a stimuli-responsive anethole dithiolethione-loaded magnetic liposome for its ability to serve as a potential tool in image-guided cancer theranostics.[116]

Further, the H_2S bubbles generated intratumorally can be imaged by real-time ultrasound imaging, thereby serving as a promising multimodal-image-guided tool for cancer therapy.[116] Polyamine levels are elevated in tumor cells. Taking advantage of this, Cheng and co-workers prepared cucurbit [7] (CB [7]) functionalized liposomes. The study was hypothesized due to the robust, biologically compatible interactions between CB [7] and polyamines. *In vitro* cytotoxic analysis was evaluated on 4T1 solid tumor cells. The study revealed that the cell death rate of 4T1 cells increased to 31.6% when the concentration of CB [7]-lipo reached 100 μg/mL. This can likely be attributed to the capture of polyamines by CB [7], which disrupts the balance of polyamines and reduces the survival of the cells. Next, the functionalized CB [7] showed improved cellular uptake. Moreover, in the *in vivo* biodistribution studies, it was noticed that CB [7] functionalized liposomes showed a higher accumulation rate, higher signal intensity, and higher fluorescence intensity at the tumor site. The study incorporated excellent bio-orthogonal interactions for chemotaxis-guided targeting using liposomes.[220] The results are presented in Figure 7.

In a study, the researchers designed a liposome (Lip-AIPH) loaded with 2,2′-azobis[2-(2-imidazolin-2-yl) propane] dihydrochloride (AIPH) that can produce gas bubbles and a high concentration of reactive oxygen species (ROS) simultaneously under ultrasound (US) irradiation. The generated gas bubbles acted as a potent US contrast agent and enhanced the anticancer efficacy of sonodynamic therapy in a hypoxic tumor microenvironment. The study demonstrated that the liposome enhanced US imaging and exhibited improved anticancer efficacy, making it a promising ultrasound (US) imaging-guided hypoxic tumor therapy with deep tissue penetration.[222] Second, in another study Dai et al. reported that a NIR absorbing dye, ICG, and the antitumor drug, DOX, were effectively encapsulated into thermosensitive liposomes based on natural phase-change material. Liposomes were functionalized with folate and conjugated Gd chelate to enhance the nanoplatforms' active targeting and magnetic resonance performance while maintaining their size. The resulting temperature-sensitive liposome nanoplatforms, ID-TSL-Gd NPs, demonstrated NIR-triggered therapeutic drug release and remarkable chemo-, photothermal, and photodynamic therapy properties. Furthermore, the ID-TSL-Gd NPs can be used for triple-modal imaging (fluorescence/photoacoustic/magnetic resonance imaging) guided combination tumor therapy (chemotherapy, photothermaltherapy, and photodynamic therapy) due to the coencapsulation of ICG, DOX, and conjugated Gd chelates. Upon tail vein injection, the ID-TSL-Gd NPs effectively accumulated in the subcutaneous HeLa tumor of mice. The tumors were accurately imaged and

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effectively suppressed using NIR triggered phototherapy and chemotherapy. No tumor regression or side effects were observed. Overall, the prepared ID-TSL-Gd NPs achieved multimodal imaging-guided cancer combination therapy, which can improve the diagnosis and treatment of cancer.[223]

