International Journal of Pharmaceutics 593 (2021) 120117

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Review

Liposomal doxorubicin as targeted delivery platform: Current trends in surface functionalization

Vivek Makwana [a,b,c], Jasmine Karanjia [d], Thomas Haselhorst [e], Shailendra Anoopkumar-Dukie [a,b,c], Santosh Rudrawar [a,b,c,*]

[a] School of Pharmacy and Pharmacology, Griffith University, Gold Coast, QLD 4222, Australia
[b] Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD 4222, Australia
[c] Quality Use of Medicines Network, Griffith University, Gold Coast, QLD 4222, Australia
[d] Clinical and Health Sciences, University of South Australia, Adelaide, SA 5000, Australia
[e] Institute for Glycomics, Griffith University, Gold Coast, QLD 4222, Australia

ARTICLE INFO

Keywords:
Liposomes
Doxorubicin
Surface functionalization
Ligands
Targeted drug delivery systems

ABSTRACT

Liposomal delivery systems have significantly enhanced the efficacy and safety of chemotherapeutic agents compared to free (non-liposomal) formulations. Liposomes are vesicles made up of lipophilic bilayer and a hydrophilic core which provides perfect opportunity for their application as transport vehicle for various therapeutic and diagnostic agents. Doxorubicin is the most exploited chemotherapeutic agent for evaluation of different liposomal applications, as its physicochemical properties permit high drug entrapment and easy remote loading in pre-formulated liposomes. Pegylated liposomal doxorubicin clinically approved and, on the market, Doxil®, exemplifies the benefits offered upon the surface modification of liposome with polyethylene glycol. This unique formulation prolonged the drug residence time in the circulation and increased accumulation of doxorubicin in tumor tissue *via* passive targeting (enhanced permeability and retention effect). However, there is ample scope for further improvement in the efficiency of targeting tumors by coupling biological active ligands onto the liposome surface to generate intelligent drug delivery systems. Small biomolecules such as peptides, fraction of antibodies and carbohydrates have the potential to target receptors present on the surface of the malignant cells. Hence, active targeting of malignant cells using functionalised nanocarrier (liposomes encapsulated with doxorubicin) have been attempted which is reviewed in this article.

1. Introduction

The phospholipid vesicles (liposomes) containing chemotherapeutic agent doxorubicin demonstrate a distinct superiority in clinical performance over free (non-liposomal) doxorubicin, considered as standard care. Unique pharmacokinetic properties offered by liposomal doxorubicin resulted in dramatic reduction of cumulative-dose related cardiotoxicity. This improvement in patient daily compliance allowed increasing the accumulated dose and extending treatment duration with doxorubicin. Targeted liposomal doxorubicin involving surface functionalization of liposomes with ligands played a key role in overcoming the limitation of doxorubicin liposomal formulations such as selectively targeting specific cancer cell (eg. endocrine) or organelle (eg. mitochondria, lysosome). Therefore, there is an increasing demand for targeted delivery systems which are able to withhold the molecules inside and transport it to the respective targeted tissue with appreciable safety (Fig. 1). This approach is particularly important for advanced stage cancers where surgical removal is not an option. In the nanotechnological advanced era, various platforms such as microspheres (Varde and Pack, 2004), solid lipid nanoparticles (Makwana et al., 2015), dendrimers (Madaan et al., 2014), micelles (Gong et al., 2012), liposomes and polymeric nanoparticles (Shukla and Gupta, 2020; Shukla et al., 2020; Vaidya et al., 2020) are created to achieve targeted deliveries. Among these carriers, liposomes have many advantages as they can be used for hydrophilic molecules (entrapped in liposomal core), hydrophobic molecules (entrapped in liposomal bilayer) and amphiphilic molecules (lipid-aqueous interface) making them suitable carriers for a wide range of therapeutic applications (Laouini et al., 2012). Liposomes are also biodegradable, non-toxic and non-immunogenic which make them ideal candidates that can be administered directly into

* Corresponding author at: School of Pharmacy and Pharmacology, Griffith University Gold Coast Campus, QLD 4222, Australia.
E-mail address: s.rudrawar@griffith.edu.au (S. Rudrawar).

https://doi.org/10.1016/j.ijpharm.2020.120117
Received 23 August 2020; Received in revised form 17 November 2020; Accepted 21 November 2020
Available online 28 November 2020

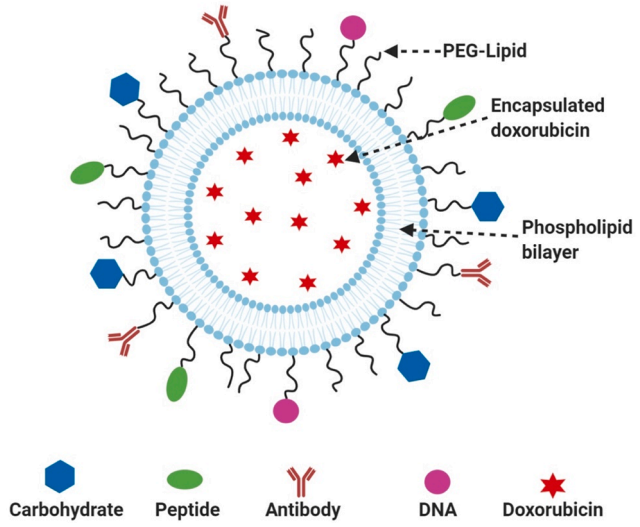

Fig. 1. Active targeting through surface functionalization of doxorubicin encapsulated liposomes.

systemic circulation.

Over the period of time as the progress in the field of targeted delivery flourished, the advancements demonstrated multiple approaches one of which is modifying the exterior surface of liposomes. This review focusses on the delivery of doxorubicin using liposomes as targeted drug delivery platform through surface functionalization approaches. It provides comprehensive note on the currently available options for ligand selection, application-based ligation reactions and the selection of appropriate route to access targeted tissue/organ.

1.1. Liposomes as drug delivery platform for doxorubicin

Liposomes were first observed under an electronic microscope by Dr Bangham and were then known as bangosomes which was later changed to liposomes as they were formulated using phospholipids (Bangham and Horne, 1964). Gregory Gregoriadis was however the first person to use liposomes for entrapping drug molecules and suggested its probable application as a vehicle (Gregoriadis, 1976). Liposomes are spherical micro-vesicles (0.05–5.0 μM) formed spontaneously when phospholipids are hydrated in aqueous medium and composed of concentric mono/multi-layers of lipids with an internal aqueous core (Wang et al., 2012a). In general, bilayer lipid membrane of liposome consists of two essential components: phospholipid and cholesterol. Phospholipids have polar heads and non-polar tails which allows the formation of bilayer vesicle above its critical micelle concentration. Phosphotidylcholine (PC) is the most widely utilized phospholipid whereas phosphotidylethanolamine (PE), phosphotidylserine (PS) and phosphotidylglycerol PG) are popular as well. Liposomes prepared from only phospholipids suffer from chemical and physical instability and hence addition of cholesterol becomes necessary to provide fluidity and stability. However, several varieties of lipids and cholesterols from natural or synthetic origins are also being exploited according to the end use applications. In general, thin-film hydration, reverse-phase evaporation, detergent dialysis, membrane extrusion or freeze–thaw are some of the common liposome preparation methods. Based on the method applied for the preparation of liposomes, they can be found in multilamellar vesicles (MLV), large unilameller vesicles (LUV) or small unilameller vesicles (SUV) (Laouini et al., 2012).

Liposomes are the most successful delivery platforms in comparison to various nanoparticle carrier systems with respect to safety and efficiency. Hence, many formulations such as Ambiosome® (Amphotericin B), Daunosome® (Doxorubicin), Doxil®/Caelyx® (Doxorubicin) are clinically available as liposomal formulations and many other are at different stages of clinical development (Elbayoumi and Torchilin, 2010). Their applicability encompasses small molecule therapeutics, gene therapy, immunotherapy, as well as diagnostic and theranostic applications. Liposome-based vaccines such as Epaxal®, Inflexal® and Mosquirix® are approved marketed products (Abu Lila and Ishida, 2017).

In the history of liposome-based delivery of doxorubicin, OLV-Dox (oligolamellar liposome) was the first to enter clinical trials but failed to deliver expected outcomes (Barenholz, 2012a). Major reasons for the failure were: (a) drug leakage before reaching the target site, (b) high dilution of liposomal preparation in plasma volume (Goren et al., 1990), (c) larger particle size to enter into tumor site (Hwang, 1987), and (d) high lipid fraction which removed them from systemic circulation by reticuloendothelial system (RES) (Gabizon and Papahadjopoulos, 1988). Recent developments in liposome technology have shown to be promising and Doxil® became the first approved marketed liposomal preparation in 1995 (Barenholz, 2012b).

2. Doxorubicin entrapped in PEGylated liposomes-Doxil®

The first attempt of using OLV-Dox in clinical trials was a failure and it took 10 more years to circumvent the obstacles leading to the successful development of **Dox**orubicin **I**n **L**iposome (**DOXIL**®). The main contributing factors were enhanced retention and permeation (EPR) achieved by downsizing the particle size to less than 100–120 nm. In addition, stable and sufficient entrapment of doxorubicin until it reached the target site was a crucial determinant to reduce drug leakage. Another factor which posed a challenge was that the required nano-scale of the particles led to a reduced volume of the aqueous media thereby affecting the relatively high concentration (approx. 50 $\mu M/m^2$) needed for doxorubicin to be efficacious. Hence, remote loading was the only solution to overcome this challenge. The use of the pH gradient method was already established for amphipathic weak base but the transmembrane gradient ammonium sulfate method was trialled for the development of Doxil®. This method was a breakthrough in the development of Doxil® as despite the low aqueous volume, it became possible to load high amounts of doxorubicin. However, the problem of extended circulation time and controlled release at targeted tumor cells remained unsolved. Both these problems were inter-connected as the drug release rate (k_{off}) and liposomal clearance (k_c) have a direct impact on the controlled release of entrapped drug into plasma/targeted tissues. This required the adjustment of the k_{off}/k_c ratio tailoring the release rate. Release of doxorubicin was further controlled by choosing hydrogenated soy phosphatidylcholine (HSPC) and cholesterol as the lipid bilayer which provided liquid order phase *via* attaining high transition temperature. Sterically stabilized liposomes were already prepared by Terry Allen using GM1 ganglioside (Allen and Chonn, 1987). Prolongation of nano-scale liposomes in systematic circulation was achieved by PEGylation using DSPE-$mPEG_{2000}$ thus generating Stealth® liposomes. Incorporating the aforementioned features DOXIL® successfully entered clinical trials and was approved by the US FDA (US Food and Drug Administration) in 1995. It became the first approved liposomal formulation with modified surface properties using PEGylation. This revolutionized the field of surface functionalization for liposomes. PEGylation offered many advantages compared to liposomes such as masking the surface charge of liposomes reducing its liver uptake as well as enhancing their tumor accumulations (Levchenko et al., 2002). A PEG coat over liposomes reduces their interaction with mononuclear phagocyte system (MPS) and hence aids in bypassing elimination in the liver (Newman et al., 1999). PEG chains can act as spacers on the surface of liposomes that also hinder vesicle aggregation thus resulting in a higher physical stability (Needham et al., 1992). It is well documented that PEGylation enhances blood circulation time of the vesicles ($t_{1/2}$ > 40 hrs) which not only overcomes the issue of rapid elimination but also allows the liposomes to accumulate in the tumor by exploiting ERP effects (Allen and Hansen, 1991). PEGylation of liposomes has paved the

way for innovative manipulations of the liposomal surface with the help of coupling reactions resulting in targeted and intelligent nanoparticulate systems.

3. Surface functionalization chemistry

The clinical efficacy of conventional liposomes is limited by rapid clearance from the blood circulation by the reticuloendothelial system, opsonisation and interaction with binding proteins thereby failing to reach targeted tissue (Yan et al., 2005). Development from OLV-Dox to Doxil® clearly highlighted the importance of surface modification of liposomes for maximum benefit as drug delivery system. PEGylation paved the way for a new generation of liposomes called 'Stealth' that are sterically stabilized liposomes. The surface modification of stealth liposomes prolonged the drug circulation time and EPR effect that allowed access to slow-growing tumors such as (soft tissue) sarcomas which are not extremely vascular. Composition of bilipid layers, preparation methods and remote loading methods have encouraged researchers to explore novel applicabilities. This exploration stretched the boundaries for the application of liposomes from delivery of therapeutic agent to diagnostic agent (MRI agents, radioactive isotopes) and gene delivery. PEG is an extremely versatile excipient with a head group tolerant to various functionalizations (—NH_2, —COOH, –CHO, —SH, —NHS). A large variety of functionalized lipids are commercially available which enable efficient surface modification of the liposomes. Easy access to functionalized lipids has encouraged more innovative surface modification to develop tissue specific targeted liposomes. Hence, it becomes important to understand the chemistry involved in ligation/conjugation methods.

In general, liposomal surface modification methods can be categorised into 'pre-formulation' and 'post-formulation'. Pre-formulation modification involves ligation of specific ligands to the phospholipid (most frequently PEGylated) by covalent bond formation (amide bond, thioether bond, hydrazone bond or disulfide bond). The final liposomal formulation is prepared by mixing ligand anchored lipids with conventional lipids followed by remote loading of the active drug (Banerjee et al., 2004; Surace et al., 2009). The major advantage of pre-formulation modification is that it involves small molecule as a ligand which is easy to manipulate and characterization. Moreover, complete control of the amount of ligand per liposome is possible by optimizing ligation conditions. As drug loading is followed by preparation of the ligand attached liposomes, drug leakage due to the insertion method is bypassed. Although it offers aforementioned merits, this approach is not suitable for those ligands having a large molecular size i.e. proteins and ligands which might lose their active conformation in organic solvents. There is a 50% chance that ligands attached to lipids may orient towards the interior of the liposomes. This approach is particularly convenient for small molecular ligands which are available in large quantities and hence may be expensive.

