Asian Pacific Journal of Tropical Disease

journal homepage: http://www.apjtcm.com

Review article https://doi.org/10.12980/apjtd.7.2017D6-182

Liposome as nanocarrier: Site targeted delivery in lung cancer

Najeeb Ullah[1*], Saba Noreen[1], Kanza Tehreem[1], Aqal Zaman[2], Zara Ahmad[1], Humaira Balouch[1], Noreen Samad[1]

[1]*Department of Biochemistry, Bahauddin Zakariya University Multan-60800, Punjab, Pakistan*

[2]*Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan-60800, Punjab, Pakistan*

ARTICLE INFO

Article history:
Received 31 May 2016
Received in revised form 5 Sep, 2nd revised form 26 Sep 2016, 3rd revised form 19 Apr 2017
Accepted 16 May 2017
Available online 10 Aug 2017

Keywords:
Targeted drug delivery system
Nebulizer
Radiolabelling
Cancers

ABSTRACT

Lung cancer is fatal and spreading rapidly worldwide. Different clinical strategies are applied to stop this cancer. As the lung is a delicate organ, special clinical applications must be used and nanodrugs delivery systems are the most important applications of all. This review discusses the lung problems such as lung cancer, lung inflammation and bronchi constrictions followed by repetitive intake of some drugs. The objective of this review is to study how nanodrug delivery systems were synthesized and used in lung disorder treatment especially in lung cancer. The authors studied some articles from 1989 to 2015. Liposome encapsulation was done in various ways for the delivery of different drugs such as metaproterenol into liposomes caused bronchodilation, immunoliposomes bearing antibodies for doxorubicin reduced 50% inhibitory effects, radioliposomes with high penetrating ability to peripheral airways, aerosol delivery systems with deep pulmonary deposition, polymeric drug delivery having potential to improve beneficial index of drug, solid lipid liposomes, liposomal gentamicin with altered different clinical susceptibilities of resistance, transferrin conjugated liposomes to deliver cytostatic drugs to site of lungs, anti-inflammatory drugs with mannosylated liposomes, liposomal suspensions with single stranded RNAs and peptide encapsulation of liposomes. This review indicates that many animals perished with intravenous administration of drugs but survived in liposomal targeting groups.

1. Introduction

The phenomenal perceptive and study of how to handle the materials at molecular, macromolecular and atomic levels, possessing characteristics which are appreciably discriminated from the higher levels, is notorious as nanoscience. The applications of these technologies for cancer handling are drug delivery modifications and overpowering some drug delivery disorders[1]. One billionth of a meter (a nanometer) and this subatomic and atomic level, has the material features which are evidently distinctive from features of larger sized forms of the same materials. The innovative traits haunted by nanomaterials, though, renowned were for its mechanical, electrical, physical, magnetic, biological and chemical applications, presently, pharmaceutical purposes, mainly in drug delivery field, are getting a much more dedication[2]. Extensive research has been done for many eras in cancer mitigation using nanotechnology that is moving ahead quickly up to now. The pharmacokinetics individuality of many chemotherapeutic medications that are potentially functioning with less aqueous solubility, can be enhanced by nanocarriers predominantly. In this way the therapeutic effect of these products is being boosted. Hence, a multiplicity of drug delivery systems based on the biocompatible polymeric resources has been anticipated[3].

The most substantial research field for the pharmaceutical drugs management and development is targeted drug delivery system (TDDS). TDDS has the major role in drug delivery to the targeted tissues while comparative medication strength is reduced in other remaining tissues subsequent this. Consequently, leaving the neighboring tissues uninfluenced, the drug is mainly confined to the targeted area. The TDDS advantages, mainly include safe and welfare consignment and perfection in therapeutic catalog. A greater variety of quantitative advantages of TDDS for disease therapy are also explored[4]. The four most frequently identified cancers are prostate, breast, colorectal and lung cancers. Plentiful hostile effects of chemotherapeutic drugs are also pragmatic. Nanotechnology has a great exploitation in medical sciences as it is the escalating filed in medicine[5]. Cancer is one of the miscellaneous and heterogeneous diseases. Drug withstanding is the consequence of the synchronized practice of multiple drugs which is known as multidrug resistance (MDR) and it can be either intrinsic or acquired. Cancerous cells are being immune to typical therapy due to MDR with many anticancer mechanisms. Now it is the foremost routine in cancer medication to

*Corresponding author: Najeeb Ullah, Department of Biochemistry, Bahauddin Zakariya University Multan-60800, Punjab, Pakistan.
Tel: +92 345 9147288
E-mails: najeeb_khattaks@yahoo.com, najeebkhattak@bzu.edu.pk
The journal implements double-blind peer review practiced by specially invited international editorial board members.

direct and diverse phenotypes such as MDR phenotypes[6].

There is an enhanced cancer recurrence probability even after the anticancer therapy by surgical tumor removal, chemotherapeutic drug administration or radiation therapy. The cancer repetition risk can be elicited among cancer patients. The most economical durable way for the cancer protection is offered for this prevention. Naturally occurring nontoxic molecules are being used in chemoprevention and it is the eminent unique way for cancer management[7]. By using the nanoparticle formulations of the drugs, a few problems related to chemotherapeutics are being solved. These narrative formulations have much substantial advantage as tumor cells are their prior target by the enhanced permeability and retention occurrence revealed by the solid tumors proportional to ordinary tissues[8]. Biodegradable polymers resembling polysaccharides as well as proteins are mechanized the nanoparticles that can behave as proficient, controlled and targeted drug delivery fomite which has the endeavor to make the therapeutic effects healthier. For few decades, it has been the main trepidation to develop drug delivery devices as proteins-based nanoparticles that are generally regarded as safe[9].

Chemotherapeutic nanoparticles acquire long blood circulation to passively target tumor tissues, as their dissemination and stipulation is high. Decamping of reticulo-endothelial system detain is their inimitable benefit. Drug delivery faces low drug-loading contents and problems in encapsulation competence because medicines against tumor have physical encapsulation of nanoparticles[10].

A drug carrier having drug with its active part is isolated, encapsulated, or possessing adsorbed or joined active elements in drug release system. Bio-macromolecule, silk fibrin, is based on protein. Biomedical area as biomaterial has its ample norms in the spheres, shape of films, electrospun fibers, hydrogels and three dimensional (3D) scaffolds. For the most part, nanoparticles that are based on silk fibrin are optimistic drug delivery system due to their aptness of cell adhesion, biodegradability, chemical modification prospective, superlative biocompatibility, profusion and cross linking panorama[11]. First nanoparticle-based drug, Doxil is approved by Food and Drug Administration for cancer cure in 1995. Doxil is liposome (about 100 nm diameter) chiefly composed of two kinds of lipids and it capsulizes the doxorubicin (DOX); an anthracycline drug. Another liposomal preparation of an anthracycline, daunorubicin, (trade name DaunoXome) is approved by the Food and Drug Administration after one year. DaunoXome also constitutes two lipids. Currently, nanoparticles of the second cohort are being formulated to bring upgrading in pharmacodynamic and pharmacokinetic drug profiles. Treatment against solid tumors was also revolutionized[12]. The supplying of adequate drug quantity to tumors to curtail the harm to normal tissues is the primary destinations of a triumphant cancer treatment. Anticancer drug dose due to fear of the severe tribulations to the patients is often bounded or restricted. These doses are lower than optimal, and are the grounds of faulty tumor responses arousal, followed by disease relapse and drug resistance. Encapsulation of drugs in particles that transport them priory to tumor sites is the most effectual format. For instance, liposome particles have been approved due to capability of delivery of the chemotherapeutic agents, radionuclides and genes to tumor areas[13].

Nanotechnology has anticipated an innovative version of lightweight materials having better-quality electrical and mechanical persona. Matrix of some other complexes has entrenched in engineered nanoparticles (NPs) to broaden certain features. Spread nanoparticles are on the whole employed in biological sciences and medicine. The engineered nanoparticles have a distinctive advantage over other polymeric or macromolecular materials due to its potential at a molecular level to correlate with cells and tissues. Nanotechnology based approaches have many progresses and discoveries, but for the treatment of neurological problems like brain tumor, Alzheimer's disease, and stroke, their effectivenesses have been chiefly enforced[14].

Recently, important consideration for a large number of biotechnological applications has been given to magnetic nanoparticles (MNPs). Different biological uses as medicine and gene delivery, specific cell detection, disparity perfection in magnetic resonance imaging (MRI), bio separation, tissue repair, magnetofection, hyperthermia, *etc*. are making headway as they are appropriate due to their superparamagnetic nature and biocompatibility[15]. Polymeric self-assemblies constitute smart nanocarriers. For tumor-targeted delivery and proscribed expel of an array of active agents such as genetic agents, tiny molecule anticancer drugs, or proteins, these smart nanocarriers have emerged as on tenterhooks nanovehicles. Many intrinsic significance including increased solubility, protracted course in the bloodstream, customized thermodynamic constancy, and partisan collection into cancerous tissue via the increased permeation and retention outcome are exhibited by amphiphile containing nanoparticles acting as anticancer drug delivery carriers. Despite all these rewards, restricted triumph is pragmatic in clinical outcomes of drug laoded polymeric self-assemblies[16].

The liposomes, nanoparticles, polymeric micelles and polymeric-drug conjugates include in the succession of nano preprations that have been man-made. Some of them from this series are used in the preclinical studies. Routine drugs with the help of the passive diffusion or active transport with tiny molecules usually enter into the cells, on the other hand the nanomedicines progress into the cells by endocytosis and get together there[17].