Dual-targeting ligands, including low-density lipoprotein receptor-related protein and RNA aptamer bound CD133, were used in the dual modified cationic liposomes (DP-CLPs) loaded with siRNA and paclitaxel (PTX-siRNA) for actively targeting imaging and treating CD133+ glioma stem cells. These DP-CLPs showed a persistent ability to bind glioma cells and brain microvascular endothelial cells (BCECs) and deliver drugs to CD133+ glioma stem cells. The prepared DP-CLPs-PTX-siRNA nano complex exhibited very low cytotoxicity to BCECs but selectively induced apoptosis of CD133+ glioma stem cells, improved their differentiation into nonstem-cell lineages, markedly inhibited tumorigenesis, induced CD133+ glioma cell apoptosis in intracranial glioma tumor-bearing nude mice, and improved survival rates. Overall, the prepared DP-CLPs-PTX-siRNA nano complex has great potential for targeted imaging and therapy of brain glioma stem cells.[221] A nanobubble paclitaxel liposome (NB-PTXLp) complex was developed for ultrasound imaging and drug delivery in cancer cells. The liposomes were submicron-sized (528.7 ± 31.7 nm) complexes that have demonstrated the potential to enhance cell membrane permeability in response to ultrasound pulses, allowing for targeted delivery of a higher intracellular payload. Our 200 nm sized liposomes were efficiently tethered (conjugation efficiency ∼98.7 ± 0.14%) with nanobubbles to form conjugates with a paclitaxel entrapment efficiency of 85.4 ± 4.39%. Treatment with nanobubbles and ultrasound resulted in sonoporation of MiaPaCa-2 cells, leading to 2.5-fold higher uptake of liposomes compared to liposome treatment alone. This resulted in over 300-fold higher anticancer activity of NB-PTXLps in MiaPaCa-2, Panc-1, MDA-MB-231, and AW-8507 cell lines compared to the commercial formulation ABRAXANE. Additionally, NB-PTXLp conjugates exhibited echogenicity comparable to the commercial ultrasound contrast agent SonoVue. The nanobubbles were found to show more than 1 week of echogenic stability, as opposed to the 6 h stability of SonoVue. Overall, the NB-PTXLps represent a promising, minimally invasive platform for cancer theranostics.[224]

Liposomes can act as versatile carriers facilitating integrated diagnostics and therapeutic agents within a single platform. This has enabled liposomes to act as theranostics agents and has allowed them to act as carrier systems for a combination of multiple cargo (imaging and therapy probes) moieties.[225] In this regard, Chauhan et al. formulated disintegrable NIR triggered gold nanorods and liposomal nanohybrids to treat cancer. These surfaces engineered liposomal hybrid structures have been investigated for localized bioimaging and dual chemo and photothermal therapeutic.[226] The dual therapeutic liposomal nanohybrid consists of plasmonic gold nanorods supported by anticancer drug DOX loaded liposomes. These gold nanorods, named GNRs, not only accomplish their photothermal response and improve the phototriggered drug release in cancer cellular environment but also provide mechanical support to the soft liposomes from both surfaces (interior and exterior surfaces) of liposomes. Hydrophilic nature of nanorods made them easy to encapsulate and attach within the inner cavity of the liposomal hybrid system. The preparation of this plasmonic nanotheranostics was achieved through a one pot sonication method producing a system for imaging guided synergistic chemo-PTT due to loaded anticancer drug and photothermally active gold nanorods. Gold nanorods for this system were prepared by the surfactant-mediated seed-growth procedure that produces toxic nanorods. To make them biocompatible, the toxic surfactant layers from gold nanorods were replaced by lipid bilayers first and then decorated with a liposomal platform along with anticancer drug encapsulation. It was also noticed that the decorated gold nanorods on the interior and exterior lipid bilayer prevent the premature leakage of loaded cargo or drug molecules. Folic acid as a targeting ligand was attached to the surface of gold nanorods supported by drug-loaded liposomes through PEG linkers targeting the folate receptors overexpressed cancer cells. The hydrodynamic diameter of engineered particles was 160 to 180 nm, demonstrating better entrapment and loading efficiencies of 85% and 39%, respectively. From electron microscopic imaging, it was possible to verify the gold nanorods (GNRs) structure and their decoration with liposomal surfaces since nanorods were determined to be linked on the interior and exterior surfaces of the liposomes. Utilizing UV−vis-NIR spectroscopy and microscopic imaging, it was also possible to identify the NIR-triggered disintegration of the gold nanorods liposome nanohybrid. Because of changes in the refractive index of the surrounding environment, the proposed nanohybrid almost completely lost its ability to show both the transverse and longitudinal bands of GNRs in the absorbance spectra following NIR exposure. The GNR liposomal nanohybrid's capability to be effectively taken up by cells overexpressing the folate receptor is demonstrated by the intense red fluorescence in the cytoplasm and nucleus of cells.[23] To the author's knowledge, this was the first report on synergistic chemo-photothermal therapy using gold nanorods decorated (inside and outside the surface) liposomal nanoparticles that demonstrate photothermal-assisted disintegration of nanohybrid, but only for *in vitro* testing, as *in vivo* testing is yet to be achieved.