On the contrary, post-formulation functionalization involves the liposome preparation and drug loading is carried out first followed by insertion of targeted ligands to the surface of pre-formulated liposomes (Palekar et al., 2013; Torchilin et al., 2001a). Ligands can be conjugated to the functionalised group exposed on the external surface of the liposomes or can be anchored to micelles with a consecutive transfer of the ligands from micelles to the surface of the liposomes. This approach is called as post-insertion method (Ishida et al., 1999). The major merit of post-formulation modification is ligands with larger molecular size i.e. proteins can be easily attached to the outer surface of liposomes. This approach is cost-effective as it requires small quantities of ligands (specifically antibodies which are available in small quantities). Drawbacks of such approach includes drug leakage as the reaction condition for insertion may destabilize the lipid bilayer and characterization for the amount of ligand on the surface is necessary for better outcomes.

Conjugation of ligand molecules to PEGylated liposomes involves basic coupling chemistry. Commonly used ligations methods in the development of targeted drug delivery systems include amide coupling, disulfide linkage conjugation, thioether formation and hydrazone bond formation (Fig. 2).

As shown in the Table 2, depending on the reactive groups of ligands and lipids any ligation strategy can be selected to conjugate ligands to the lipids or liposomes. The commercial availability of a large variety of functionalized lipids (suppliers Avantis® Polar lipids, Merck) has been highly useful in efficient and fast synthesis. For example, DSPE-PEG_{2000} is the most common lipid used for functionalization (DSPE-PEG_{2000} enlisted in Table 1). Folate receptor alpha (FRα), is a tumor-associated antigen overexpressed on the surface of epithelial cells in many cancers (ovarian, breast and lung cancers). It's low and restricted distribution on normal tissues offered an attractive approach for targeted delivery. The folate-conjugated cytotoxic agents such as vinblastine and mitomycin C are under clinical development. Hence, targeting the folate receptor on cancer cells by conjugating folic acid (FA) on the surface of liposomes serves as a rationalized targeted drug delivery system. Lee and Low were the first to demonstrate folic acid covalently conjugated to liposomes for targeting folate receptors particularly found on human nasopharyngeal epidermal carcinoma cells (Lee and Low, 1994). Malhi et al. reported dual functionalised mitocancerotropic liposomes (FA-MTLs) in which the liposomal surface was modified with dual ligands, FA for cancer cell targeting and triphenylphosphonium (TPP) cations for mitochondrial targeting. Mitocancerotropic liposomes demonstrated enhanced cytotoxicity due to their cellular and mitochondrial delivery of doxorubicin in FR (+) KB cells (Malhi et al 2012). Transferrin (TfR) is another receptor overexpressed on tumor cells and hence transferrin or anti-TfR antibody have been selected as ligands and the liposomal surface was decorated to target TfR expressing cells (Hatakeyama et al., 2004). This rational approach was further explored for the targeted delivery of doxorubicin in glioma. Results demonstrated that the enhanced cytotoxic effect of TfR attached liposomes was due to their high binding affinity towards the glioma cells (Eavarone et al., 2000).

Following the selection of ligands and lipids for liposomes, choosing the right reaction conditions for the conjugation of ligand to lipids or preformulated liposomes is critical. Coupling reactions should be simple, easy, efficient and reproducible. It should be devoid of harsh conditions for the ligand in order to maintain its biological activity. The reaction temperature, pH, catalysts and organic solvents should also be considered as crucial factors for the successful completion of coupling reactions.

The conjugation reaction temperature is a critical factor when preformulation modification approach is selected as after conjugation of ligand to the lipid, liposomes might need to be prepared or loaded with drug remotely at high temperature. Clearly, this condition is not suitable for ligands such as peptide or antibody but works well for saccharides. To evaluate the biodistribution of galactose attached liposomes *in vivo*, first Gal-PEG-lipids were synthesized. After that, liposomes were prepared and down sized using extrusion at 60 °C (Shimada et al., 1997). Hence post-insertion approach was applied as the method of choice for peptides and antibodies. The $\alpha_v\beta 6$-specific H2009.1 peptide was conjugated to preformulated liposome loaded with doxorubicin using such approach. Here, liposomes were prepared using DSPE-PEG_{2000} and DSPE-PEG_{2000}-Mal (2:1) at 60 °C and doxorubicin was loaded at the same temperature. The peptide was conjugated to the surface of liposome using the maleimide-thiol conjugation reaction at ambient temperature (Gray et al., 2013). However, another study demonstrated the synthesis of cyclic-NGR peptide and its conjugation to liposomes. These targeted liposomes not only target the CD13(+) cells but also act as temperature sensitive liposomes (Negussie et al., 2010). Depending upon the nature of ligand appropriate temperature conditions can be selected.

The pH is also one of the most critical factors affecting stability and functionality of ligand conjugated liposomes. In the preparation of targeted liposomes entrapped with therapeutic agents (such as doxorubicin) the pH has a central role to play as it is involved in both, remote

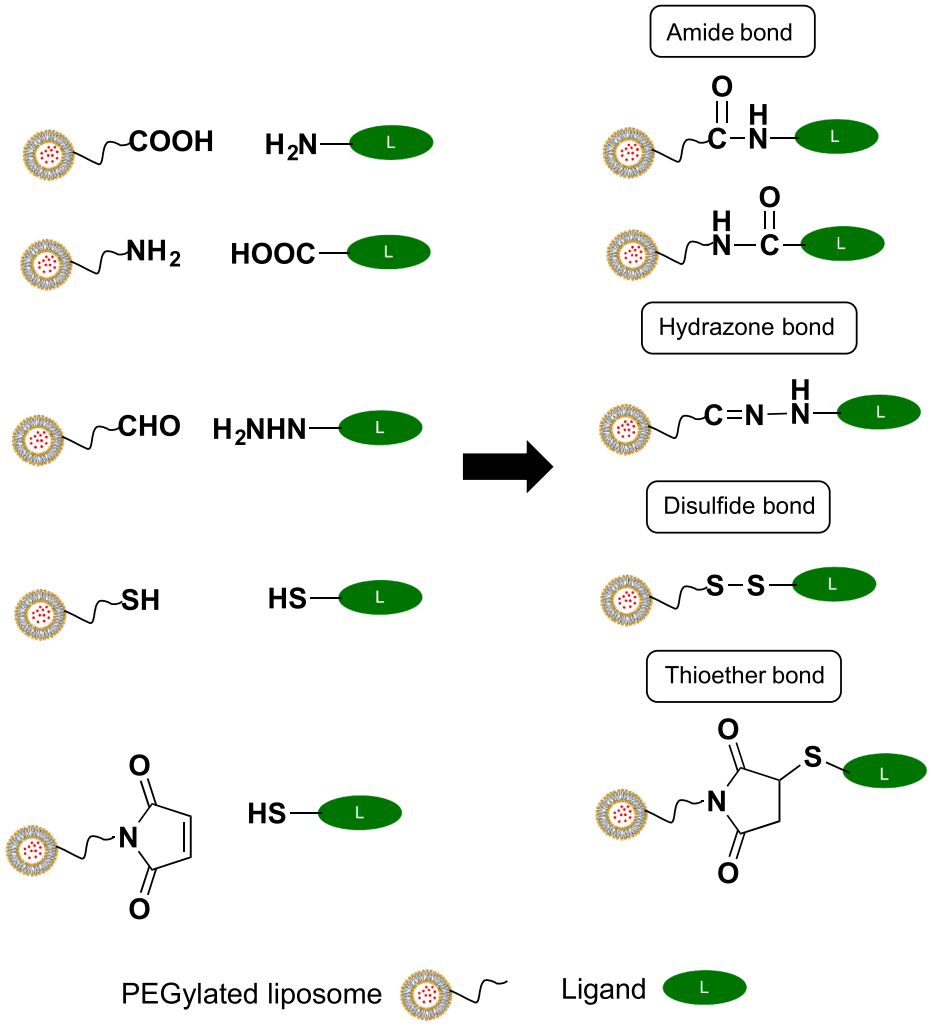

Fig. 2. Classical conjugation strategies.

Table 1
Commercially available functionalized DSPE-PEG_{2000}.

Sr. No.	Functionalized DSPE-PEG_{2000}
1	DSPE PEG_{2000}-*N*-Cy5
2	DSPE PEG_{2000}-*N*-Cy7
3	DSPE-PEG_{2000}-Amine
4	DSPE-PEG_{2000}-Azide
5	DSPE-PEG_{2000}-Biotin
6	DSPE-PEG_{2000}-Carboxy NHS
7	DSPE-PEG_{2000}-Carboxylic acid
8	DSPE-PEG_{2000}-Cyanur
9	DSPE-PEG_{2000}-Folate
10	DSPE-PEG_{2000}-Maleimide
11	DSPE-PEG_{2000}-PDP
12	DSPE-PEG_{2000}-Succinyl
13	DSPE-PEG_{2000}-DBCO
14	DSPE-PEG_{2000}-square
15	DSPE-PEG_{2000}-TMS

loading of drug into prepared liposomes using pH gradient method as well as conjugation of ligand to preformulated liposomes. There was a significant difference in the reaction kinetics when DSPE-Mal and DSPE-Br-Acetyl derivatives were attempted to be ligated to the thiolated peptides at pH 6.5 and 9 (Schelté et al., 2000).

Such environmental factors can also be used as a stimulus for controlled/targeted release of encapsulated drug and has created a new class of modified liposomes, called as stimuli-responsive liposomes. For example, pH of the micro-environment surrounding the tumor is slightly acidic in comparison to the normal tissue. Hence, pH-labile ligands such as peptides can be safeguarded using protective groups that are stable at neutral pH but can be removed under acidic conditions allowing the exposed ligand peptide to interact with the tumor cells followed by consequent efficient internalization. TAT and 2C5 mAb decorated liposomes were protected by forming pH-sensitive hydrazone bond with PEG_{2000} and PE. Once inside the acidic environment, the shield is removed allowing the liposomes to enter into the cells *via* receptor mediated endocytosis (Koren et al., 2012). Stimuli-responsive liposomes are enlisted in miscellaneous Section 4.4.

4. Ligand targeted liposomes for tissue specific delivery of doxorubicin

The anticancer drug doxorubicin is most commonly encapsulated into liposomal preparation as a targeted delivery system for cancer therapy providing enhanced therapeutic efficacy and reduced systemic toxicity (Fig. 3). Doxorubicin has been the first drug of choice for liposomal delivery to treat solid tumors for multiple reasons. Firstly, doxorubicin is a highly effective anticancer drug with a broad spectrum of activity against various kind of cancers such as breast, uterine, ovarian, lymphoma, lung cancer and leukemias (Rivankar, 2014; Weiss, 1992). Doxorubicin has more than one mechanism of action to act as a cytotoxic agent such as inhibition of topoisomerase-II and generation of iron-mediated free radicals (Rahman et al., 1980; Rivankar, 2014).

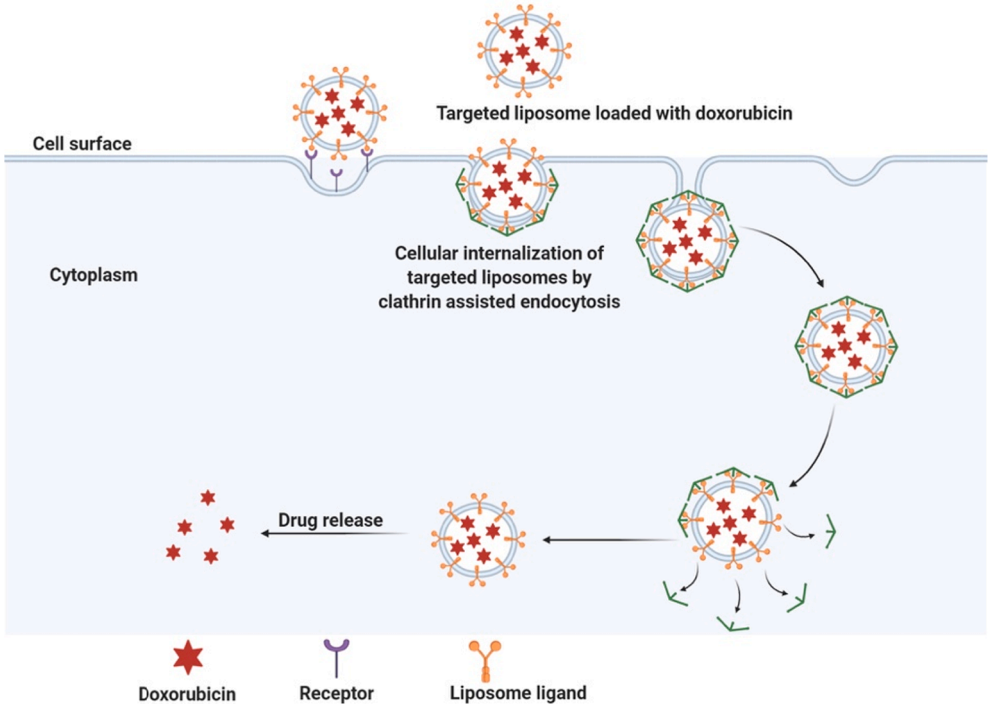

Fig. 3. Doxorubicin encapsulated liposomes for targeted delivery.