In the microenvironment of lump, cellular free drug uptake follows the seepage of encapsulated chemotherapeutic. There are limitations in drug efficacy as these preparations are full of some cytotoxic agents commonly. For example, DOX, releases from its nanocarrier as there is high empathy or kinship between the drug and a variety of extracellular elements, as a result it has constrained cancer tissue permeability[18]. An innovative delivery podium that ensures the direct cytosolic release of a mammoth miscellany of therapeutics, for example protein, chemotherapy drugs and small interfering RNAs, is known as nanoparticle-stabilized capsule (NPSC)[19].

A levelheaded potential possessing RNA interference (RNAi) is molecular therapeutic paraphernalia as it covers an extensive array of applications; currently including efficient image-guided siRNA delivery to breast tumors and its role as a new cancer nanodrug by settling of a fervent therapeutic effect. It is also acting as a therapeutic tool due to its obligate specificity[20]. For the proscribed release of drugs and bioactive proteins, the natural polysaccharide is used which is injected through variety of routes in the body. In the pharmaceutical appliance, the diverse kinds of natural polysaccharides, including gelatin, pectin, guar gum, chitosan, alginate, dextran, starch, xanthan gum, arabic gum, cellulose, insulin and carrageenans are used[21]. In a contracted therapeutic range, the high up augmentation is made in distribution of high doses to the hearty tissues. Cardiotoxicity, mucositis and myelosuppression are the major unsympathetic effects of DOX as a drug restraining their clinical utilization. The nanotechnology provisions to consumer products have their admittance, however, their potent medical requisitions are little known[22].

A variety of biomolecular markers have been discovered which

are undeniably expressed itself in the cancer, and are helpful in improving the cancer understandings and as a result the targeted cancer therapies obtained[23]. Gold nanoparticles (AuNPs) possess tuneable shape, size, surface chemistry and many therapeutic implementations. They are permitting the attachment of a capricious medical drug and diagnostic agents. So, they are particularly noticeable regarding this issue. Recently, an AuNP being used for patients of complex stage cancer has passed the phase I clinical trials as it constitutes surface bounded thiolated polyethylene glycol and recombinant human tumor necrosis factor alpha (rhTNF)[24]. Gold nanoparticles (AuNPs) due to their matchless plasmonic features and admirable biocompatibility are frequently betrothed as analytical as well as recuperative agents for cancer. For the cancerous cells, AuNPs are being utilized as salutary and incongruous agent for cancer disclosure. NPs have charged surface that can fasten arbitrarily to biological molecules through the electrostatic interactions[25]. For the configuration of electrochemical and other types of biosensors, nanoparticle-based materials are used as they give the great sensitivity and specificity. The gold nanoparticles have effective use due to their stability against oxidation along with the metallic nanoparticles[26].

Need of nanomaterials is tinted at the beginning for transport and target based drug delivery. To make the treatment up to standard and to achieve the healing, there must be silent slinking of drug to the tainted or infected segment, voiding any commotion. Furthermore, drug must have prejudice for healthy and diseased portions. Drug should have the selective and exclusive interaction with disturbed area for its apt remedial accomplishment[27].

To invoke Sq-based multifunctional nanoparticles such as: (i) rhodamine–squalene due to pragmatic properties (*e.g.* good photostability, increased water solubility and the fluorescence retention emanation at abroad range of pH of its derivatives of tertiary amide, (ii) gemcitabine–squalene on account of the established broad range activity of Gemcitabine against solid tumors and (iii) biotin–squalene so as to exclusively target cancer cells through hyper-expression of biotin receptors on these cells, an adept and simple strategy is reported[28]. 2-dimensionally layered nanomaterials as layered double hydroxide (LDH) are the types of certain formulations of nanomaterials, which are being interestingly used as decisive cellular delivery nanocarriers for anionic antitumor drugs containing methotrexate along with 5-fluorouracil (5-FU) and showing low resemblance to the plasma membrane that is negatively charged[29].

Encapsulation of drugs within nanoparticles provides advantages when using hydrophobic, water-insoluble anticancer drugs by incorporating them into the matrix of nanoparticles. A hydrophobic anticancer drug called camptothecin, incorporation with the luminous mesoporous silica nanoparticles orifice to get rid of the insolubility issues as a medium[30].

In photodynamic therapy, photochemical reactions between light and cancer tissues are also moving ahead with exogenous photosensitizing entities which are clinically acknowledged for alleviation of plentiful non-malignant and malevolent diseases using different photosensitizers[31].

For gene delivery the recombinant viral vectors due to the inheritability and relatively high transfection aptitude have been predominantly utilized. On the other hand, non-viral vectors have large delivery capacity, resilient structures, and low immunogenicity, building them best alternatives to viral vectors. Polyethylenimine because of its high transfection efficiency in diverse cell lines has become a gold standard of non-viral vectors[32].

For the cure of a heap of diseases like Parkinson's and cancer to specific brain regions, administration of therapeutic agents is most important. Intranasal strategy for delivery of drugs to the brain appears as a revenue crammed course. For treating the cognitive impairments in Alzheimer's disease, recently approved drugs are based on neurotransmitter or enzyme inflection[33]. The intracellular delivery progress including lysosomal and mitochondrial targeting has also searched[34].

Oral route has major focus as compared to parenteral route for drug delivery. Many clinically relevant aspects are affected by oral anticancer therapy[35]. The starch-based nanoparticles are constrained due to their hydrophilicity. This hardship is solved by hydrophilic backbone which is transplanted by hydrophobic side chains. Organic solvents, such as dichloromethane or dimethyl sulfoxide are employed for the preparation of these derivatives that have appallingly toxicological and other fortification coercion. Resultantly, there are boundaries in the use of these formulations[36]. Hydrophobic anticancer drugs are easily encapsulated by liposomal or micelle nanoparticles due to their amphiphilic configuration increasing their targeting aptitude to tumor sites. Long-time blood stream circulation and interception of renal clearance is achieved by the nanometer-sized nanoparticles. Particularly, in angiogenic tumor sites, NPs can easily pervade the punctured blood vessels[37]. Presently, the most effectual indicative technique for molecular imaging is MRI. It works by avoiding the ionizing radiations. MRI, largely due to its non-invasive character interrelated to other compensations, as like direct multiplane metaphors forming capability, discriminates soft tissues and obtains anatomical statistics[38]. To target a wide range of cancer types, different antitumor agents ranging from diminutive chemicals to bio macromolecules have been turned out which are promoted by intravenous pathway due to swift onset time, advanced propensity to reach the tumor section and slightest drug loss at the administration time[39].

Clinical trials are made by nano-sized anticancer drug administration showing that some drugs are not utterly effectual as widely used PEGylation cause a halt of nanomedicines from getting into tumor cells. However, PEGylation imparts proper pharmacokinetic properties to the nanoparticles escaping the reticuloendothelial system and immune cells scrutiny[40].

Polydopamine coatings with both supported films on electrodes and nanotubes of carbon and unconnected films were generated as a result of this work possessing a range of implementations[41].

Tissue selection is a major issue pertaining to specific effectiveness of pharmacological resources. Augmentation in the endurance time and the eminence of one's life by sinking chemotherapeutic toxicity is the decisive venture of cancer cure[42].

Prodrug solubility dilemma can be solved by nanoparticle encapsulation. This mixture of prodrugs and nanoparticles provides a successful cancer treatment as well as anti-inflammation and anti-viral cure[43].

Daunorubicin-loaded magnetic nanoparticles (DNR-MNPs) formulations constituted by iron oxide core are firstly covered with oleic acid and then made unfluctuated by Pluronic F-127, which are made for frequent discharge of DNR to curtail the superfluous effects and exploit the utility. DNR is stored there[44]. Many pH-induced drug delivery systems anticipate to be accessible to a discrete pH state. They can target either the extracellular or the intracellular pH forms of cancerous states[45].

Surface of cancer cells have sialic acid (SA) over-expressed discriminately renowned by phenylboronic acid (PBA) forming a

complex between PBA and SA that is firm even at pH negligible than its pKa. PBA also forms complexes with other regular sugars that are unbalance if not formed at a pH higher than that of pKa value[46].

Minute molecular antitumor drugs are exposed to some predicaments such as low solubility, high toxicity, untargeted biodistribution or rapid secretion that are resolved by PEGylation approach. In current years, four PEGylated small organic drugs are undergoing clinical inquest, however, still no product has access to the markets[47].

SLN are used in number of applications like enhancing drugs solubility, improving bioavalability, controlling drug release, drug targeting, reduction in therapeutic dose, and increase stability of the drug. SLN have been used for administration by number of routes such as paroral, topical, parenteral, and pulmonary[48]. Nanoparticles morphology was examined by SEM[49]. Nanoparticles covered with target ligands comprise the peptides, antibodies, aptamers, little molecules and proteins as lectin-carbohydrates, or antigenic molecules that are decipherable to definite sites. Antibodies that are endogenous in nature were also used to formulate drug delivery intracellularly. Hence, energetic targeted delivery advancement was equipped[50].

