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Review

Second generation liposomal cancer therapeutics: Transition from laboratory to clinic

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ABSTRACT

Recent innovations and developments in nanotechnology have revolutionized cancer therapeutics. Engineered nanomaterials are the current workhorses in the emerging field of cancer nano-therapeutics. Lipid vesicles bearing anti-tumor drugs have turned out to be a clinically feasible and promising nano-therapeutic approach to treat cancer. Efficient entrapment of therapeutics, biocompatibility, biodegradability, low systemic toxicity, low immunogenicity and ability to bypass multidrug resistance mechanisms has made liposomes a versatile drug/gene delivery system in cancer chemotherapy. The present review attempts to explore the recent key advances in liposomal research and the vast arsenal of liposomal formulations currently being utilized in treatment and diagnosis of cancer.

Contents

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1. Introduction

Liposomes, uni-lamellar or multi-lamellar spherical vesicles, primarily comprising phospholipids, either from plant or animal source (Torchilin, 2005; Zhang et al., 2008; Zhang and Granick, 2006) were first discovered by A.D. Bangham at the Agricultural Research Council Institute of Animal Physiology at Babraham, Cambridge (Duzgunes and Gregoriadis, 2005) in 1961, when he and his colleagues observed that phospholipids upon dispersion in water spontaneously formed spherical, self-closed vesicles consisting of concentric lipid bilayers (Bangham et al., 1965a). These vesicles initially called 'smectic mesophases', were later renamed as 'liposomes' (Sessa and Weissman, 1968). The resemblance of the lamellar structure of the vesicles with natural membranes, the capability to discriminate ions (cations diffuse poorly from membranes which are permeable to univalent anions and water) and susceptibility to stabilization or labilization by bioactive molecules similar to biological membranes have rendered liposomes versatile tool in the field of biology, biochemistry and medicine (Bangham et al., 1965a). The ability of the vesicles to swell osmotically, the possibility to vary membrane composition and surface potential and availability of several analytical techniques to study these systems have made liposomes a preferred lipid matrix model of living cells (Bangham et al., 1965b).

With the recognition of the biocompatibility, biodegradability, low toxicity and immunogenicity and the capability to entrap molecules, liposomes have moved a long way from being just another exotic object of biophysical research to becoming a pharmaceutical carrier of choice for numerous practical applications (Black and Gregoriadis, 1976; Gregoriadis, 1976; Juliano and Mccullough, 1980; Neerunjun and Gregoriadis, 1976; Torchilin, 2005).

The size of the liposomes range from 20 nm to more than 1 μm (Samad et al., 2007). Each microscopic vesicle has a hydrophilic core and hydrophobic bilayer which enables the entrapment of both hydrophilic and hydrophobic drugs (Medina et al., 2004; Zhang et al., 2008). These self-assembled lipid vesicles protect the cargo by encapsulating hydrophilic drugs within the aqueous core and hydrophobic drugs within lipid bilayers (Portney and Ozkan, 2006) which leads to the isolation of the drug molecules from the surrounding environment (Zhang and Granick, 2006).

Liposomes are generally classified based on lamellarity of the vesicles and can be distinguished into unilamellar and multilamellar vesicles (Fig. 1). While multilamellar vesicle comprises of several concentric bilayers arranged in an onion peel pattern with aqueous layer between them, unilamellar vesicles contains a single bilayer (Hofheinz et al., 2005; Perezsoler, 1989).

2. Liposomal uptake and role in cancer chemotherapy

2.1. Passive and active targeting

Liposomal formulations of several key active molecules were developed in order to overcome the problems associated with conventional drug therapy such as inefficient bio-distribution throughout the body and lack of specific delivery, by encapsulating the molecules within the vesicles to prevent degradation and passively targeting tissues and organs that have discontinuous endothelium (*e.g.* liver, spleen and bone marrow) (Immordino et al., 2006).

Passive targeting of tumor by drug-loaded liposomes employs the chaotic tumor-vessel architecture to its advantage. Tumors characteristically have leaky vasculature and dysfunctional lymphatic drainage (Underwood and Carr, 1972). The liposomes thus can escape into the tumor tissue *via* the fenestrations of the leaky vasculature. This phenomenon is termed as Enhanced Permeation and Retention (EPR) effect (Fig. 3) whereby there is extravasation followed by increased accumulation of the drug loaded nano-vector in the tumor tissue (Peer et al., 2007; Phillips et al., 2010). The "Enhanced Permeation and Retention" (EPR) effect was coined by Maeda in 1989 (Maeda and Matsumura, 1989).

In 1987, Jain reported that the osmotic pressure within tumors is high (Jain, 1987). Since tumor interstitial fluid pressure (IFP) is high in most solid tumors it causes a significant impediment in efficient anti-cancer drug delivery (Jain, 1987, 1994). The movement of high molecular weight anti-cancer drugs/nanoparticles from the circulatory system through the interstitial space takes place by convection rather than by diffusion unlike low molecular weight drugs. Increased IFP inhibits convection which contributes to decreased uptake of drugs into tumor. Moreover, IFP at the center of the solid tumor is higher in comparison to its periphery (Danhier et al., 2010). By adopting active targeting strategies, liposomes can overcome the high IFP barrier within tumors (Chang et al., 2009). Ligand-targeted therapy, which involves the use of ligand with affinity for the receptor expressed on plasma membrane of cancer cells or tumor neovasculature, may increase the accumulation of anti-cancer drugs in high IFP environment of the tumor tissues and improve the therapeutic efficacy (Lee et al., 2007). Therapeutic interventions designed to reduce IFP could be used in conjugation of

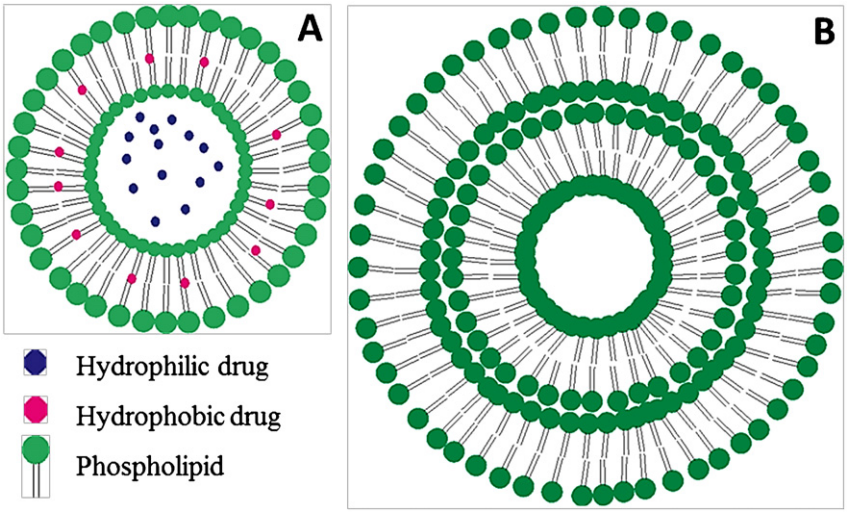

Fig. 1. Schematic representation of (A) unilamellar and (B) multilamellar liposomes.

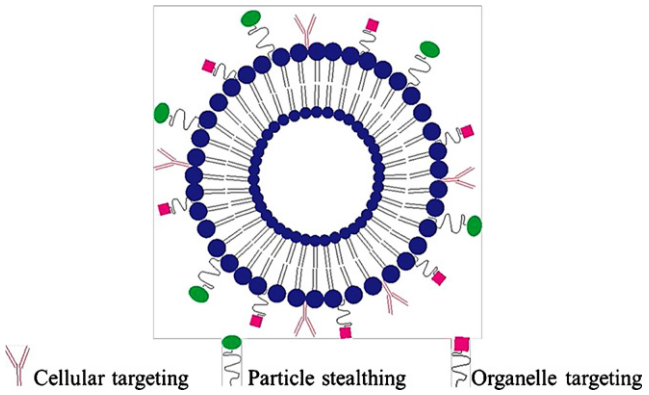

Fig. 2. Schematic representation of an engineered liposome for long circulation and ligand/antibody mediated targeted delivery.

nanotherapeutics in order to augment the conventional treatments of cancer (Cairns et al., 2006).

In order to circumvent the problem of increased toxicity in normal cells, decreased retention of drug loaded nanocarriers due to higher IFP and development of drug resistance caused by passive targeting, liposomes have to be explicitly directed to bind to specific target cells (Medina et al., 2004; Sapra and Allen, 2003). Targeted drug delivery involves ligand-mediated or antibody (Ab)-mediated targeting of the therapeutics to the cancer cell. Ligand targeting therapeutics (LTT) improves the therapeutic index of the drug by increasing the drug's efficacy (therapeutic effect) and reducing the drug's toxicity (side effects) (Juliano and Daoud, 1990; Lian and Ho, 2001; Malam et al., 2009; Perumal et al., 2011). A wide spectrum of ligand targeting agents such as protein (antibody or antibody fragments) (Heath et al., 1983), peptides (arginine-glycine-aspartic acid or RGD) (Schiffelers et al., 2003), vitamin (folic acid) (Rui et al., 1998), nucleic acid (aptamer) (Brody and Gold, 2000; Floege et al., 1999; White et al., 2000) and glycoprotein (transferrin) (Juliano and Stamp, 1976) are currently available. While, RGD targets cellular adhesion molecules like integrin $\alpha_V\beta_3$ (Suri et al., 2007) which is important in cancer progression (Cooper et al., 2002) due to its angiogenic role (Danhier et al., 2012) its significant upregulation in some tumors (Desgrosellier and Cheresh, 2010), growth factor receptors are specifically targeted by transferrin (Weinzimer et al., 2001) and folate ligands (Herbert et al., 1962).

Non-antibody ligand-targeted delivery like folate and transferrin-mediated drug delivery systems are attracting major attention lately (Fig. 2). Folate-mediated liposome targeting is increasingly gaining importance due to the frequent overexpression of folate receptors (FR) in a wide variety of tumor cells (Lu and Low, 2002). Similarly, targeting tumors with transferrin-modified liposomes also provides a suitable approach due to the increased frequency of transferrin receptors (TfR) in cancer cells (Hatakeyama et al., 2004). Peptides (arginine-glycine-aspartic acid or RGD) (Schiffelers et al., 2003), glycan (Xie et al., 2012) and nucleic acid (aptamer) (Leamon et al., 2003) have also been reported as other forms of non-Ab ligand liposome-based targeted drug delivery systems. Second generation liposomes bearing dual-ligands enhances targeting selectivity of drug-loaded nanocarriers and are designed to target multiple receptors for reduced toxicity on non-target cells (Saul et al., 2006).

The success of the targeted drug delivery is based on the density of the expressed targeted receptor/antigen on the cell. The enhanced effectivity of antibody-mediated drug delivery in comparison to non-antibody mediated targeted drug delivery arises from its increased specificity. However, the high cost and production time of antibody-anchored liposomes significantly limits its application in targeted delivery (Allen, 2002).

Table 1
Advantages of liposomes as pharmaceutical carriers.

Biocompatibility (Mufamadi et al., 2011)
Prevents premature degradation of encapsulated cargo (Goyal et al., 2005; Petros and DeSimone, 2010)
Entrapment of both hydrophilic and hydrophobic drugs (Medina et al., 2004; Zhang et al., 2008)
Targeted delivery—Can be functionalized with ligands to deliver therapeutic agents into cells or cellular components (Torchilin, 2005)
Site avoidance—The entrapped drug is prevented from reaching the healthy tissue (Hofheinz et al., 2005)
Size or lipid component variation helps in regulating bio-distribution of liposomes (Iinuma et al., 2002).

2.2. Liposomes and cancer therapeutics

Gregoria et al. (1974) first proposed liposomes for delivery of cancer therapeutics. Liposomes have been widely reported to be a therapeutic tool of choice since they have numerous advantages as pharmaceutical carriers (Table 1). However, the major associated limitation of conventional liposomes (Table 2) for therapeutic use lies in its fast elimination from the blood and recognition by the reticulo-endothelial system (RES) (Torchilin, 2005).

The efficient uptake of liposomes by macrophages and subsequent removal from systemic circulation upon intravenous administration is however, severely affected when the target site is beyond the mononuclear phagocyte system (MPS). The binding of opsonins (such as immunoglobulins, fibronectin and C-reactive protein) (Falcone, 1986a; Patel, 1992a) (Volanakis and Narkates, 1981) on the surface of liposomes, results in MPS recognizing these serum proteins rather than the vesicles and translates into removal of the liposomes from the circulation. Complement components such as C5b-9 complexes (membrane attack complex: MAC) which acts as an immediate host defense against invading foreign particles also recognize liposomes (Hamada et al., 2008; Moghimi and Patel, 1998) and initiate membrane lysis through pore formation and enhances uptake by neutrophils, monocytes, and macrophages (MPS cells) (Immordino et al., 2006). However, the presence of dysopsonins such as human serum albumin and IgA on the vesicle surface reduces recognition and inhibits phagocytosis of liposomes (Ishida et al., 2002). In fact, a fine balance between the blood opsonic and suppressive proteins regulates the rate of liposome clearance (Moghimi and Patel, 1998). The conventional liposomes were also observed to demonstrate profound instability in plasma which resulted in the rapid release of the encapsulated cargo due to their interactions with both high and low density (HDL and LDL) lipoproteins (Immordino et al., 2006).

In order to bypass the low-systemic circulation time of conventional liposomes, synthesis of long circulating liposomes (Stealth liposomes) has been attempted by coating the liposome surface with polymers, such as polyethylene glycol (PEG) (Klibanov et al., 1990), poly(vinyl pyrrolidone) (PVP), poly(acryl amide) (PAA) (Torchilin et al., 1994), poly[*N*-(2-hydroxypropyl) methacrylamide] and amphiphilic poly-*N*-vinylpyrrolidones (Torchilin et al., 2001) (Fig. 2). This resulted in significantly increased liposome stability which prolonged, by several orders of magnitude their blood

Table 2
Limitations of liposomes in drug/gene delivery.

High production cost–raw material (lipids) cost is high (Barenholz, 2001; Peer et al., 2007)
Oxidation of some phospholipids (Peer et al., 2007)
Rapid clearance by the Reticulo-endothelial system (RES) (Mufamadi et al., 2011)
Removal from the circulatory system (Peer et al., 2007)
Non-specific uptake (Peer et al., 2007)
Physiochemical instability (aggregation, sedimentation, hydrolysis) (Gurley, 2011)

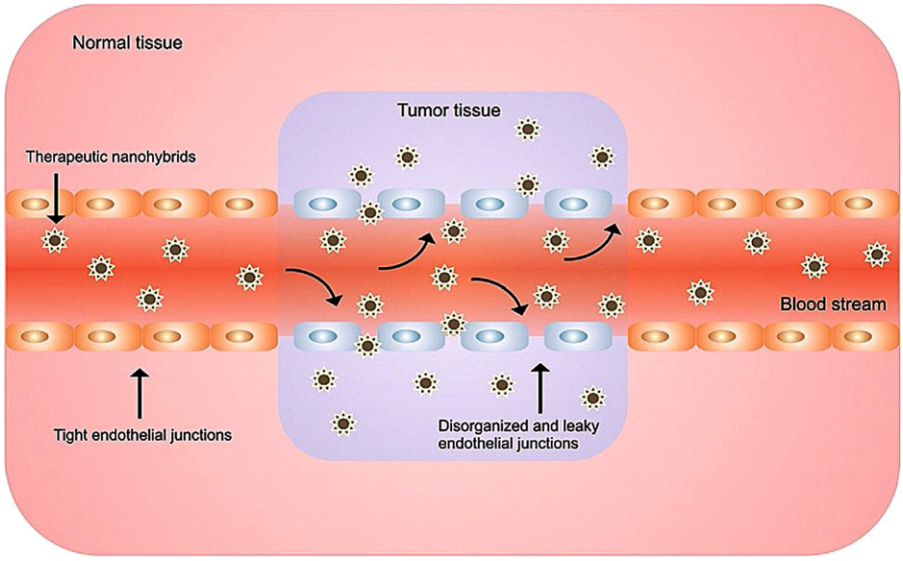

Fig. 3. Schematic representation of tumor targeting by nanohybrids *via* Enhanced Permeation and Retention (EPR) effect. [Reproduced with permission from Prakash et al. (2011)].

circulation times after systemic administration (Immordino et al., 2006) and ultimately led to the development of tailor-made liposomal formulations with increased stability both *in vitro* and *in vivo*, improved bio-distribution and optimized residence time in systemic circulation (Allen et al., 1991; Klibanov et al., 1990).