Secondly, the primary amino-functional group of doxorubicin allows it to be entrapped into liposomes easily using remote loading methods (Zucker et al., 2009). Furthermore, doxorubicin is easily quantified by robust analytical methods for its assay as well as its metabolites (or relative substances as impurity) estimation (Andrews et al., 1980). Despite such beneficial qualities, doxorubicin has several toxicities including dose dependent cardiotoxicity, (Minotti et al., 2004) myelosuppression, hand foot syndrome, alopecia, nausea and vomiting (Peng et al., 2005). Therefore, targeted drug delivery of doxorubicin becomes pivotal for its effective and safe applications in cancer patients and that was the primary driving force behind the clinical development of Doxil®. Considering all physicochemical characteristics of ligands, a wide range of targeted liposomes have been developed using various ligands and a brief discussion on some of the most frequently used ligands for development of functionalized liposomes for targeted delivery of doxorubicin is discussed here.

4.1. Antibodies

Liposomes that are conjugated to antibody fragments or whole intact antibody are called immunoliposomes. Hybridoma technology has provided a platform isolating a wide range of monoclonal antibodies with binding affinity towards specific antigenic sites. The existence of disulfide bonds and free hydroxyl group of the polysaccharide in Fc region of antibodies, provides a perfect opportunity for chemical modification or conjugation. Antibodies consist of two heavy chains (hinge region) that are linked to each other by disulfide bonds. Reduction of these disulfide bonds is most commonly achieved using dithiothreitol (DTT) or mercaptoethylamine (MEA), resulting in two equal parts of antibodies with sulfhydryl (-SH) moieties that are reactive for conjugation to the liposomes through thioether bond formation. On the other hand, oxidation of the hydroxyl groups to aldehyde residues provides an opportunity to synthesize hydrazone bonds using hydrazine as a functionalized coupling agent (Chua et al., 1984).

The most common method for coupling of antibodies to liposome is either using available (Cys)-SH or thiolation of antibodies (Manjappa et al., 2011). Thiolation using Traut's reagent (2-iminothiolane) is the most widely employed method for antibodies containing primary amine. Thiolated antibodies are then conjugated with Mal-PEG-lipid/liposomes through thioether formation. Allen et al. applied this coupling method for conjugation of CD19 mAb on the surface of functionalized liposomes. CD19 are overly expressed on malignant B-cells and hence anti-CD19 mAb is a perfect ligand to target B-cell lymphoma. They prepared liposomes using Mal-PEG-DSPE with other neutral phospholipids and remotely loaded doxorubicin. CD19 mAb was thiolated first using 2-iminothiolane and coupled by sulfhydryl-maleimide coupling reaction (Lopes de Menezes et al., 1999).

Conjugation of PEGylated liposomes to CD22 mAb loaded with doxorubicin (Doxil®) resulted in immunoliposomes that delivered doxorubicin more efficiently to non-Hodgkin lymphoma specific B cells than Doxil® alone. Anti-CD22 monoclonal antibody (HB22.7) has specific affinity towards CD22 (B-lymphocytes specific glycoprotein) expressed on B-lymphocytes. Surface modification of Doxil® using CD22 mAb reduced its IC_{50} by 3.1–5.4-fold in CD22-positive cell lines (O'Donnell et al., 2010). Another modification of Doxil® involved attachment of anti-GD_2 (disialoganglioside) antibody or its Fab'-fragment to the Mal-PEG-liposomes using post-insertion approach. Neuroblastoma tumor expressed GD_2 and immunoliposomes with anti- GD_2 antibody prevents metastasis of human blastoma in nude mouse metastatic model *in-vivo* (Pastorino et al., 2003b). GAH antibody (MCC-465, targeting GAH binding site) (Hosokawa et al., 2003; Matsumura et al., 2004), mesotheline mAb (targeting mesotheline antigen) (Deng et al., 2012), anti-β1 Fab' mAb (targeting β1-integrins) (Sugano et al., 2000) and rhumAb HER2 (targeting $_p185^{HER2}$) (Park et al., 1995), anti-CD22 mAb, LL2 (Targeting CD22 on B-lymphocytes) (Lundberg et al., 2000), Fab'-fraction C225 mAb (Cetuximab, targeting anti-EGFR, Phase-II clinical trial) (Mamot et al., 2011) have been conjugated to preformulated liposomes (with maleimide functionalization) to enhance internalization of immunoliposomes in specific target cells and demonstrated improved cytotoxicity in comparison to Doxil®.

Heterobifunctional crosslinking agents such as succinimidyl 6-(3́[2-pyridyldithio]propionamido)hexanoate (SPDP), 4-succinimidyloxycarbonyl-methyl-a-(2-pyridylthio)toluene (SMPT), *N*-succinimidyl *S*-acetylthioacetate (SATA) and *N*-hydroxysuccinimide ester (SATP) have

reactive functional groups at both ends and find their applicability as linkers in conjugation methods. These crosslinking agents have NHS ester at one end and 2-pyridyldithiol or protected sulfhydryl at the other end. Primary amine of antibody reacts with the NHS ester to form amide bonds with them and the reduction of this modified antibody with DTT (SPDP/SMPT) or hydroxylamine (SATA/SATP) provides free -SH. Heterobifunctional PEG are also available commercially. PDP/MPB-PEG-DSPE are more popular in the field of immunoliposome development. A monoclonal antibody against MUC-1 (B27.29) was conjugated to the preformulated liposomes with PDP-PEG-DSPE *via* thioether linkage (Moase et al., 2001). CD30 is specifically overexpressed on malignant T-cells and hence after attachment of anti-CD30 mAb on the surface of Doxil® proved more efficient for CD30-expressing malignancies. Accordingly, nano-carriers were developed for improved delivery of the chemotherapeutic agent in which Doxil® was conjugated with anti-CD30 mAb using post-insertion method. Evaluation of the therapeutic efficiency of Doxil® conjugated with anti-CD30 antibodies (CD30-targeted Doxil®) *vs* Doxil® in CD30-positive malignancy anaplastic large cell lymphoma (ALCL) demonstrated that CD30-targeted Doxil® had a 5-times higher binding affinity to ALCL cells than Doxil®. Hence, more target specific delivery of doxorubicin was achieved in comparison to Doxil® *in-vitro* as well *in-vivo* (Molavi et al., 2013). Moreover, Gupta et al. demonstrated the process of conjugation of mAb 2C5 to the external surface of Doxil® using micelle transfer method. Conjugation of 2C5 involved installation of antibody to p-nitrophenyl-carbonyl-PEG-PE to formulate micelles at 4 °C which was followed by transfer of antibody from micelles to Doxil® after 24 hr incubation period. The 2C5-immunoliposomes demonstrated significantly higher accumulation in U-87 MG tumor compared to Doxil® in subcutaneous model (Gupta and Torchilin, 2007). These studies clearly demonstrated that immunoliposomes offer better tumor tissue specific targeted delivery than stealth liposomes.

Several lines of evidences support the application of immunoliposomes loaded with different drug substances/diagnostics but are not included here as the current review highlights the advancements in liposomal delivery systems of doxorubicin (see Tables 1–3)

4.2. Peptides

Recent advancements in proteomics through phase display screening and solid phase peptide synthesis have encouraged a deeper exploration of the application of specific peptide sequences in the field of targeted drug delivery. Active functional groups such as —COOH, —NH_2 and —SH in peptides provides a perfect opportunity for their easy ligation to the liposomes, specially DSPE-PEG-Mal/NHS. Conjugation of peptides as ligands onto the liposomes shares almost similar coupling reactions as the antibody (thioether bond and amide bond formation). Although PEGylation of liposomes (such as Doxil®) prolonged residential time in blood, passive targeting showed insufficient internalization into the targeted tissue/cells. The process of transduction of host cells using phase guided a new path to access visceral area of tumors. For example, Doxil® coated with phase fusion pVIII induced more apoptosis and cell killing efficiency in MCF-7 cells owing to a more efficient internalization than Doxil® (Wang et al., 2010). Different sequences have been

Table 2
Conjugation strategies for surface modification.

Sr. No.	Ligand with reactive group	Functional group of lipid/liposomes	Ligation	Pros and Cons	References
1	L-CHO/ —C═NH	Amino group Lipid-NH_2	Amine-amine conjugation (Imine/ imidine formation)	Wide range of amino-lipids are commercially available, more suitable for antibodies as reaction conditions preserves their affinity and specificity towards targeted antigen. Homobifunctionality of linkers available in excess results into side reaction and loss of targeting ligand.	(Torchilin et al., 1978), (Torchilin et al., 1979), (Torchilin, 1997)
2	L-COOH		Amide bond	Naturally occurring lipids are utilized without further modifications. Homopolymerization of ligands or liposomes might lead to aggregation	(Zhao et al., 2013), (Sugiyama et al., 2013), (Yin et al., 2018), (Paoli et al., 2014), (Lee and Low, 1995)
3	L-CHO		Amide bond		(Banerjee et al., 2004)
4	L-NH_2	Carboxylic group Lipid —COOH	Amide bond Carbonyl amine bond	Prior ligand modification is not required and hence reduces chances of denaturation Due to availability of multiple amino groups (peptides and antibodies) random attachment is possible.	(Ndinguri et al., 2012), (Muñoz et al., 1998) (Palekar et al., 2013)
		NHS-Lipid	Amide bond		(Wang et al., 2012b), (Haghiralsadat et al., 2018)
		Lipid-COO-Phe-NO_2	Amide bond	Single step reaction which forms stable and non-toxic bond Dimerization due to bis(p-nitrophenylcarbonyl)- PEG which adds additional purification step.	(Torchilin et al., 2001a), (Torchilin et al., 2001b)
5	L-SPDP/PDP	SH-Lipid	Disulfide bond	Relatively easy with quantitative yield Cross-linking of reactive ligand or liposome due to presence of excess free thiol	(Martin et al., 1981), (Leserman et al., 1980), (Muñoz et al., 1998)
6	L-NHS/MPB		Thioether bond		(Muñoz et al., 1998)
7	L-SH/Cys-SH	Maleimide group Mal-Lipid	Thioether bond	Catalyst-free, quick, quantitative yield and requires mild ligation conditions (ambient temperature and neutral pH) It might produce immunogenic reactions	(Grange et al., 2010), (Cheng et al., 2014), (Hussain et al., 2007), (Béduneau et al., 2007), (Garnier et al., 2009), (O'Donnell et al., 2010)
8	L-NH-NH_2	Aldehyde group Lipid-CHO	Hydrazone bond	Hydrazone bond formation is spontaneous and does not require catalyst most of the time. Addition of hydrazine group can be challenging specially for antibodies and other complex ligands.	(Bourel-Bonnet et al., 2005), (Chenevier et al., 2003)

*L = ligand, reactive groups are either already present in ligand or attached.

Table 3
Different antibodies for surface modification of Dox-liposomes.

Sr. No.	Type of ligand	Targeted Receptor	Chemical reaction/Bond	Functionalization	Reference
1	Anti-CD30 Ab	CD30	Amide bond formation	Post-formulation Functionalization	(Molavi et al., 2013)
2	mAb 2C5 or non-specific IgG Ab TAT-peptide	Nucleosome on cell surface in response to apoptically dying neighbouring cells	Ab modified with p-nitrophenyl carbonyl-PEG-PE (pNP-PEG-PE) Groups. Ab-PEG-PE was incubated with Doxil®	Post-formulation Functionalization	(Gupta and Torchilin, 2007), (Apte et al., 2014)
3	F(Ab')2/GAH antibody	GAH-binding site	Thioether bond formation	Post-formulation Functionalization	(Hosokawa et al., 2003); (Matsumura et al., 2004)
4	Anti-CD22 mAb (HB22.7)	B-lymphocyte specific glycoprotein	Thioether bond formation	Post-formulation Functionalization Post-insertion	(O'Donnell et al., 2010)
5	Anti-CD19 mAb	B-lymphocyte specific glycoprotein	Thioether bond formation	Post-formulation Functionalization Post-insertion	(Lopes de Menezes et al., 1999)
6	Anti-GD2 (whole or fraction of antibody)	Disialoganglioside (GD2)	Thioether bond formation	Post-formulation Functionalization Post-insertion	(Pastorino et al., 2003b)
7	Mesotheline mAb	Mesotheline antigen	Thioether bond formation	Pre- and Post-formulation Functionalization Post-insertion	(Deng et al., 2012)
8	Anti- β1 Fab mAb	β1-integrins	Thioether bond formation	Post-formulation Functionalization Post-insertion	(Sugano et al., 2000)
9	Anti-MUC-1 B27.29 mAb	MUC-1	Thiol-maleimide coupling reaction	Post-formulation Functionalization Post-insertion	(Moase et al., 2001)
10	rhuMAbHER2	P185HER2	Thioether bond formation	Post-formulation Functionalization	(Park et al., 1995)
11	Apo2L/TRAIL	TNF-related apoptosis inducing ligand	His-Tag chelation catalysed by Ni	Post-formulation Functionalization	(De Miguel et al., 2013), (Martinez-Lostao et al., 2010), (De Miguel et al., 2019)

identified which allow access into specific cell types either by acting as cell penetrating peptides (CPP) or interacting with integrins for broad range of substances (see Table 4).