2. Discussion

2.1. The effects of encapsulation of the beta-2-adrenergic agonist drug

McCalden *et al.*[51] performed experiment for the determination of encapsulation effects of drug metaproterenol sulfate (MPS), after anesthesia the beta-2-adrenergic agonist into a variety of liposomes, on guinea pigs. The encapsulation involved egg phosphatidylglycerol (EPG), dipalmitoylphosphatidylglycerol, dipalmitoylphosphatidylcholine (DPPC), egg phosphatidylcholine (EPC), cholesterol (CH) and plus alpha-tocopherol (a-T)[51]. The reduction in broncho-constriction is the bronchodilator activity that is the consequence of frequent inhalation of aerosolized histamine solution and it is measured as the ability of inhaled aerosolized MPS by Ney[52]. Firstly, intramuscular injection of xylazine/acepromazine/ketamine was administered for the rationale of anesthesia to Guinea pigs (weighing 350–500 g). Systemic arterial blood pressure (systolic, diastolic, and mean) together with heart rate were measured and recorded[53]. The data were collected during impetuous breathing before and after governing the MPS. Subsequently, the animals were inhaled with a solution having either 1% MPS in saline (F-MPS), or an MPS-liposome preparation with 1% MPS (L-MPS). The similar animals were used for the comparison of results that were pretreated with inhaled saline or drug-free liposomes, administered through a Pulmosonic nebulizer with respirator working at 60 breaths/min for 2 min. Histamine administration the time scale was the end of the whole procedure. After bronchodilator inhalation, histamine (0.05% solution) was likewise administered by a 15 s length aerosol at 15, 45, 75, and 105 min.

McCalden *et al.*[51] concluded that all three liposome-encapsulated MPS formulations produced deliberately trivial cardiovascular side effects. As like free drug, one preparation also showed immediate stipulation of the histamine bronchoconstriction. The obstinacy of 10–12 h study period suggests that the hiatus of activity was greater than that of free drugs. Thus, drug liposome encapsulation can increase time of bronchodilator activity of inhaled MPS and decrease its undesired cardiovascular impacts. Besides, the outcomes with the EPC/EPG/CH preparations explicate that liposome formulations are valuable to handle the rate of drug release and to achieve an unmitigated period of bronchodilator feat with a bridged cardiovascular side effects[51].

2.2. Use of monoclonal antibodies against the squamous lung cancer cell

Ahmad and Allen[54] premeditated the monoclonal antibodies used in opposition to the squamous lung cancer cell line KLN-205. Then they ligated them to the liposomes that showed distinct, competitive and specific uptake by KLN-205 cells than the liposomes having nonspecific antibody-free liposomes or isotype-matched antibodies. They have done radiolabelling by Allen *et al.*[55] method of these liposomes after preparing them by the use of hydrogenated soy phosphatidylcholine (HSPC). The remote-loading method by Mayer *et al.*[56] was utilized for DOX encapsulation. Thymidine incorporation assay technique by Ding *et al.*[57] was used after cell culture preparation for the uptake and cytotoxicity testing. Ahmad and Allen[54] concluded that antibody-liposomes with DOX encapsulation, when compared with free DOX or antibody-free encapsulated DOX, lessened as much as 15-fold in the 50% inhibitory concentration for DOX against KLN-205 cells.

2.3. Characterization of aerosols

Barker *et al.*[58] studied categorization of aerosols formed by EPC and multilamellar vesicles (MLVs) atomization in respirgard jet nebulisers from radiolabelled liposomes. Phospholipids and cholesterol mixing was done for liposome preparation and then dissolved in small volume of chloroform. Aerosols were generated instantly by means of a mouth piece containing Respirgard-II nebuliser. Radioactivity was measured with the help of an accurate counter at each step. Gamma-scintigraphy was used to study inhaled dipalmitoylphosphatidylcholine/cholesterol liposomes clearance having the aqueous phase marker 99mTc-DTPA (diethylenetriamine pentacetic acid)[59] with three healthy participants between 18 and 30 years using a Respirgard-II nebulizer. Camera heads were rotated by laying down the volunteer and tomographic images were taken by photon emission. 2–5 h post-nebulisation was done giving 2 min frames in 20 min duration.

Barker *et al.*[58] concluded that the deposited radiolabelled liposomes with DPPC/Chol in the multi-stage liquid impinge gave a mean MMAD mass median aerodynamic diameter] (n = 4) of 1.25 pm which designated that liposomes given through a Respirgard II nebulizer had a grand prospect of penetrating for the peripheral airways. Radioactively entrapped 58.5%, 54.8% and 44.7% liposome, initially deposited in the lung areas in the 5 h, 6 h or 24 h of post-inhalation, still are comparable to 16.8% of free activity at the same time point. This illustrates us that the part of the radiolabeling still remained intact in alveolar deposited liposomes. The consequences point out the liposomes capability for the drug release to the respiratory zone in the body. Moreover, the amendment of the bilayer composition of the above preparations was manipulated to adjust the release rate of radiolabelled hydrophilic products and it will proffer advance valuable information about inhaled liposomal drug delivery systems[58].

2.4. The effectiveness of pulmonary targeting of the CsA–DLPC liposome aerosol

Waldrep *et al.*[60] studied that in the lungs, instead of conformist intravenous or oral routes of administration, the selective drug

delivery of salutary cyclosporine-A (CsA) concentrations would stipulate lesser dosages. The probable and potent benefits from targeted lung delivery are the decreased systemic toxicity and the persistent immunosuppressive action. Aerosol delivery systems are settled at the lung disease site to settle down the drugs on the pulmonary surfaces[61]. The new HPLC method established by Waldrep *et al.*[62] was used for tissue investigation of (CsA)–liposomes, and to measure cyclosporine-A retrieved from BALB/c mouse lung tissues. After 15-min aerosol disclosure, diminutive cyclosporine-A was seen in the blood, kidney, liver or spleen. Waldrep *et al.*[60] concluded that lung restrained the uppermost organ cyclosporine-A levels with elevated immunosuppressive working and this designates the efficient targeting of the Cyclosporin-A–dilauroylphosphatidylcholine (DLPC) liposome aerosol for lungs.

2.5. Comparison of the intrapulmonary distribution and clearance of DLPC and DPPC

Saari *et al.*[63] compared the intrapulmonary allocation and clearance regarding beclomethasone dipropionate (Bec) that are 99mTC-labelled, dilauroyl phosphatidyl choline and DPPC liposomes in hale and hearty participants. DPPC is the core phospholipid derivative for the treatment of respiratory distress syndrome of the neonate. Phosphatidylcholine subsidiary products dipalmitoyl phophatidyl choline (DPPC) and dilauroyl phophatidyl choline (DLPC) were used to produce multilamellar beclomethasone dipropionate liposomes[64]. Firstly, the reconstructed liposomes were labelled with 99mTc as described by Richardson *et al.*[65]. Then, the pulmonary delivery and clearance of 99mTc-labelled beclomethasone dipropionate were analogized in 11 fit volunteers[66]. Both liposome aerosols have pertinent droplet size (diameter 1.3 mm) that are administered by Aerotech jet nebulizer and intend reflective pulmonary deposition. However, DLPC and DPPC of 11.4 and 3.1 mg exhibit a profound difference of drug output when inhaled, respectively.

Saari *et al.*[63] concluded that in a gamma camera analysis, no momentous difference is found during a deposition in the central/peripheral lung for DLPC and DPPC preparations. Continuing clearance of Tc-labelled Bec liposomes were pragmatic, 24 h after inhalation. The persistence of 79% of DLPC liposomes and 83% of that of DPPC liposomes in the lungs was also seen. Thus, it was concluded that clearance of inhaled liposomes with DPPC instead of DLPC was slower and both liposome constitutions are appropriate for nebulization, though aerosol clouds composed of DLPC liposome suspension were additionally proficient. These results prop up the study that drug-liposome encapsulation can proffer continual liberation and drug accomplishment in the inferior airways[63].

2.6. The incorporation of paclitaxel (Ptx) in nanoparticles

Fonseca *et al.*[67] studied that for paclitaxel to build up a polymeric medicine delivery system and to have the potential to extend the valuable drug index and deficient the contradictory impacts, Cremophor-EL, Paclitaxel (Ptx)-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles were prepared by a method of Fessi *et al.*[68]. The amalgamation competence of paclitaxel in the nanoparticles was calculated by different experimental parameters. Preparation of the organic phase and the organic phase/aqueous phase ratio by Szoka and Papahadjopoulos[69] influenced the assimilation efficacy of paclitaxel in nanoparticles. The data specified that the preparation method permitted the development of negatively charged spherical nanometric and homogenized particles (200 nm) that are paramount when used for intravenous administration. The emancipation sculpt of paclitaxel from the nanoparticles showed a biphasic model which has a characteristic that initially releases fast in 24 h, and then tracked by a slower and steady discharge. Their studies showed that using a small human cancer cell line (NCI-H69 SCLC), the *in-vitro* anti-tumoral activity of Ptx-PLGA-Nps was evaluated and related to the laboratorial anti-tumoral functioning of the lucrative preparation Taxol. The Cremophor EL effect on the cell feasibility was examined as well. Fonseca *et al.*[67] concluded that introduction of NCI-H69 cells to 25 μg/mL Taxol showed a sharp decline in cell viability which demonstrated that amalgamation of paclitaxel[70] in nanoparticles has vigorously enhanced the cytotoxic activity of the drug in contrast to the Taxol.