On the other hand, another surface-engineered liposomal theranostics system, viz., emissive and photothermal active graphene oxide flakes wrapped liposomes functionalized with folic acid targeting ligands, has been tested for solid tumor imaging, phototriggered tumor ablation, and light-triggered drug delivery applications. As discussed, this small-sized graphene oxide support improves the stability and strength of anticancer drug-loaded liposomal particles, preventing the premature leakage of loaded anticancer drugs from the liposomal cavity before reaching the target site.[31] This surface-engineered liposomal hybrids system (graphene oxide flake decorated liposomes) has been designed at the ambient condition and was tested systematically for light-mediated tissue visualization and localized solid tumor regression. The designed system has been reported with specific characteristics such as spherical morphology with uniform particle size distribution (in the range of 200−250 nm), good colloidal stability, aqueous dispersion, NIR emission and photothermal performance, and better wrapping of soft liposomes which help in the prevention of premature leakage of loaded cargo in extracellular conditions. Further, folic acid improves the targeting performance of designed nanotheranostics systems with significant performance for noninvasive targeted tumor imaging and deep tissue visualization, localized breast tumor ablation under external stimuli with minimum dose injection.

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Overall, it has been noticed that developing a nanotheranostic agent for better image resolution and high accumulation into solid tumor microenvironment is challenging. To achieve such properties, multifunctional and photothermally active liposomal nanotheranostics were engineered, demonstrating better tumor accumulation and binding ability within a solid tumor environment.[25] In the literature, low entry of nanoparticles (less than 10%) in either imaging or therapeutics or theranostics systems in the solid tumor environment has been noticed. There are several reasons why nanoparticles do not accumulate in tumors, although solid tumors have good vascular systems with leaky blood vessels. In these studies, better tumor accumulation was reported, possibly due to the photothermal effect of engineered nanotheranostics systems where generated photothermal heat expands the cellular gap within leaky blood vessels in a solid tumor environment. However, this concept is not fully proven and is still under investigation. In terms of eliminating solid tumors, an anticancer therapeutics drug like DOX hydrochloride was encapsulated along with photothermal active therapeutics probes in this lipid-based theranostics system which demonstrated phototriggered combined chemotherapy for tumor reduction without showing any side effects on surrounding healthy tissues. Encapsulated different imaging probes showed imaging bimodality for *in vivo* tumor diagnosis based on their high atomic number, electron density, X-ray attenuation, and red emissive properties. Solid tumor reduction was seen in small animals because of the effect produced by photothermal heat and highly toxic ROS. But the generated ROS are nonspecific and uncontrolled which may produce/or cause eschars, inflammation, mutation, protein denaturation, cell apoptosis, or necrosis in the healthy tissues. Critical challenges in cancer nanomedicine include selective tissue imaging and targeted tumor regression without damaging the nearby healthy tissues. Theranostics for cancer have been developed explicitly for selective targeting and localized tumor growth suppression. Thus, safe and biodegradable nanohybrids have been the main focus of theranostics designs. Recent studies have focused on NIR responsive integrated materials such as graphene oxide, GNR, organic NIR dyes, and gold-coated polymeric nanoparticles-loaded liposomes to demonstrate both imaging and therapeutics for solid tumors.[25]