CPP are peptide sequences that have a net positive charge and enhance cell membrane permeation as the overall negative charge of the cell-membrane allows easy access. Although the exact mechanism of cell penetration is poorly understood, endocytosis is considered to be the major pathway. TAT (Yuan et al., 2016; Zhu et al., 2014), Penetratin, poly-arginine (R7 and R8) peptide (Biswas et al., 2013a, 2013b), Transportan and MAP are excellent examples of CPPs. CPP are gaining popularity as they are easy to synthesize and characterize; non-toxic and non-immunogenic in nature. TAT and polyarginine-peptides are more exploited CPP, especially for delivery of doxorubicin entrapped liposomes. Commercially available doxorubicin containing PEGylated liposome, Doxil® was coated with TAT and 2C5 mAb to prepare multifunctional immunoliposome. It was observed that multifunctional liposomes showed significantly high antitumor effect *in-vitro* and *in-vivo* in comparison to non-modified liposomes (Koren et al., 2012). Enhanced cell penetration and antibody-based targeting features significantly improved therapeutic outcomes and also seemed a promising approach to resolve multidrug resistance in ovarian cancer (Apte et al., 2014). Torchilin et al. prepared TAT-peptide conjugated Doxil® and micelles to highlight the impact of enzymatic activity of trypsin on physical stability. This study demonstrated that TATp-Doxil® showed enhanced tumor cell internalization and cytotoxicity in B16-F10 and HeLa cancer cell lines. Trypsin induced significant proteolysis of TAT which affected the stability of peptides and it has been demonstrated that the application of a longer spacer can overcome this problem (Koren et al., 2011). Dual-ligand attached liposomes for co-delivery of doxorubicin and paclitaxel were designed to kill melanoma cells. This double-drug liposomal delivery systems showed synergistic anti-tumor activity whereas better biodistribution *in-vivo* was the result of efficient targeting capabilities (Yuan et al., 2016). Apart from targeting cancer cells, TAT-liposomes with doxorubicin have been also used as a model to study enhanced skin penetration using CPP-attached liposome where doxorubicin acts as a fluorophore to explore the dermatological application of nanocarriers (Boakye et al., 2015).

Mammalian aminopeptidase N (CD13) is a tumor marker overexpressed on the cell surface of all major tumors. Presence of CD13 or integrin is another receptor for the selection of peptides as ligands for targeted drug delivery to tumour vasculature. Linear or cyclic peptides containing Asn-Gly-Arg (NGR) and Arg-Gly-Asp (RGD) motifs show affinity towards CD13 and integrin respectively and can therefore be used as ligands. This concept is based on the fact that solid tumors require initiating vascularization for rich supply of blood. Endothelial cells of such vessels expressing integrins and NGR/RGD peptides attached ligand can interact easily with them which leads to more uptake of liposomes in tumor cells. Using a maleimide-thiol conjugation strategy, the NGR-peptide was attached to preformulated liposome with Mal-PEG_{2000}-DSPE. Results showed that circulation time in blood of NGR-liposomes was significantly improved and tumor uptake was increased by 10-folds compared to non-targeted Dox-liposomes. To test effectiveness of the formulation, treatment with high dose of NGR-peptide or ARA-liposomes (mismatch attached liposomes) was administered to the mice which resulted in either blockade in NGR coupled liposomes or no uptake of liposome respectively (Pastorino et al., 2003a). Additionally, based on a hypothesis that addition of CPP (tandem insert nona-arginine, tiR_9) to cyclic-NGR can increase the efficiency of target

Table 4
Different peptides/proteins for surface modification of Dox-liposomes.

Sr. No.	Type of ligand	Targeted Receptor	Chemical reaction/Bond	Functionalization	Reference
1	RGERPPR	Neuropilin-1	Thiol-maleimide coupling reaction	Pre-formulation Functionalization	(Yang et al., 2013)
2	APRPG and GRGDS	VEGFR-1 and integrin $\alpha_v\beta_3$	Amide bond formation	Pre-formulation Functionalization	(Sugiyama et al., 2013), (Murase et al., 2010)
3	cGRD	Integrine	Amide bond formation	Pre-formulation Functionalization	(Dicheva et al., 2015)
4	cGRD	Integrine $\alpha_v\beta_3$, $\alpha_v\beta_5$	Thiol-maleimide coupling reaction	Post-formulation Functionalization Post-insertion	(Chen et al., 2012)
5	cNGR and tiR_9	Aminopeptidase and CPP	Michael addition reaction	Pre-formulation Functionalization	(Shi et al., 2018)
6	($_D$-Arg^6, $_D$-Trp^{7,9,} -N^{me}Phe^8) Antagonist-G	Specific receptors on Small cell lung cancer	Thioether bond formation	Post-formulation Functionalization	(Moreira et al., 2001)
7	Carboxy terminated CRPPR	Neuropilin-1 Receptor	Amide bond formation	Pre-formulation Functionalization	(Paoli et al., 2014)
8	α1(IV)1263–1277 peptide ampiphiles peptide chain	Chondroitin Sulfate Proteoglycan receptor (CD44)	Amide bond formation	Pre-formulation Functionalization	(Ndinguri et al., 2012)
9	TAT	CPP	Post-formulation Doxil® were incubated with TATp and made complex (Adsorption) Thioether bond formation	Pre-formulation Functionalization Post-formulation Functionalization	(Koren et al., 2011)
10	Transferrin TAT	Tf-Receptor CPP	Thioether bond formation	Pre-formulation Functionalization	(Yuan et al., 2016)
11	TAT	CPP	His-Tag chelation catalysed by Ni	Post-formulation Functionalization	(Boakye et al., 2015)
12	Short peptide Amyloid β 25–35 (A β25-35)	Exchangeable Apolipoprotein	Thioether bond formation	Pre-formulation Functionalization	(Zhang et al., 2019)
13	Phase protein DMPGTVLP	Tumor specific phase fusion	Coating on lipid membrane	Post-formulation Functionalization	(Wang et al., 2010)
14	YSA peptide (YSAYPDSVPMMS)	EphA2 receptor	Amide bond formation	Pre-formulation Functionalization	(Wang et al., 2012b), (Haghiralsadat et al., 2018)
15	C3d Gadolinium	Neural cell adhesion molecule (NCAM) MRI agent	Thioester bond formation	Post-formulation Functionalization	(Grange et al., 2010)
16	GE-11 Cys-YHWYGYTPQNVI	Epidermal growth factor receptor	Michael addition reaction	Post-formulation Functionalization	(Cheng et al., 2014)
17	HER targeting peptide	HER2 receptor	Amide bond formation	Pre-formulation Functionalization	(Geng et al., 2016)
18	Elastin-like polypeptide ELP	Temperature stimulus	Amide bond formation	Post-formulation Functionalization	(Na et al., 2012)

ligand peptide, cNGR-tiR_9-PEG_{2000}-DSPE was synthesized (Shi et al., 2018). A combination of anti-neovascular and cancer chemotherapeutic drug enhanced the efficacy of chemotherapy due to anti-angiogenic drug-induced normalization of tumor blood vessels. A peptide 5-mer (Ala-Pro-Arg-Pro-Gly, APRPG-peptide) that was isolated using phage displayed peptide library, has been shown to have specific binding affinity towards tumor angiogenic vasculatures (specifically, VEGFR-1). APRPG-PEG_{2000}-DSPE was prepared prior to liposome formulation and doxorubicin loading using pre-formulation modification approach. *In-vitro* study using human umbilical vein endothelial cells (HUVECs) cells showed that APRPG-PEG_{2000}-DSPE liposomes were able to bind to the vascular endothelial growth factor. *In-vivo* investigations revealed that there was a significant elevation in localization of liposomes in tumor tissue and enhanced circulation period for peptide conjugated liposomes in colon 26 NL-17 carcinoma bearing mice (Maeda et al., 2004) could be detected. It was later reported that dual-targeting liposomes using APRPG and GNGRG peptides significantly suppressed the growth of HUVECs compared with that in single-targeting liposomes (Murase et al., 2010). GE-11 (Cys-YHWYGYTPQNVI) which also act as ligand to VEGFR-1 was attached to liposome for targeted delivery of doxorubicin in Non-Small Cell Lung Carcinoma (NSCLC) *ex vivo* and *in vivo* (Cheng et al., 2014). Growth factor antagonist peptide sequence, named as antagonist-G also found its application to also target lung cancer (Moreira et al., 2001). YAS (YSAYPDSVPMMS) is ephrin-mimetic peptide and also a ligand for EphA2 receptor. Liposomes decorated with YAS peptide were used to treat chordial neovascularization (Wang et al., 2012b) and osteosarcoma (Haghiralsadat et al., 2018).

The Arg-Gly-Asp (RGD) tripeptide sequence is a ligand for integrin $\alpha_v\beta_3$ which is also overexpressed on the tumor vasculatures and vascular endothelial cells. Post-formulation modification of liposome with Cyclo-Arg-Gly-Asp-D-Phe-Cys using thiol-maleimide coupling reaction produces cRGD decorated liposomes. *In-vitro* results showed that cellular internalization was elevated for peptide-attached liposomes compared to non-decorated liposomes whereas blood circulation time were similar for both liposome types in rats (Chen et al., 2012). Thermosensitive liposomes anchored with cRGD-pentapeptide (Arg-Cys-D-Phe-Asp-Gly) were prepared to gain advantages of targeting receptor as well as hyperthermic condition and enhance overall benefits of liposomal drug

delivery (Dicheva et al., 2015).

Neuropilin-1 (NRP-1) is another receptor overexpressed on glioblastoma and tumor vasculatures that acts as a co-receptor for VEGF in angiogenesis related processes. C-end-R peptides such as CRGERPPR or CRPPR peptides are the ligands for NRP-1 and hence find their application in decoration of liposomes for targeting specific endothelial and cancer cells. CPPRP conjugated liposomes encapsulating doxorubicin were able to bind with NPR-positive prostate cancer cell-line but not NPR-negative cells. Accumulation, internalization and therapeutic efficiency of NPR-1 targeted liposomes using peptide ligand were significantly higher than conventional Dox-liposome in tumor bearing mice model (Paoli et al., 2014). Similarly, another group developed NPR-1 targeted liposomes using RGERPPR peptide after attachment to DSPE-mPEG (Yang et al., 2013). HER-1/2/3 are legitimate members of epidermal growth factor receptor and HER-2 is especially studied more in breast cancer pathology as it also considered a biomarker. Geng et al. showed a computational peptide library to screen selective peptide sequence for HER-2. They found PYL/YYL/PPL***NP as sequence motif and formulated peptide conjugated liposome using novel peptide P25 and P51 which demonstrated strong affinity for HER-2 *ex vivo* and *in vivo* (Geng et al., 2016).

Selective nature of blood brain barrier (BBB) tightly regulates entry of any substance to the brain. Exchangeable apolipoproteins such as Apo-A1 and Apo-E are capable to cross BBB due to the presence of lipid-binding and receptor binding domains. These apolipoproteins recognize amyloid $\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) and are involved in the clearance of Aβ plaque into blood circulation. A non-toxic short peptide sequence, $A\beta_{25\text{-}35}$ has been synthesized which can be used as peptide ligand as it has similar attraction for apolipoproteins. Modified liposomes with $A\beta_{25\text{-}35}$ were prepared using pre-formulation modification approach. Modified liposomes exhibited significant binding efficiency toward rhu-ApoE antibodies and delivered doxorubicin specifically to brain-tumors which showcases its applicability as brain-targeting delivery system (Zhang et al., 2019).

4.3. Carbohydrates

The first generation of Stealth® liposomes were prepared using monosialoganglioside GM_1 as a linker to overcome issue of uptake by mononuclear phagocyte system (MPS) (Allen and Chonn, 1987). It was the first study which showed that stealth liposomes in which inclusion of GM1 ganglioside as steric stabilizer, are able to escape from MPS and remain in systemic circulation for extended period of time (Allen and Hansen, 1991). The conjugation of sialyl-Lewis × (sLe^x) to PEG-DSPE liposomes demonstrated E-selectin mediated cell adhesion of liposomes. Sialic acid as carbohydrate ligand has capabilities to interact with membrane receptors overexpressed on tumor cells and therefore liposomes conjugated with sialic acid-octadecylamine were designed to treat S180-bearing Kunming mice. Sialyl modified liposomes demonstrated better pharmacokinetic profile as well as enhanced cytotoxic effects in mouse models (Sun et al., 2016). Immunoglobulins (IgM) have also been attached to liposomes using the IgM's carbohydrate residues present on the heavy chains of antibody. The hydroxyl groups of carbohydrates are oxidized using mild oxidizing agents such as sodium periodate or enzyme catalysed reaction using galactose oxidase to convert the hydroxyl groups into aldehydes. Subsequent conjugation of oxidized IgM using hydrazone ligation to hydrazide-containing liposomes was carried out to form immunoglobulin attached vesicles (Chua et al., 1984) (see Table 5).

Galactose and mannose have demonstrated their applicability as a ligand for the targeted delivery of doxorubicin using liposomes (Shimada et al., 1997). Generally, liposomes decorated with carbohydrates target in particular liver cancers as they have a higher specificity for asialoglycoprotein receptor (ASGP-R) (Ashwell and Harford, 1982). ASGP-receptors are frequently found on the surface of malignant liver cells and have selective binding affinity for carbohydrates, in particular galactose and *N*-acetylgalactosamine. Such ligand-receptor interactions have been exploited to achieve liver targeted delivery of doxorubicin as it also offers clathrin-mediated endocytosis for their hepatocellular internalization. Galactosylated liposomes were prepared using Gal-DPPE with neutral lipids to selectively target hepatocellular carcinoma. Local targeted delivery of doxorubicin using galactosylated liposomes demonstrated better uptake *in vivo* and *in-vitro* than unconjugated liposomes (Zhao et al., 2013). Cluster glycoside effect has significant impact on binding affinity of oligosaccharide and ASGP-R. To evaluate the selectivity of galactosylated liposomes, tetravalent galactosylated diethylenetriaminepentaacetic acid-distearoyl phosphatidylethanolamine (4Gal-DTPA-DSPE) were synthesized to prepare galactosylated liposomes. Pharmacokinetic evaluation showed that treatment with 4Gal-liposomes implied prolonged circulation time in

Table 5
Different carbohydrates for surface modification of Dox-liposomes.