2.7. Antiproliferative effect of SLN formulations

Serpe *et al.*[71] studied previously SLN with DOX, cholesteryl butyrate (chol-but) and paclitaxel. The antiproliferative effect of SLN formulations evaluated against conventional drug formulations on HT-29 cells. Growth curves and cell viability were taken by trypan blue (tb)-exclusion assay[72]. Interpolated growth curves gave inhibitory concentration (IC_{50}) values that were 50%. *In vitro* cytotoxicity of conventional drug formulations by Singla *et al.*[70] was lesser than that of SLN carrying cholesteryl-butyrate and DOX. After 24 h exposure to loaded SLN intracellular DOX was double, against the traditional drug formulations, through flow cytometry evaluation of the maximum concentration. There was likeliness among *in-vitro* paclitaxel (Ptx) full SLN cytotoxicities and conformist drug formulation. Serpe *et al.*[71] concluded that at 24 h exposures, the small concentrations of cholesteryl butyrate-SLN and paclitaxel or DOX was joined that gave a cosmic preservative antiproliferative influence that was not obtained from sodium butyrate and DOX or paclitaxel grouping. The initial *in vitro* upshot suggested that SLN offered as another drug delivery system.

3. Assessment of the antibacterial activities of liposomal

3.1. Gentamicin

Mugabe *et al.*[73] assessed antibacterial activity of the liposomal gentamicin against clinically isolated *Pseudomonas aeruginosa* (*P. aeruginosa*). Diverse lipids and cholesterol were used with liposomes to encapsulate gentamicin that were in the 2:1 molar ratio through sonication method[74]. The stability of this gentamicin was studied *in-vitro* for 48 h period and at 37 ˚C in collective plasma. The free and liposomal gentamicin was analyzed by the technique of broth dilution for clinical isolates of *P. aeruginosa*[75]. Mugabe *et al.*[73] concluded that the encapsulation competence of liposomal products was 4%–5.18% of the early quantity of the drug in solution. The gentamicin-liposomes existed 60%–70% for 48 h, when incubated in normal pooled plasma of human at 4 ˚C or 37 ˚C. The MICs of liposomal gentamicin for all *P. aeruginosa* were lesser than the free gentamicin MICs. Spectacularly, liposomal gentamicin altered the probability of the clinically secluded strains from gentamicin opposing or resistant to either intermediate or susceptible[73].

3.2. Aerosolization of amphotericin-B (AMB)

Gavalda *et al.*[76] studied the aptitude of remedial aerosolized amphotericin-B in a steroid-immune compromised murine model of attacking pulmonary aspergillosis. They compared a murine model of invasive pulmonary aspergillosis (IPA), for the competence of nebulized deoxycholate-amphotericin-B or liposomal AMB and then the banal intravenous (*i.v.*) dosage of both preparations. The feasible revenue of inhalation plus *i.v.* direction was searched. The intravenous treatments were given to central venous[77]. In opposition to the *Aspergillus* infectivity, the capability of AMB prophylaxis was studied extensively. For the treatment of IPA, they investigated the efficiency of nebulized AMB. Gavalda *et al.*[76] concluded that for the cure of experimental disturbing IPA, AMB is a valuable method, with more apposite domino effect attained with nebulized-L amphotericin-B (NL-AMB) than with nebulized deoxycholate-amphotericin-B (ND-AMB). In the lungs, the suitable concentrations of both ND-AMB and NL-AMB were seen, though with liposomal formulation concentrations were perchance elevated. Pooled management of nebulized plus *i.v.* L-AMB reveal the consequences like NL-AMB alone.

3.3. Study of the expression levels and location of transferrin receptor (Tf-R)

Anabousi *et al.*[78] studied miscellaneous types of lung epithelial cells, the expression levels and zone of transferrin receptor (Tf-R) by the flow cytometery and the confocal laser scanning microscopy (CLSM)[79]. The *in-vitro* assessment of transferrin (Tf)-conjugated liposomes uptake values and cytotoxicity was done. The transferrin receptor (Tf-R) exposed at a considerable eminent level in epithelial cells of the bronchi in comparison to their alveolar counterparts. Cancerous cells (*i.e*. A549 cell line) exposed an advanced transferrin receptor (Tf-R) expression level instead of primary culture containing alveolar epithelial type-II strong cells. Confocal laser scanning microscopy (CLSM) results indicate that the transferrin receptor (Tf-R) showed thrashing at the epithelial membranes. Anabousi *et al.*[78] concluded that higher expression levels of transferrin receptor (Tf-R) associated with greater transferrin (Tf)-liposomes uptake and enhanced cytotoxicity levels. Liposome uptake was constrained due to inconsistent free transferrin and it is temperature-dependent. (Tf)-liposomes act as suitable contender for cytostatic drug delivery to lungs.

3.4. Dexamethasone palmitate-mannosylated liposomes (DPML) effect

Wijagkanalan *et al.*[80] studied the communal effect of DPML at 0.5 mg/kg via intra tracheal direction in rat lung swelling aggravated by lipopolysaccharide. They initially prepared liposomes by Kawakami *et al.*[81] and then they disengage the alveolar macrophages. After that in these isolated alveolar macrophages, lipopolysaccharides were enthused by co-incubation method. Then they induced lung inflammation by the lipopolysaccharides and equipped the broncho alveolar lavage (BAL) samples. After all, cytokine and chemokine measurements were done, then western blot analysis was performed by the method of Hohmann and Faulkner[82]. Wijagkanalan *et al.*[80] concluded that, dexamethasone palmitate included in mannosylated liposomes (DPML) perceptibly introverted tumor necrosis factor, interleukin 1, neutrophil chemoattractant-1 concentrations induced by cytokines, neutrophil that are deprived in filtration and myeloperoxidase activity, repressed nuclear factor-B(NFB) and p-38 mitogen induced protein kinase endeavor in lung. These consequences proved the strong targeting systems of mannosylated liposomes for anti-inflammatory drug release to alveolar macrophages and to get healthier their effectiveness to cure lung inflammation.

3.5. Preparation of nano-sized carriers for single stranded RNA

Taetz *et al.*[83] used single stranded RNA and bordered against a section between the mRNA nucleotides that instruct the human telomerase reverse transcriptase. Then they performed radiolabeling of single stranded RNA[84] by labeling the 5'-end of coding strand and the labeled sense strand strengthened through the antisense strand. The cationic hyaluronic acid (HA) conjugate with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) was prepared and after that 1,2-dioleoy-l,3-trimethylammonium-propane (DOTAP)/1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) liposomes were synthesized via injecting the ethanol by Kremer *et al*.[85]. Lipoplexes were arranged at various ratios by speedily injecting various volumes of liposomal (200 mm or below) suspension of ssRNA solution and their zeta potentials based on the amendment level and the ± charge ratio. Lipoplexes binding efficiencies were resolute before the step of centrifugation. Lipoplexes and 15% cationic hyaluronic acid-(DOPE) liposomes (2:1 and 8:1 ratio) were set with radiolabeled siRNA and it was confirmed that sample processing will not mortify after incubation; it is a post-incubation dilapidation control. Presence of HA improved the properties, *i.e.* protection of siRNA from degradation, binding and composite stabilities in serum[83]. Taetz *et al.*[83] concluded that liposome cytotoxicity, examined by the MTT assay (a colorimetry assay for assessing metabolic activity)[86] and lactate dehydrogenase ejection after treatment of CD44–Calu-3 and CD44+A549 cells, was established at elevated concentrations only. In contrast with all other formulations, the addition of single stranded RNA to cationic (HA)-adapted liposomes proscribed cytotoxic effects. By flow cytometry and by using a quantitative real-time PCR (qPCR)-telomeric repeat amplification protocol assay by Herbert *et al.*[87], telomerase activity was tested with no proved difference in the effectiveness in between tailored and non-tailored HA preparations. A few diminutions in telomerase function was detected only with liposomes, lipoplexes; structured with lipofectamine and nonsense ssRNA illuminating for few lipids and single stranded RNA direct resistive effects on enzyme expression. They successfully prepared cationic (DOTAP)/(DOPE) liposomes adapted with the polymer having higher negative charge and nano-sized carrier cationic hyaluronic acid (HA) for single stranded RNA[83].

3.6. Isolation from a phage-displayed peptide library

Chang *et al.*[88] deliberated the separation from a phage-exposing peptide library by the method of Zwick *et al.*[89], a novel peptide ligand that attached to non-small lung cancer cell lines. The phage was ordained to a number of NSCLC cell lines excluding typical cells. The destined page and the artificial peptide accredited the surgical sample of (NSCLC) with a 75% positive rate. In severe combined immunodeficiency (SCID) mice containing (NSCLC)

xenografts by Sandler *et al*.[90], the targeting phage specially bounded to tumor grass roots. The targeting phage's tumor homing ability by the analogous synthetic peptide was repressed[88]. Chang *et al.*[88] concluded that when the targeted peptide fixed with DOX or vinorelbine with liposomes, the counteractive manifestation of the chemotherapeutic entities and the resilience rates of mice elevated with human lung cancer xenografts. Liposomes augmented drug count in tumor cells 5.7 times compared to free drugs and boosted cancer cell death caused by a bioavailable DOX high level[88].