Repeated injections of sterically stabilized liposomes over a short duration leads to their rapid elimination from the system. This reduction in half-life of PEG liposomes has been termed as accelerated blood clearance (ABC) (Ishida et al., 2004; Ishida et al., 2003b). The ABC effect has been observed in animal models (rat, rabbit, mice, Rhesus monkey, Beagle) and reflects a major change in pharmacokinetics of consecutive injections of PEG liposomes (Dams et al., 2000; Ishida et al., 2003a; Zhao et al., 2012). Repeated injections of empty PEGylated liposomes elicit immune response and lead to production of anti-PEG IgM, which enhances blood clearance of subsequently injected PEGylated liposomes *via* anti-PEG IgM-mediated complement activation under certain conditions (Ishida et al., 2005; Ishida et al., 2006b). However, it has been recently reported that encapsulated Dox in drug loaded PEGylated liposomes causes selective damage of T cell-independent B cell mediated ABC phenomenon (Koide et al., 2010).

However, these pharmacokinetic changes were most distinct at dosing frequencies (1–3 weeks) which is higher than those used in current clinical practice of approved formulation Doxil® 180 (3–6 weeks). PEG liposomal doxorubicin formulation has a recommended low injection frequency of (3–6 weeks) in order to prevent the occurrence of cutaneous toxicity (Muggia et al., 1997) thus, the occurrence of ABC effect has not yet been reported in humans (Dams et al., 2000).

The magnitude of the induction of the ABC phenomenon is directly dependent on the interval between injections (Dams et al., 2000) and inversely related to the dose (Ishida et al., 2005). Thus, it is very important to design optimal dosing schedules in order to enhance the therapeutic efficacy and reduce the induced toxicity or immunological responses (Ishida et al., 2006a).

Recent combinatorial approaches aim to achieve greater circulation time of the vesicles (*via* PEGylation), specific delivery of encapsulated pay-load and synergistic uptake *via* dual-ligand targeting (Takara et al., 2010). *In vitro* and *in vivo* intracellular delivery of doxorubicin with RGD-modified pegylated liposomes exhibited increased cytotoxicity against melanoma (Xiong et al., 2005). Cationic liposomes have been effectively utilized in the treatment of resistant forms of cancer which have been unresponsive to conventional chemotherapy and other forms of treatment (Campbell et al., 2009). Combinatorial treatment regimens involving cationic nano-systems and other cancer therapeutic approaches such as hyperthermia or application of magnetic fields are being currently assessed (Campbell et al., 2009) for enhanced cancer chemotherapy. Cationic liposomes are effective, but they strongly interact with the blood components before they can reach the therapeutic target (Nicolazzi et al., 2003). Latest second-generation liposomal strategy aiming at conjugating lipoplex technology with PEGylation has reported to have substantial increase in circulation time. While this succeeds in enhancing the effectivity of these formulations (Nicolazzi et al., 2003), its limitation lies in reduced transfection rates (Xu et al., 2011).

Recent advances report the emergence of a new class of liposomes for cancer specific therapy which successfully overcomes the limitation of conventional liposomes. In contrast to conventional liposomes, stimuli-responsive vesicles undergo relatively large and abrupt physical and chemical changes in sharp response to applied stimuli. This becomes of particular interest when the stimuli to which these vesicles react are disease or systemic-biochemistry specific (such as pH). Solid tumors are characterized by poor vasculature which causes prevalence of anaerobic conditions and the extracellular pH is also significantly acidic (~6–7) than systemic pH (7.4). The pH-shift of the specific tissues can act as internal stimuli of chemical and biochemical origin that trigger drug release from the stimuli responsive nanocarriers. External physical stimuli triggering release of encapsulated cargo include heat, light and magnetic field (Deok Kong et al., 2012; Fleige et al., 2012; Ganta et al., 2008).With primary cancer prevention being the goal of the present day cancer chemotherapy, cancer vaccines have been developed to significantly reduce the incidence of cancer caused by microorganisms such as hepatocellular carcinoma (hepatitis B virus) and cervical carcinoma [human papilloma viruses (HPV)] (Goymer, 2005; Villa et al., 2005). However, the anti-tumor vaccine studies have been limited to *in vivo* models and transition to the clinical trials have not been very fulfilling (Lollini et al., 2006). Liposomal vaccine formulation bearing antigenic peptide derived from choriomeningitis virus and immune-stimulatory oligonucleotides has been reported to elicit antiviral and antitumor immunity (Ludewig et al., 2000). One of the most recent additions to the repertoire of liposomes is the multifunctional theranostic liposome which can be

Table 3
Approved anti-cancer liposomal formulations.

Product	Company	Drug	Disease
DaunoXome®	Galen	Daunorubicin	Advanced Kaposi's sarcoma
DepoCyt™	DepoTech Corporation	Cytarabine	Lymphomatous meningitis
Doxil®/Caelyx®	Johnson&Johnson	Doxorubicin	Metastatic ovarian cancer and advanced Kaposi's sarcoma
Lipo-Dox	Taiwan Liposome Company	Doxorubicin	Kaposi's sarcoma, breast and ovarian cancer
Marqibo®	Talon Therapeutics	Vincristin sulfate	Philadelphia chromosome negative (Ph-) acute lymphoblastic leukemia (ALL)
Myocet®	Cephalon/Sopherion Therapeutics	Doxorubicin	Metastatic breast cancer

considered as a key advancement in nanomedicine and has opened up a plethora of possibilities for simultaneous cancer therapy and diagnosis.

Approved liposomal drug formulations in cancer therapeutics have gone a long way and evolved from classical conventional liposomes (Myocet®/DaunoXome®) (Batist et al., 2001; Petre and Dittmer, 2007) to PEGylated forms (Doxil® and Lipo-dox™) (Allen and Cullis, 2012; Barenholz, 2012). Second generation liposomal drug delivery system endeavors for clinic use ranges from dual-drug loaded liposomes (CPX-1/CPX-351) (Dicko et al., 2010; Riviere et al., 2011) to stimuli-sensitive liposomes (ThermoDox) (Poon and Borys, 2011). The current focus of drug delivery research in clinical trials has been on active targeted drug delivery (MM-302/MBP-436) (Drummond et al., 2006; McDonagh et al., 2012) or utilization of cationic liposomes for drug delivery (EndoTAG™-1) (Fasol et al., 2012). Liposomal cancer vaccines being tested clinically include the Anti-MUC1 cancer vaccine (Bradbury and Shepherd, 2008) and L-BLP25 (Butts et al., 2005). Other approaches include RNAi based therapies which involve the delivery of siRNA (ALN-VSP/TKM-PLK1/TKM-ApoB) (Allen and Cullis, 2012; Semple et al., 2010). By tracking the evolution of liposomes as potent pharmaceutical carriers for anti-cancer drugs one can assimilate that liposomes have gone a long way and currently numerous attractive and diversified strategies are being successfully applied pre-clinically or clinically for enhanced and effective delivery of drugs.

3. Types of liposomes in treating cancer

3.1. Conventional liposomes

Liposomes act as reservoirs encapsulating the drug and protecting it from the degradation (Goyal et al., 2005) and reducing the unintended side effects such as cardio- (Forssen and Tokes,

Table 4
Liposome-based anti-cancer therapeutics undergoing clinical trials.

Product	Company/Organization	Drug	Disease
Annamycin	Aronex Pharmaceuticals	Annamycin	Breast cancer
Aroplatin	Antigenics Inc.	*cis*-bis-neodeca-noato-*trans*-R,R-1,2-diaminocyclohexane platinium(II) [Analogue of oxaliplatin]	Advanced solid malignancies, B-cell lymphoma
Atragen®	Aronex Pharmaceuticals	All-trans-retinoic acid (tretinoin)	Leukamia
CPX-1	Celator Pharmaceuticals	Fixed combination of irinotecan and floxuridine	Advanced Colorectal Cancer
CPX-351	Celator Pharmaceuticals	Fixed combination of cytarabine and daunorubicin	Advanced Hematologic Cancer
IHL-305	Yakult Honsha Co., LTD	Irinotecan	Treat Advanced Solid Tumors
INX-0125	Inex Pharm	Vinorelbine	Advanced breast cancer
JNS002	Janssen Pharmaceutical K.K.	Doxorubicin	Epithelial ovarian carcinoma, primary carcinoma of fallopian tube, peritoneal carcinoma
L9NC	University of New Mexico	9-nitro-20 (S)-camptothecin	Metastatic or Recurrent Cancer of the Endometrium or the Lung
LEM	Insys Therapeutics Inc	Mitoxantrone	Advanced Cancer
LEP-ETU	NeoPharm	Paclitaxel	Ovarian, breast, and lung cancer
LE-SN38	NeoPharm	SN-38 active metabolite of irinotecan	Advanced colorectal cancer
Lipoplatin™	Regulon	Cisplatin	Colon cancer, gastric tumor
Lipoxal™	Regulon	Oxaliplatin	Colorectal cancer
L-NDDP	New York University School of Medicine and National Cancer Institute (NCI)	Cisplatin Analog-Aroplatin	Malignant Mesothelioma
MBP-426	Mebiopharm Co., Ltd	Oxaliplatin	Treat Advanced or Metastatic Solid Tumors
NL CPT-11	University of California, San Francisco	CPT-11	Recurrent High-Grade Gliomas
OSI-211	OSI Pharmaceuticals	Lurtotecan	Ovarian cancer
OSI-7904L	OSI Pharmaceuticals	(S-2-[-5-[[[1,2-dihydro-3-methyl-1-oxobenzo[f]-quinazolin-9-yl] methyl] amino]-1-oxo-2-isoinso-linyl] glutaric acid) [Thymidylate synthase Inhibitor]	Gastric or Gastroesophageal (GEJ) Cancer
PEP02	PharmaEngine	Irinotecan	Metastatic Pancreatic Cancer
PNU-93914	Memorial Sloan-Kettering Cancer Center and National Cancer Institute (NCI)	Paclitaxel	Locally Advanced or Metastatic Cancer of the Esophagus
S-CKD602	Johnson&Johnson	CKD-602 [semi-synthetic analogue of camptothecin]	Advanced Malignancies
SPI-77	New York University School of Medicine and National Cancer Institute (NCI)	Cisplatin	Recurrent Ovarian Cancer
Telcyta®	Telik, Inc.	Canfosfamide HCl	Advanced ovarian, non-small cell lung, colon and breast cancers
ThermoDox®	Celsion	Doxorubicin	Hepatocellular carcinoma
TLC ELL-12	The Liposome Company	L-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine [L-ET-18-OCH3 (EL)]	Advanced solid tumors, including non-small cell lung, prostate cancer and melanoma
TLI	Talon Therapeutics, Inc	Topotecan	Small Cell Lung Cancer (SCLC), Ovarian Cancer and Other Advanced Solid Tumors
VLI	Talon Therapeutics, Inc	Vinorelbine	Advanced Solid Tumors, Non-Hodgkin's Lymphoma or Hodgkin's Disease

1981), nephro- (Smeesters et al., 1988), neuro- (Park et al., 2008; Rosentha and Kaufman, 1974) or dermal- (Boman et al., 1996) toxicity. Numerous liposomal formulations bearing cancer therapeutics have been approved or are currently undergoing clinical trials (Tables 3 and 4).

Liposomal formulation of Doxorubicin, an anthracycline-class drug and topoisomerase inhibitor with reported irreversible cardiotoxicity (Lipshultz et al., 1995; Vonhoff et al., 1979), has been successfully developed to effectively treat cancers with much lesser-associated side effects. Other noted examples of conventional liposomes in clinical use include Myocet® (Sopherion Therapeutics or Cephalon in USA and Europe respectively) loaded with doxorubicin (Alberts et al., 2004), DaunoXome® (Galen) encapsulating daunorubicin (Allen et al., 1991) and Marqibo® (Talon Therapeutics) carrying vincristin sulfate (Boehlke and Winter, 2006; Rodriguez et al., 2009). Liposomal daunorubicin formulation DaunoXome® is a pure lipid formulation which efficiently bypasses the RES and has reduced cardiotoxicity (Batist et al., 2001).

Vincristine sulfate has been successfully used in treatment of childhood and adolescent leukemia (Crom et al., 1994) and lymphoma (Jackson et al., 1984). However, the associated toxicity of vincristine sulfate is clinically manifested by mixed sensory-motor neuropathy. Other side effects include seizures, mental changes, orthostatic hypotension, inappropriate secretion of antidiuretic hormone (Rosentha and Kaufman, 1974). Vincristine-induced dermal toxicity is significantly reduced when the drug is delivered *via* liposomes (Boman et al., 1996).

Aroplatin® (Antigenics Inc., Lexington, MA, USA), a multilamellar liposomal formulation of saturated phospholipids dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG) bearing oxaliplatin analogue is undergoing clinical trials (Immordino et al., 2006) and has been reported to have reduced nephrotoxicity (Farrell, 2011), a side effect attributed to the drug, without compromising on its tumoricidal activity.

Liposomal annamycin (3′-deamino-4′-epi-3′-hydroxy-2′-iodo-4-demethoxy doxorubicin) composed of DMPC and DMPG (Wasan and Kwong, 1997), exhibited increased encapsulation of the drug within the vesicles and increased its therapeutic potential (Priebe and Perez-Soler, 1993).

While conventional drug-bearing liposomes have only been described to be loaded with single drugs, second generation liposomes have been reported to be loaded with two or more different drugs simultaneously for enhanced cytotoxicity in cancer cells (Agrawal et al., 2005; Cosco et al., 2012). This strategy aims at association of two or more antitumoral compounds for reduced effective dosages and associated-side effects (Fig. 4) (Colomer, 2005; Theodossiou et al., 1998). The liposomal multidrug carrier (MDC) can either be loaded with both water soluble (in the aqueous core) and lipophilic (entrapped in the bilayers) (Cosco et al., 2012) drugs or multiple drugs with same affinity

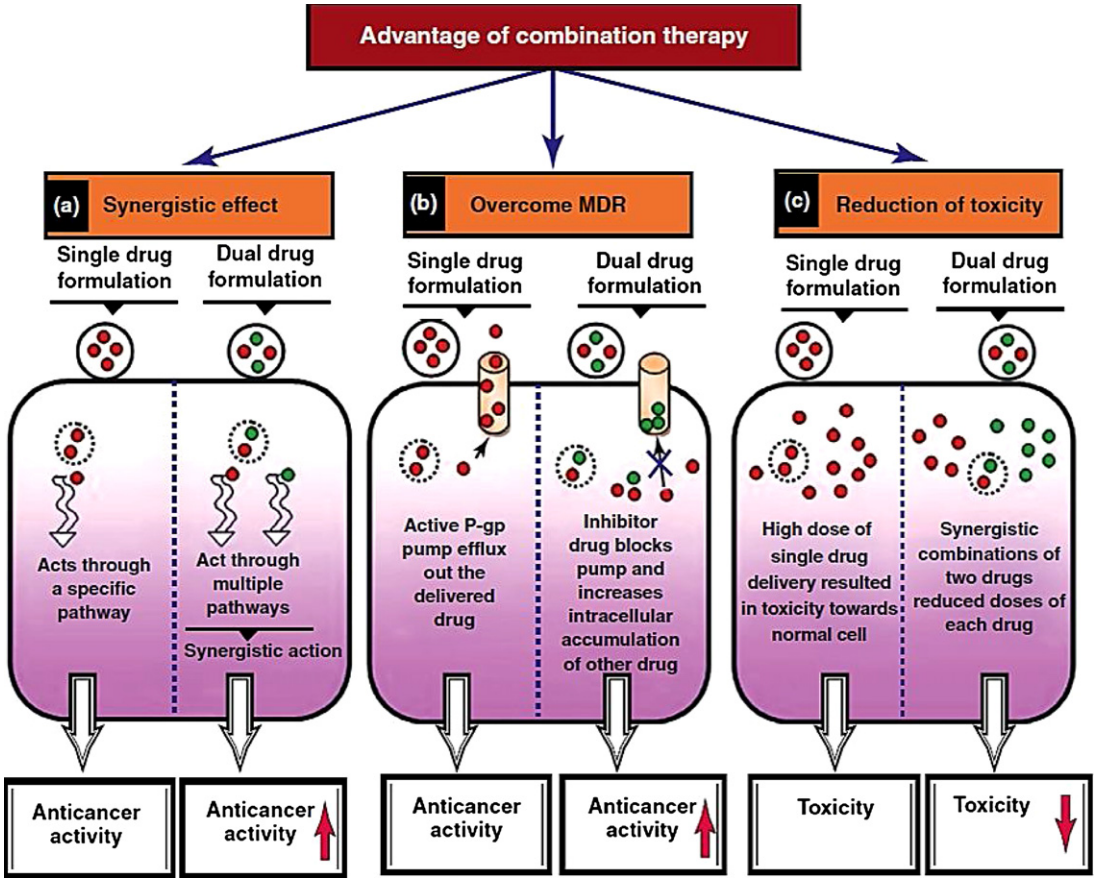

Fig. 4. Schematic representation depicting numerous advantages of combination drug delivery for cancer therapy. (A) Single drug acts through a particular pathway, whereas multiple drugs can show enhanced anticancer activity by acting through several pathways. (B) In the case of single drug treatment, MDR proteins such as P-gp efflux drug out of the cell, whereas for dual formulations P-gp inhibitor blocks the role of MDR proteins and increases the intracellular concentration of other co-administered drugs resulting in higher efficacy by overcoming the MDR phenotype. (C). High dose is required for single drug treatment and consequently results in toxicity to the normal cells, whereas treatment with different drug combinations leads to synergistic action which can reduce the dose of each single drug and thereby decrease the toxicity. [Reproduced with permission from Parhi et al. (2012)].