Sr. No.	Type of ligand	Targeted Receptor	Chemical reaction/Bond	Functionalization	Reference
1	β-D-Galactoside	Asialo-glycoprotein receptor (ASGP-R)	Amide bond formation	Pre-formulation Functionalization	(Zhao et al., 2013)
2	$(\beta\text{-D-Galactoside})_4$	ASGP-R	Amide bond formation	Pre-formulation Functionalization	(Xiao et al., 2013)
3	Chitooligosaccharide Estrogen	Mucoadhesive Estrogen Receptor	Disulfide and amide bond formation	Pre- and Post-formulation Functionalization	(Yin et al., 2018)
4	Sucrose/Maltose	Lectins	Amide bond formation	Pre-formulation Functionalization	(Song et al., 2009)
5	Sialic acid	Membrane receptors	Amide bond formation	Pre-formulation Functionalization	(Sun et al., 2016)
6	Lactoferrin	ASGP-R	Amide bond formation	Post-formulation Functionalization	(Wei et al., 2015)
7	*p*-Aminophenyl-α-D-mannopyranoside	Mannose Receptor	Crosslinking of 1° Amine using Glutaraldehyde	Post-formulation Functionalization Post-insertion	(Kole et al., 1999)
8	D-Mannose	CD206 Mannose receptor	Amide bond formation	Pre-formulation Functionalization	(Li et al., 2019)
9	L-Fucose	E-selectin	Amide bond formation	Pre-formulation Functionalization	(Li et al., 2019)
10	9BPC-Neu5Ac	CD22 (Siglec-2)	Amide bond formation	Post-formulation Functionalization post-insertion	(Chen et al., 2010)

plasma and significant accumulation of doxorubicin in the liver was estimated from frozen liver section study in mice (Xiao et al., 2013). Lactoferrin, a mammalian cationic iron-binding glycoprotein also exhibits significant binding affinity to ASGP-R. This binding characteristic was explored as a ligand for modification of liposomes loaded with doxorubicin and were evaluated for its targeting efficiency in hepatocellular carcinoma cell lines overexpressing ASGP-R. Lactoferrin conjugated liposomes showed significantly higher internalization and antitumor effects both *in-vitro* (ASGPR(+) HCC*)* and *in vivo* (HepG2 bearing nude mice xenograft model) compared to conventional liposomes (Wei et al., 2015). Galactosylated liposomes are also applied for dual delivery of doxorubicin and siRNA in hepatocellular carcinoma to be benefited by both, chemotherapy as well as gene therapy (Oh et al., 2016).

Mannose receptors are involved in inflammation and immunogenic response through interaction of different lectins. Mannosyled liposomes were developed to target mannose receptor expressing cells. DSPE-PEG_{2000}-Mannose was synthesized after reacting mannose to isocyanate and DSPE-PEG_{2000}-NH_2. Using this modified DSPE-PEG, liposomes were decorated with mannose and loaded with doxorubicin and dihydroartemisinin to overcome multidrug resistance in colon cancer. Treatment of doxorubicin-resistant cells (HCT8/ADR) with this targeted liposome demonstrated enhanced intracellular AUC *in-vivo* and increased cell-death in resistant cell lines (Kang et al., 2017). Liposomes loaded with doxorubicin were prepared and then mannosylated using *p-aminophenyl-α-D-mannopyranoside.* This mannosylated liposomes were used in treatment of leishmaniasis to evaluate combined effect of doxorubicin and interferon-γ in rodents (Kole et al., 1999). In another study, a multi-ligand targeted liposome using D-mannose (targeting mannose receptor, CD206) and L-fucose (targeting E-selectin) were prepared to enhance anti-tumor efficacy of doxorubicin in S180 tumor bearing mouse (Li et al., 2019).

Lectins are carbohydrate binding proteins, expressed on the plasma membrane of many malignant cells, which makes disaccharides such as maltose or sucrose as potential ligand to be introduced to the surface of the liposomes. Lectin assisted internalization of drug loaded polymeric nanoparticles is an interesting approach suggested by Song et al. (2009). The author attempted to validate this relationship by formulating doxorubicin loaded liposome modified with mannose or sucrose. Maltose and sucrose were aminated first and then attached to DSPE-PEG_{2000} using amide coupling reaction. Disaccharide-containing liposomes loaded with doxorubicin had higher cytotoxic effect on cancer cells as they internalized more due to lectin mediated endocytosis in comparison of PEGylated liposomes loaded with doxorubicin (Song et al., 2009).

Carbohydrates are not only used as ligands for targeted delivery but have also been used as mucoadhesive agents for localized delivery with chitosan being the most popular agent used for this application. On hydrolysis, chitosan produces chitooligosaccharide (COS) which has found applications in ocular drug delivery. A multifunctional liposome (Chol-SS-COS/ES/DOX) having estrogen (ES) as targeting ligand and glutathione responsive COS as dose dumping mechanism were prepared. COS was covalently linked to the liposomal surface through a disulfide bond (—SS—) and estrogen was grafted via DSPE-PEG(2000) chain. Selective entry to osteosarcoma cells was gained with the help of estrogen receptor present on the surface osteosarcoma cells whereas dumping of doxorubicin was achieved due to intracellular glutathione mediated disruption of liposome at COS-S-S-Cholesterol (Yin et al., 2018).

4.4. Miscellaneous

Apart from small molecular ligands, stimuli-responsive targeted liposomes using different environmental stimulus such as pH (Ding et al., 2015; Koren et al., 2012; Mamasheva et al., 2011; Paliwal et al., 2012), temperature (Dong et al., 2005; Negussie et al., 2010; Ninomiya et al., 2014; Pradhan et al., 2010), redox reaction (Goldenbogen et al., 2011; Park et al., 2010; Tang et al., 2016) and magnetism (Kubo et al., 2000) are also gaining popularity for the targeted delivery of doxorubicin. Thermodox® is a successful example of stimuli-responsive targeted liposomes of doxorubicin which has entered into phase III clinical trial (Zagar et al., 2014). Specific receptor such as folate receptor (de Oliveira Silva et al., 2019; Saul et al., 2003; Sriraman et al., 2016; Watanabe et al., 2012), transferrin receptor (Kobayashi et al., 2007; Li et al., 2009; Sriraman et al., 2016), sigma receptor (Banerjee et al., 2004) and estrogen receptor (Rai et al., 2008; Yin et al., 2018) are also being targeted for specific delivery of doxorubicin. DNA/RNA aptamers are also used for decoration of doxorubicin encapsulated liposomes (Dou et al., 2018; Moosavian et al., 2016; Ninomiya et al., 2014; Song et al., 2015; Xing et al., 2013). 2B3-101 is a glutathione coated Doxil®/Caelyx® which is in phase I/IIA clinical trial (Gaillard et al., 2014). Different vitamins such as tocopherol (Tan et al., 2019) and vitamin B12 (Gupta et al., 2011) have also displayed their ability to be used as a ligand onto the surface of liposome for better internalization efficiency.

5. Clinical applications of liposomal doxorubicin

Successful approval of Doxil® paved the path for further clinical applications of liposomal formulations. Myocet®, a non-pegylated liposomal preparation of doxorubicin is another approved liposomal formulation which offered the same benefits as Doxil® without demonstrating hand-foot syndrome, a major side-effect of pegylated liposomal doxorubicin formulations. Lipodox® is an approved generic product of doxorubicin hydrochloride liposomal injection. Thermodox®, Nudoxa®, 2B3-101 and C225-ILS-Dox are some other examples of liposomal preparation encapsulating doxorubicin which have undergone clinical investigations (see Table 6). Besides doxorubicin, there are also other therapeutic agents which find liposomes as suitable carriers for targeted deliveries with significant clinical applications for various conditions such as Amphotec® and Ambiosome® (Amphotericin-B), DepoDur (Morphine sulfate), DaunoXome (Daunorubicin), Mepact (Mifamurtide), DepoCyt (Cytarabine), Marqibo (Vincristine), Epaxal® (Hepatitis-A vaccine), Inflexal® (Influenza vaccine).

6. Pharmacokinetics of marketed liposomal doxorubicin formulations

A pilot study conducted to investigate plasma pharmacokinetic (PK) and accumulation of doxorubicin in cancer patients after intravenous administration of Doxil® and free doxorubicin (non-liposomal) included 53 courses of Doxil® (Barenholz, 2012b). The results demonstrated a much higher concentration of doxorubicin in tumor cells after

Table 6
List of doxorubicin liposomal preparation and their clinical status.

Sr. No.	Trade Name	Clinical Status	Company/ Sponsor	Reference
1	Myocet®	Approved	Cephalon	Nogueira et al., 2015
2	Doxil®/Caelyx®	Approved	Janssen	
3	Lipodox®	Approved	Sun Pharmaceutical Industries Ltd.	
4	Thermodox®	Phase-III (NCT00346229, NCT00826085)	Celsion Corporation	Zagar et al., 2014
5	Nudoxa®	Phase-II/III CTRI/2009/091/ 1,000,795	Zydus & Bharat Serum and vaccine Pvt	Rivankar, 2014
6	2B3-101	Phase-I/IIA (NCT01386580)	BBB Technologies BV	Gaillard et al., 2014
7	C225-ILS-Dox (Anti-EGFR-Immunoliposome-Dox)	Phase-I (NCT01702129)	University Hospital, Switzerland	Mamot et al., 2011

administration of Doxil® over free doxorubicin. PK parameters were determined for 25 and 50 mg/m^2 doses of doxorubicin. Plasma elimination time for Doxil® was found to follow bi-exponential curve with two half-lives (2 and 45 h). There was a huge difference in the values of volume of distribution of Doxil® (4 L) and free doxorubicin (254 L). Clearance rate of doxorubicin from Doxil® was found to be 0.1 L/h which was significantly slower than free doxorubicin (45 L/h). In a brief summary, the PK of Doxil® in humans at does between 10 and 80 mg/m^2 demonstrated two half-lives in two phases (phase-1: 1–3 h and phase-2: 30–90 h). Area under curve (AUC) was found to be approximately 300-fold greater over free doxorubicin after administration of 50 mg/m^2 of liposomal doxorubicin. Volume of distribution (Vd) and clearance were found to be at least 60-fold and 250-fold lower than free doxorubicin respectively (Barenholz, 2012b). On the other hand, in a clinical investigation of Myocet®, there was a 9-fold and 25-fold reduction in the clearance and volume of distribution of doxorubicin whereas a 25-fold increment in the AUC over free doxorubicin was observed (Batist et al., 2002) (see Tables 7 and 8).

7. Conclusion

Though doxorubicin is a broad-spectrum chemotherapeutic agent, its therapeutic applicability is limited due to dose dependent toxicities. Hence, it is necessary to load doxorubicin in a special vehicle and unload it in targeted tumor sites thereby minimizing its toxicities and achieving maximum therapeutic benefit. This has been successfully achieved by Doxil®, a liposomal targeted drug delivery system. Although Doxil® enhanced the accumulation of drug in tumor sites *via* the EPR effect, its passive targeting still limited the true potential of liposome-based doxorubicin delivery. Active targeting of liposomes using receptor-ligand navigation systems on the surface of cancer cells has shown great promise in delivering chemotherapeutic agents to target tissue sites. This comprehensive review has highlighted the ligands, ligation reaction conditions based on available reactive group of ligands and factors affecting those coupling reactions. Depending upon the active target present on the tumor surface various antibodies, peptides, carbohydrates, vitamins and other bioactive substances have been deployed as ligands.

7.1. Future directions and prospects

Despite the success of ligand targeted liposomes in improving the therapeutic output over conventional preparations, there are certain challenges with respect to its interference in diffusion and permeation through the targeted tissues, complex release patterns, immunogenic responses and unwanted interaction with serum proteins that reduces the availability of ligand to the binding site. Systematic studies on the optimization of process variables such as particle size and charge of liposomes, selection of specific ligands, receptor expression level, density of ligand, type and length of spacer etc. can provide useful information that will assist easy and reproducible scale up.

Besides cancer, liposomal drug delivery platform is being explored for a wide range of therapeutic applications such as treatment of eye, skin and respiratory diseases. Furthermore, liposomes are also being developed as multidrug or multifunctional carrier systems. The encapsulation of DNA/RNA/aptamer into liposomes has made gene therapy development easier and non-destructive. Apart from their therapeutic application, liposomal preparations are also gaining significant momentum in the field of diagnostics, vaccination, cosmetics and photodynamic therapy. The versatile nature of liposomes has translated their successful approvals in the clinic and clinically approved products or products in advanced clinical trials. In the future also this trend of probing diversified utility will continue to deliver more advanced and target specific liposomal preparation on the pharmaceutical market.

Table 7
Pharmacokinetic parameters of marketed liposomal doxorubicin.