3.7. Novel aerosolized liposome formulation development for pulmonary delivery of anti-asthmatic medication

Chen *et al*.[91] developed a narrative aerosolized liposomal preparation for anti-asthmatic medication to explicate the association between bioavailability and anti-asthmatic effectiveness of this product. Salbutamol sulfate (SBS) was also used as similar drug due to advanced water solubility and brisk assimilation rate. SBS was proficiently encapsulated in liposomes through vesicular phospholipid gel processes of Huang *et al.*[92]. SBS penetration crossways to the pulmonary membrane of Asian toad was resoluted by *in-vitro* amendment according to Okumura *et al.*[93]. The small animal imaging system and liposomes having fluorescent dye 1,1′-dioctadecyltetramethyl indotricarbo cyanine iodide (DiR) labeling were used for intratracheal liposome targeting in rats. Chen *et al.*[91] concluded that liposomes competently encapsulate SBS with 70% effectiveness and SBS-liposomes showed a low transport rate than free SBS solution. After pulmonary delivery of liposomes in rats, triumphant diffusion in the respiratory tract was made. SBS released from liposomes was continued for no less than 48 h. Pharmacodynamic recognition in a guinea pig explained that the anti-asthmatic influence of SBS liposomes remained up to 18 h, whereas free SBS shows less than 8 h. *In vivo* analysis made sure that liposome formulation may permit establishment of SBS in the tissues of lungs contributory to persistent liberation of SBS and thus impinging an extended antiasthmatic effect[91].

3.8. Combination of inhalation therapy and drug targeting as an efficient lung cancer therapy

Gaspar *et al*.[94] studied that a valuable remedy is attained by combining the gasp treatment and drug targeting methods (Tf)-conjugated PEG liposomes encumbered with DOX were given by intracorporeal nebulizing tube to lung cancer of rat model[95]. Various DOX products and dosage (0.2 and 0.4 mg/kg) were experienced for their effect on cancer progression. Rats' life time was compared to administration of Tf-PEG-liposomes encapsulating DOX (2 mg/kg). Gaspar *et al.*[94] concluded that control group rats showed the incredible weight decrease 2 weeks after tumor initiation and died in days 19 and 29. Animal lungs showed diverse foci of neoplastic insertions, up to 20 mm in place of the intact lobe. Vacant Tf-liposomes explained a momentous effect on endurance time due to the secondary cytotoxicity through the impetus of macrophages in pulmonary demeanor. No important infringement in survival was seen among the treated animals with free drug aerosols, Tf-modified liposomes and DOX encapsulated in a plain. The basic principle of study was to scrutinize if the active drug destined through Tf receptor-mediated uptake, can additionally recover the curative proficiency of localized cure of lung cancer[94].

3.9. Targeting mechanism of the ligand-mediated drug delivery system

Chang *et al*.[96] studied the targeted working of ligand-mediated drug liberation system with a peptide, SP5-2 that is an imitated peptide, which predominantly attached to (NSCLC) cells[97]. Liposomal conjugation of SP5-2 improved the magnitude of drug directly added into NSCLC cells via receptor-controlled endocytosis. Lipo-Dox ingenious catalog was made better by active SP5-2 that reduced the undesired effects and increased the enduring rate of mice with tumor in metastatic, synergistic and orthotopic animal samples. Chang *et al*.[96] concluded that cell tumor had cSP5-2-conjugated liposomal DOX (SP5-2-LD) that was 11.2 times elevated than free DOX and the environs of concentration-time curve was amplified 159.2-fold. The bioavailability testing was performed to verify the efficacy of SP5-2 for *in-vivo* liposomal drugs uptake in tumor tissues. The pharmacokinetic properties are elevated by SP5-2-conjugated liposomes that also proceed the effectiveness and safety profiles allowing the well managed drug discharge and biodistribution[96].

3.10. Determination of the effectiveness of targeted delivery of miR–ephrin-A1–LNP

Lee *et al*.[98] determined the expediency of targeted escape of ephrin-A1 encapsulated with let-7a miR and joined LNP (miR–ephrin-A1–LNP) on malignant pleural mesothelioma and NSCLC *in-vitro* tumor growth. To heave the liberation of miR, initially the miRs were covered in the DOTAP (N-[1-(2.3-dioleoyloxy) propyl] -N,N,N-trimethyl ammonium)/DSPE (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[cyanur(polyethylene glycol)-2000]) -PEG (polyethylene glycol)-cyanur liposomal nanoparticles (LNP)/ cholesterol. Then receptor EphA-2 on lung cells was targeted by ephrin-A1 conjugated on the LNP facade. Cellular uptake was observed by flow cytometry explained by Kinch *et al*.[99] after the 40-min incubation of MPM and NSCLC cells with LNP. After 24 h, the transfection effectiveness of miR–ephrin-A1–LNP was estimated by observing the emergence levels of let-7a miR in lung cancerous cells. The LNP complexes effects on cell proliferation of LCCs (lung cancer cells) as well as cytotoxicity were exposed. Lee *et al*.[98] got end result that the miR–ephrin-A1–LNP transfected cells have proficient uptake while comparing with LNP unaided tranfection. Encapsulation value of let-7a miR was evaluated by using centrifugal filtration method that was above 95% and confirmed by gel electrophoresis which implies that almost 10% miR was immersed on the LNP face somewhat than piercing inside the LNPs whilst comparing with free miR (100%). LNP up to an elevated levels of 100 μg/mL indicated no major cytotoxicity on all the three tested cell lines. No cytotoxic influence was observed by miR-con–LNP or miR-con-ephrin-A1–LNP on LLCs while analyzing with LNP alone. The fallout suggested that the engineered LNPs on increased cellular uptake effectiveness along with less cytotoxic effect. The complex is also an effective carrier of small RNA ordained for pulmonary areas in lung cancer[98].

3.11. Preparation of grafted single stranded (siRNA) nanoconstructs

Khatri *et al.*[100] performed the experiment in which cyclic Arg-Gly-Asp (cRGD) grafted single stranded (siRNA) nanoconstructs

were made for well-organized and intracellularly destined release of single stranded RNA. They prepared liposomes from hydrogenated soy phosphotidylcholine (HSPC), DOTAP, DOPE, cholesterol. They developed formulations by using a gel retardation assay of Scott *et al.* method[101], cryo transmission electron microscopy by Almgren *et al.* method[102], and zeta potential, particle size, serum stability, *in-vitro* cytotoxicity, qualitative and quantitative cell uptake, gene expression, and chemo sensitization tactic method of Kerbel[103]. Khatri *et al.*[100] concluded that entire complexation of single stranded RNA with cyclic Arg-Gly-Asp (cRGD) grafted nanoconstructs was observed at 2.0 N/P ratio. Nanoconstructs secluded the complexed single stranded RNA after 24 h while naked single stranded RNA was decided to destroy in 50% serum within the time period of 6 h. Ribonucleotide reductase subunit (RRM1) level, knowingly decreased when siRNA was transported in nanoconstruct forms in comparison with uncovered siRNA. A-549 cells showed decreased Gemcitabine hydrochloride IC_{50} value when pre-exposed to RRM1 single stranded RNA up to 5 folds in comparing with only Gemcitabine hydrochloride[100].

3.12. In-vitro investigation of the feasibility of carbonic anhydrase-IX for targeted delivery of docetaxel to human lung cancer

Wong *et al.*[104] investigated the prospect of carbonic anhydrase-IX for targeted deliverance of docetaxel to human lung cancer cells *in-vitro* to direct immunoliposomes. The DTX-encapsulated liposomes were organized when ethanol mixed in soybean phosphatidyl choline (SPC) and docetaxel (DTX). The mixture was injected by using a syringe needle into phosphate buffered saline solution (PBS) as described by Jaafar-Maalej *et al.*[105]. Using the post insertion technique, immunoliposomes were generated[106]. Anti-carbonic anhydrase-IX antibody was conjugated with the DSPE-PEG-MAL in micelle after the DSPE-PEG-MAL micelles N-[(3-maleimide-1-oxopropyl) aminopropyl polyethyleneglycol-carbamyl] distearoylphosphatidyl-ethanolamine (DSPE-PEG-MAL) formation. The antibody cleaved by reacting with the reducing agent dithiotreitol (DTT). At the room temperature, the reduced antibody was conjugated to DSPE-PEG-MAL micelles. The antibody-conjugated micelles were incubated with the preformed liposomes at a SPC:DSPE-PEG-MAL molar ratio of 31:1 at 60 ˚C for 2 h to prepare the carbonic anhydrase-IX directed the targeted liposomes. By incubating unmodified micelles with the preformed liposomes, nontargeted liposomes were obtained, and then by using Western blot analysis, the antibody-liposomes coupling efficiency were evaluated by Nielsen *et al.*[107]. By using the fluorescence-based flow cytometry method, the association of the binding affinities of nontargeted DTX liposomes and targeted liposomes to carbonic anhydrase-IX positive and carbonic anhydrase-IX negative A549 cells were performed. To remove the cell-free form, the cells were then washed with PBS that could interfere with cell binding. The cytotoxic effects of nontargeted liposomal DTX, targeted liposomal DTX, and free DTX on carbonic anhydrase-IX positive and carbonic anhydrase-IX negative A549 cells were determined. In 96-well culture plates, A549 cells were seeded per well and permitted to grow overnight. Carbonic anhydrase-IX positive anegative cells were obtained after equivalent incubations in hypoxia and normoxia. The cells were washed with the PBS to confiscate cell-free carbonic anhydrase-IX and then incubated with targeted DTX liposomes, nontargeted DTX liposomes, or free DTX solution at different concentrations at 4 ˚C. The cells were incubated with fresh medium at 37 ˚C for ancillary 46 h after exclusion of the unbound liposomes. From untreated cells the cell viability was expressed as a percentage comparative to the absorbance value[104].