It should be noted that proliferation and metastasis influence tumor malignancies which also depend on small population of cancer stem cells which are resistant to many conventional therapeutics approaches.[227] It is well-studied that cancer stem cells have inherent ability for self-renewal, proliferation, and differentiation into other tumor cells. Many surface markers such as CD133, EpCAM, CD44, etc. have been identified for cancer stem cells.[228−230] Hence, apart from small molecule targeting ligands, aptamer-based approaches have been tested for binding these markers in cancer therapeutics applications.[231] Aptamers are short, single-stranded nucleic acid molecules (such as DNA or RNA) that can bind to specific target molecules with high affinity and specificity.[232−234] They are often called "chemical antibodies" due to their ability to recognize and bind to target molecules, including proteins, peptides, small molecules, and even cells.[235] It should be noted that the biomarker-like CD44 receptor is a naturally available surface protein expressed in many tumors such as breast, colon, pancreas, head, and neck which makes this an attractive receptor for aptamer-mediated targeted therapeutics.[236,237] Compared to other targeting molecules, aptamers hold great advantages like easy functionalization and conjugation, good biocompatibility, low immunogenicity, better half-life, etc.[238] For the first time in 2006, FDA has approved aptamer-based therapeutics for Age Macular Degeneration treatment. It has been studied that aptamers can be used for both (i) carrier systems for drug molecules by encapsulating nucleic acid-intercalating drugs and (ii) engineered pro-drug system by covalently conjugating the aptamers with the drug molecules.[239] However, aptamer-drug complex systems face major limitations of insufficient drug release and aptamer binding to the target site. Hence, to overcome these limitations aptamer-modified nanoparticles have been proposed in cancer therapeutics.[240,241] Among them, liposomal nanoparticles have been recognized as versatile systems in targeted cancer therapeutics.[242] Aptamer attached liposomes exhibit better targeting and therapeutic response to cancer cells as compared to nontargeting liposomes. For example, the AS1411 aptamer (a 26-mer DNA aptamer) has been attached on the surface of drug-loaded liposomes.[243] These engineered platforms specifically binds to nucleolin, a type of protein which is highly expressed in the plasma membrane of different cancer cells (breast, melanoma, and prostate) and absent in normal cells.

Recently, thiol-maleimide click chemistry has been applied to conjugate the 2′-F-pyrimidine-containing RNA aptamer (Apt1) on PEGylated liposomes.[244] These aptamers conjugated liposomal therapeutics system has been tested against CD44 overexpressed tumors. On the other hand, drug encapsulated liposome modified with DNA aptamers have been tested for targeting (i) breast cancer cells expressed with transmembrane glycoprotein mucin 1 antigen (MUC1) and (ii) cancer stem cells expressed with glycoprotein CD44 antigen (CD44). Further, aptamer-modified liposomes have also been developed to deliver imaging agents to cancer cells or tumor tissues specifically.[244] This approach takes advantage of aptamers' high binding affinity and specificity to target molecules overexpressed on cancer cells' surfaces. By conjugating aptamers to the surface of liposomes, the modified liposomes can selectively recognize and bind to cancer cells, enabling targeted delivery of imaging agents.[245] These aptamers-modified liposomal nanoparticles influence the overall targeting and tumor binding affinity response by altering physicochemical properties of used nanoparticles. The aptamers used in such systems are selected through a process called SELEX (systematic evolution of ligands by exponential enrichment).[246] SELEX involves iterative cycles of binding, separation, and amplification, ultimately identifying aptamers with high affinity and specificity for the desired target molecule.[247]

PATENTABILITY AND COMMERCIALIZATION CHALLENGES OF LIPOSOMAL THERANOSTICS AGENTS

In terms of intellectual property (IP) there is a need for clear definitions of the types of constituents involved while protecting nanomedicine/theranostics-related IPs.[248] A wide range of variable components like the types of therapeutic drug and imaging agent, types of engineering systems and methodologies, types of surface coating and targeting agents, characteristics of the designed nanotheranostics can be protected during IP filing.[249] However, the IP process has become complicated or confusing in the case of developing multistep nanotheranostics or nanomedicine systems which are also owned by several companies. Involvement of multiple