Parameters	Myocet®[a]	Doxil®[b]	Lipodox®[b]
Formulation type	Non-pegylated liposomes	Pegylated liposomes	Pegylated liposomes
Peak plasma concentration (μg/mL)	7.33 ± 4.09	4.12 ± 0.215	4.12 ± 0.215
Plasma clearance (L/h/m^2)	5.58 ± 2.95	0.056 ± 0.01	0.056 ± 0.01
Steady state volume of distribution (L/m^2)	81.73 ± 72.51	2.83 ± 0.145	2.83 ± 0.145
AUC (mcg/mL.h)	35.61 ± 26.28	277 ± 32.9	277 ± 32.9
First phase half-life (h)	52.63 ± 20 0.67	4.7 ± 1.1	4.7 ± 1.1
Second phase half-life (h)	–	52.3 ± 5.6	52.3 ± 5.6

Table 8
Comparison of pharmacokinetic parameters of marketed liposomal doxorubicin.

Parameters	Myocet®[a]	Caelyx®[c]
Formulation type	Non-pegylated liposomes	Pegylated liposomes
C_{max} (μM)	16.0	36.6
$AUC_{0-\infty}$ (μM/h)	79.2	1555.2
Terminal half-life $t_{½}$ (h)	16.4	45.9
Clearance (mL/min/m^2)	50.8	0.88
Steady state volume of distribution (L/m^2)	34.2	3.5

[a]The pharmacokinetic parameters are for 60 mg/m^2 strength of formulation (Ref: Mross et al., 2004).
[b]The pharmacokinetic parameters are for 10 mg/m^2 strength of formulation and as per described in the approved label at USFDA.
[c]The pharmacokinetic parameters are for 50 mg/m^2 strength of formulation (Ref: Mross et al., 2004).

CRediT authorship contribution statement

Vivek Makwana: Writing - original draft. **Jasmine Karanjia:** Writing - review & editing. **Thomas Haselhorst:** Supervision, Writing - review & editing. **Shailendra Anoopkumar-Dukie:** Supervision, Writing - review & editing. **Santosh Rudrawar:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Financial support to SR from an Australian Research Council – Discovery Early Career Researcher Award (DE140101632) is gratefully acknowledged. VM is supported by the Griffith University International Postgraduate Research Scholarship (GUIPRS) and the Griffith University Postgraduate Research Scholarship (GUPRS). JK is supported by the University of South Australia President's Scholarship (UPS). Images are created with BioRender.com under paid subscription.

References

Abu Lila, A.S., Ishida, T., 2017. Liposomal Delivery systems: design optimization and current applications. Biol. Pharm. Bull. 40, 1–10.
Allen, T., Chonn, A., 1987. Large unilamellar liposomes with low uptake into the reticuloendothelial system. FEBS Lett. 223, 42–46.
Allen, T.M., Hansen, C., 1991. Pharmacokinetics of stealth versus conventional liposomes: effect of dose. Biochim. Biophys. Acta 1068, 133–141.
Andrews, P.A., Brenner, D.E., Chou, F.T., Kubo, H., Bachur, N.R., 1980. Facile and definitive determination of human adriamycin and daunorubicin metabolites by high-pressure liquid chromatography. Drug Metab. Dispos. 8, 152–156.

Apte, A., Koren, E., Koshkaryev, A., Torchilin, V.P., 2014. Doxorubicin in TAT peptide-modified multifunctional immunoliposomes demonstrates increased activity against both drug-sensitive and drug-resistant ovarian cancer models. Cancer Biol. Ther. 15, 69–80.
Ashwell, G., Harford, J., 1982. Carbohydrate-specific receptors of the liver. Annu. Rev. Biochem. 51, 531–554.
Banerjee, R., Tyagi, P., Li, S., Huang, L., 2004. Anisamide-targeted stealth liposomes: a potent carrier for targeting doxorubicin to human prostate cancer cells. Int. J. Cancer 112, 693–700.
Bangham, A.D., Horne, R.W., 1964. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J. Mol. Biol. 8, 660–668.
Barenholz, Y.C., 2012a. Doxil®—The First FDA-Approved Nano-Drug: From an Idea to a Product. Handbook of Harnessing Biomaterials in Nanomedicine: Preparation, Toxicity, and Applications. Pan Standford Publishing, Singapore, pp. 335–398.
Barenholz, Y., 2012b. Doxil®—The first FDA-approved nano-drug: lessons learned. J. Control. Release 160, 117–134.
Batist, G., Barton, J., Chaikin, P., Swenson, C., Welles, L., 2002. Myocet (liposome-encapsulated doxorubicin citrate): a new approach in breast cancer therapy. Expert Opin. Pharmacother. 3, 1739–1751.
Béduneau, A., Saulnier, P., Hindré, F., Clavreul, A., Leroux, J.C., Benoit, J.P., 2007. Design of targeted lipid nanocapsules by conjugation of whole antibodies and antibody Fab' fragments. Biomaterials 28, 4978–4990.
Biswas, S., Deshpande, P.P., Perche, F., Dodwadkar, N.S., Sane, S.D., Torchilin, V.P., 2013a. Octa-arginine-modified pegylated liposomal doxorubicin: an effective treatment strategy for non-small cell lung cancer. Cancer Lett. 335, 191–200.
Biswas, S., Dodwadkar, N.S., Deshpande, P.P., Parab, S., Torchilin, V.P., 2013b. Surface functionalization of doxorubicin-loaded liposomes with octa-arginine for enhanced anticancer activity. Eur. J. Pharm. Biopharm. 84, 517–525.
Boakye, C.H., Patel, K., Singh, M., 2015. Doxorubicin liposomes as an investigative model to study the skin permeation of nanocarriers. Int. J. Pharm. 489, 106–116.
Bourel-Bonnet, L., Pécheur, E.I., Grandjean, C., Blanpain, A., Baust, T., Melnyk, O., Hoflack, B., Gras-Masse, H., 2005. Anchorage of synthetic peptides onto liposomes via hydrazone and alpha-oxo hydrazone bonds. Preliminary functional investigations. Bioconjug. Chem. 16, 450–457.
Chen, W.C., Completo, G.C., Sigal, D.S., Crocker, P.R., Saven, A., Paulson, J.C., 2010. In vivo targeting of B-cell lymphoma with glycan ligands of CD22. Blood 115, 4778–4786.
Chen, Z., Deng, J., Zhao, Y., Tao, T., 2012. Cyclic RGD peptide-modified liposomal drug delivery system: enhanced cellular uptake in vitro and improved pharmacokinetics in rats. Int. J. Nanomed. 7, 3803–3811.
Chenevier, P., Bourel-Bonnet, L., Roux, D., 2003. Chemical characterization of alpha-oxohydrazone ligation on colloids: toward grafting molecular addresses onto biological vectors. J. Am. Chem. Soc. 125, 16261–16270.
Cheng, L., Huang, F.Z., Cheng, L.F., Zhu, Y.Q., Hu, Q., Li, L., Wei, L., Chen, D.W., 2014. GE11-modified liposomes for non-small cell lung cancer targeting: preparation, ex vitro and in vivo evaluation. Int. J. Nanomed. 9, 921–935.
Chua, M.M., Fan, S.T., Karush, F., 1984. Attachment of immunoglobulin to liposomal membrane via protein carbohydrate. Biochim. Biophys. Acta 800, 291–300.
De Miguel, D., Basáñez, G., Sánchez, D., Malo, P.G., Marzo, I., Larrad, L., Naval, J., Pardo, J., Anel, A., Martinez-Lostao, L., 2013. Liposomes decorated with Apo2L/TRAIL overcome chemoresistance of human hematologic tumor cells. Mol. Pharm. 10, 893–904.
De Miguel, D., Gallego-Lleyda, A., Martinez-Ara, M., Plou, J., Anel, A., Martinez-Lostao, L., 2019. Double-edged lipid nanoparticles combining liposome-bound TRAIL and encapsulated doxorubicin showing an extraordinary synergistic pro-apoptotic potential. Cancers (Basel) 11, 1948.
De Oliveira Silva, J., Fernandes, R.S., Ramos Oda, C.M., Ferreira, T.H., Machado Botelho, A.F., Martins Melo, M., De Miranda, M.C., Assis Gomes, D., Dantas Cassali, G., Townsend, D.M., Rubello, D., Oliveira, M.C., de Barros, A.L.B., 2019. Folate-coated, long-circulating and pH-sensitive liposomes enhance doxorubicin antitumor effect in a breast cancer animal model. Biomed. Pharmacother. 118, 109323.
Deng, L., Ke, X., He, Z., Yang, D., Gong, H., Zhang, Y., Jing, X., Yao, J., Chen, J., 2012. A MSLN-targeted multifunctional nanoimmunoliposome for MRI and targeting therapy in pancreatic cancer. Int. J. Nanomed. 7, 5053–5065.
Dicheva, B.M., ten Hagen, T.L., Seynhaeve, A.L., Amin, M., Eggermont, A.M., Koning, G. A., 2015. Enhanced specificity and drug delivery in tumors by cRGD-anchoring thermosensitive liposomes. Pharm. Res. 32, 3862–3876.
Ding, Y., Sun, D., Wang, G.L., Yang, H.G., Xu, H.F., Chen, J.H., Xie, Y., Wang, Z.Q., 2015. An efficient PEGylated liposomal nanocarrier containing cell-penetrating peptide and pH-sensitive hydrazone bond for enhancing tumor-targeted drug delivery. Int. J. Nanomed. 10, 6199–6214.
Dong, L.F., Mei, H.S., Song, S.X., Lu, Z.J., 2005. Synergistic role between rhIL-2 and adriamycin long circulating temperature-sensitive liposome in targeting therapy on tumor. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 21, 296–300.
Dou, X.Q., Wang, H., Zhang, J., Wang, F., Xu, G.L., Xu, C.C., Xu, H.H., Xiang, S.S., Fu, J., Song, H.F., 2018. Aptamer-drug conjugate: targeted delivery of doxorubicin in a HER3 aptamer-functionalized liposomal delivery system reduces cardiotoxicity. Int. J. Nanomed. 13, 763–776.
Eavarone, D.A., Yu, X., Bellamkonda, R.V., 2000. Targeted drug delivery to C6 glioma by transferrin-coupled liposomes. J. Biomed. Mater. Res. 51, 10–14.
Elbayoumi, T., Torchilin, V., 2010. Current trends in liposome research. In: Weissig, V. (Ed.), Liposomes, Methods in Molecular Biology, 605. Human Press, Clifton, NJ, pp. 1–27.
Gabizon, A., Papahadjopoulos, D., 1988. Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. Proc. Natl. Acad. Sci. U. S. A. 85, 6949–6953.
Gaillard, P.J., Appeldoorn, C.C., Dorland, R., van Kregten, J., Manca, F., Vugts, D.J., Windhorst, B., van Dongen, G.A., de Vries, H.E., Maussang, D., van Tellingen, O., 2014. Pharmacokinetics, brain delivery, and efficacy in brain tumor-bearing mice of glutathione pegylated liposomal doxorubicin (2B3-101). PLoS One 9, e82331.
Garnier, B., Bouter, A., Gounou, C., Petry, K.G., Brisson, A.R., 2009. Annexin A5-functionalized liposomes for targeting phosphatidylserine-exposing membranes. Bioconjug. Chem. 20, 2114–2122.
Geng, L., Wang, Z., Jia, X., Han, Q., Xiang, Z., Li, D., Yang, X., Zhang, D., Bu, X., Wang, W., Hu, Z., Fang, Q., 2016. HER2 targeting peptides screening and applications in tumor imaging and drug delivery. Theranostics 6, 1261–1273.
Goldenbogen, B., Brodersen, N., Gramatica, A., Loew, M., Liebscher, J., Herrmann, A., Egger, H., Budde, B., Arbuzova, A., 2011. Reduction-sensitive liposomes from a multifunctional lipid conjugate and natural phospholipids: reduction and release kinetics and cellular uptake. Langmuir 27, 10820–10829.
Gong, J., Chen, M., Zheng, Y., Wang, S., Wang, Y., 2012. Polymeric micelles drug delivery system in oncology. J. Control. Release 159, 312–323.
Goren, D., Gabizon, A., Barenholz, Y., 1990. The influence of physical characteristics of liposomes containing doxorubicin on their pharmacological behavior. Biochim. Biophys. Acta 1029, 285–294.
Grange, C., Geninatti-Crich, S., Esposito, G., Alberti, D., Tei, L., Bussolati, B., Aime, S., Camussi, G., 2010. Combined delivery and magnetic resonance imaging of neural cell adhesion molecule-targeted doxorubicin-containing liposomes in experimentally induced Kaposi's sarcoma. Cancer Res. 70, 2180–2190.
Gray, B.P., Li, S., Brown, K.C., 2013. From phage display to nanoparticle delivery: functionalizing liposomes with multivalent peptides improves targeting to a cancer biomarker. Bioconjug. Chem. 24, 85–96.
Gregoriadis, G., 1976. The carrier potential of liposomes in biology and medicine (first of two parts). N. Engl. J. Med. 295, 704–710.
Gupta, B., Torchilin, V.P., 2007. Monoclonal antibody 2C5-modified doxorubicin-loaded liposomes with significantly enhanced therapeutic activity against intracranial human brain U-87 MG tumor xenografts in nude mice. Cancer Immunol. Immunother. 56, 1215–1223.
Gupta, Y., Ganesh, N., Veer Kohli, D., Jain, S.K., 2011. Development and characterization of doxorubicin bearing vitamin B12 coupled sterically stabilized liposomes for tumor targeting. Curr. Nanosci. 7, 427–435.
Haghiralsadat, F., Amoabediny, G., Naderinezhad, S., Zandieh-Doulabi, B., Forouzanfar, T., Helder, M.N., 2018. Codelivery of doxorubicin and JIP1 siRNA with novel EphA2-targeted PEGylated cationic nanoliposomes to overcome osteosarcoma multidrug resistance. Int. J. Nanomed. 13, 3853–3866.
Hatakeyama, H., Akita, H., Maruyama, K., Suhara, T., Harashima, H., 2004. Factors governing the in vivo tissue uptake of transferrin-coupled polyethylene glycol liposomes in vivo. Int. J. Pharm. 281, 25–33.
Hosokawa, S., Tagawa, T., Niki, H., Hirakawa, Y., Nohga, K., Nagaike, K., 2003. Efficacy of immunoliposomes on cancer models in a cell-surface-antigen-density-dependent manner. Br. J. Cancer 89, 1545–1551.
Hussain, S., Plückthun, A., Allen, T.M., Zangemeister-Wittke, U., 2007. Antitumor activity of an epithelial cell adhesion molecule targeted nanovesicular drug delivery system. Mol. Cancer Ther. 6, 3019–3027.
Hwang, K., 1987. In: Liposomes: From Biophysics to Therapeutics. Marcel Dekker Inc., New York and Barsel, pp. 109–156.
Ishida, T., Iden, D.L., Allen, T.M., 1999. A combinatorial approach to producing sterically stabilized (Stealth) immunoliposomal drugs. FEBS Lett. 460, 129–133.
Kang, X.J., Wang, H.Y., Peng, H.G., Chen, B.F., Zhang, W.Y., Wu, A.H., Xu, Q., Huang, Y. Z., 2017. Codelivery of dihydroartemisinin and doxorubicin in mannosylated liposomes for drug-resistant colon cancer therapy. Acta Pharmacol. Sin. 38, 885–896.
Kobayashi, T., Ishida, T., Okada, Y., Ise, S., Harashima, H., Kiwada, H., 2007. Effect of transferrin receptor-targeted liposomal doxorubicin in P-glycoprotein-mediated drug resistant tumor cells. Int. J. Pharm. 329, 94–102.
Kole, L., Das, L., Das, P.K., 1999. Synergistic effect of interferon-gamma and mannosylated liposome-incorporated doxorubicin in the therapy of experimental visceral leishmaniasis. J. Infect. Dis. 180, 811–820.
Koren, E., Apte, A., Jani, A., Torchilin, V.P., 2012. Multifunctional PEGylated 2C5-immunoliposomes containing pH-sensitive bonds and TAT peptide for enhanced tumor cell internalization and cytotoxicity. J. Control. Release 160, 264–273.
Koren, E., Apte, A., Sawant, R.R., Grunwald, J., Torchilin, V.P., 2011. Cell-penetrating TAT peptide in drug delivery systems: proteolytic stability requirements. Drug Deliv. 18, 377–384.
Kubo, T., Sugita, T., Shimose, S., Nitta, Y., Ikuta, Y., Murakami, T., 2000. Targeted delivery of anticancer drugs with intravenously administered magnetic liposomes in osteosarcoma-bearing hamsters. Int. J. Oncol. 17, 309–315.
Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I., Sfar, S., Charcosset, C., Fessi, H., 2012. Preparation, characterization and applications of liposomes: state of the art. J. Colloid Sci. Biotechnol. 1, 147–168.
Lee, R.J., Low, P.S., 1994. Delivery of liposomes into cultured KB cells via folate receptor-mediated endocytosis. J. Biol. Chem. 269, 3198–3204.
Lee, R.J., Low, P.S., 1995. Folate-mediated tumor cell targeting of liposome-entrapped doxorubicin in vitro. Biochim. Biophys. Acta 1233, 134–144.
Leserman, L.D., Barbet, J., Kourilsky, F., Weinstein, J.N., 1980. Targeting to cells of fluorescent liposomes covalently coupled with monoclonal antibody or protein A. Nature 288, 602–604.