(hydrophilic/hydrophobic) without any interactions between the two compounds (Tardi et al., 2007).

3.2. Stealth liposomes

One of the major limitations of the conventional liposome is the rapid clearance by the RES. The clearance behavior and tissue distribution of nanocarriers like liposomes are greatly influenced by their size (Klibanov et al., 1991; Liu et al., 1991), charge (Chonn et al., 1991) and surface characteristics (Moghimi and Davis, 1994). The stealth liposomal technology is an ingenious solution which successfully resolves the drawbacks of the conventional liposome of diminished circulation longevity through steric stabilization (Mayer et al., 2000). Stealth liposomes show reduced uptake by the RES and increased accumulation in tumours (Papahadjopoulos et al., 1991).

In order to achieve prolonged and sustained drug delivery, conventional liposomes are being surface modified with inert, biocompatible, hydrophilic polymers such as PEG (Drummond et al., 1999). The presence of PEG molecules on the liposome surface results in the exclusion of other macromolecules from the 'periliposomal layer' by occupying the space immediately adjacent to the vesicles through its flexible chains and forms a protective layer which reduces the liposome recognition by 'opsonins' (Blume and Cevc, 1993; Klibanov et al., 1990). Some of the opsonizing proteins that are responsible for recognizing liposomes have been identified (Immordino et al., 2006) as immunoglobulins (Patel, 1992b), fibronectin (Falcone, 1986b; Patel, 1992b), beta2-glycoprotein (Chonn et al., 1995), C-reactive protein (CRP) (Volanakis and Narkates, 1981) and beta 2-macroglobulin (Murai et al., 1995).

Polymers like poly(vinyl pyrrolidone) (PVP), poly(acryl amide) (PAA) (Torchilin et al., 1994), poly[*N*-(2-hydroxypropyl) methacrylamide], amphiphilic poly-*N*-vinylpyrrolidones (Torchilin et al., 2001), L-amino-acid-based biodegradable polymer-lipid conjugate (Metselaar et al., 2003), polyvinyl alcohol (Takeuchi et al., 1996), amphipatic polymers poly(2-methyl-2-oxazoline) and (PMOZ) poly(2-ethyl-2-oxazoline) (PEOZ) (Woodle et al., 1994) have also been reported to be utilized for preparation of long circulating liposomes.

Pegylated liposome containing doxorubicin named Doxil®/Caelyx® is the first stealth liposomal formulation to be approved in USA and Europe for treatment of Kaposi's sarcoma (Krown et al., 2004) and recurrent ovarian cancer (Rose, 2005). Several other formulations are currently undergoing clinical trials, and a few more formulations are expected to be available in the market very soon (Katsaros et al., 2005; Hau et al., 2004).

Even though stealth liposomes show reduced uptake by the RES and increased accumulation in tumours (Papahadjopoulos et al., 1991), they are eventually cleared at some point from the blood circulation by macrophages of the RES (Moghimi, 1998; Moghimi and Gray, 1997; Moghimi and Murray, 1996).

3.3. Immunoliposomes

Immunoliposomes are considered to be a promising new candidate for targeted delivery of anti-cancer drugs (Table 5). Immunoliposomes have monoclonal antibodies (mAb) or antibody fragments conjugated to their surface. Conjugation with whole mAb leads to greater binding avidity and higher stability. However, the increased immunogenicity of whole mAb due to the presence of the fragment crystallizable (F_c) domain (Allen, 2002) severely limits its application.

Targeted drug delivery utilizing immunoliposomes involves two phases: the transport phase, where the immunoliposomes traverse from the site of administration to the target cells, and the effector phase which includes specific binding of immunoliposomes to the target cells and the subsequent delivery of encapsulated cargo (Mastrobattista et al., 1999).

Table 5
Antibody mediated drug delivery using liposomes.

Ligand	Cancer	Reference
Anti-CD74 antibody	Malignant B lymphoma	Lundberg et al. (2004)
Monoclonal nucleosome (NS)-specific 2C5 antibody (mAb 2C5)	Mammary adenocarcinoma	Lukyanov et al. (2004)
F(ab′)2 fragment of human monoclonal antibody GAH	Metastatic stomach cancer	Matsumura et al. (2004)
Fab' fragments of a humanized anti-p185HER2 monoclonal antibody (rhuMAbHER2)	Breast cancer	Park et al. (1995)
Anti-transferrin receptor single-chain antibody fragment (TfRscFv)	Advanced Solid Tumors	Xu et al. (2002)

The immunoliposome preparation is based on the following chemical strategies: (i) use of free functional groups like amino groups, carboxyl groups and carbohydrate chains present in the antibody molecule, (ii) modification of existing functional groups (disulfide, amine, carboxyl, and carbohydrate groups) in the antibodies with appropriate crosslinking reagents bearing reactive functional groups, (iii) utilization of free functional groups present in phospholipids (like hydroxyl and amine groups), (iv) modification of the existing functional groups of the phospholipids using suitable crosslinking reagents containing reactive functionalities, and (v) utilization of various functionalized PEG derivatives, which act as a linker between antibodies and liposomes (Manjappa et al., 2011). An interesting example relates to monoclonal nucleosome (NS)-specific antibody 2C5, which has been modified with poly(ethylene glycol)-phosphatidyl ethanolamine conjugate (PEG-PE) with the free PEG terminus activated by p-nitrophenylcarbonyl group (pNP-PEG-PE) for incorporation onto the liposomal surface (Lukyanov et al., 2004).

Long-circulating liposomes coated with hydrophilic polymer PEG conjugated with monoclonal antibodies (MAb N-12A5) directed against erbB-2 oncoprotein, a functional surface antigen in breast cancer has been reported (Goren et al., 1996). Another study reports the production of sterically stabilized immunoliposomal drugs (SIL) useful in 'mix and match' combinatorial applications of a variety of anti-cancer drugs (Ishida et al., 1999). Sterically stabilized liposomes containing doxorubicin- Doxil® (Johnson&Johnson) modified with monoclonal nucleosome (NS)-specific 2C5 antibody (mAb 2C5) showed improved antitumor efficacy *in vitro* in comparison to non-targeted conventional doxorubicin-loaded liposomes (Lukyanov et al., 2004).

3.4. Folate mediated liposomal targeting

Folic acid is a dietary vitamin that is required by eukaryotic cells for DNA synthesis and one-carbon metabolism (Ke et al., 2003). Folate receptor (FR) has been identified as cellular surface marker for a wide variety of malignant tumor types ranging from hematological to solid tumor. FR is a tumor-associated antigen which is over expressed in the advanced stages of cancer (Lu and Low, 2002). With the progression of cancer, the FR density have been reported to increase (Lu and Low, 2002).

In the case of folate-mediated liposomal targeting, the folate ligand-drug interactions are barred, since the folate ligand is in the outer leaflet of the bilayer rather than being in the liposomal interior (Zalipsky et al., 2004). Although, eukaryotic cells lack a

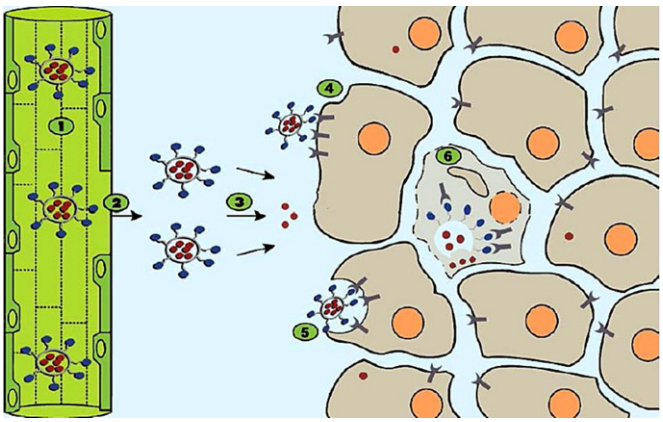

Fig. 5. Schematic representation illustrating the concept of folate targeting of liposomes to tumor cells. The blue dots represent the liposomal folate ligands. The red dots represent the drug molecules encapsulated in the liposome water phase. The various steps involved in the targeting process are numerically designated from 1 to 6. Steps 1–3 are common to non-targeted and targeted liposomes. Steps 4–6 are specific to FTL. (1) Liposomes with long-circulating properties increase the number of passages through the tumor microvasculature. (2) Increased vascular permeability in tumor tissue enables properly downsized liposomes to extravasate and reach the tumor interstitial fluid. (3) Drug is gradually released from liposomes remaining in the interstitial fluid and enters tumor cells as free drug to exert a cytotoxic effect. (4) Other liposomes bind to the FR expressed on the tumor cell membrane *via* the folate ligand. Because of the limited diffusion capacity of liposomes, binding is likely to be limited to those tumor cells in closest vicinity to blood vessels. (5) Liposomes are internalized by tumor cells *via* FRME. (6) Internalized liposomes release their drug content in the cytosol enabling the drug to exert its cytotoxic effect. [Reproduced with permission from Gabizon et al. (2003)].

pathway for folate biosynthesis (Ke et al., 2003), the folate targeted liposomes are internalized *via* FR mediated endocytosis (Fig. 5) (Gabizon et al., 2003; Lee and Low, 1994).

Liposomal folic acid targeted drug delivery is an attractive drug delivery vehicle because its size exceeds the critical glomerular filtration threshold which minimizes the loss by excretion (Gabizon et al., 2003). Folate liposomal formulations have been lately described as efficient delivery vehicles (Gabizon et al., 1999; Lee and Low, 1994) in cancer therapeutic studies *in vitro* (Lu and Low, 2002; Rui et al., 1998). However, the tendency of free folate ligand to compete for binding with targeted therapy presents a significant challenge (Allen, 2002). Second generation liposomes bearing both folic acid and a monoclonal antibody against endothelial growth factor receptors (EGFR) have been reported for effective and specific *in vitro* delivery of doxorubicin (Saul et al., 2006).

3.5. Transferrin mediated liposomal targeting

Rapidly proliferating tumor cells over-express transferrin receptor (Tf-R) (Yamada et al., 2005) due to increased iron requirement (Bellocq et al., 2003). A recent strategy reports coupling of transferrin (a non-heme iron-binding glycoprotein) with PEG of PEGylated liposomes, in order to achieve prolonged circulation and targeted drug delivery to solid tumors (Ishida et al., 2001a).

The effectiveness of transferrin-targeted PEGylated liposomes has (Qian et al., 2002) been described in colon and gastric cancer models (Iinuma et al., 2002; Ishida et al., 2001a). MBP-426 a novel oxaliplatin-encapsulated transferrin (Tf)-conjugated N-glutaryl phosphatidylethanolamine (NGPE) liposome has recently entered clinical trials for the treatment of advanced or metastatic solid tumors (Allen and Cullis, 2012).

Another *in vitro* study with doxorubicin-loaded transferrin-conjugated stealth liposomes (Tf-SL-DOX) showed enhanced intracellular uptake of the encapsulated DOX by HepG2 cells while results from *in vivo* tumor models reported that Tf-SL-DOX had similar pharmacokinetic behavior comparable to SL-DOX. Tf-SL-DOX administration led to targeted delivery and reduced DOX concentration in heart and kidney (Li et al., 2009). Thus, combinatorial strategy focusing on transferrin-mediated targeted delivery and stealth technology is a very promising approach having increased circulation time and reduced side effects on non-target organs. In addition anti-transferrin receptor single-chain antibody Fv fragment (scFv)-immunoliposomes for gene therapy have also been described lately, which amalgamates transferrin mediated targeted delivery with immuno-mediated targeting for effective delivery of gene (Xu et al., 2002).

The main challenge in effective brain glioma chemotherapy lays in the transport of drugs across the blood-brain barrier (BBB) and penetration of drugs into the tumor. In order to overcome this challenge second generation dual-targeting liposomes bearing daunorubicin have been developed conjugated with two different ligands p-aminophenyl-α-D-mannopyranoside (MAN) and transferrin, which successfully transports the drug across the BBB and specifically targets brain glioma (Ying et al., 2010).

3.6. Cationic liposomes

One of the most effective non-viral systems for oligonucleotide or gene delivery is the cationic lipid-based liposomes. The resulting complex of cationic lipid-based vesicles with oligonucleotides is termed lipoplex. Lipoplexes have been described to treat cancer efficiently (Table 6) and are reported to encapsulate high amount of nucleotides (Felgner and Ringold, 1989). Cationic liposome bearing paclitaxel (MBT-0206) showed selective uptake by angiogenic tumoral endothelial cells abundant in solid tumor and metastases (Immordino et al., 2006). EndoTAG^{TM}-1 (MediGene A.G., Martinsried, Germany) is currently undergoing Phase II clinical trial against advanced pancreatic cancer (Eichhorn et al., 2006; Schuch, 2005).

Recent research initiatives have reported lipoplexes to effectively transfect cells with DNA (Neves et al., 2009) or microRNAs (miRNAs) (Malone et al., 1989; Wu et al., 2011). Moreover, the transfection efficiency of cationic liposomes have been known to increase when their surface is tagged with a ligand that is recognized by a cell surface receptor through the initial binding of the ligand to the cell (Pirollo et al., 2000). Cationic liposomes target the anionic functional groups which line the tumor vasculature and ultimately help in arresting tumor angiogenesis (Figs. 6 and 7). Although, RNA interference (RNAi) therapeutics is an emerging novel approach against cancer, the key challenge lies in effective delivery to target tissues. The preclinical development and toxicological profiling of lipid nanoparticle (LNP)-formulated siRNA chemotherapeutic has thus been a major thrust area of investigation recently (Barros and Gollob, 2012; Ozpolat et al., 2010).

Combination of stealth and ligand targeted therapeutics with antisense delivery has proved to be a promising strategy.

Table 6
Lipoplexes undergoing clinical trials.