Wong *et al.*[104] analyzed the quantity of antibody observed immunoliposomes and gave the outcome that the liposome-antibody coupling effectiveness was about 50%. After the enclosure of the antibody-conjugated micelles, there was a diminutive however substantial raise in the particle size of the DTX liposomes suggesting the existence of antibody molecules on the liposome surface. Both targeted and nontargeted liposomes demonstrate binding affinities to A549 cells due to the lack of carbonic anhydrase-IX antigen. The targeted liposomes were found to divulge 1.65-fold higher binding affinity when carbonic anhydrase (CA)-IX was induced. To the liposomal surface, antibody coupling could boost up the liposomal uptake in lung cancer cells by immunobinding to CA-IX on the cell surface and CA-IX mediated liposomal uptake in lung cancer cells could enhance the drug internalization and thus contribute to a higher anticancer activity. Therefore, *in-vivo* studies founded on various liposomal antibody densities are anticipated to further resolve whether CA-IX-directed immunoliposomes can increase drug accumulation in the tumor, leading to a higher therapeutic response[104].

3.13. Biotherapeutic agent evaluation for in-vitro and in-vivo effectiveness

Zhao *et al.*[108] evaluated the *in-vivo* and *in-vitro* efficiency of biotherapeutic entities made up of proteins of lysosome, phospholipid [dioleoyl phosphatidyl serine (DOPS), Saposin-C (SapC)]. This biotherapeutic agent was accumulated into nanovesicle (SapC–DOPS) for antitumor activity on of lungs cancer. Lung cancer is the deadlist and most pervasive type of cancer in the global according to Torre *et al.*[109]. Anionic phospholipid (phosphatidyl serine) is particularly exposed on the surface of cancer cells and tumor-associated vasculature targeted by the SapC–DOPS. As the binding of SapC to phosphatidyl serine is ideal at acidic pH, and then the effect of pH on the binding potential of SapC–DOPS to lung tumor cells was checked[108]. Zhao *et al.*[108] accomplished that the acidic pH promoted the binding of SapC–DOPS to human tumor cells. Viability assays by Mahata *et al.*[86] on a section of human lung cancer cells observed that cytotoxcity of SapC–DOPS was positively allied with cell peripheral phosphatidylserine concentrations, while mitochondrial membrane prospective detections were reliable with apoptotic ways. Fluorescence tracking method of Wojton *et al.*[110] in alive mice explained that SapC–DOPS precisely fixed for human lung cancer xenografts[111], and the systemic treatment with SapC–DOPS provoked tumor cell demise and mainly resisted tumor expansion. These consequences suggested that (SapC–DOPS) nanovesicles are astonishing choice for therapy of lung cancer[108].

4. Conclusion

The nanoparticles which are conceded by the liposomes (carriers), target the cancer cells more immaculately than the other conformist carriers. Drugs are encapsulated on the liposomes then it is injected into the body for cancer treatment. The pH affects the binding of the

drugs to the liposome and also the release of drugs from liposome at cancer target cells. The liposomes are used as a most effective carrier than others because of its greater constancy, enhanced circulation time and thermodynamical stablility. The liposomes release drugs most effectively so that normal cell damage is negligible. So, liposomes as nanocarriers are effective for the targeted delivery in lung cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are very thankful to Prof. Dr. Najam Ul Haq, Chairman Department of Biochemistry, BZU Multan, Pakistan for his continuous support as he is expert in cancer research.

References

[1] Gupta S, Yadav BS, Kesharwani R, Mishra KP, Singh NK. The role of nanodrugs for targeted drug delivery in cancer treatment. *Arch Appl Sci Res* 2010; **2**(1): 37-51.

[2] Rodrigues A, Emeje M. Recent applications of starch derivatives in nanodrug delivery. *Carbohydr Polym* 2012; **87**: 987-94.

[3] Wang Z, Lee TY, Ho PC. A novel dextran-oleate-cRGDfK conjugate for self-assembly of nanodrug. *Basic Sci* 2011; **8**: 194-203.

[4] Kumar A, Zhang X, Liang XJ. Gold nanoparticles: emerging paradigm for targeted drug delivery system. *Biotechnol Adv* 2013; **31**: 593-606.

[5] Rastegari A, Atyabi F. Nanotechnology approaches in cancer therapy, research gate. In: Akhoundi MM, Mohammadi Z, Rasekh M, editors. *Nanotechnology in diagnosis and treatment of hard to treat diseases.* Tehran: Avicenna Research Institute; 2015, p. 198-224.

[6] Mistry SK, Govender T, Srivastava R, Yergeri M. Nanodrug delivery in reversing multidrug resistance in cancer cells. *Pharmacology* 2014; **5**: 159.

[7] Majumdar D, Jung KH, Zhang H, Nannapaneni S, Wang X, Amin ARMR, et al. Luteolin nanoparticle in chemoprevention: in vitro and in vivo anticancer activity. *Cancer Prev Res* 2014; **7**: 65-73.

[8] Díaz MR, Mejia PEV. Nanoparticles as drug delivery systems in cancer medicine: emphasis on RNAi-containing nanoliposomes. *Int J Pharm* 2013; **6**: 1361-80.

[9] Elzoghby AO. Gelatin-based nanoparticles as drug and gene delivery systems: reviewing three decades of research. *J Control Release* 2013; **172**: 1075-91.

[10] Liu R, Lai Y, He B, Li Y, Wang G, Chang S, et al. Supramolecular nanoparticles generated by the self-assembly of polyrotaxanes for antitumor drug delivery. *Int J Nanomed* 2012; **7**: 5249-58.

[11] Zhao Z, Li Y, Xie MB. Silk fibroin-based nanoparticles for drug delivery. *Int J Mol Sci* 2015; **16**: 4880-903.

[12] Pearce TR, Shroff K, Kokkoli E. Peptide targeted lipid nanoparticles for anticancer drug delivery. *Adv Mater* 2012; **24**: 3803-22.

[13] Wu HC, Chang DK. Peptide-mediated liposomal drug delivery system targeting tumor blood vessels in anticancer therapy. *Int J Oncol* 2010; doi: 10.1155/2010/723798.

[14] Bhaskar S, Tian F, Stoeger T, Kreyling W, Fuente JMDL, Grazú V, et al. Multifunctional nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: perspectives on tracking and neuroimaging. *Part Fibre Toxicol* 2010; **7**: 3.

[15] Sahoo B, Devi KSP, Banerjee R, Maiti TK, Pramanik P, Dhara D. Thermal and pH responsive polymer-tethered multifunctional magnetic nanoparticles for targeted delivery of anticancer drug. *ACS Appl Mater Interfaces* 2013; **5**: 3884-93.

[16] Han HS, Thambi T, Choi KY, Son S, Ko H, Lee MC, et al. Bioreducible shell-cross-linked hyaluronic acid nanoparticles for tumor-targeted drug delivery. *Biomacromolecules* 2015; **16**: 447-56.

[17] Kou LF, Sun J, Zhai YL, He ZG. The endocytosis and intracellular fate of nanomedicines: implication for rational design. *Asian J Pharm Sci* 2013; doi: 0.1016/j.ajps.2013.07.001.

[18] Khan DR. The use of nanocarriers for drug delivery in cancer therapy. *J Cancer Sci Ther* 2010; **2**(3): 58-62.

[19] Jeong YD, Saha K, Yan B, Rotello VM. Nanoparticle-dendrimer hybrid nanocapsules for therapeutic delivery. *Nanomedicine* 2016; doi: 10.2217/nnm-2016-0034.

[20] Kumar M, Yigit M, Dai G, Moore A, Medarova Z. Image-guided breast tumor therapy using a small interfering RNA nanodrug. *Cancer Res* 2010; **70**(19): 7553-61.

[21] Yadav G, Sharma N, Bansal M, Thakur N. Application of natural polysaccharide for delivery of biopharmaceuticals. *Int J Pharm Life Sci* 2013; **4**(6): 2756-65.

[22] Kamba SA, Ismail M, Hussein-Al-Ali SH, Ibrahim TAT, Zakaria ZAB. *In-vitro* delivery and controlled release of doxorubicin for targeting osteosarcoma bone cancer. *Molecules* 2013; **18**(9): 10580-98.

[23] Lim EK, Jang EJ, Lee K, Haam S, Huh YM. Delivery of cancer therapeutics using nanotechnology. *Pharmaceutics* 2013; **5**: 294-317.

[24] Alexander CM, Dabrowiak JC, Maye MM. Investigation of the drug binding properties and cytotoxicity of DNA-capped nanoparticles designed as delivery vehicles for the anticancer agents doxorubicin and actinomycin D. *Bioconjugate Chem* 2011; **23**(10): 2061-70.

[25] Martins AF, Facchi SP, Monteiro JP, Nocchi SR, Silva CTP, Nakamura CV, et al. Preparation and cytotoxicity of N,N,N-trimethyl chitosan/alginate beads containing gold nanoparticles. *Int J Biol Macromol* 2015; **72**: 466-71.

[26] Vigneshvar S, Sudhakumari C, Senthilkumaran B, Prakash H. Recent advances in biosensors technology fopr potential applications - an overview. *Front Bioeng Biotechnol* 2016; **4**: 11.

[27] Khanna VK. Targeted delivery of nanomedicines. *Pharmacology* 2012; doi: 10.5402/2012/571394.

[28] Bui DT, Nicolas J, Maksimenko A, Desmaele D, Couvreur P. Multifunctional squalene-based prodrug nanoparticles for targeted cancer therapy. *Chem Commun* 2014; **50**(40): 5336-8.