Levchenko, T.S., Rammohan, R., Lukyanov, A.N., Whiteman, K.R., Torchilin, V.P., 2002. Liposome clearance in mice: the effect of a separate and combined presence of surface charge and polymer coating. Int. J. Pharm. 240, 95–102.
Li, C., Lai, C., Qiu, Q., Luo, X., Hu, L., Zheng, H., Lu, Y., Liu, M., Zhang, H., Liu, X., Deng, Y., Song, Y., 2019. Dual-ligand modification of PEGylated liposomes used for targeted doxorubicin delivery to enhance anticancer efficacy. AAPS PharmSciTech 20, 188.
Li, X., Ding, L., Xu, Y., Wang, Y., Ping, Q., 2009. Targeted delivery of doxorubicin using stealth liposomes modified with transferrin. Int. J. Pharm. 373, 116–123.
Lopes de Menezes, D.E., Kirchmeier, M.J., Gagne, J.-F., Pilarski, L.M., Allen, T.M., 1999. Cellular trafficking and cytotoxicity of anti-CD19-targeted liposomal doxorubicin in B lymphoma cells. J. Liposome Res. 9, 199–228.
Lundberg, B.B., Griffiths, G., Hansen, H.J., 2000. Specific binding of sterically stabilized anti-B-cell immunoliposomes and cytotoxicity of entrapped doxorubicin. Int. J. Pharm. 205, 101–108.
Madaan, K., Kumar, S., Poonia, N., Lather, V., Pandita, D., 2014. Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues. J. Pharm. Bioallied Sci. 6, 139–150.
Maeda, N., Takeuchi, Y., Takada, M., Sadzuka, Y., Namba, Y., Oku, N., 2004. Anti-neovascular therapy by use of tumor neovasculature-targeted long-circulating liposome. J. Control. Release 100, 41–52.
Malhi, S.S., Budhiraja, A., Arora, S., Chaudhari, K.R., Nepali, K., Kumar, R., Sohi, H., Murthy, R.S.R., 2012. Intracellular delivery of redox cycler-doxorubicin to the mitochondria of cancer cells by folate receptor targeted mitocancerotropic liposomes. Int. J. Pharm. 432, 63–74.
Makwana, V., Jain, R., Patel, K., Nivsarkar, M., Joshi, A., 2015. Solid lipid nanoparticles (SLN) of Efavirenz as lymph targeting drug delivery system: Elucidation of mechanism of uptake using chylomicron flow blocking approach. Int. J. Pharm. 495, 439–446.
Mamasheva, E., O'Donnell, C., Bandekar, A., Sofou, S., 2011. Heterogeneous liposome membranes with pH-triggered permeability enhance the in vitro antitumor activity of folate-receptor targeted liposomal doxorubicin. Mol. Pharm. 8, 2224–2232.
Mamot, C., Ritschard, R., Vogel, B., Dieterle, T., Bubendorf, L., Hilker, C., Deuster, S., Herrmann, R., Rochlitz, C., 2011. A phase I study of doxorubicin-loaded anti-EGFR immunoliposomes in patients with advanced solid tumors. J. Clin. Oncol. 29, 3029–3029.
Manjappa, A.S., Chaudhari, K.R., Venkataraju, M.P., Dantuluri, P., Nanda, B., Sidda, C., Sawant, K.K., Murthy, R.S., 2011. Antibody derivatization and conjugation strategies: application in preparation of stealth immunoliposome to target chemotherapeutics to tumor. J. Control. Release 150, 2–22.
Martin, F.J., Hubbell, W.L., Papahadjopoulos, D., 1981. Immunospecific targeting of liposomes to cells: a novel and efficient method for covalent attachment of Fab' fragments via disulfide bonds. Biochemistry 20, 4229–4238.
Martinez-Lostao, L., García-Alvarez, F., Basáñez, G., Alegre-Aguarón, E., Desportes, P., Larrad, L., Naval, J., Martínez-Lorenzo, M.J., Anel, A., 2010. Liposome-bound APO2L/TRAIL is an effective treatment in a rabbit model of rheumatoid arthritis. Arthritis Rheum. 62, 2272–2282.
Matsumura, Y., Gotoh, M., Muro, K., Yamada, Y., Shirao, K., Shimada, Y., Okuwa, M., Matsumoto, S., Miyata, Y., Ohkura, H., Chin, K., Baba, S., Yamao, T., Kannami, A., Takamatsu, Y., Ito, K., Takahashi, K., 2004. Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer. Ann. Oncol. 15, 517–525.
Minotti, G., Menna, P., Salvatorelli, E., Cairo, G., Gianni, L., 2004. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol. Rev. 56, 185–229.
Moase, E.H., Qi, W., Ishida, T., Gabos, Z., Longenecker, B.M., Zimmermann, G.L., Ding, L., Krantz, M., Allen, T.M., 2001. Anti-MUC-1 immunoliposomal doxorubicin in the treatment of murine models of metastatic breast cancer. Biochim. Biophys. Acta-Biomembr. 1510, 43–55.
Molavi, O., Xiong, X.B., Douglas, D., Kneteman, N., Nagata, S., Pastan, I., Chu, Q., Lavasanifar, A., Lai, R., 2013. Anti-CD30 antibody conjugated liposomal doxorubicin with significantly improved therapeutic efficacy against anaplastic large cell lymphoma. Biomaterials 34, 8718–8725.
Moosavian, S.A., Abnous, K., Badiee, A., Jaafari, M.R., 2016. Improvement in the drug delivery and anti-tumor efficacy of PEGylated liposomal doxorubicin by targeting RNA aptamers in mice bearing breast tumor model. Colloids Surf. B Biointerfaces 139, 228–236.
Moreira, J.N., Hansen, C.B., Gaspar, R., Allen, T.M., 2001. A growth factor antagonist as a targeting agent for sterically stabilized liposomes in human small cell lung cancer. Biochim. Biophys. Acta 1514, 303–317.
Mross, K., Niemann, B., Massing, U., Drevs, J., Unger, C., Bhamra, R., Swenson, C.E., 2004. Pharmacokinetics of liposomal doxorubicin (TLC-D99; Myocet) in patients with solid tumors: an open-label, single-dose study. Cancer Chemother. Pharmacol. 54, 514–524.
Muñoz, M., García, M., Reig, F., Alsina, M.A., Haro, I., 1998. Physico-chemical characterization of liposomes with covalently attached hepatitis A VP3 (101–121) synthetic peptide. Analyst 123, 2223–2228.
Murase, Y., Asai, T., Katanasaka, Y., Sugiyama, T., Shimizu, K., Maeda, N., Oku, N., 2010. A novel DDS strategy, "dual-targeting", and its application for antineovascular therapy. Cancer Lett. 287, 165–171.
Na, K., Lee, S.A., Jung, S.H., Hyun, J., Shin, B.C., 2012. Elastin-like polypeptide modified liposomes for enhancing cellular uptake into tumor cells. Colloids Surf. B Biointerfaces 91, 130–136.
Ndinguri, M.W., Zheleznyak, A., Lauer, J.L., Anderson, C.J., Fields, G.B., 2012. Application of collagen-model triple-helical peptide-amphiphiles for CD44-targeted drug delivery systems. J. Drug Deliv. 2012, 592602.
Needham, D., McIntosh, T.J., Lasic, D.D., 1992. Repulsive interactions and mechanical stability of polymer-grafted lipid membranes. Biochim. Biophys. Acta 1108, 40–48.
Negussie, A.H., Miller, J.L., Reddy, G., Drake, S.K., Wood, B.J., Dreher, M.R., 2010. Synthesis and in vitro evaluation of cyclic NGR peptide targeted thermally sensitive liposome. J. Control. Release 143, 265–273.
Newman, M.S., Colbern, G.T., Working, P.K., Engbers, C., Amantea, M.A., 1999. Comparative pharmacokinetics, tissue distribution, and therapeutic effectiveness of cisplatin encapsulated in long-circulating, pegylated liposomes (SPI-077) in tumor-bearing mice. Cancer Chemother. Pharmacol. 43, 1–7.
Ninomiya, K., Yamashita, T., Kawabata, S., Shimizu, N., 2014. Targeted and ultrasound-triggered drug delivery using liposomes co-modified with cancer cell-targeting aptamers and a thermosensitive polymer. Ultrason. Sonochem. 21, 1482–1488.
Nogueira, E., Gomes, A.C., Preto, A., Cavaco-Paulo, A., 2015. Design of liposomal formulations for cell targeting. Colloids Surf. B Biointerfaces 136, 514–526.
O'Donnell, R.T., Martin, S.M., Ma, Y., Zamboni, W.C., Tuscano, J.M., 2010. Development and characterization of CD22-targeted pegylated-liposomal doxorubicin (IL-PLD). Invest. New Drugs 28, 260–267.
Oh, H.R., Jo, H.Y., Park, J.S., Kim, D.E., Cho, J.Y., Kim, P.H., Kim, K.S., 2016. Galactosylated liposomes for targeted co-delivery of doxorubicin/vimentin siRNA to hepatocellular carcinoma. Nanomaterials (Basel) 6.
Palekar, R.U., Myerson, J.W., Schlesinger, P.H., Sadler, J.E., Pan, H., Wickline, S.A., 2013. Thrombin-targeted liposomes establish a sustained localized anticlotting barrier against acute thrombosis. Mol. Pharm. 10, 4168–4175.
Paliwal, S.R., Paliwal, R., Pal, H.C., Saxena, A.K., Sharma, P.R., Gupta, P.N., Agrawal, G. P., Vyas, S.P., 2012. Estrogen-anchored pH-sensitive liposomes as nanomodule designed for site-specific delivery of doxorubicin in breast cancer therapy. Mol. Pharm. 9, 176–186.
Paoli, E.E., Ingham, E.S., Zhang, H., Mahakian, L.M., Fite, B.Z., Gagnon, M.K., Tam, S., Kheirolomoom, A., Cardiff, R.D., Ferrara, K.W., 2014. Accumulation, internalization and therapeutic efficacy of neuropilin-1-targeted liposomes. J. Control. Release 178, 108–117.
Park, J.W., Hong, K., Carter, P., Asgari, H., Guo, L.Y., Keller, G.A., Wirth, C., Shalaby, R., Kotts, C., Wood, W.I., et al., 1995. Development of anti-p185HER2 immunoliposomes for cancer therapy. Proc. Natl. Acad. Sci. U. S. A. 92, 1327–1331.
Park, K.M., Lee, D.W., Sarkar, B., Jung, H., Kim, J., Ko, Y.H., Lee, K.E., Jeon, H., Kim, K., 2010. Reduction-sensitive, robust vesicles with a non-covalently modifiable surface as a multifunctional drug-delivery platform. Small 6, 1430–1441.
Pastorino, F., Brignole, C., Marimpietri, D., Cilli, M., Gambini, C., Ribatti, D., Longhi, R., Allen, T.M., Corti, A., Ponzoni, M., 2003a. Vascular damage and anti-angiogenic effects of tumor vessel-targeted liposomal chemotherapy. Cancer Res. 63, 7400–7409.
Pastorino, F., Brignole, C., Marimpietri, D., Sapra, P., Moase, E.H., Allen, T.M., Ponzoni, M., 2003b. Doxorubicin-loaded Fab' fragments of anti-disialoganglioside immunoliposomes selectively inhibit the growth and dissemination of human neuroblastoma in nude mice. Cancer Res. 63, 86–92.
Peng, X., Chen, B., Lim, C.C., Sawyer, D.B., 2005. The cardiotoxicology of anthracycline chemotherapeutics: translating molecular mechanism into preventative medicine. Mol. Interv. 5, 163–171.
Pradhan, P., Giri, J., Rieken, F., Koch, C., Mykhaylyk, O., Döblinger, M., Banerjee, R., Bahadur, D., Plank, C., 2010. Targeted temperature sensitive magnetic liposomes for thermo-chemotherapy. J. Control. Release 142, 108–121.
Rahman, A., Kessler, A., More, N., Sikic, B., Rowden, G., Woolley, P., Schein, P.S., 1980. Liposomal protection of adriamycin-induced cardiotoxicity in mice. Cancer Res. 40, 1532–1537.
Rai, S., Paliwal, R., Vaidya, B., Khatri, K., Goyal, A.K., Gupta, P.N., Vyas, S.P., 2008. Targeted delivery of doxorubicin via estrone-appended liposomes. J. Drug Target. 16, 455–463.
Rivankar, S., 2014. An overview of doxorubicin formulations in cancer therapy. J. Cancer Res. Ther. 10, 853–858.
Saul, J.M., Annapragada, A., Natarajan, J.V., Bellamkonda, R.V., 2003. Controlled targeting of liposomal doxorubicin via the folate receptor in vitro. J. Control. Release 92, 49–67.
Schelté, P., Boeckler, C., Frisch, B., Schuber, F., 2000. Differential reactivity of maleimide and bromoacetyl functions with thiols: application to the preparation of liposomal diepitope constructs. Bioconjug. Chem. 11, 118–123.
Shi, N.Q., Li, Y., Zhang, Y., Li, Z.Q., Qi, X.R., 2018. Deepened cellular/subcellular interface penetration and enhanced antitumor efficacy of cyclic peptidic ligand-decorated accelerating active targeted nanomedicines. Int. J. Nanomed. 13, 5537–5559.
Shimada, K., Kamps, J.A., Regts, J., Ikeda, K., Shiozawa, T., Hirota, S., Scherphof, G.L., 1997. Biodistribution of liposomes containing synthetic galactose-terminated diacylglyceryl-poly(ethyleneglycol)s. Biochim. Biophys. Acta 1326, 329–341.
Shukla, S.K., Gupta, V., 2020. Utilizing nanotechnology to recuperate sorafenib for lung cancer treatment: challenges and future perspective. Ther. Deliv. 11, 213–215.
Shukla, S.K., Kulkarni, N.S., Farrales, P., Kanabar, D.D., Parvathaneni, V., Kunda, N.K., Muth, A., Gupta, V., 2020. Sorafenib loaded inhalable polymeric nanocarriers against non-small cell lung cancer. Pharm. Res. 37, 67.
Song, C.K., Jung, S.H., Kim, D.D., Jeong, K.S., Shin, B.C., Seong, H., 2009. Disaccharide-modified liposomes and their in vitro intracellular uptake. Int. J. Pharm. 380, 161–169.
Song, X., Ren, Y., Zhang, J., Wang, G., Han, X., Zheng, W., Zhen, L., 2015. Targeted delivery of doxorubicin to breast cancer cells by aptamer functionalized DOTAP/DOPE liposomes. Oncol. Rep. 34, 1953–1960.
Sriraman, S.K., Salzano, G., Sarisozen, C., Torchilin, V., 2016. Anti-cancer activity of doxorubicin-loaded liposomes co-modified with transferrin and folic acid. Eur. J. Pharm. Biopharm. 105, 40–49.