Product	Company	Description
SGT-53	SynerGene Therapeutics, Inc.	Liposome encapsulates plasmid DNA coding for p53 wild type gene
FANG^{TM} Vaccine	Gradalis, Inc.	Expresses rhGMCSF, bifunctional RNAi effector and bi-shRNAfurin
Pbi-shRNA STMN1 LP	Gradalis, Inc.	Encapsulates bifunctional short hairpin RNAs (shRNA) against human stathmin 1 (STMN1)
BP-100-1.01	Bio-Path Holdings, Inc.	Delivers antisense oligodeoxynucleotide (ODN) growth factor receptor-bound protein 2 (Grb2), with potential antineoplastic activity

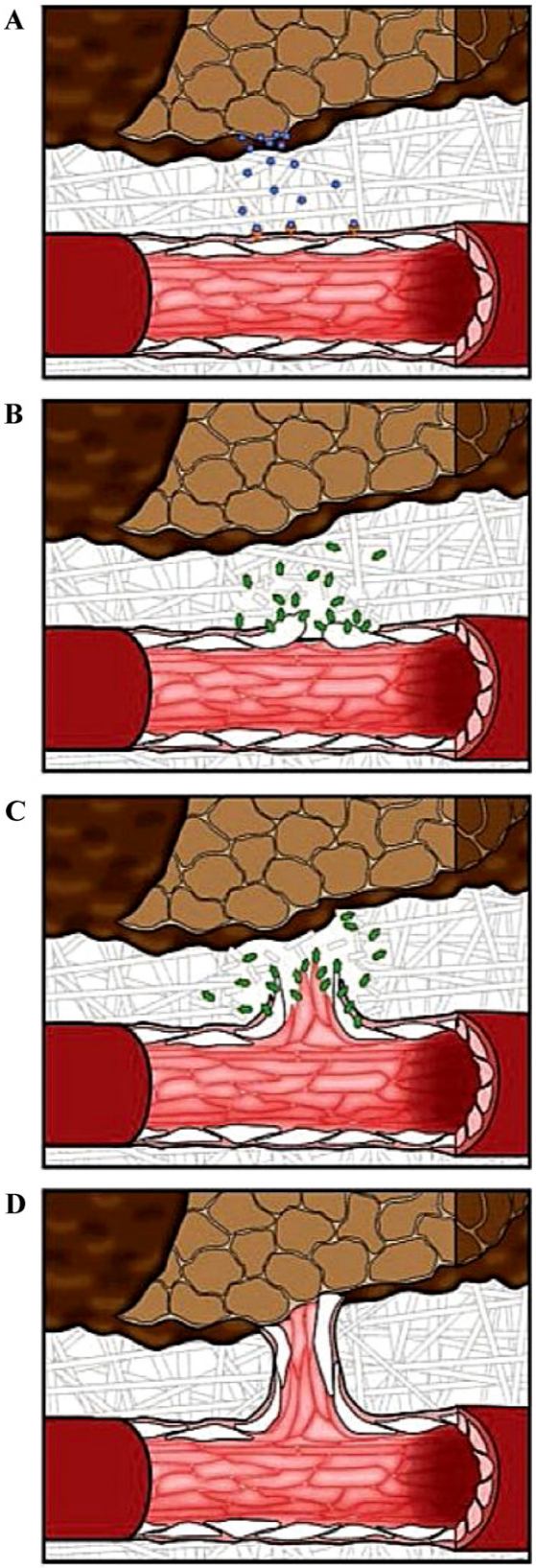

Fig. 6. Tumor angiogenesis: a step by step approach: An angiogenic stimulus is secreted by a developing tumor an a vessel sprouts in the direction of the stimulus (A), proteases begin to degrade the basement membrane (B), while endothelial cells migrate in the direction of the stimulus formed through the newly formed openings in the basement membrane (C), and a new vessel sprouts forms (D). [Reproduced with permission from Campbell et al. (2009)].

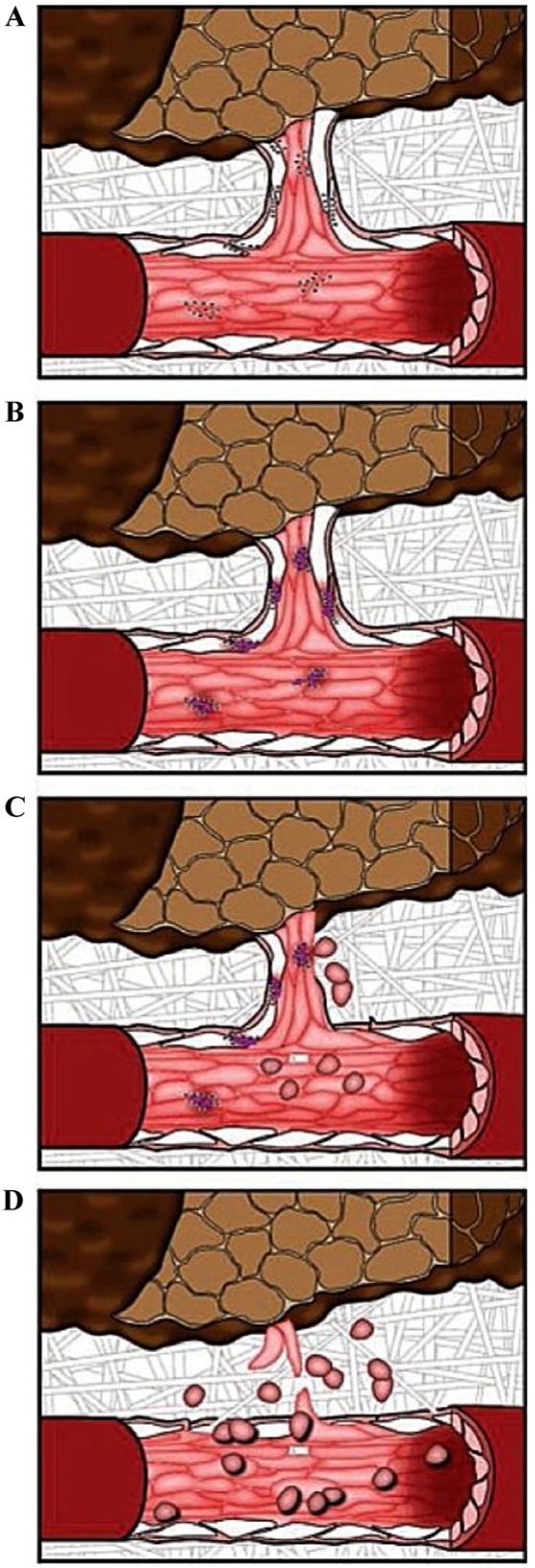

Fig. 7. Vascular targeting with cationic liposomal therapeutics: The tumor vasculature is lined with an overexpression of anionic functional groups (A), cationic liposomal therapeutics interact with tumor vessels (B), injury to the tumor microvasculature results in damage to the endothelial cells (C), and eventual loss of tumor vessel function results in the death of thousands of cancer cells owing to severe oxygen and nutrient deprivation (D). [Reproduced with permission from Campbell et al. (2009)].

Conjugating stealth liposomal technology with Tf-mediated delivery of drug has been described for the delivery of a phosphorothioate antisense oligodeoxyribonucleotide (ODN) (G3139, oblimerson sodium, or Genasense™) in leukemia cells *in vitro* for effective treatment *via* Bcl-2 regulation (Chiu et al., 2006). *In vivo* combination treatment of PEGylated siBcl-2-lipoplex and S-1(5-FU) pro-drug has reported to exhibit enhanced antineoplastic activity in a DLD-1 xenograft model (Nakamura et al., 2011).

3.7. Stimuli responsive liposomes

Engineered liposomes that provide therapeutic control of pathological states by an enhanced enrichment of therapeutic or diagnostic agents in diseased tissues have currently emerged as workhorses in nano-medicine (Fleige et al., 2012). Stimuli-responsive liposomes are active delivery vehicles that evolve with an external signal and are equipped with "load-and-release" modalities within their constituting units. The central operating principle lies in the fact that a specific cellular/extracellular stimulus of chemical, biochemical, or physical origin can modify the structural composition or conformation of the liposomes, thereby promoting release of the active species to specific biological environment (Calderon et al., 2010; Kost and Langer, 2001). The specificity allows liposomes to release the encapsulated cargo in a temporal or spatial pattern in response to particular pathological triggers (Ganta et al., 2008) present in the diseased tissues with substantially reduced side effects (Drummond et al., 2000). These liposomes mimic numerous feed-back controlled biological events prevailing in nature where the enrichment or absence of any physical, chemical or physico-chemical factors regulates a series of biochemical processes (Fleige et al., 2012).

The tumor microenvironment is characterized by oxygen depletion or hypoxia and certain aberrations in temperature and pH. These triggers are currently being widely exploited to design stimuli responsive liposomes for site-specific delivery of drugs.

3.7.1. pH-sensitive liposomes

The heterogeneity of tumor tissue is one of the major challenges in intratumoral drug delivery. Abnormalities in vessel and tumor microenvironment often result in reduced therapeutic efficiency and drug delivery (Jain, 2005). However, varied approaches and strategies are currently being applied to utilize the disordered tumor microenvironment to one's advantage for enhanced anti-neoplastic drug delivery. pH-sensitive liposomes hold great promise in cancer therapy because they are tailored to combat tumor, based on their distinctive hypoxic, acidic and nutrient impoverished microenvironment which arises due to the high metabolic rate, invasiveness and hyper-proliferating nature of the cancer cells (Cho et al., 2008). pH-sensitive liposomes are tailored with pH-sensitive components such as unsaturated lipid molecules like phosphatidylethanolamine (PE), amphiphilic molecules (cholesteryl hemisuccinate or oleic acid) (Cho et al., 2009) or polymers containing ionizable groups like amines or carboxylic acids (Banerjee et al., 2012a,b; Fleige et al., 2012).

Active targeting by pH responsive nano-liposomal carriers has been achieved by covalent coupling of different ligands to the liposome surface or to the distal end of PEG–lipid conjugates. H-$2K^k$ receptor (expressed in numerous types of tumor cells) (Wang and Huang, 1987), CD-19 (expressed on B-lymphoma cells) (Ishida et al., 2001b), CD3 (expressed by T-leukemia cells) (Turner et al., 2002) have been targeted by coupling mAb with second generation pH-sensitive liposomes. pH-sensitive liposomes also achieve folate-mediated active targeting by conjugating folic acid to the distal end of PEG molecules and have been reported recently to deliver anti-neoplastic drugs (Shi et al., 2002; Sudimack et al., 2002) as well as plasmid DNA (Chan et al., 2012; Reddy and Low, 2000) for enhanced cancer chemotherapy. However, the transfection efficiency of pH-sensitive vehicles is reported to be lower than cationic liposomes under the same experimental conditions (Legendre and Szoka, 1992).

3.7.2. Thermo-sensitive liposomes

A potential form of cancer therapy currently attracting enhanced interest involves the use of thermo-sensitive liposomes in combination with mild hyperthermia. Mild hyperthermia is considered as a clinically feasible approach in cancer therapy because of its reported therapeutic benefits. Moreover, hyperthermia has been described to be cytotoxic to tumor cells. (Dewhirst et al., 1997; Karino et al., 1988). The permissible range of applicable mild hyperthermia has been reported to be between 41 and 42 °C. Mild hyperthermia increases the micro-vascular permeability of tumor tissue which causes increased extravasation and accumulation of liposomes bearing the bioactive molecules (Karino et al., 1988; Kong et al., 2000; Koning et al., 2010; Ponce et al., 2006). Temperature-sensitive liposomes (TSL) were first formulated by Yatvin et al. (1978). Recent attractive strategies for the development of thermo-sensitive vesicles includes utilization of phospholipids having phase transition temperature (T_m) between 41 and 42 °C (Chiu et al., 2005) and undergoing gel-to-liquid crystalline transitions (Wagner and Vorauer-Uhl, 2011) or with leucine zipper sequence peptide which dissociates above its melting temperature (~40 °C) into a disordered conformation (Al-Ahmady et al., 2012).

Thermo-sensitive liposomes with long circulating properties have also been formulated using PEG (Li et al., 2010; Needham et al., 2000) or oligoglycerol-moieties (Lindner et al., 2004). The membrane permeability of TSLs has lately been enhanced by incorporating additional lipid compounds like lysolipid (Needham et al., 2000) or oligoglycerol-PG (Lindner et al., 2004). An interesting approach involving targeted temperature sensitive magnetic liposomes for thermo-chemotherapy has recently been reported which focusses on magnetic hyperthermia-triggered drug release (Pradhan et al., 2007, 2010; Shinkai et al., 1996). Recently FR-targeted thermo-sensitive magnetic liposomes serving dual role of drug targeting and magnetic hyperthermia-triggered drug release have also been reported (Pradhan et al., 2010). Stealth liposomal technology has been effectively combined with hyperthermia mediated release of chemotherapeutic drug in a recent report describing PEGylated cationic thermosensitive liposomes (CTSL) (Dicheva et al., 2012). This approach achieves dual-targeting by cationic lipids with temperature-triggered release (Dicheva et al., 2012).

Both *in vitro* and *in vivo* studies involving the delivery of anti-cancer drug encapsulated into thermo-sensitive liposomal formulations have been reported (Chiu et al., 2005; Tagami et al., 2011).

3.8. Liposomal vaccines

Cancer vaccines have generated a major interest since they achieve active specific immunotherapy (ASI). ASI involves the administration of an antigen to elicit an immune response against that antigen (Palmer et al., 2001). Moreover they have the potential to combat tumor as well as its metastasis. An ideal vaccine should preferably be biocompatible, storable with long shelf life and potentially be able to stimulate both humoral as well as cell-mediated immune systems.

Micro-organisms have been reported to be the causative agent of 10–20% tumor incidences (Lollini et al., 2006). Primary cancer prevention targets the reduction of infection with viruses like hepatitis B and human papilloma virus in order to reduce the incidence

of hepatocellular carcinoma (Chang et al., 2000) and cervical carcinoma (Goymer, 2005; Villa et al., 2005) respectively.

Liposomal peptide vaccines have been described to activate dendritic cells which elicit anti-tumor immune responses (Ludewig et al., 2000). Human synthetic peptide MUC1 has been reported to elicit strong antigen-specific T-response when incorporated in or attached to the surface of liposomes (Guan et al., 1998). Oncothyreon Inc. is currently sponsoring Phase I Study of ONT-10, a liposomal MUC1 cancer vaccine, in patients with solid tumors (Bradbury and Shepherd, 2008). Phase IIB Trial of BLP25 liposome vaccine (L-BLP25) is being currently conducted against Stage IIIB and IV Non-Small-Cell Lung Cancer (Butts et al., 2005).

3.9. Virosomes

In 1975 Almeida et al. first reported liposomal formulations which have viral envelope proteins integrated into the lipid membrane of vesicles known as virosomes (Almeida et al., 1975). The fusogenic viral envelope proteins are either anchored in the liposomal lipid membrane or attached to the liposomal surface (Kaneda, 2000). Virosomes provide a platform for the effective delivery of antigens, nucleic acids and cytotoxic drugs (Gluck et al., 2004; Kaneda, 2000; Moser et al., 2003; Sarkar et al., 2002).

Although virosomes have been reported to have anti-tumorigenic applications, there are recent reports of the use of virosomes in preclinical/clinical trials or approved formulations for immunization against influenza (Intranasal Virosomal Influenza Vaccine) (Gluck et al., 1999; Marchisio et al., 2002), malaria (AMA49-C1 and FFM ME-TRAP + PEV3A) (Cech et al., 2011; Okitsu et al., 2007; Thompson et al., 2008), HIV (HIV-1 gp41 subunit virosomes) (Bomsel et al., 2011) and viral bronchiolitis (Virosomal Respiratory Syncytial Virus Vaccine) (Kamphuis et al., 2012). Virosomes have also been utilized in vaccine based adjuvant therapy against tuberculosis (Tuberculosis Subunit Vaccination Ag85B-ESAT-6/CAF01 where ESAT-6 is antigenic target and CAF01 is cationic adjuvant formulation) (Christensen et al., 2010; Lindenstrom et al., 2009), HIV (applying novel cationic adjuvant CAF01) (Fomsgaard et al., 2011; Gram et al., 2009) or influenza (Inflexal® V) (Herzog et al., 2009).

'Vir' (reconstituted fusion-active viral envelopes) delivers cytotoxic drugs by binding and penetrating tumor cells. rNeu-overexpressing breast tumors have been reported to be inhibited by Fab fragments of antirat Neu (anti-rNeu) mAb-conjugated to Vir (Waelti et al., 2002).

3.10. Theranostic liposomes

Theranostic liposomes are currently being extensively studied for their dual utility in nanomedicine. The primary evolution of theranostic liposomes took place in order to achieve synchronous diagnosis and treatment. The liposomal bilayer structure facilitates the compartmentalization of imaging and therapeutic agents. Currently theranostic liposomes are either being designed bearing non-invasive multimodality imaging agents like fluorescent probes, radio-isotopes and nanoparticle like magnetic nanoparicles or quantum dots (QDs). Diagnostics by theranostic liposomes have been reportedly done by utilizing (i) magnetic resonance (MR) imaging (Negussie et al., 2011), (ii) positron emission tomography (PET) imaging (Petersen et al., 2012), (iii) single-photon emission computed tomography (SPECT) (Li et al., 2011) and iv) near infrared resonance (NIR) fluorescent imaging (Li et al., 2012).

3.10.1. MRI contrast agents

Liposome loaded with MRI contrast agents were first described in the 1980s (Tilcock et al., 1989). Co-loading drug and MR contrast agent using liposomes have been reported for enhanced therapeutics, drug dosage manipulation and imaging (Ponce et al., 2007; Viglianti et al., 2006). Moreover, magnetic resonance image guided drug release is also possible in the case of temperature-sensitive liposomes (de Smet et al., 2010; Viglianti et al., 2004).

3.10.2. Radio-isotopes

Gadolinium (Gd)-based contrast agents have been reported to be loaded in the liposome aqueous core (Ghaghada et al., 2008) or conjugated to their membrane (Laurent et al., 2008; Mulder et al., 2004) or both (Ghaghada et al., 2009). Theranostic drug loaded liposomes could also be stably radiolabeled with ^{99m}Tc (Li et al., 2011) or ^{64}Cu (Petersen et al., 2012) for single-photon emission computed tomography (SPECT) or positron emission tomography (PET) imaging, respectively.