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Table 4. Various Patents Filed Incorporating Liposomes for Cancer Thernaostics and Diagnosis

patent number	granting agency	application year	disease	key claims	ref
EP1233752A2	Europe patent office	2000	cancer	polymerizable liposome radiation sensitive liposome	262
CN105617407A	Chinese patent office	2016	lung cancer	liposome consisting of molecular imaging probes and composed of lecithin or cholesterol	263
CN107556367A	Chinese patent office	2017	liver cancer and breast cancer	liposome consisting of a therapeutic and molecular imaging agent	264
CN102350002A	Chinese patent office	2011	glioma	liposomes consisting of paramagnetic substances for molecular imaging	265
CN110393810A	Chinese patent office	2019	nonsmall lung cell cancer	liposomes, along with a perfluorocarbon probe	266
CN110746489A	Chinese patent office	2019	breast cancer	coupling moiety is selected from a radionucleotide the drug delivery system used is liposome	267
CN112876538A	Chinese patent office	2021	breast cancer	molecular imaging agent was prepared to estimate the abnormal expression of CD105 the drug carrier used is liposome	268
CN105693821A	Chinese patent office	2016	various cancers	the active substance also consists of a radiolabeled substance drug delivery system consisting of liposomes	269
US2010105139A1	US patent office	2008	various cancers	ligand targeted polycation liposome diagnostic agent entrapped along with molecular imaging agent	270
WO2009008489A1	PCT	2008	various cancers	antibody modified liposomes the molecular imaging agent constitutes a certain range of the concentration of liposomes	271
CN101080240A	Chinese patent office	2005	various cancers	liposomes containing iodinated compounds the preparation is also suitable for molecular imaging	272
CN112028968A	Chinese patent office	2019	various cancers	use of nanocarriers such as liposomes magnetic resonance contrast agent consisting of a molecular agent	273
EP1827508A2	European patent office	2005	various cancers	liposomes as carriers for paramagnetic ions targeting contrasting agents that are used for molecular imaging	274
CN114835710A	Chinese patent office	2021	various cancers	carrier consisting of liposomes radiopharmaceutical is a diagnostic agent for molecular imaging	275
CN107638574A	Chinese patent office	2011	various cancers	liposomes consist of gas a molecular imaging agent loaded into the liposome	276
CN112028969A	Chinese Patent Office	2019	various cancers	nanocarrier composed of liposome loaded with a molecular imaging agent	277
WO03106675A1	PCT	2003	various cancers	composed of liposomes loaded with a molecular imaging agent	278

patent associations and cross-licensing arrangements again complicate the IP process.[250,251]

To overcome these issues there is a need of new IP practices and related protocols which simplify the whole process of laboratory invention to commercialization. These simplified IP regulations also reduce the process time and expense along with easy collaboration and licensing agreements. More importantly, patent examiners should have expertise and training with related fields of nanoimaging, nanotherapuetics, nanomedicine, nanotheranostics, and nanotechnology.[252] Various regulatory factors and government policies decide the commercialization of nanotechnology-related products.[253,254] Additionally, initiating collaborations between academia and industry and regulatory agencies smooth the commercialization. Understanding the market potential of liposomes as theranostics for molecular imaging requires considering patent applications submitted through various issuing agencies.[255] The filing of numerous patents using liposomes as theranostics agents highlight the significance of liposomes in aiding in therapeutic delivery and as a theranostics agent for diagnosing and treating various types of cancer. Considering the stability issues related to liposomes, it was noticed that radiolabeled liposomes were able to provide and establish an excellent proof of concept that liposomes can be used as theranostics for cancer treatment. The increasing number of patent applications for theranostics liposomes confirms the positive outlook on the growing market potential of these vesicular drug delivery agents.[256] It strengthens the dedication to leveraging scientific expertise to develop advanced modular platforms integrating therapy and diagnostics. We have discussed an overview of clinical translation and commercialization challenges of liposomal nanotheranostics systems with their intellectual properties that represent the typical primary theranostics design shown in Table 4. This indicates they have not yet been fully developed and optimized to their maximum potential.