Sugano, M., Egilmez, N.K., Yokota, S.J., Chen, F.A., Harding, J., Huang, S.K., Bankert, R. B., 2000. Antibody targeting of doxorubicin-loaded liposomes suppresses the growth and metastatic spread of established human lung tumor xenografts in severe combined immunodeficient mice. Cancer Res. 60, 6942–6949.
Sugiyama, T., Asai, T., Nedachi, Y.M., Katanasaka, Y., Shimizu, K., Maeda, N., Oku, N., 2013. Enhanced active targeting via cooperative binding of ligands on liposomes to target receptors. PLoS One 8, e67550.
Sun, J., Song, Y., Lu, M., Lin, X., Liu, Y., Zhou, S., Su, Y., Deng, Y., 2016. Evaluation of the antitumor effect of dexamethasone palmitate and doxorubicin co-loaded liposomes modified with a sialic acid-octadecylamine conjugate. Eur. J. Pharm. Sci. 93, 177–183.
Surace, C., Arpicco, S., Dufaÿ-Wojcicki, A., Marsaud, V., Bouclier, C., Clay, D., Cattel, L., Renoir, J.M., Fattal, E., 2009. Lipoplexes targeting the CD44 hyaluronic acid receptor for efficient transfection of breast cancer cells. Mol. Pharm. 6, 1062–1073.
Tan, X., Fang, Y., Ren, Y., Li, Y., Wu, P., Yang, X., Liu, W., 2019. D-α-tocopherol polyethylene glycol 1000 succinate-modified liposomes with an siRNA corona confer enhanced cellular uptake and targeted delivery of doxorubicin via tumor priming. Int. J. Nanomed. 14, 1255–1268.
Tang, J., Zhang, L., Gao, H., Liu, Y., Zhang, Q., Ran, R., Zhang, Z., He, Q., 2016. Co-delivery of doxorubicin and P-gp inhibitor by a reduction-sensitive liposome to overcome multidrug resistance, enhance anti-tumor efficiency and reduce toxicity. Drug Deliv. 23, 1130–1143.
Torchilin, V.P., 1997. Targeting of liposomes within cardiovascular system. J. Liposome Res. 7, 433–454.
Torchilin, V.P., Goldmacher, V.S., Smirnov, V.N., 1978. Comparative studies on covalent and noncovalent immobilization of protein molecules on the surface of liposomes. Biochem. Biophys. Res. Commun. 85, 983–990.
Torchilin, V.P., Khaw, B.A., Smirnov, V.N., Haber, E., 1979. Preservation of antimyosin antibody activity after covalent coupling to liposomes. Biochem. Biophys. Res. Commun. 89, 1114–1119.
Torchilin, V.P., Levchenko, T.S., Lukyanov, A.N., Khaw, B.A., Klibanov, A.L., Rammohan, R., Samokhin, G.P., Whiteman, K.R., 2001a. p-Nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups. Biochim. Biophys. Acta 1511, 397–411.
Torchilin, V.P., Rammohan, R., Weissig, V., Levchenko, T.S., 2001b. TAT peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. Proc. Natl. Acad. Sci. U. S. A. 98, 8786–8791.
Vaidya, B., Kulkarni, N.S., Shukla, S.K., Parvathaneni, V., Chauhan, G., Damon, J.K., Sarode, A., Garcia, J.V., Kunda, N., Mitragotri, S., Gupta, V., 2020. Development of inhalable quinacrine loaded bovine serum albumin modified cationic nanoparticles: Repurposing quinacrine for lung cancer therapeutics. Int. J. Pharm. 577, 118995.
Varde, N.K., Pack, D.W., 2004. Microspheres for controlled release drug delivery. Expert Opin. Biol. Ther. 4, 35–51.
Wang, A.Z., Langer, R., Farokhzad, O.C., 2012a. Nanoparticle delivery of cancer drugs. Annu. Rev. Med. 63, 185–198.
Wang, J.L., Liu, Y.L., Li, Y., Dai, W.B., Guo, Z.M., Wang, Z.H., Zhang, Q., 2012b. EphA2 targeted doxorubicin stealth liposomes as a therapy system for choroidal neovascularization in rats. Invest. Ophthalmol. Vis. Sci. 53, 7348–7357.
Wang, T., D'Souza, G.G., Bedi, D., Fagbohun, O.A., Potturi, L.P., Papahadjopoulos-Sternberg, B., Petrenko, V.A., Torchilin, V.P., 2010. Enhanced binding and killing of target tumor cells by drug-loaded liposomes modified with tumor-specific phage fusion coat protein. Nanomedicine (London) 5, 563–574.
Watanabe, K., Kaneko, M., Maitani, Y., 2012. Functional coating of liposomes using a folate- polymer conjugate to target folate receptors. Int. J. Nanomed. 7, 3679–3688.
Wei, M., Guo, X., Tu, L., Zou, Q., Li, Q., Tang, C., Chen, B., Xu, Y., Wu, C., 2015. Lactoferrin-modified PEGylated liposomes loaded with doxorubicin for targeting delivery to hepatocellular carcinoma. Int. J. Nanomed. 10, 5123–5137.
Weiss, R.B., 1992. The anthracyclines: will we ever find a better doxorubicin? Semin. Oncol. 670.
Xiao, Y., Zhang, H., Zhang, Z., Yan, M., Lei, M., Zeng, K., Zhao, C., 2013. Synthesis of novel tetravalent galactosylated DTPA-DSPE and study on hepatocyte-targeting efficiency in vitro and in vivo. Int. J. Nanomed. 8, 3033–3050.
Xing, H., Tang, L., Yang, X., Hwang, K., Wang, W., Yin, Q., Wong, N.Y., Dobrucki, L.W., Yasui, N., Katzenellenbogen, J.A., Helferich, W.G., Cheng, J., Lu, Y., 2013. Selective delivery of an anticancer drug with aptamer-functionalized liposomes to breast cancer cells in vitro and in vivo. J. Mater. Chem. B 1, 5288–5297.
Yan, X., Scherphof, G.L., Kamps, J.A., 2005. Liposome opsonization. J. Liposome Res. 15, 109–139.
Yang, Y., Yan, Z., Wei, D., Zhong, J., Liu, L., Zhang, L., Wang, F., Wei, X., Xie, C., Lu, W., 2013. Tumor-penetrating peptide functionalization enhances the anti-glioblastoma effect of doxorubicin liposomes. Nanotechnology 24, 405101.
Yin, X., Feng, S., Chi, Y., Liu, J., Sun, K., Guo, C., Wu, Z., 2018. Estrogen-functionalized liposomes grafted with glutathione-responsive sheddable chotooligosaccharides for the therapy of osteosarcoma. Drug Deliv. 25, 900–908.
Yuan, M., Qiu, Y., Zhang, L., Gao, H., He, Q., 2016. Targeted delivery of transferrin and TAT co-modified liposomes encapsulating both paclitaxel and doxorubicin for melanoma. Drug Deliv. 23, 1171–1183.
Zagar, T.M., Vujaskovic, Z., Formenti, S., Rugo, H., Muggia, F., O'Connor, B., Myerson, R., Stauffer, P., Hsu, I.C., Diederich, C., Straube, W., Boss, M.K., Boico, A., Craciunescu, O., Maccarini, P., Needham, D., Borys, N., Blackwell, K.L., Dewhirst, M. W., 2014. Two phase I dose-escalation/pharmacokinetics studies of low temperature liposomal doxorubicin (LTLD) and mild local hyperthermia in heavily pretreated patients with local regionally recurrent breast cancer. Int. J. Hyperthermia 30, 285–294.
Zhang, Z., Guan, J., Jiang, Z., Yang, Y., Liu, J., Hua, W., Mao, Y., Li, C., Lu, W., Qian, J., Zhan, C., 2019. Brain-targeted drug delivery by manipulating protein corona functions. Nat. Commun. 10, 3561.
Zhao, C., Feng, Q., Dou, Z., Yuan, W., Sui, C., Zhang, X., Xia, G., Sun, H., Ma, J., 2013. Local targeted therapy of liver metastasis from colon cancer by galactosylated liposome encapsulated with doxorubicin. PLoS One 8, e73860.
Zhu, Y., Cheng, L., Cheng, L., Huang, F., Hu, Q., Li, L., Tian, C., Wei, L., Chen, D., 2014. Folate and TAT peptide co-modified liposomes exhibit receptor-dependent highly efficient intracellular transport of payload in vitro and in vivo. Pharm. Res. 31, 3289–3303.
Zucker, D., Marcus, D., Barenholz, Y., Goldblum, A., 2009. Liposome drugs' loading efficiency: a working model based on loading conditions and drug's physicochemical properties. J. Control. Release 139, 73–80.