3.10.3. Magnetoliposomes/ferrosomes

Liposomes bearing magnetic nanoparticles are now being preferred for drug delivery because of their structural and pharmacokinetic advantages for drug delivery (Bogdanov et al., 1994; Bulte et al., 1999; Di Paolo et al., 2009; Zhang et al., 2005). The advantages of theranostic strategy *via* ferri-liposomes include non-invasiveness (Mikhaylov et al., 2011), deeper penetration (Diou et al., 2012), improved sensitivity (Diou et al., 2012) and favorable biodistribution (Zhang et al., 2005). There has been a recent report which describes the enhanced efficacy of ferri-liposomes bearing cathepsin inhibitor JPM-565 against peri-tumoral region of mouse breast cancer (Mikhaylov et al., 2011). Combinatorial approaches in cancer therapeutics using magnetoliposomes have recently emerged which simultaneously involve hyperthermia and chemotherapy (Kulshrestha et al., 2012) or folate targeting and enhanced drug delivery (Bothun et al., 2011).

3.10.4. Quantum dots

QDs facilitate multiplex imaging under a single light source since they have narrow band emissions and large ultraviolet absorption spectra (Ozkan, 2004; Wang et al., 2010). Moreover, recent advances report semi-conductor-based QDs which have successfully overcome the limitations of organic fluorophores (Wang et al., 2010). Drug-conjugated QDs have their own limitations which include longer internalization time (Ozkan, 2004) and limited drug molecule loading on the surface by covalent and ionic bonding. Hence, liposomes are being designed as a carrier of choice bearing both QDs and drugs for therapeutic and diagnostic applications. Recently targeted co-delivery of QDs and drugs for enhanced therapeutic efficacy and sustained imaging have also been reported in *in vitro* studies involving breast cancer cell lines (Muthu et al., 2012; Schroeder et al., 2007).

4. Conclusion

The evolution of new generation pharmaceutical liposomes has marked a new era in drug delivery systems in cancer therapeutics. Liposomes are versatile drug delivery systems which can be designed and modified in order to enhance the effectivity of the therapeutic drug. The wide array of liposomal drug formulations approved and undergoing clinical trials for cancer therapeutics (Table 3) points to the translation of liposomes from an object of research to preferred pharmaceutical carrier for clinical applications. Other important liposomal formulations approved for use in applications other than tumorigenic therapy include-AmBisome (fungal infections and Leishmaniasis), Amphotec (invasive aspergillosis), Abelcet (aspergillosis), DepoDur (pain following surgery), Diprivan (anesthesia), Estrasorb (menopausal therapy), Visudyne (wet macular degeneration) (Allen and Cullis, 2012). A better understanding of liposomal-drug interaction with biological system will facilitate the emergence of a novel class of

anticancer therapeutics with improved efficacy and safety. The vast array of liposome based therapeutics in pre-clinical/clinical trials and marketed formulations provide a new paradigm in cancer nano-therapeutics with focus towards diagnosis, treatment and prevention.

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References

Agrawal, V., Paul, M.K., Mukhopadhyay, A.K., 2005. 6-mercaptopurine and daunorubicin double drug liposomes – preparation, drug–drug interaction and characterization. J. Liposome Res. 15, 141–155.

Al-Ahmady, Z.S., Al-Jamal, W.T., Bossche, J.V., Bui, T.T., Drake, A.F., Mason, A.J., Kostarelos, K., 2012. Lipid-Peptide Vesicle Nanoscale Hybrids for Triggered Drug Release by Mild Hyperthermia in Vitro and in Vivo. Acs Nano 6 (10), 9335–9346.

Alberts, D.S., Muggia, F.M., Carmichael, J., Winer, E.P., Jahanzeb, M., Venook, A.P., Skubitz, K.M., Rivera, E., Sparano, J.A., Dibella, N.J., Stewart, S.J., Kavanagh, J.J., Gabizon, A.A., 2004. Efficacy and safety of liposomal anthracyclines in phase I/II clinical trials. Semin. Oncol. 31, 53–90.

Allen, T.M., 2002. Ligand-targeted therapeutics in anticancer therapy. Nat. Rev. Cancer 2, 750–763.

Allen, T.M., Cullis, P.R., 2012. Liposomal drug delivery systems: From concept to clinical applications. Adv. Drug Deliv. Rev. 65, 36–48.

Allen, T.M., Hansen, C., Martin, F., Redemann, C., Yauyoung, A., 1991. Liposomes containing synthetic lipid derivatives of poly(ethylene glycol) show prolonged circulation half-lives in vivo. Biochim. Biophys. Acta 1066, 29–36.

Almeida, J.D., Edwards, D.C., Brand, C.M., Heath, T.D., 1975. Formation of virosomes from influenza subunits and liposomes. Lancet 2, 899–901.

Banerjee, S., Pal, T.K., Guha, S.K., 2012a. Probing molecular interactions of poly(styrene-co-maleic acid) with lipid matrix models to interpret the therapeutic potential of the co-polymer. Biochim. Biophys. Acta 1818, 537–550.

Banerjee, S., Sen, K., Pal, T.K., Guha, S.K., 2012b. Poly(styrene-co-maleic acid)-based pH-sensitive liposomes mediate cytosolic delivery of drugs for enhanced cancer chemotherapy. Int. J. Pharm. 436, 786–797.

Bangham, A.D., Standish, M.M., Watkins, J.C., 1965a. Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 13, 238–252.

Bangham, A.D., Standish, M.M., Weissman, G., 1965b. Action of steroids and streptolysin S on permeability of phospholipid structures to cations. J. Mol. Biol. 13, 253–259.

Barenholz, Y., 2001. Liposome application: problems and prospects. Curr. Opin. Colloid Interface Sci. 6, 66–77.

Barenholz, Y., 2012. Doxil (R)—The first FDA-approved nano-drug: Lessons learned. J. Control. Release 160, 117–134.

Barros, S.A., Gollob, J.A., 2012. Toxicity profile of RNAi nanomedicines. Adv. Drug Deliv. Rev.

Batist, G., Ramakrishnan, G., Rao, C.S., Chandrasekharan, A., Gutheil, J., Guthrie, T., Shah, P., Khojasteh, A., Nair, M.K., Hoelzer, K., Tkaczuk, K., Park, Y.C., Lee, L.W., Grp, M.S., 2001. Reduced cardiotoxicity and preserved antitumor efficacy of liposome-encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer. J. Clin. Oncol. 19, 1444–1454.

Bellocq, N.C., Pun, S.H., Jensen, G.S., Davis, M.E., 2003. Transferrin-containing, cyclodextrin polymer-based particles for tumor-targeted gene delivery. Bioconjug. Chem. 14, 1122–1132.

Black, C.D.V., Gregoriadis, G., 1976. Interaction of Liposomes with Blood-Plasma Proteins. Biochem. Soc. Trans. 4, 253–256.

Blume, G., Cevc, G., 1993. Molecular mechanism of the lipid vesicle longevity in vivo. Biochim. Biophys. Acta 1146, 157–168.

Boehlke, L., Winter, J.N., 2006. Sphingomyelin/cholesterol liposomal vincristine: a new formulation for an old drug. Expert. Opin. Biol. Ther. 6, 409–415.

Bogdanov, A.A., Martin, C., Weissleder, R., Brady, T.J., 1994. Trapping of dextran-coated colloids in liposomes by transient binding to aminophospholipid—preparation of ferrosomes. Bba Biomembr. 1193, 212–218.

Boman, N.L., Tron, V.A., Bally, M.B., Cullis, P.R., 1996. Vincristine-induced dermal toxicity is significantly reduced when the drug is given in liposomes. Cancer Chemother. Pharmacol. 37, 351–355.

Bomsel, M., Tudor, D., Drillet, A.S., Alfsen, A., Ganor, Y., Roger, M.G., Mouz, N., Amacker, M., Chalifour, A., Diomede, L., Devillier, G., Cong, Z., Wei, Q.A., Gao, H., Qin, C.A., Yang, G.B., Zurbriggen, R., Lopalco, L., Fleury, S., 2011. Immunization with HIV-1 gp41 subunit virosomes induces mucosal antibodies protecting nonhuman primates against vaginal SHIV challenges. Immunity 34, 269–280.

Bothun, G.D., Lelis, A., Chen, Y., Scully, K., Anderson, L.E., Stoner, M.A., 2011. Multicomponent folate-targeted magnetoliposomes: design, characterization, and cellular uptake. Nanomed. UK 7, 797–805.

Bradbury, P.A., Shepherd, F.A., 2008. Immunotherapy for lung cancer. J. Thorac. Oncol. 3, S164–S170.

Brody, E.N., Gold, L., 2000. Aptamers as therapeutic and diagnostic agents. J. Biotechnol. 74, 5–13.

Bulte, J.W.M., de Cuyper, M., Despres, D., Frank, J.A., 1999. Short- vs. long-circulating magnetoliposomes as bone marrow-seeking MR contrast agents. J. Magn. Reson. Imaging 9, 329–335.

Butts, C., Murray, N., Maksymiuk, A., Goss, G., Marshall, E., Soulieres, D., Cormier, Y., Ellis, P., Price, A., Sawhney, R., Davis, M., Mansi, J., Smith, C., Vergidis, D., MacNeil, M., Palmer, M., 2005. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. J. Clin. Oncol. 23, 6674–6681.

Cairns, R., Papandreou, I., Denko, N., 2006. Overcoming physiologic barriers to cancer treatment by molecularly targeting the tumor microenvironment. Mol. Cancer Res. 4, 61–70.

Calderon, M., Quadir, M.A., Strumia, M., Haag, R., 2010. Functional dendritic polymer architectures as stimuli-responsive nanocarriers. Biochimie 92, 1242–1251.

Campbell, R.B., Ying, B., Kuesters, G.M., Hemphill, R., 2009. Fighting cancer: from the bench to bedside using second generation cationic liposomal therapeutics. J. Pharma. Sci. 98, 411–429.

Cech, P.G., Aebi, T., Abdallah, M.S., Mpina, M., Machunda, E.B., Westerfeld, N., Stoffel, S.A., Zurbriggen, R., Plueschke, G., Tanner, M., Daubenberger, C., Genton, B., Abdulla, S., 2011. Virosome-formulated *Plasmodium falciparum* AMA-1 & CSP derived peptides as malaria vaccine: randomized phase 1b trial in semi-immune adults & children. PloS One 6.

Chan, C.L., Majzoub, R.N., Shirazi, R.S., Ewert, K.K., Chen, Y.J., Liang, K.S., Safinya, C.R., 2012. Endosomal escape and transfection efficiency of PEGylated cationic liposome-DNA complexes prepared with an acid-labile PEG-lipid. Biomaterials 33, 4928–4935.

Chang, D.K., Chiu, C.Y., Kuo, S.Y., Lin, W.C., Lo, A., Wang, Y.P., Li, P.C., Wu, H.C., 2009. Antiangiogenic targeting liposomes increase therapeutic efficacy for solid tumors. J. Biol. Chem. 284, 12905–12916.

Chang, M.H., Shau, W.Y., Chen, C.J., Wu, T.C., Kong, M.S., Liang, D.C., Hsu, H.M., Chen, H.L., Hsu, H.Y., Chen, D.S., TCHS Group, 2000. Hepatitis B vaccination and hepatocellular carcinoma rates in boys and girls. J. Am. Med. Assoc. 284, 3040–3042.

Chiu, G.N.C., Abraham, S.A., Ickenstein, L.M., Ng, R., Karlsson, G., Edwards, K., Wasan, E.K., Bally, M.B., 2005. Encapsulation of doxorubicin into thermosensitive liposomes via complexation with the transition metal manganese. J. Control. Release 104, 271–288.

Chiu, S.J., Liu, S., Perrotti, D., Marcucci, G., Lee, R.J., 2006. Efficient delivery of a Bcl-2-specific antisense oligodeoxyribonucleotide (G3139) via transferrin receptor-targeted liposomes. J. Control. Release 112, 199–207.

Cho, E.C., Lim, H.J., Kim, H.J., Son, E.D., Choi, H.J., Park, J.H., Kim, J.W., Kim, J., 2009. Role of pH-sensitive polymer-liposome complex in enhancing cellular uptake of biologically active drugs. Mater. Sci. Eng. C Biol. Sci. 29, 774–778.

Cho, K., Wang, X., Nie, S., Chen, Z.G., Shin, D.M., 2008. Therapeutic nanoparticles for drug delivery in cancer. Clin. Cancer Res. 14, 1310–1316.

Chonn, A., Cullis, P.R., Devine, D.V., 1991. The role of surface-charge in the activation of the classical and alternative pathways of complement by liposomes. J. Immunol. 146, 4234–4241.

Chonn, A., Semple, S.C., Cullis, P.R., 1995. Beta 2 glycoprotein I is a major protein associated with very rapidly cleared liposomes in vivo, suggesting a significant role in the immune clearance of non-self particles. J. Biol. Chem. 270, 25845–25849.

Christensen, D., Lindenstrom, T., van de Wijdeven, G., Andersen, P., Agger, E.M., 2010. Syringe free vaccination with CAF01 adjuvated Ag85B-ESAT-6 in bioneedles provides strong and prolonged protection against tuberculosis. PloS One 5.

Colomer, R., 2005. What is the best schedule for administration of gemcitabine-taxane? Cancer Treat. Rev. 31, S23–S28.

Cooper, C.R., Chay, C.H., Pienta, K.J., 2002. The role of alpha(v)beta(3) in prostate cancer progression. Neoplasia 4, 191–194.

Cosco, D., Paolino, D., Cilurzo, F., Casale, F., Fresta, M., 2012. Gemcitabine and tamoxifen-loaded liposomes as multidrug carriers for the treatment of breast cancer diseases. Int. J. Pharm. 422, 229–237.

Crom, W.R., Degraaf, S.S.N., Synold, T., Uges, D.R.A., Bloemhof, H., Rivera, G., Christensen, M.L., Mahmoud, H., Evans, W.E., 1994. Pharmacokinetics of vincristine in children and adolescents with acute lymphocytic-leukemia. J. Pediatr. 125, 642–649.

Dams, E.T.M., Laverman, P., Oyen, W.J.G., Storm, G., Scherphof, G.L., Van der Meer, J.W.M., Corstens, F.H.M., Boerman, O.C., 2000. Accelerated blood clearance and altered biodistribution of repeated injections of sterically stabilized liposomes. J. Pharmacol. Exp. Ther. 292, 1071–1079.

Danhier, F., Breton, A.L., Preat, V., 2012. RGD-based strategies to target alpha(v) beta(3) integrin in cancer therapy and diagnosis. Mol. Pharma. 9, 2961–2973.

Danhier, F., Feron, O., Preat, V., 2010. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J. Control. Release 148, 135–146.

de Smet, M., Langereis, S., van den Bosch, S., Grull, H., 2010. Temperature-sensitive liposomes for doxorubicin delivery under MRI guidance. J. Control. Release 143, 120–127.

Deok Kong, S., Sartor, M., Jack Hu, C.M., Zhang, W., Zhang, L., Jin, S., 2012. Magnetic field activated lipid-polymer hybrid nanoparticles for stimuli-responsive drug release. Acta Biomater.