[29] Kim TH, Lee GJ, Kang JH, Kim HJ, ilKim T, Oh JM. Anticancer drug-incorporated layered double hydroxide nanohybrids and their enhanced anticancer therapeutic efficacy in combination cancer treatment. *BioMed Res Int* 2014; doi: 10.1155/2014/193401.

[30] Lu J, Li ZX, Zink JI, Tamanoi F. *In vivo* tumor suppression efficacy of mesoporous silicananoparticles-based drug delivery system: enhanced efficacy by folate modification. *Nanomedicine* 2012; **8**(2): 212-20.

[31] Paszko E, Ehrhardt C, Senge MO, Kelleher DP, Reynolds JV. Nanodrug applications in photodynamic therapy. *Photodiag Photodyn Ther* 2011; **8**(1): 14-29.

[32] Zhang M, Kim YK, Cui PF, Zhang JL, Qiao JB, He YJ, et al. Folate-conjugated polyspermine for lung cancer-targeted gene therapy. *Acta Pharm Sin B* 2016; **6**(4): 336-43.

[33] Fonseca-Santos B, Gremião MPD, Chorilli M. Nanotechnology-based drug delivery systems for the treatment of Alzheimer's disease. *Int J Nanomed* 2015; **10**: 4981-5003.

[34] Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. *Adv Drug Deliv Rev* 2013; **65**(1): 36-48.

[35] Yadav SK, Parvez N, Sharma PK. An overview and new strategies to improve formulation development of anticancer drugs. *Adv Biol Res*

2014; **8**(6): 233-43.

[36] Ortega MJS, Stauner T, Loretz B, Ortega-Vinuesa JL, Bastos-González D, Wenz G, et al. Nanoparticles made from novel starch derivatives for transdermal drug delivery. *J Control Release* 2010; **141**(1): 85-92.

[37] Koo H, Min KH, Lee SC, Park JH, Park K, Jeong SY, et al. Enhanced drug-loading and therapeutic efficacy of hydrotropic oligomer-conjugated glycol chitosan nanoparticles for tumor-targeted paclitaxel delivery. *J Control Release* 2013; **172**(3): 823-31.

[38] Mouffouk F, Simao T, Dornelles DF, Lopes AD, Sau P, Martins J, et al. Self-assembled polymeric nanoparticles as new, smart contrast agents for cancer early detection using magnetic resonance imaging. *Int J Nanomed* 2015; **10**: 63-76.

[39] Lee JY, Chung SJ, Cho HJ, Dukkim D. Bile acid-conjugated chondroitin sulfate A-based nanoparticles for tumor-targeted anticancer drug delivery. *Eur J Pharm Biopharm* 2015; **94**: 532-41.

[40] Song WT, Tang Z, Zhang D, Zhang Y, Yu H, Li MQ, et al. Anti-tumor efficacy of c(RGDfK)-decorated polypeptide-based micelles co-loaded with docetaxel and cisplatin. *Biomaterials* 2014; **35**(9): 3005-14.

[41] Ochs CJ, Hong T, Such GK, Cui J, Postma A, Caruso F. Dopamine-mediated continuous assembly of biodegradable capsules. *Chem Mater* 2011; **23**(13): 3141-3.

[42] Danhier F, Feron O, Preat V. To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *J Control Release* 2010; **148**(2): 135-46.

[43] Fang JU, Al-Suwayeh SA. Nanoparticles as delivery carriers for anticancer prodrugs. *Expert Opin Drug Deliv* 2012; **9**(6): 657-69.

[44] Wang J, Cai X, Zhang Y. Synthesis and antitumor efficacy of daunorubicin-loaded magnetic nanoparticles. *Int J Nanomed* 2011; **6**: 203-11.

[45] Du JZ, Du XJ, Mao CQ, Wang J. Tailor-made dual pH-sensitive polymer doxorubicin nanoparticles for efficient anticancer drug delievery. *J Am Chem Soc* 2011; **133**(44): 17560-3.

[46] Deshayes S, Cabral H, Ishii H, Miura Y, Kobayashi S, Yamashita T, et al. Phenyl boronic acid-installed polymeric micelles for targeting sialylated epitopes in solid tumors. *J Am Chem Soc* 2013; **135**(41): 15501-7.

[47] Li W, Zhana P, Clercqb ED, Loua H, Liu X. Current drug research on PE-Gylation with small molecular agents. *Prog Polym Sci* 2013; **38**: 421-44.

[48] Yassin AEB, Albekairy A, Alkatheri A, Sharma RK. Anticancer-loaded solid lipid nanoparticles: high potential advancement in chemotherapy. *Dig J Nanomater Bios* 2013; **8**(2): 905-16.

[49] Wu J, Song C, Jiang C, Shen X, Qiao Q, Hu Y. Nucleolin targeting AS1411 modified protein nanoparticle for antitumor drugs delivery. *Mol Pharm* 2013; **10**(10): 3555-63.

[50] Kanapathipillai M, Brock A, Ingber DE. Nanoparticle targeting of anti-cancer drugs that alter intracellular signaling or influence the tumor microenvironment. *Adv Drug Deliv Rev* 2014; **15**(79-80): 107-18.

[51] McCalden TA, Abra RM, Mihalko PJ. Bronchodilator efficacy of liposome formulations of metaproterenol sulfate in the anesthetized Guinea pig. *J Liposome Res* 1989; **1**(2): 211-22.

[52] Ney UM. Propranolol-induced airway hyperreactivity in guinea-pigs. *Br J Pharmacol* 1983; **79**(4): 1003-9.

[53] Ogedegbe G, Pickering T. Principles and techniques of blood pressure measurement. *Cardiol Clin* 2010; **28**(4): 571-86.

[54] Ahmad I, Allen TM. Antibody-mediated specific binding and cytotoxicity of liposome-entrapped doxorubicin to lung cancer cells *in vitro*. *Cancer Res* 1992; **52**: 4817-20.

[55] Allen TM, Hansen C, Martin F, Redemann C, Young AY. Liposomes containing synthetic lipid derivatives of poly (ethylene glycol) show prolonged circulation half-lives *in vivo*. *Biochim Biophys Acta* 1991; **1066**(1): 29-36.

[56] Mayer LD, Bally MB, Cullis PR. Uptake of adriamycin into large unilamellar vesicles in response to a pH gradient. *Biochim Biophys Acta* 1986; **857**(1): 123-6.

[57] Ding L, Samuel J, MacLean GD, Noujaim AA, Diener E, Longenecker BM. Effective drug-antibody targeting using a novel monoclonal antibody against the proliferative compartment of mammalian squamous carcinoma. *Cancer Immunol Immunother* 1990; **31**: 105-9.

[58] Barker SA, Taylor KMG, Short MD. The deposition and clearance of liposome entrapped 99m Tc-DTPA in the human respiratory tract. *Int J Pharm* 1994; **102**(1-3): 159-65.

[59] Farr SJ, Kellaway IW, Jones DRP, Woolfrey SG. 99m-Technetium as a marker of liposomal deposition and clearance in the human lungs. *Int J Pharm* 1995; **26**(3): 303-16.

[60] Waldrep JC, Arppe J, Jansa KA, Vidgren M. Experimental pulmonary delivery of cyclosporin A by liposome aerosol. *Int J Pharm* 1998; **160**(2): 239-49.

[61] Waldrep JC, Scherer PW, Keyhani K, Knight V. Cyclosporin A liposomal aerosol: particle size and calculated respiratory deposition. *Int J Pharm* 1993; **97**(1-3): 205-12.

[62] Waldrep JC, Arppe J, Jansa KA, Knight V. High dose cyclosporine A and budesonide-liposome aerosols. *Int J Pharm* 1997; **152**(1): 27-36.

[63] Saari M, Vidgren MT, Koskinen MO, Turjanmaa VMH, Nieminen MM. Pulmonary distribution and clearance of two beclomethasone liposome formulations in healthy volunteers. *Int J Pharm* 1999; **181**(1): 1-9.

[64] Waldrep JC, Keyhani K, Black M, Knight V. Operating characteristics of 18 different continuous-flow jet nebulizers with beclomethasone dipropionate liposome aerosol. *Chest* 1994; **105**: 106-10.

[65] Richardson VJ, Ryman BE, Jewkes RF, Jeyasingh K, Tattersall ANH, Newlands ES, et al. Tissue distribution and tumor localization of 99m-technetium-labelled liposomes in cancer patients. *Br J Cancer* 1979; **40**(1): 35-43.

[66] Saari SM, Vidgren MT, Koskinen MO, Turjanmaa VMH, Waldrep JC, Nieminen MM. Regional lung deposition and clearance of 99mTc-labeled beclomethasone-DLPC liposomes in mild and severe asthma. *Chest* 1998; **113**: 1573-9.

[67] Fonseca C, Simoes S, Gaspar R. Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and *in vitro* anti-tumoral activity. *J Control Release* 2002; **83**: 273-86.

[68] Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita BS. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int J Pharm* 1989; **55**(1): R1-R4.

[69] Szoka F Jr., Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. *Prod Nat Acad Sci U S A* 1978; **75**(9): 4194-8.

[70] Singla AK, Garg A, Aggarwal D. Paclitaxel and its formulations. *Int J Pharm* 2002; **235**(1-2): 179-92.

[71] Serpe L, Catalano MG, Cavalli R, Ugazio E, Bosco O, Canaparo R, et al. Cytotoxicity of anticancer drugs incorporated in solid lipid nanoparticles on HT-29 colorectal cancer cell line. *Eur J Pharm Biopharm* 2004; **58**(3): 673-80.