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Next, it has been reported that liposomal medicines have great potential in human health for the prevention, diagnosis, and treatment of diseases like cancer. Various liposomal formulations (surface coated and uncoated; drug loaded and empty, etc.) have been studied to improve the delivery of imaging and therapeutics or both in some cases to specific sites of disease. For targeting a wide range of cancers and tumors, the majority of engineered liposomal medicines have been implemented in preclinical and clinical use. But, recently, it has been noticed that the injected liposomal imaging and therapeutics or theranostics systems demonstrate easy targeting to noncancerous cells or tissues in preclinical and clinical models. On the other hand, the clinical translation of liposomal based theranostics and nanomedicines have been reported as an expensive and time-consuming process. Additionally, liposome formulations are reported with complicated surface engineering and encapsulation with diagnostics and therapeutics systems that may affect product yield, batch-to-batch variability, repeatability, biocompatibility, and overall theranostics response when we take them at large scale preparation.[252,257] It should be noted that storage conditions and environmental factors can also influence the physicochemical properties of engineered liposomal medicines. Parameters like administration dose and route, low complexity in formulation engineering, final dosage concentration form for human use, engineering expertise and involved cost, reproducibility, techniques for validation, quality control characterization, biosafety and biocompatibility pharmaceutical (pharmacokinetics and dynamics), and physicochemical stability need to be taken care of when liposomal formulations are designed at large-scale production according to GMP standards.[258] There is a lack of clear understanding on engineering, clinical trial design, evaluation of therapeutic efficacy in patients, regulatory guidelines specific for liposomes, biological interaction of liposomes with the biological substance and complexity in liposomes, and related patents and IP. It is easy to design lab scale medicines with better theranostics performances in preclinical models, but large-scale production is always challenging. To the best of our knowledge, hurdles in large scale production or manufacturing is one of the important factors contributing to the clinical translation progress of liposomal medicines.[254] Developed products at large scale face many structural and physicochemical complexity formulation engineering itself.[259] Overall, manufacturing of liposomal theranostics face potential challenges related to lack of infrastructure and in-house expertise, low scalability and high complexities, poor quality control, contaminations of byproducts and starting materials, high manufacturing costs, low reproducibility, poor storage stability and low physicochemical instability, premature leakage and denaturation of the encapsulated diagnostics and therapeutics probes during the manufacturing process.[257,259] Especially for engineering liposomal theranostics, integrating imaging and therapeutics components into single nanosized lipid particles compromises with a complicated production process which faces major issues of large-scale good manufacturing (GMP) production, high production cost, the choice of organic solvent, quality assurance, and quality control challenges.[260] Both short-term and long-term toxicity, half-lives circulation, and retention times should be considered while engineering liposomal medicines for clinical trials.[260] There is also need of regulatory validations for sophisticated assays related to *in vitro*, *ex vivo*, and preclinical examinations to evaluate the successive design of liposomal theranostics at the early stages of clinical development. Most importantly, a detailed understanding on parameters like surface modification, absorption, specific distribution, metabolism, and body clearance of liposomal nanoparticles in preclinical models is essential to predict their biocompatible responses.[256] Real-time imaging techniques have been recently conceptualized for better understanding of injected nanotheranostics in the body and the interaction of liposomal nanoparticles/theranostics with organs and tissues. However, clear mechanisms of site-selective targeting, specific biodistribution, biocompatibility, immunotoxicity, etc. are not well studied. The aforementioned issue hampers the clinical translational performance of liposomal-based medicines for cancer diagnosis and treatment. In summary, liposomal theranostics agents, which combine both therapeutic and diagnostic functions, can indeed be complex and require a combination of various technologies and intellectual properties. These liposomal theranostics agents may fall under the regulatory purview of both drug and diagnostic agencies. Navigating the regulatory landscape can be complex, as different regulatory bodies may have varying requirements for approval and commercialization.[261] The process of obtaining regulatory approvals for liposomal theranostics can be time-consuming and expensive, which can pose significant barriers to clinical translation and commercialization.