Desgrosellier, J.S., Cheresh, D.A., 2010. Integrins in cancer: biological implications and therapeutic opportunities. Nat. Rev. Cancer 10, 9–22.
Dewhirst, M.W., Prosnitz, L., Thrall, D., Prescott, D., Clegg, S., Charles, C., MacFall, J., Rosner, G., Samulski, T., Gillette, E., LaRue, S., 1997. Hyperthermic treatment of malignant diseases: Current status and a view toward the future. Semin. Oncol. 24, 616–625.
Di Paolo, D., Loi, M., Pastorino, F., Brignole, C., Marimpietri, D., Becherini, P., Caffa, I., Zorzoli, A., Longhi, R., Gagliani, C., Tacchetti, C., Corti, A., Allen, T.M., Ponzoni, M., Pagnan, G., 2009. Liposome-mediated therapy of neuroblastoma. Methods Enzymol. 465, 225–249.
Dicheva, B.M., Hagen, T.L., Li, L., Schipper, D., Seynhaeve, A.L., Rhoon, G.C., Eggermont, A.M., Lindner, L.H., Koning, G.A., 2012. Cationic thermosensitive liposomes: a novel dual targeted heat-triggered drug delivery approach for endothelial and tumor cells. Nano Lett.
Dicko, A., Mayer, L.D., Tardi, P.G., 2010. Use of nanoscale delivery systems to maintain synergistic drug ratios in vivo. Exp. Opin. Drug Deliv. 7, 1329–1341.
Diou, O., Tsapis, N., Fattal, E., 2012. Targeted nanotheranostics for personalized cancer therapy. Exp. Opin. Drug Deliv. 9, 1475–1487.
Drummond, D.C., Meyer, O., Hong, K.L., Kirpotin, D.B., Papahadjopoulos, D., 1999. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. Pharmacol. Rev. 51, 691–743.
Drummond, D.C., Noble, C.O., Guo, Z.X., Hong, K., Park, J.W., Kirpotin, D.B., 2006. Development of a highly active nanoliposomal irinotecan using a novel intraliposomal stabilization strategy. Cancer Res. 66, 3271–3277.
Drummond, D.C., Zignani, M., Leroux, J.C., 2000. Current status of pH-sensitive liposomes in drug delivery. Prog. Lipid Res. 39, 409–460.
Duzgunes, N., Gregoriadis, G., 2005. Introduction: the origins of liposomes: Alec Bangham at Babraham. Liposomes 391 (Pt E), 1–3.
Eichhorn, M.E., Becker, S., Strieth, S., Werner, A., Sauer, B., Teifel, M., Ruhstorfer, H., Michaelis, U., Griebel, J., Brix, G., Jauch, K.W., Dellian, M., 2006. Paclitaxel encapsulated in cationic lipid complexes (MBT-0206) impairs functional tumor vascular properties as detected by dynamic contrast enhanced magnetic resonance imaging. Cancer Biol. Ther. 5, 89–96.
Falcone, D.J., 1986a. Fluorescent opsonization assay—binding of plasma fibronectin to fibrin-derivatized fluorescent particles does not enhance their uptake by macrophages. J. Leukoc. Biol. 39, 1–12.
Falcone, D.J., 1986b. Fluorescent opsonization assay: binding of plasma fibronectin to fibrin-derivatized fluorescent particles does not enhance their uptake by macrophages. J. Leukoc. Biol. 39, 1–12.
Farrell, N.P., 2011. Platinum formulations as anticancer drugs clinical and pre-clinical studies. Curr. Top. Med. Chem. 11, 2623–2631.
Fasol, U., Frost, A., Buchert, M., Arends, J., Fiedler, U., Schan, D., Scheuenpflug, J., Mross, K., 2012. Vascular and pharmacokinetic effects of EndoTAG-1 in patients with advanced cancer and liver metastasis. Ann. Oncol. 23, 1030–1036.
Felgner, P.L., Ringold, G.M., 1989. Cationic liposome-mediated transfection. Nature 337, 387–388.
Fleige, E., Quadir, M.A., Haag, R., 2012. Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: Concepts and applications. Adv. Drug Deliv. Rev. 64, 866–884.
Floege, J., Ostendorf, T., Janssen, U., Burg, M., Radeke, H.H., Vargeese, C., Gill, S.C., Green, L.S., Janjic, N., 1999. Novel approach to specific growth factor inhibition in vivo—Antagonism of platelet-derived growth factor in glomerulonephritis by aptamers. Am. J. Pathol. 154, 169–179.
Fomsgaard, A., Karlsson, I., Gram, G., Schou, C., Tang, S., Bang, P., Kromann, I., Andersen, P., Andreasen, L.V., 2011. Development and preclinical safety evaluation of a new therapeutic HIV-1 vaccine based on 18 T-cell minimal epitope peptides applying a novel cationic adjuvant CAF01. Vaccine 29, 7067–7074.
Forssen, E.A., Tokes, Z.A., 1981. Use of anionic liposomes for the reduction of chronic doxorubicin-induced cardiotoxicity. Proc. Natl. Acad. Sci. Biol. 78, 1873–1877.
Gabizon, A., Horowitz, A.T., Goren, D., Tzemach, D., Mandelbaum-Shavit, F., Qazen, M.M., Zalipsky, S., 1999. Targeting folate receptor with folate linked to extremities of poly(ethylene glycol)-grafted liposomes: in vitro studies. Bioconjug. Chem. 10, 289–298.
Gabizon, A., Horowitz, A.T., Goren, D., Tzemach, D., Shmeeda, H., Zalipsky, S., 2003. In vivo fate of folate-targeted polyethylene-glycol liposomes in tumor-bearing mice. Clin. Cancer Res. 9, 6551–6559.
Ganta, S., Devalapally, H., Shahiwala, A., Amiji, M., 2008. A review of stimuli-responsive nanocarriers for drug and gene delivery. J. Control. Release 126, 187–204.
Ghaghada, K., Hawley, C., Kawaji, K., Annapragada, A., Mukundan, S., 2008. T1 relaxivity of core-encapsulated gadolinium liposomal contrast agents—Effect of liposome size and internal gadolinium concentration. Acad. Radiol. 15, 1259–1263.
Ghaghada, K.B., Ravoori, M., Sabapathy, D., Bankson, J., Kundra, V., Annapragada, A., 2009. New dual mode gadolinium nanoparticle contrast agent for magnetic resonance imaging. PloS One 4.
Gluck, R., Moser, C., Metcalfe, I.C., 2004. Influenza virosomes as an efficient system for adjuvanted vaccine delivery. Exp. Opin. Biol. Ther. 4, 1139–1145.
Gluck, U., Gebbers, J.O., Gluck, R., 1999. Phase 1 evaluation of intranasal virosomal influenza vaccine with and without *Escherichia coli* heat-labile toxin in adult volunteers. J. Virol. 73, 7780–7786.
Goren, D., Horowitz, A.T., Zalipsky, S., Woodle, M.C., Yarden, Y., Gabizon, A., 1996. Targeting of stealth liposomes to erbB-2 (Her/2) receptor: in vitro and in vivo studies. Br. J. Cancer 74, 1749–1756.
Goyal, P., Goyal, K., Vijaya Kumar, S.G., Singh, A., Katare, O.P., Mishra, D.N., 2005. Liposomal drug delivery systems—clinical applications. Acta Pharm. 55, 1–25.
Goymer, P., 2005. In the news – Gardasil – the perfect guard? Nat. Rev. Cancer 5, 840.
Gram, G.J., Karlsson, I., Agger, E.M., Andersen, P., Fomsgaard, A., 2009. A novel liposome-based adjuvant CAF01 for induction of CD8(+) Cytotoxic T-Lymphocytes (CTL) to HIV-1 Minimal CTL Peptides in HLA-A*0201 Transgenic Mice. PloS One 4.
Gregoria, G., Wills, E.J., Swain, C.P., Tavill, A.S., 1974. Drug-carrier potential of liposomes in cancer chemotherapy. Lancet 1, 1313–1316.
Gregoriadis, G., 1976. Carrier potential of liposomes in biology and medicine. New Engl. J. Med. 1, 704–710.
Guan, H.H., Budzynski, W., Koganty, R.R., Krantz, M.J., Reddish, M.A., Rogers, J.A., Longenecker, B.M., Samuel, J., 1998. Liposomal formulations of synthetic MUC1 peptides: Effects of encapsulation versus surface display of peptides on immune responses. Bioconjug. Chem. 9, 451–458.
Gurley, B.J., 2011. Emerging Technologies for Improving Phytochemical Bioavailability: Benefits and Risks. Clin. Pharmacol. Ther. 89, 915–919.
Hamada, I., Hunter, A.C., Szebeni, J., Moghimi, S.M., 2008. Poly(ethylene glycol)s generate complement activation products in human serum through increased alternative pathway turnover and a MASP-2-dependent process. Mol. Immunol. 46, 225–232.
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.
Hau, P., Fabel, K., Baumgart, U., Rummele, P., Grauer, O., Bock, A., Dietmaier, C., Dietmaier, W., Dietrich, J., Dudel, C., Hubner, F., Jauch, T., Drechsel, E., Kleiter, I., Wismeth, G., Zellner, A., Brawanski, A., Steinbrecher, A., Marienhagen, J., Bogdahn, U., 2004. Pegylated liposomal doxorubicin-efficacy in patients with recurrent high-grade glioma. Cancer 100, 1199–1207.
Heath, T.D., Montgomery, J.A., Piper, J.R., Papahadjopoulos, D., 1983. Antibody-targeted liposomes: increase in specific toxicity of methotrexate-gamma-aspartate. Proc. Natl. Acad. Sci. U. S. A. 80, 1377–1381.
Herbert, V., Larrabee, A.R., Buchanan, J.M., 1962. Studies on the identification of a folate compound of human serum. J. Clin. Invest. 41, 1134–1138.
Herzog, C., Hartmann, K., Kunzi, V., Kursteiner, O., Mischler, R., Lazar, H., Gluck, R., 2009. Eleven years of Inflexal (R) V-a virosomal adjuvanted influenza vaccine. Vaccine 27, 4381–4387.
Hofheinz, R.D., Gnad-Vogt, S.U., Beyer, U., Hochhaus, A., 2005. Liposomal encapsulated anti-cancer drugs. Anti-Cancer Drug 16, 691–707.
Iinuma, H., Maruyama, K., Okinaga, K., Sasaki, K., Sekine, T., Ishida, S., Ogiwara, N., Johkura, K., Yonemura, Y., 2002. Intracellular targeting therapy of cisplatin-encapsulated transferrin-polyethylene glycol liposome on peritoneal dissemination of gastric cancer. Int. J. Cancer 99, 130–137.
Immordino, M.L., Dosio, F., Cattel, L., 2006. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. Int. J. Nanomed. 1, 297–315.
Ishida, O., Maruyama, K., Tanahashi, H., Iwatsuru, M., Sasaki, K., Eriguchi, M., Yanagie, H., 2001a. Liposomes bearing polyethyleneglycol-coupled transferrin with intracellular targeting property to the solid tumors in vivo. Pharm. Res. Dordr. 18, 1042–1048.
Ishida, T., Harashima, H., Kiwada, H., 2002. Liposome clearance. Biosci. Rep. 22, 197–224.
Ishida, T., Iden, D.L., Allen, T.M., 1999. A combinatorial approach to producing sterically stabilized (Stealth) immunoliposomal drugs. FEBS Lett. 460, 129–133.
Ishida, T., Kirchmeier, M.J., Moase, E.H., Zalipsky, S., Allen, T.M., 2001b. Targeted delivery and triggered release of liposomal doxorubicin enhances cytotoxicity against human B lymphoma cells. Bba Biomembr. 1515, 144–158.
Ishida, T., Atobe, K., Wang, X.Y., Kiwada, H., 2006a. Accelerated blood clearance of PEGylated liposomes upon repeated injections: Effect of doxorubicin-encapsulation and high-dose first injection. J. Control Release 115, 251–258.
Ishida, T., Harada, M., Wang, X.Y., Ichihara, M., Irimura, K., Kiwada, H., 2005. Accelerated blood clearance of PEGylated liposomes following preceding liposome injection: Effects of lipid dose and PEG surface-density and chain length of the first-dose liposomes. J. Control Release 105, 305–317.
Ishida, T., Ichihara, M., Wang, X., Yamamoto, K., Kimura, J., Majima, E., Kiwada, H., 2006b. Injection of PEGylated liposomes in rats elicits PEG-specific IgM, which is responsible for rapid elimination of a second dose of PEGylated liposomes. J. Control Release 112, 15–25.
Ishida, T., Ichikawa, T., Ichihara, M., Sadzuka, Y., Kiwada, H., 2004. Effect of the physicochemical properties of initially injected liposomes on the clearance of subsequently injected PEGylated liposomes in mice. J. Control Release 95, 403–412.
Ishida, T., Maeda, R., Ichihara, M., Irimura, K., Kiwada, H., 2003a. Accelerated clearance of PEGylated liposomes in rats after repeated injections. J. Control Release 88, 35–42.
Ishida, T., Masuda, K., Ichikawa, T., Ichihara, M., Irimura, K., Kiwada, H., 2003b. Accelerated clearance of a second injection of PEGylated liposomes in mice. Int. J. Pharm. 255, 167–174.
Jackson, D.V., Paschold, E.H., Spurr, C.L., Muss, H.B., Richards, F., Cooper, M.R., White, D.R., Stuart, J.J., Hopkins, J.O., Rich, R., Wells, H.B., 1984. Treatment of advanced non-hodgkins lymphoma with vincristine infusion. Cancer 53, 2601–2606.
Jain, R.K., 1987. Transport of molecules in the tumor interstitium—a review. Cancer Res. 47, 3039–3051.
Jain, R.K., 1994. Barriers to drug-delivery in solid tumors. Sci. Am. 271, 58–65.
Jain, R.K., 2005. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 307, 58–62.