[72] Avelar-Freitas BA, Almeida VG, Pinto MCX, Mourão FAG, Massensini AR, Martins-Filho OA, et al. Trypan blue exclusion assay by flow cytometry. *Braz J Med Biol Res* 2014; **47**(4): 307-15.

[73] Mugabe C, Azghani AO, Omri AW. Liposome-mediated gentamicin delivery: development and activity against resistant strains of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients. *J Antimicrob Chemother* 2005; **55**(2): 269-71.

[74] Kallinteria P, Antimisiarisa SG, Karnabatidisb D, Kalogeropouloub C, Tsotab I, Siablis D. Dexamethasone incorporating liposomes: an in

vitro study of their applicability as a slow releasing delivery system of dexamethasone from covered metallic stents. *Biomaterials* 2002; **23**(24): 4819-26.

[75] Omri A, Suntres ZE, Shek PN. Enhanced activity of liposomal polymyxin B against *Pseudomonas aeruginosa* in a rat model of lung infection. *Biochem Pharmacol* 2002; **64**(9): 1407-13.

[76] Gavaldà J, María-Teresa M, López P, Gomis X, José-Luís R, Rodríguez D, et al. Efficacy of nebulized liposomal amphotericin B in treatment of experimental pulmonary aspergillosis. *J Virol* 2005; **49**(7): 3028-30.

[77] Taylor RW, Palagiri AV. Central venous catheterization. *Crit Care Med* 2007; **35**(5): 1390-6.

[78] Anabousi S, Bakowsky U, Schneider M, Huwer H, Lehr CM, Ehrhardt C. *In vitro* assessment of transferrin-conjugated liposomes as drug delivery systems for inhalation therapy of lung cancer. *Eur J Pharm Sci* 2006; **29**(5): 367-74.

[79] Michael CW, King JAC, Hester BR. Confocal laser scanning microscopy and three-dimensional reconstruction of cell clusters in serous fluids. *Diagn Cytopathol* 1997; **17**(4): 272-9.

[80] Wijagkanalan W, Higuchi Y, Kawakami S, Teshima M, Sasaki H, Hashida M. Enhanced anti-inflammation of inhaled dexamethasone palmitate using mannosylated liposomes in an endotoxin-induced lung inflammation model. *Mol Pharmacol* 2008; **74**(5): 1183-92.

[81] Kawakami S, Wong J, Sato A, Hattori Y, Yamashita F, Hashida M. Biodistribution characteristics of mannosylated, fucosylated, and galactosylated liposomes in mice. *Biochim Biophys Acta* 2000; **1524**(2-3): 258-65.

[82] Hohmann AW, Faulkner P. Monoclonal antibodies to baculovirus structural proteins: determination of specificities by Western blot analysis. *Virology* 1983; **125**(2): 432-44.

[83] Taetz S, Bochot A, Surace C, Arpicco S, Renoir JM, Schaefer UF, et al. Hyaluronic acid-modified DOTAP/DOPE liposomes for the targeted delivery of anti-telomerase siRNA to CD44-expressing lung cancer cells. *Oligonucleotides* 2009; **19**(2): 103-16.

[84] Haley B, Tang G, Zamore PD. *In vitro* analysis of RNA interference in drosophila melanogaster. *Science* 2003; **30**(4): 330-6.

[85] Kremer JMH, Esker MWJvd, Pathmamanoharan C, Wiersema PH. Vesicles of variable diameter prepared by a modified injection method. *Biochemistry* 1977; **16**(17): 3932-5.

[86] Mahata S, Maru S, Shukla S, Pandey A, Mugesh G, Das BC, et al. Anticancer property of *Bryophyllum pinnata* (Lam.) Oken, leaf on human cervical cells. *BMC Complement Altern Med* 2012; **12**: 15-25.

[87] Herbert BS, Hochreiter AE, Wright WE, Shay JW. Nonradioactive detection of telomerase activity using the telomeric repeat amplification protocol. *Nat Protoc* 2006; **1**(3): 1583-90.

[88] Chang DK, Lin CT, Wu CH, Wu HC. A novel peptide enhances therapeutic efficacy of liposomal anti-cancer drugs in mice models of human lung cancer. *PLoS One* 2009; **4**(1): e4171.

[89] Zwick MB, Shen J, Scott JK. Phage-displayed peptide libraries. *Curr Opin Biotechnol* 1998; **9**(4): 427-36.

[90] Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel–carboplatin alone or with bevacizumab for non–small-cell lung cancer. *N Eng J Med* 2006; **335**(24): 2542-50.

[91] Chen X, Huang W, Wong BC, Yin L, Wong YF, Xu M, et al. Liposomes prolong the therapeutic effect of anti-asthmic medication via pulmonary delivery. *Int J Nanomed* 2012; **7**: 1139-48.

[92] Huang WH, Yang ZJ, Wong YF, Zhao ZZ, Liu L. Development of liposomal salbutamol dry powder inhaler formulation. *Biol Pharm Bull* 2010; **33**(3): 512-7.

[93] Okumura S, Fukuda Y, Takahashi K, Fujita T, Yamamoto A, Muranish S. Transport of drugs across the *Xenopus* pulmonary membrane and their absorption enhancement by various absorption enhancers. *Pharm Res* 1996; **13**(8): 1247-51.

[94] Gaspar MM, Bakowsky U, Radomska A, Radomski MW, Gobbo OL, Ehrhardt C. Targeted delivery of transferrin-conjugated liposomes to an orthotopic model of lung cancer in nude rats. *J Aerosol Med Pulm Drug Deliv* 2012; **25**(6): 310-8.

[95] Li B, Torossian A, Li W, Schleicher S, Niu K, Giacalone NJ, et al. A novel bioluminescence orthotopic mouse model for advanced lung cancer. *Radiat Res* 2011; **176**(4): 486-93.

[96] Chang DK, Li PC, Lu RM, Jane WN, Wu HC. Peptide-mediated liposomal doxorubicin enhances drug delivery efficiency and therapeutic efficacy in animal models. *PLoS One* 2013; **8**(12): e83239.

[97] Chang DK, Lin CT, Wu CH, Wu HC. A novel peptide enhances therapeutic efficacy of liposomal anti-cancer drugs in mice models of human lung cancer. *PLoS One* 2009; **4**(1): e4171.

[98] Lee HY, Mohammed KA, Kaye F, Sharma P, Moudgil BM, Clapp WL, et al. Targeted delivery of let-7a micro RNA encapsulated ephrin-A1 conjugated liposomal nanoparticles inhibits tumor growth in lung cancer. *Int J Nanomed* 2013; **8**(1): 4481-94.

[99] Kinch MS, Moore MB, Harpole DH. Predictive value of the EphA2 receptor tyrosine kinase in lung cancer recurrence and survival. *Clin Cancer Res* 2003; **9**(2): 613-8.

[100]Khatri N, Rath M, Baradia D, Misra A. cRGD grafted siRNA nano-constructs for chemosensitization of gemcitabine hydrochloride in lung cancer treatment. *Pharm Res* 2013; **32**(3): 806-18.

[101]Scott V, Clark AR, Docherty K. The gel retardation assay, methods in molecular biology. *Protocols Gene Anal* 1994; **31**: 339-47.

[102]Almgren M, Edwards K, Karlsson G. Cryo transmission electron microscopy of liposomes and related structures. *Colloids Surf A Physicochem Eng Asp* 2000; **174**(1-2): 3-21.

[103]Kerbel RS. Antiangiogenic therapy: a universal chemo sensitization strategy for cancer. *Science* 2006; **312**(5777): 1171-5.

[104]Wong BCK, Zhang H, Qin L, Chen H, Fang C, Lu A, et al. Carbonic anhydrase IX-directed immunoliposomes for targeted drug delivery to human lung cancer cells *in vitro*. *Drug Des Dev Ther* 2014; **8**: 993-1001.

[105]Jaafar-Maalej C, Diab R, Anrieu V, Elaissari A, Fessi H. Ethanol injection method for hydrpophilic and lipophilic drug-loaded liposome preparation. *J Liposome Res* 2010; **20**(3): 228-43.

[106]Iden DL, Allen TM. *In vitro* and *in vivo* comparison of immunoliposomes made by conventionaal coupling techniques with those made by a new post insertion approach. *Biochem Biophys Acta* 2001; **1513**(2): 207-16.

[107]Nielsen UB, Kirpotin DB, Pickering EM, Drummond DC, Marks JD. A novel assay for monitoring internalization of nanocarrier coupled antibodies. *BMC Immunology* 2006; **7**: 24-38.

[108]Zhao S, Chu Z, Blanco VM, Nie Y, Hou Y, Qi X. SapC–DOPS nanovesicles as targeted therapy for lung cancer. *Clin Cancer Res* 2015; **14**: 491-9.

[109]Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *Cancer J Clin* 2015; **65**: 87-108.

[110]Wojton J, Chu Z, Mathsyaraja H, Meisen WH, Denton N, Kwon CH, et al. Systemic delivery of SapC-DOPS has antiangiogenic and antitumor effects against glioblastoma. *Mol Ther* 2013; **21**(8): 1517-25.

[111]Chu Z, Abu-Baker S, Palascak MB, Ahmad SA, Franco RS, Qi X. Targeting and cytotoxicity of SapC-DOPS nanovesicles in pancreatic cancer. *PLoS One* 2013; **8**(10): e75507.