CONCLUDING REMARKS AND FUTURE PERSPECTIVE

In the past few years, the onco-nanomedicine field has become an integrative junction for biomedical engineers, oncologists, nanobiotechnologists, biochemists, physicists, and biologists who work closely with clinicians to resolve multiple cancer diagnosis/care and treatment issues. Engineering safe and targeted medicines for specific cellular and tumor microenvironment needs has become critical in translational research. Liposomes are the first platform for drug delivery systems translated into clinical applications. This article covers the theranostics importance and clinical relevance of liposomal-based nanomedicines for targeted bioimaging and therapeutics. We have discussed why and how liposomal systems demonstrate their significant role in *in vivo*/preclinical models, especially in the case of solid tumor-bearing models.

Further, the importance of surface functionalization for localized diagnosis and therapeutics of solid tumors has been highlighted here. The advantages and limitations of liposomes for site-selective imaging and therapeutics are explained with various examples. This area offers enormous potential for targeted drug delivery and localized therapeutics. Liposomes have improved diagnostic performance for CT, MRI, and ultrasound technologies and administered medicines and genetic material to various disease states. Liposomes can improve therapeutic outcomes in animal disease models for multiple formulations regarding drug delivery to the circulatory system. Encapsulation can be particularly helpful for poorly soluble pharmaceuticals like statins and effective, bulky medications like bisphosphonates to increase cellular permeability and therapeutic effectiveness. Alternatively, it has been suggested that liposomes composed of more than one type of ligand offer better tumor penetration and can enter the vasculature, facilitating a better diagnosis than other nontargeting liposomes. Despite significant advancements, liposomes also face certain limitations, such as a low rate of cellular uptake, a lack of specificity toward cancer cells, poor

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tumor accumulation, and a short retention time. Future studies will aid in elucidating liposomes as potential carriers for nanodelivery and diagnosis. It has been proposed that external stimuli, such as temperature, NIR light, and ultrasound, may control the release of active substances, boost tissue perfusion, and increase cellular permeability to increase the success of liposome administration. Ultrasonography has frequently been employed by causing heat or mechanical effects to alter the distribution profile of active compounds from liposomes. Compared to oncology applications, the use of liposome-based technologies to diagnose and treat various disease states has received little attention from the research community. This is because of the clinical implications of liposomes as a drug delivery system.

The number of control groups required is higher than in other drug delivery systems, notwithstanding the cost-to-benefit ratio. Changing the physicochemical properties of the formulation may cause a significant and unpredictable change in the final formulation's pharmacokinetics, pharmacodynamics, and toxicological properties. Apart from the above-mentioned issues, disease-dependent anatomical and physiological barriers, target accessibility expression, and formulation stability are critical parameters a formulation scientist should consider while developing liposomal theranostics and warrant further study. However, significant challenges exist with such nanoimaging and therapeutic systems for site-specific tumor targeting with high tumor entry and preclinical validations at the pilot scale. Apart from this, the brightness of nanoimaging particles is an important parameter to study before designing a safe nanotheranostics platform.

Overall, significant progress in theranostics for targeted tumor imaging and therapeutics of solid tumors has opened new hope and direction of nanoparticles in human health. Among various theranostics systems, liposomal has been considered clinically relevant systems. These liposomal hybrid nanotheranostics demonstrate better imaging (applicable for multimode imaging too) and therapeutics (both standalone and combination) for preclinical models and in some clinical models, where they also have FDA approval but fail in human trials. Additionally, there are many concerns related to nanoparticles in imaging and treatment of solid tumors in preclinical and clinical models like promising tumor accumulation, tumor binding efficacy, prolonged circulation, biocompatibility, specific biodistribution, biodegradation, biosafety, reliability, repeatability, scalability, and stability of nanoparticle medicines. On the other hand, we still face some ongoing arguments in nanotheranostics research such as (i) number of publications are higher than translating nanomedicine to the clinic, (ii) medical oncologist and radio-oncologist or radiologist are still following traditional contrast and therapeutics agents, (iii) a proper bridge between biomedical scientists, medical doctors, clinicians, and industries is still lacking. Hence, this article may provide an overall view of theranostics medicines in preclinical and clinical applications with several advantages and disadvantages of liposomal nanotheranostics.

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Notes

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