Juliano, R.L., Daoud, S.S., 1990. Liposomes as a delivery system for membrane-active antitumor drugs. J. Control. Release 11, 225–232.
Juliano, R.L., Mccullough, H.N., 1980. Controlled delivery of an anti-tumor drug—localized action of liposome encapsulated cytosine-arabinoside administered via the respiratory system. J. Pharmacol. Exp. Ther. 214, 381–387.
Juliano, R.L., Stamp, D., 1976. Lectin-mediated attachment of glycoprotein-bearing liposomes to cells. Nature 261, 235–238.
Kamphuis, T., Meijerhof, T., Stegmann, T., Lederhofer, J., Wilschut, J., de Haan, A., 2012. Immunogenicity and protective capacity of a virosomal respiratory syncytial virus vaccine adjuvanted with monophosphoryl lipid A in Mice. PloS One 7.
Kaneda, Y., 2000. Virosomes: evolution of the liposome as a targeted drug delivery system. Adv. Drug Deliv. Rev. 43, 197–205.
Karino, T., Koga, S., Maeta, M., 1988. Experimental studies of the effects of local hyperthermia on blood-flow, oxygen-pressure and pH in tumors. Jpn. J. Surg. 18, 276–283.
Katsaros, D., Oletti, M.V., de la Longrais, I.A.R., Ferrero, A., Celano, A., Fracchioli, S., Donadio, M., Passera, R., Cattel, L., Bumma, C., 2005. Clinical and pharmacokinetic phase II study of pegylated liposomal doxorubicin and vinorelbine in heavily pretreated recurrent ovarian carcinoma. Ann. Oncol. 16, 300–306.
Ke, C.Y., Mathias, C.J., Green, M.A., 2003. The folate receptor as a molecular target for tumor-selective radionuclide delivery. Nucl. Med. Biol. 30, 811–817.
Klibanov, A.L., Maruyama, K., Beckerleg, A.M., Torchilin, V.P., Huang, L., 1991. Activity of amphipathic poly(ethylene glycol)-5000 to prolong the circulation time of liposomes depends on the liposome size and is unfavorable for immunoliposome binding to target. Biochim. Biophys. Acta 1062, 142–148.
Klibanov, A.L., Maruyama, K., Torchilin, V.P., Huang, L., 1990. Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. FEBS Lett. 268, 235–237.
Koide, H., Asai, T., Hatanaka, K., Akai, S., Ishii, T., Kenjo, E., Ishida, T., Kiwada, H., Tsukada, H., Oku, N., 2010. T cell-independent B cell response is responsible for ABC phenomenon induced by repeated injection of PEGylated liposomes. Int. J. Pharm. 392, 218–223.
Kong, G., Anyarambhatla, G., Petros, W.P., Braun, R.D., Colvin, O.M., Needham, D., Dewhirst, M.W., 2000. Efficacy of liposomes and hyperthermia in a human tumor xenograft model: Importance of triggered drug release. Cancer Res. 60, 6950–6957.
Koning, G.A., Eggermont, A.M.M., Lindner, L.H., ten Hagen, T.L.M., 2010. Hyperthermia and thermosensitive liposomes for improved delivery of chemotherapeutic drugs to solid tumors. Pharm. Res. Dordr. 27, 1750–1754.
Kost, J., Langer, R., 2001. Responsive polymeric delivery systems. Adv. Drug Deliv. Rev. 46, 125–148.
Krown, S.E., Northfelt, D.W., Osoba, D., Stewart, J.S., 2004. Use of liposomal anthracyclines in Kaposi's sarcoma. Semin. Oncol. 31, 36–52.
Kulshrestha, P., Gogoi, M., Bahadur, D., Banerjee, R., 2012. In vitro application of paclitaxel loaded magnetoliposomes for combined chemotherapy and hyperthermia. Colloid. Surf. B Biointerfaces 96, 1–7.
Laurent, S., Elst, L.V., Thirifays, C., Muller, R.N., 2008. Paramagnetic liposomes: Inner versus outer membrane relaxivity of DPPC liposomes incorporating lipophilic gadolinium complexes. Langmuir 24, 4347–4351.
Leamon, C.P., Cooper, S.R., Hardee, G.E., 2003. Folate-liposome-mediated antisense oligodeoxynucleotide targeting to cancer cells: Evaluation in vitro and in vivo. Bioconjug. Chem. 14, 738–747.
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, T.Y., Lin, C.T., Kuo, S.Y., Chang, D.K., Wu, H.C., 2007. Peptide-mediated targeting to tumor blood vessels of lung cancer for drug delivery. Cancer Res. 67, 10958–10965.
Legendre, J.Y., Szoka, F.C., 1992. Delivery of plasmid DNA into mammalian-cell lines using pH-sensitive liposomes—comparison with cationic liposomes. Pharm. Res. Dordr. 9, 1235–1242.
Li, L., ten Hagen, T.L.M., Schipper, D., Wijnberg, T.M., van Rhoon, G.C., Eggermont, A.M.M., Lindner, L.H., Koning, G.A., 2010. Triggered content release from optimized stealth thermosensitive liposomes using mild hyperthermia. J. Control. Release 143, 274–279.
Li, S.H., Goins, B., Phillips, W.T., Saenz, M., Otto, P.M., Bao, A., 2011. Post-lumpectomy intracavitary retention and lymph node targeting of Tc-99m-encapsulated liposomes in nude rats with breast cancer xenograft. Breast Cancer Res. Tr. 130, 97–107.
Li, S.H., Goins, B., Zhang, L.J., Bao, A.D., 2012. Novel multifunctional theranostic liposome drug delivery system: construction, characterization, and multimodality mr, near-infrared fluorescent, and nuclear imaging. Bioconjug. Chem. 23, 1322–1332.
Li, X.M., Ding, L.Y., Xu, Y.L., Wang, Y.L., Ping, Q.N., 2009. Targeted delivery of doxorubicin using stealth liposomes modified with transferrin. Int. J. Pharm. 373, 116–123.
Lian, T., Ho, R.J.Y., 2001. Trends and developments in liposome drug delivery systems. J. Pharma. Sci. 90, 667–680.
Lindenstrom, T., Agger, E.M., Korsholm, K.S., Darrah, P.A., Aagaard, C., Seder, R.A., Rosenkrands, I., Andersen, P., 2009. Tuberculosis subunit vaccination provides long-term protective immunity characterized by multifunctional CD4 memory T Cells. J. Immunol. 182, 8047–8055.
Lindner, L.H., Eichhorn, M.E., Eibl, H., Teichert, N., Schmitt-Sody, M., Issels, R.D., Dellian, M., 2004. Novel temperature-sensitive liposomes with prolonged circulation time. Clin. Cancer Res. 10, 2168–2178.
Lipshultz, S.E., Lipsitz, S.R., Mone, S.M., Goorin, A.M., Sallan, S.E., Sanders, S.P., Orav, E.J., Gelber, R.D., Colan, S.D., 1995. Female sex and higher drug dose as risk-factors for late cardiotoxic effects of doxorubicin therapy for childhood-cancer. New Engl. J. Med. 332, 1738–1743.
Liu, D.X., Mori, A., Huang, L., 1991. Large liposomes containing ganglioside Gm1 accumulate effectively in spleen. Biochim. Biophys. Acta 1066, 159–165.
Lollini, P.L., Cavallo, F., Nanni, P., Forni, G., 2006. Vaccines for tumour prevention. Nat. Rev. Cancer 6, 204–216.
Lu, Y.J., Low, P.S., 2002. Folate-mediated delivery of macromolecular anticancer therapeutic agents. Adv. Drug Deliv. Rev. 54, 675–693.
Ludewig, B., Barchiesi, F., Pericin, M., Zinkernagel, R.M., Hengartner, H., Schwendener, R.A., 2000. In vivo antigen loading and activation of dendritic cells via a liposomal peptide vaccine mediates protective antiviral and anti-tumour immunity. Vaccine 19, 23–32.
Lukyanov, A.N., Elbayoumi, T.A., Chakilam, A.R., Torchilin, V.P., 2004. Tumor-targeted liposomes: doxorubicin-loaded long-circulating liposomes modified with anticancer antibody. J. Control. Release 100, 135–144.
Lundberg, B.B., Griffiths, G., Hansen, H.J., 2004. Cellular association and cytotoxicity of anti-CD74-targeted lipid drug-carriers in B lymphorna cells. J. Control Release 94, 155–161.
Maeda, H., Matsumura, Y., 1989. Tumoritropic and lymphotropic principles of macromolecular drugs. Crit. Rev. Ther. Drug 6, 193–210.
Malam, Y., Loizidou, M., Seifalian, A.M., 2009. Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends Pharmacol. Sci. 30, 592–599.
Malone, R.W., Felgner, P.L., Verma, I.M., 1989. Cationic liposome-mediated RNA transfection. Proc. Natl. Acad. Sci. U. S. A. 86, 6077–6081.
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.
Marchisio, P., Cavagna, R., Maspes, B., Gironi, S., Esposito, S., Lambertini, L., Massimini, A., Herzog, C., Principi, N., 2002. Efficacy of intranasal virosomal influenza vaccine in the prevention of recurrent acute otitis media in children. Clin. Infect. Dis. 35, 168–174.
Mastrobattista, E., Koning, G.A., Storm, G., 1999. Immunoliposomes for the targeted delivery of antitumor drugs. Adv. Drug Deliv. Rev. 40, 103–127.
Matsumura, Y., Gotoh, M., Muro, K., Yamada, Y., Shirao, K., Shimada, Y., Okuwa, M., Matsumoto, S., Miyata, Y., Ohkura, H., Chin, K., Baba, S., Yama, 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.
Mayer, L.D., Krishna, R., Webb, M., Bally, M., 2000. Designing liposomal anticancer drug formulations for specific therapeutic applications. J. Liposome Res. 10, 99–115.
McDonagh, C.F., Huhalov, A., Harms, B.D., Adams, S., Paragas, V., Oyama, S., Zhang, B., Luus, L., Overland, R., Nguyen, S., Gu, J.M., Kohli, N., Wallace, M., Feldhaus, M.J., Kudla, A.J., Schoeberl, B., Nielsen, U.B., 2012. Antitumor activity of a novel bispecific antibody that targets the ErbB2/ErbB3 oncogenic unit and inhibits heregulin-induced activation of ErbB3. Mol. Cancer Ther. 11, 582–593.
Medina, O.P., Zhu, Y., Kairemo, K., 2004. Targeted liposomal drug delivery in cancer. Curr. Pharm. Design 10, 2981–2989.
Metselaar, J.M., Bruin, P., de Boer, L.W.T., de Vringer, T., Snel, C., Oussoren, C., Wauben, M.H.M., Crommelin, D.J.A., Storm, G., Hennink, W.E., 2003. A novel family of L-amino acid-based biodegradable polymer-lipid conjugates for the development of long-circulating liposomes with effective drug-targeting capacity. Bioconjug. Chem. 14, 1156–1164.
Mikhaylov, G., Mikac, U., Magaeva, A.A., Itin, V.I., Naiden, E.P., Psakhye, I., Babes, L., Reinheckel, T., Peters, C., Zeiser, R., Bogyo, M., Turk, V., Psakhye, S.G., Turk, B., Vasiljeva, O., 2011. Ferri-liposomes as an MRI-visible drug-delivery system for targeting tumours and their microenvironment. Nat. Nanotechnol. 6, 594–602.
Moghimi, S.M., 1998. Humoral-mediated recognition of 'phagocyte-resistant' beads by lymph node macrophages of poloxamine-treated rats. Clin. Sci. 95, 389–391.
Moghimi, S.M., Davis, S.S., 1994. Innovations in avoiding particle clearance from blood by Kupffer cells—cause for reflection. Crit. Rev. Ther. Drug 11, 31–59.
Moghimi, S.M., Gray, T., 1997. A single dose of intravenously injected poloxamine-coated long-circulating particles triggers macrophage clearance of subsequent doses in rats. Clin. Sci. 93, 371–379.
Moghimi, S.M., Murray, J.C., 1996. Poloxamer-188 revisited: A potentially valuable immune modulator. J. Natl. Cancer I 88, 766–768.
Moghimi, S.M., Patel, H.M., 1998. Serum-mediated recognition of liposomes by phagocytic cells of the reticuloendothelial system—The concept of tissue specificity. Adv. Drug Deliv. Rev. 32, 45–60.
Moser, C., Metcalfe, I.C., Viret, J.F., 2003. Virosomal adjuvanted antigen delivery systems. Exp. Rev. Vac. 2, 189–196.
Mufamadi, M.S., Pillay, V., Choonara, Y.E., Du Toit, L.C., Modi, G., Naidoo, D., Ndesendo, V.M., 2011. A review on composite liposomal technologies for specialized drug delivery. J. Drug Deliv. 939851.
Muggia, F.M., Hainsworth, J.D., Jeffers, S., Miller, P., Groshen, S., Tan, M., Roman, L., Uziely, B., Muderspach, L., Garcia, A., Burnett, A., Greco, F.A., Morrow, C.P., Paradiso, L.J., Liang, L.J., 1997. Phase II study of liposomal doxorubicin in refractory ovarian cancer: Antitumor activity and toxicity modification by liposomal encapsulation. J. Clin. Oncol. 15, 987–993.
Mulder, W.J.M., Strijkers, G.J., Griffioen, A.W., van Bloois, L., Molema, G., Storm, G., Koning, G.A., Nicolay, K., 2004. A liposomal system for contrast-enhanced magnetic resonance imaging of molecular targets. Bioconjug. Chem. 15, 799–806.

Murai, M., Aramaki, Y., Tsuchiya, S., 1995. Identification of the serum factor required for liposome-primed activation of mouse peritoneal-macrophages—modified alpha(2)-macroglobulin enhances Fc-gamma receptor-mediated phagocytosis of opsonized sheep red-blood-cells. Immunology 86, 64–70.
Muthu, M.S., Kulkarni, S.A., Raju, A., Feng, S.S., 2012. Theranostic liposomes of TPGS coating for targeted co-delivery of docetaxel and quantum dots. Biomaterials 33, 3494–3501.
Nakamura, K., Abu Lila, A.S., Matsunaga, M., Doi, Y., Ishida, T., Kiwada, H., 2011. A double-modulation strategy in cancer treatment with a chemotherapeutic agent and siRNA. Mol. Ther. 19, 2040–2047.
Needham, D., Anyarambhatla, G., Kong, G., Dewhirst, M.W., 2000. A new temperature-sensitive liposome for use with mild hyperthermia: Characterization and testing in a human tumor xenograft model. Cancer Res. 60, 1197–1201.
Neerunjun, E.D., Gregoriadis, G., 1976. Tumor regression with liposome-entrapped asparaginase—some immunological advantages. Biochem. Soc. Trans. 4, 133–134.
Negussie, A.H., Yarmolenko, P.S., Partanen, A., Ranjan, A., Jacobs, G., Woods, D., Bryant, H., Thomasson, D., Dewhirst, M.W., Wood, B.J., Dreher, M.R., 2011. Formulation and characterisation of magnetic resonance imageable thermally sensitive liposomes for use with magnetic resonance-guided high intensity focused ultrasound. Int. J. Hyperther. 27, 140–155.
Neves, S., Faneca, H., Bertin, S., Konopka, K., Duzgunes, N., Pierrefite-Carle, V., Simoes, S., Pedroso de Lima, M.C., 2009. Transferrin lipoplex-mediated suicide gene therapy of oral squamous cell carcinoma in an immunocompetent murine model and mechanisms involved in the antitumoral response. Cancer Gene Ther. 16, 91–101.
Nicolazzi, C., Mignet, N., de la Figuera, N., Cadet, M., Ibad, R.T., Seguin, J., Scherman, D., Bessodes, M., 2003. Anionic polyethyleneglycol lipids added to cationic lipoplexes increase their plasmatic circulation time. J. Control. Release 88, 429–443.
Okitsu, S.L., Silvie, O., Westerfeld, N., Curcic, M., Kammer, A.R., Mueller, M.S., Sauerwein, R.W., Robinson, J.A., Genton, B., Mazier, D., Zurbriggen, R., Pluschke, G., 2007. A virosomal malaria peptide vaccine elicits a long-lasting sporozoite-inhibitory antibody response in a phase 1a clinical trial. PloS One 2.
Ozkan, M., 2004. Quantum dots and other nanoparticles: what can they offer to drug discovery? Drug Discov. Today 9, 1065–1071.
Ozpolat, B., Sood, A.K., Lopez-Berestein, G., 2010. Nanomedicine based approaches for the delivery of siRNA in cancer. J. Internal Med. 267, 44–53.
Palmer, M., Parker, J., Modi, S., Butts, C., Smylie, M., Meikle, A., Kehoe, M., MacLean, G., Longenecker, M., 2001. Phase I study of the BLP25 (MUC1 peptide) liposomal vaccine for active specific immunotherapy in stage IIIB/IV non-small-cell lung cancer. Clin. Lung Cancer 3, 49–57 (discussion 58).
Papahadjopoulos, D., Allen, T.M., Gabizon, A., Mayhew, E., Matthay, K., Huang, S.K., Lee, K.D., Woodle, M.C., Lasic, D.D., Redemann, C., Martin, F.J., 1991. Sterically stabilized liposomes—improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc. Natl. Acad. Sci. U. S. A. 88, 11460–11464.
Parhi, P., Mohanty, C., Sahoo, S.K., 2012. Nanotechnology-based combinational drug delivery: an emerging approach for cancer therapy. Drug Discov. Today 17, 1044–1052.
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, S.B., Krishnan, A.V., Lin, C.S.Y., Goldstein, D., Friedlander, M., Kiernan, M.C., 2008. Mechanisms underlying chemotherapy-induced neurotoxicity and the potential for neuroprotective strategies. Curr. Med. Chem. 15, 3081–3094.
Patel, H.M., 1992a. Serum opsonins and liposomes—their interaction and opsonophagocytosis. Crit. Rev. Ther. Drug 9, 39–90.
Patel, H.M., 1992b. Serum opsonins and liposomes: their interaction and opsonophagocytosis. Crit. Rev. Ther. Drug Carrier Syst. 9, 39–90.
Peer, D., Karp, J.M., Hong, S., FaroKHzad, O.C., Margalit, R., Langer, R., 2007. Nanocarriers as an emerging platform for cancer therapy. Nat. Nanotechnol. 2, 751–760.
Perezsoler, R., 1989. Liposomes as carriers of antitumor agents—toward a clinical reality. Cancer Treat. Rev. 16, 67–82.
Perumal, V., Banerjee, S., Das, S., Sen, R., Mandal, M., 2011. Effect of liposomal celecoxib on proliferation of colon cancer cell and inhibition of DMBA-induced tumor in rat model. Cancer Nanotechnol. 2, 67–79.
Petersen, A.L., Binderup, T., Jolck, R.I., Rasmussen, P., Henriksen, J.R., Pfeifer, A.K., Kjaer, A., Andresen, T.L., 2012. Positron emission tomography evaluation of somatostatin receptor targeted 64Cu-TATE-liposomes in a human neuroendocrine carcinoma mouse model. J. Control. Release 160, 254–263.
Petre, C.E., Dittmer, D.P., 2007. Liposomal daunorubicin as treatment for Kaposi's sarcoma. Int. J. Nanomed. 2, 277–288.
Petros, R.A., DeSimone, J.M., 2010. Strategies in the design of nanoparticles for therapeutic applications. Nat. Rev. Drug. Discov. 9, 615–627.
Phillips, M.A., Gran, M.L., Peppas, N.A., 2010. Targeted nanodelivery of drugs and diagnostics. Nano Today 5, 143–159.
Pirollo, K.F., Xu, L., Chang, E.H., 2000. Non-viral gene delivery for p53. Curr. Opin. Mol. Ther. 2, 168–175.
Ponce, A.M., Viglianti, B.L., Yu, D.H., Yarmolenko, P.S., Michelich, C.R., Woo, J., Bally, M.B., Dewhirst, M.W., 2007. Magnetic resonance imaging of temperature-sensitive liposome release: Drug dose painting and antitumor effects. J. Natl. Cancer I 99, 53–63.
Ponce, A.M., Vujaskovic, Z., Yuan, F., Needham, D., Dewhirst, M.W., 2006. Hyperthermia mediated liposomal drug delivery. Int. J. Hyperther. 22, 205–213.
Poon, R.T.P., Borys, N., 2011. Lyso-thermosensitive liposomal doxorubicin: an adjuvant to increase the cure rate of radiofrequency ablation in liver cancer. Future Oncol. 7, 937–945.
Portney, N.G., Ozkan, M., 2006. Nano-oncology: drug delivery, imaging, and sensing. Anal. Bioanal. Chem. 384, 620–630.
Pradhan, P., Giri, J., Banerjee, R., Bellare, J., Bahadur, D., 2007. Preparation and characterization of manganese ferrite-based magnetic liposomes for hyperthermia treatment of cancer. J. Magn. Magn. Mater. 311, 208–215.
Pradhan, P., Giri, J., Rieken, F., Koch, C., Mykhaylyk, O., Doblinger, M., Banerjee, R., Bahadur, D., Plank, C., 2010. Targeted temperature sensitive magnetic liposomes for thermo-chemotherapy. J. Control. Release 142, 108–121.
Prakash, S., Malhotra, M., Shao, W., Tomaro-Duchesneau, C., Abbasi, S., 2011. Polymeric nanohybrids and functionalized carbon nanotubes as drug delivery carriers for cancer therapy. Adv. Drug Deliv. Rev. 63, 1340–1351.
Priebe, W., Perez-Soler, R., 1993. Design and tumor targeting of anthracyclines able to overcome multidrug resistance: a double-advantage approach. Pharmacol. Ther. 60, 215–234.
Qian, Z.M., Li, H.Y., Sun, H.Z., Ho, K., 2002. Targeted drug delivery via the transferrin receptor-mediated endocytosis pathway. Pharmacol. Rev. 54, 561–587.
Reddy, J.A., Low, P.S., 2000. Enhanced folate receptor mediated gene therapy using a novel pH-sensitive lipid formulation. J. Control. Release 64, 27–37.
Riviere, K., Kieler-Ferguson, H.M., Jerger, K., Szoka, F.C., 2011. Anti-tumor activity of liposome encapsulated fluoroorotic acid as a single agent and in combination with liposome irinotecan. J. Control. Release 153, 288–296.
Rodriguez, M.A., Pytlik, R., Kozak, T., Chhanabhai, M., Gascoyne, R., Lu, B., Deitcher, S.R., Winter, J.N., Investigators, M., 2009. Vincristine sulfate liposomes injection (Marqibo) in heavily pretreated patients with refractory aggressive non-hodgkin lymphoma report of the pivotal phase 2 study. Cancer 115, 3475–3482.
Rose, P.G., 2005. Pegylated liposomal doxorubicin: optimizing the dosing schedule in ovarian cancer. Oncologist 10, 205–214.
Rosentha, S., Kaufman, S., 1974. Vincristine neurotoxicity. Ann. Intern. Med. 80, 733–737.
Rui, Y.J., Wang, S., Low, P.S., Thompson, D.H., 1998. Diplasmenylcholine-folate liposomes: an efficient vehicle for intracellular drug delivery. J. Am. Chem. Soc. 120, 11213–11218.
Samad, A., Sultana, Y., Aqil, M., 2007. Liposomal drug delivery systems: an update review. Curr. Drug Deliv. 4, 297–305.
Sapra, P., Allen, T.M., 2003. Ligand-targeted liposomal anticancer drugs. Prog. Lipid Res. 42, 439–462.
Sarkar, D.P., Ramani, K., Tyagi, S.K., 2002. Targeted gene delivery by virosomes. Methods Mol. Biol. 199, 163–173.
Saul, J.M., Annapragada, A.V., Bellamkonda, R.V., 2006. A dual-ligand approach for enhancing targeting selectivity of therapeutic nanocarriers. J. Control. Release 114, 277–287.
Schiffelers, R.M., Koning, G.A., ten Hagen, T.L., Fens, M.H., Schraa, A.J., Janssen, A.P., Kok, R.J., Molema, G., Storm, G., 2003. Anti-tumor efficacy of tumor vasculature-targeted liposomal doxorubicin. J. Control. Release 91, 115–122.
Schroeder, J.E., Shweky, I., Shmeeda, H., Banin, U., Gabizon, A., 2007. Folate-mediated tumor cell uptake of quantum dots entrapped in lipid nanoparticles. J. Control. Release 124, 28–34.
Schuch, G., 2005. EndoTAG-1. MediGene. Curr. Opin. Investig. Drugs 6, 1259–1265.
Semple, S.C., Akinc, A., Chen, J.X., Sandhu, A.P., Mui, B.L., Cho, C.K., Sah, D.W.Y., Stebbing, D., Crosley, E.J., Yaworski, E., Hafez, I.M., Dorkin, J.R., Qin, J., Lam, K., Rajeev, K.G., Wong, K.F., Jeffs, L.B., Nechev, L., Eisenhardt, M.L., Jayaraman, M., Kazem, M., Maier, M.A., Srinivasulu, M., Weinstein, M.J., Chen, Q.M., Alvarez, R., Barros, S.A., De, S., Klimuk, S.K., Borland, T., Kosovrasti, V., Cantley, W.L., Tam, Y.K., Manoharan, M., Ciufolini, M.A., Tracy, M.A., de Fougerolles, A., MacLachlan, I., Cullis, P.R., Madden, T.D., Hope, M.J., 2010. Rational design of cationic lipids for siRNA delivery. Nat. Biotechnol. 28, 172–176.
Sessa, G., Weissman. G, 1968. Phospholipid spherules (liposomes) as a model for biological membranes. J. Lipid Res. 9, 310–318.
Shi, G.F., Guo, W.J., Stephenson, S.M., Lee, R.J., 2002. Efficient intracellular drug and gene delivery using folate receptor-targeted pH-sensitive liposomes composed of cationic/anionic lipid combinations. J. Control. Release 80, 309–319.
Shinkai, M., Yanase, M., Honda, H., Wakabayashi, T., Yoshida, J., Kobayashi, T., 1996. Intracellular hyperthermia for cancer using magnetite cationic liposomes: In vitro study. Jpn. J. Cancer Res. 87, 1179–1183.
Smeesters, C., Giroux, L., Vinet, B., Arnoux, R., Chaland, P., Corman, J., Stlouis, G., Daloze, P., 1988. Efficacy of incorporating cyclosporine into liposomes to reduce its nephrotoxicity. Can. J. Surg. 31, 34–36.
Sudimack, J.J., Guo, W.J., Tjarks, W., Lee, R.J., 2002. A novel pH-sensitive liposome formulation containing oleyl alcohol. Bba Biomembr. 1564, 31–37.
Suri, S.S., Fenniri, H., Singh, B., 2007. Nanotechnology-based drug delivery systems. J. Occup. Med. Toxicol. 2, 16.
Tagami, T., Ernsting, M.J., Li, S.D., 2011. Optimization of a novel and improved thermosensitive liposome formulated with DPPC and a Brij surfactant using a robust in vitro system. J. Control. Release 154, 290–297.
Takara, K., Hatakeyama, H., Ohga, N., Hida, K., Harashima, H., 2010. Design of a dual-ligand system using a specific ligand and cell penetrating peptide, resulting in a synergistic effect on selectivity and cellular uptake. Int. J. Pharm. 396, 143–148.
Takeuchi, M., Imada, T., Shinkai, S., 1996. Highly selective and sensitive sugar tweezer' designed from a boronic-acid-appended mu-oxobis[porphinatoiron(III)]. J. Am. Chem. Soc. 118, 10658–10659.
Tardi, P.G., Gallagher, R.C., Johnstone, S., Harasym, N., Webb, M., Bally, M.B., Mayer, L.D., 2007. Coencapsulation of irinotecan and floxuridine into low

cholesterol-containing liposomes that coordinate drug release in vivo. Biochim. Biophys. Acta 1768, 678–687.
Theodossiou, C., Cook, J.A., Fisher, J., Teague, D., Liebmann, J.E., Russo, A., Mitchell, J.B., 1998. Interaction of gemcitabine with paclitaxel and cisplatin in human tumor cell lines. Int. J. Oncol. 12, 825–832.
Thompson, F.M., Porter, D.W., Okitsu, S.L., Westerfeld, N., Vogel, D., Todryk, S., Poulton, I., Correa, S., Hutchings, C., Berthoud, T., Dunachie, S., Andrews, L., Williams, J.L., Sinden, R., Gilbert, S.C., Pluschke, G., Zurbriggen, R., Hill, A.V.S., 2008. Evidence of blood stage efficacy with a virosomal malaria vaccine in a phase IIa clinical trial. PloS One 3.
Tilcock, C., Unger, E., Cullis, P., Macdougall, P., 1989. Liposomal Gd-Dtpa—preparation and characterization of relaxivity. Radiology 171, 77–80.
Torchilin, V.P., 2005. Recent advances with liposomes as pharmaceutical carriers. Nat. Rev. Drug Discov. 4, 145–160.
Torchilin, V.P., Levchenko, T.S., Whiteman, K.R., Yaroslavov, A.A., Tsatsakis, A.M., Rizos, A.K., Michailova, E.V., Shtilman, M.I., 2001. Amphiphilic poly-N-vinylpyrrolidones: synthesis, properties and liposome surface modification. Biomaterials 22, 3035–3044.
Torchilin, V.P., Shtilman, M.I., Trubetskoy, V.S., Whiteman, K., Milstein, A.M., 1994. Amphiphilic vinyl-polymers effectively prolong liposome circulation time in-vivo. Bba Biomembr. 1195, 181–184.
Turner, C., Weir, N., Catterall, C., Baker, T.S., Jones, M.N., 2002. The transfection of Jurkat T-leukemic cells by use of pH-sensitive immunoliposomes. J. Liposome Res. 12, 45–50.
Underwood, J.C., Carr, I., 1972. The ultrastructure and permeability characteristics of the blood vessels of a transplantable rat sarcoma. J. Pathol. 107, 157–166.
Viglianti, B.L., Abraham, S.A., Michelich, C.R., Yarmolenko, P.S., MacFall, J.R., Bally, M.B., Dewhirst, M.W., 2004. In vivo monitoring of tissue pharmacokinetics of liposome/drug using MRI: Illustration of targeted delivery. Magn. Reson. Med. 51, 1153–1162.
Viglianti, B.L., Ponce, A.M., Michelich, C.R., Yu, D.H., Abraham, S.A., Sanders, L., Yarmolenko, P.S., Schroeder, T., MacFall, J.R., Barboriak, D.P., Colvin, O.M., Bally, M.B., Dewhirst, M.W., 2006. Chemodosimetry of in vivo tumor liposomal drug concentration using MRI. Magn. Reson. Med. 56, 1011–1018.
Villa, L.L., Costa, R.L., Petta, C.A., Andrade, R.P., Ault, K.A., Giuliano, A.R., Wheeler, C.M., Koutsky, L.A., Malm, C., Lehtinen, M., Skjeldestad, F.E., Olsson, S.E., Steinwall, M., Brown, D.R., Kurman, R.J., Ronnett, B.M., Stoler, M.H., Ferenczy, A., Harper, D.M., Tamms, G.M., Yu, J., Lupinacci, L., Railkar, R., Taddeo, F.J., Jansen, K.U., Esser, M.T., Sings, H.L., Saah, A.J., Barr, E., 2005. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol. 6, 271–278.
Volanakis, J.E., Narkates, A.J., 1981. Interaction of C-reactive protein with artificial phosphatidylcholine bilayers and complement. J. Immunol. 126, 1820–1825.
Vonhoff, D.D., Layard, M.W., Basa, P., Davis, H.L., Vonhoff, A.L., Rozencweig, M., Muggia, F.M., 1979. Risk-factors for doxorubicin-induced congestive heart-failure. Ann. Intern. Med. 91, 710–717.
Waelti, E., Wegmann, N., Schwaninger, R., Wetterwald, A., Wingenfeld, C., Rothen-Rutishauser, B., Gimmi, C.D., 2002. Targeting her-2/neu with antirat Neu virosomes for cancer therapy. Cancer Res. 62, 437–444.
Wagner, A., Vorauer-Uhl, K., 2011. Liposome technology for industrial purposes. J. Drug Deliv., 591325.
Wang, C., Gao, X., Su, X.G., 2010. In vitro and in vivo imaging with quantum dots. Anal. Bioanal. Chem. 397, 1397–1415.
Wang, C.Y., Huang, L., 1987. pH-sensitive immunoliposomes mediate target-cell-specific delivery and controlled expression of a foreign gene in mouse. Proc. Natl. Acad. Sci. U. S. A. 84, 7851–7855.
Wasan, K.M., Kwong, M., 1997. Blood and plasma lipoprotein distribution and gender differences in the plasma pharmacokinetics of lipid-associated annamycin. Pharmacol. Toxicol. 80, 301–307.
Weinzimer, S.A., Gibson, T.B., Collett-Solberg, P.F., Khare, A., Liu, B.R., Cohen, P., 2001. Transferrin is an insulin-like growth factor-binding protein-3 binding protein. J. Clin. Endocr. Metab. 86, 1806–1813.
White, R.R., Sullenger, B.A., Rusconi, C.P., 2000. Developing aptamers into therapeutics. J. Clin. Investig. 106, 929–934.
Woodle, M.C., Engbers, C.M., Zalipsky, S., 1994. New amphipatic polymer lipid conjugates forming long-circulating reticuloendothelial system-evading liposomes. Bioconjug. Chem. 5, 493–496.
Wu, Y., Crawford, M., Yu, B., Mao, Y., Nana-Sinkam, S.P., Lee, L.J., 2011. MicroRNA delivery by cationic lipoplexes for lung cancer therapy. Mol. Pharma. 8, 1381–1389.
Xie, R., Hong, S., Feng, L., Rong, J., Chen, X., 2012. Cell-selective metabolic glycan labeling based on ligand-targeted liposomes. J. Am. Chem. Soc. 134, 9914–9917.
Xiong, X.B., Huang, Y., Lu, W.L., Zhang, X., Zhang, H., Nagai, T., Zhang, Q., 2005. Intracellular delivery of doxorubicin with RGD-modified sterically stabilized liposomes for an improved antitumor efficacy: In vitro and in vivo. J. Pharma. Sci. 94, 1782–1793.
Xu, L., Huang, C.C., Huang, W.Q., Tang, W.H., Rait, A., Yin, Y.Z., Cruz, I., Xiang, L.M., Pirollo, K.F., Chang, E.H., 2002. Systemic tumor-targeted gene delivery by anti-transferrin receptor scFv-immunoliposomes. Mol. Cancer Ther. 1, 337–346.
Xu, L., Wempe, M.F., Anchordoquy, T.J., 2011. The effect of cholesterol domains on PEGylated liposomal gene delivery in vitro. Ther. Deliv. 2, 451–460.
Yamada, Y., Shinohara, Y., Kakudo, T., Chaki, S., Futaki, S., Kamiya, H., Harashima, H., 2005. Mitochondrial delivery of mastoparan with transferrin liposornes equipped with a pH-sensitive fusogenic peptide for selective cancer therapy. Int. J. Pharm. 303, 1–7.
Yatvin, M.B., Weinstein, J.N., Dennis, W.H., Blumenthal, R., 1978. Design of liposomes for enhanced local release of drugs by hyperthermia. Science 202, 1290–1293.
Ying, X., Wen, H., Lu, W.L., Du, J., Guo, J., Tian, W., Men, Y., Zhang, Y., Li, R.J., Yang, T.Y., Shang, D.W., Lou, J.N., Zhang, L.R., Zhang, Q., 2010. Dual-targeting daunorubicin liposomes improve the therapeutic efficacy of brain glioma in animals. J. Control. Release 141, 183–192.
Zalipsky, S., Mullah, N., Qazen, M., 2004. Preparation of poly(ethylene glycol)-grafted liposomes with ligands at the extremities of polymer chains. Method Enzymol. 387, 50–69.
Zhang, J.Q., Zhang, Z.R., Yang, H., Tan, Q.Y., Qin, S.R., Qiu, X.L., 2005. Lyophilized paclitaxel magnetoliposomes as a potential drug delivery system for breast carcinoma via parenteral administration: In vitro and in vivo studies. Pharm. Res. Dordr. 22, 573–583.
Zhang, L., Gu, F.X., Chan, J.M., Wang, A.Z., Langer, R.S., Farokhzad, O.C., 2008. Nanoparticles in medicine: Therapeutic applications and developments. Clin. Pharmacol. Ther. 83, 761–769.
Zhang, L.F., Granick, S., 2006. How to stabilize phospholipid liposomes (using nanoparticles). Nano Lett. 6, 694–698.
Zhao, Y.X., Wang, L., Yan, M.N., Ma, Y.L., Zang, G.X., She, Z.N., Deng, Y.H., 2012. Repeated injection of PEGylated solid lipid nanoparticles induces accelerated blood clearance in mice and beagles. Int. J. Nanomed. 7, 2891–2900.