International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 1, 2023

Review Article

LIPOSOMAL DRY POWDER INHALER: NOVEL PULMONARY TARGETED DRUG DELIVERY SYSTEM FOR THE TREATMENT OF LUNG CANCER

MANSING PATIL*, PINKAL PATEL

Department of Quality Assurance, Parul Institute of Pharmacy & Research, Parul University, P.O. Limda, Tal. Waghodia, Dist. Vadodara, Gujarat 391760, India.
***Email: mansingpatil007@gmail.com**

Received: 15 Oct 2022, Revised and Accepted: 19 Nov 2022

ABSTRACT

Lung cancer is a great evil doer behind mortality around the world. The degree of lung cancer patients in developing nations has grown from 31% to 49.9% over the recent 20 y. Despite current upgrades in lung cancer chemotherapy, the death rate in lung cancer patients is high. Generally, cancer chemotherapy is accompanied by most side effects. If an anticancer drug could deliver only the right site in the right concentration at the right time, cancer could be cured without side effects. A liposomal dry powder inhaler (LDPI) is an innovative strategy to convey drug particles. A dry powder inhaler (DPI) has unique features such as targeted drug delivery, improved bioavailability, and the better therapeutic efficacy of the embedded drug's ability to deliver the drug at a constant rate. This paper emphasizes the utility of liposomes and DPI in lung cancer therapy, commonly used formulation techniques for manufacturing LDPI, various devices used to deliver the therapeutic formulation, and ongoing and recently concluded clinical trials. Patents filed by multiple researchers and the future perspective of LDPI in an innovative drug delivery system and promising systems for administering a wide variety of drugs, including anti-cancer drugs, are described for lung cancer.

Keywords: Lung cancer, Lung targeted pulmonary drug delivery, Liposomal dry powder inhalers, Inhalation devices, Clinical trials, and Patents

DOI: https://dx.doi.org/10.22159/ijap.2023v15i1.46611. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Lung cancer

Lung cancer is the commonest malignancy worldwide [1] and also a devil for a society that killed more than billions of humans, and 135,720 more are estimated deaths by 2020 in the U. S [2, 3]. Human lung cancer is categorized into two significant types, small cell lung cancer (SCLC) and non-small cell lung carcinoma (NSCLC), the last comprising many types [4]. NSCLC is the most widely recognized category worldwide, and different clinical examinations are presently evaluating new chemotherapeutic combinations. Also, protection from cytotoxic operators has been observed, including the apoptotic system in NSCLC cell lines. In SCLC, five-year endurance stays under 10%, regardless of the utilization of different medications [5]. We do not focus on a single preliminary examination to present a broad picture of the association between lung cancer and smoking. Yet, we evaluate the association with each of the smoking lists. Let's see how these relationships differ according to attributes, for example, gender, age, area, study plan, period considered, the definition of the exhibition, and the extent of the confounder adjustment [6]. Surgery is the current standard for the care of patients in stage-1 NSCLC, yet this may be to considerable morbidity and even mortality, especially since patients experiencing lung cancer are regularly old age with high comorbidity rates [7]. Current therapeutic techniques, for example, radiation or chemotherapy treatment, are just operative in the preliminary phases of treatment of SCLC. At the same time, NSCLC is less sensitive to such treatment modalities, leaving surgery and gene therapy as other conceivable choices to handle NSCLC and lung cancer stem cells. Thus, the complete removal of this illness requires another approach, for example, nanoscale materials utilization [8]. A maximum fraction other f the drugs delivered to the targeted (diseased) site with minimizing exposure of the drug to the normal tissue can be achieved by a site-specific drug delivery system [9]. Targeted Pulmonary Drug Delivery is an effective way for lung diseases and the objective of this drug delivery system is to target specific cells or regions of the lung and provide high retention of the drug for longer periods [10]. The dry powder inhaler (DPI) has been a very effective technique for the delivery of drugs to the lung and more prepared over pressurized metered dose inhalers and nebulizers in the last two decades [11]. Liposomes are an especially attractive drug delivery to the lung for inhalation application with controlled release properties that potentiate the maximum effect of the drug over a prolonged period [12]. The advantage of liposomal carrier systems in the form of inhalation includes lower toxicity of the drug with increasing stability of drug [13]. Apart from this, this article summarizes the worldwide clinical studies and the granted patents on lung cancer treatment; before this, clarity on the causes of lung cancer is essential, detailed in the below section.

A common route of causes of lung cancer

A complete understanding of the causes of lung cancer has long been a ridiculous goal of physicians and basic scientists. Unlike many other deadly diseases, most lung cancer cases may be prevented by avoiding incriminated environmental factors. In many cases, the quantitative hazard is related to long-haul low-dose exposure to a few of these equivalent cancer-causing agents in circumstances in which voluntary avoidance is increasingly complex. More population-based investigations should directly examine these lesser exposure theories [14]. There are various routes of causes or risk factors [fig. 1] for lung cancer, some of the very frequent are discussed below:

Fig. 1: Risk factors for lung cancer [Authors creation]

Smoking

Smoking, i.e., tobacco is the common ground hazard for most diseases. On account of lung disease, this relationship has been very much archived with several years of proof, mainly through epidemiological investigations. In the United States, an expected 90.3% passing in men

and 78.5% of women directly result from smoking. It has been guessed that the risk of malignant lung growth builds more when the connection is balanced for the number of cigarettes smoked and the length of the propensity. Smoking enhances the chances of forming a primary lung tumor. For early-stage tumors, smoking cessation is linked with a substantial reduction in the risk of death; most of this benefit is probably because of cancer progression rather than a decline in cardiorespiratory deaths [15, 16].

Metals

The hazardous cellular breakdown is raised by chromium [VI] in the lungs among chromate creation, chromate shading producers, chromium platers, and ferrochromium creators. No such threat has been seen among workers introduced, especially chromium [III]. Assessments of nickel excavators, smelters, electrolysis laborers, and high-nickel composite makers demonstrated an extended danger of cellular breakdown in the lungs [17].

Silica dust

Increased danger of a cellular breakdown in the lungs has been expressed *in silico*tic patients' partners. Various assessments investigated glasslike silica-uncovered laborers in foundries, ceramics, and so forth, many of whom may create silicosis. A couple represented the more danger of a cellular breakdown in lung development; at any rate, not all, were considered. The expansion was nearly nothing in the upbeat assessments, proving an openness reaction relationship in the high-openness range [18].

Air pollution

Indoor or outdoor air pollution is a significant environmental risk for lung cancer. Long-distance exposure to foul air brought by formaldehyde from industry and automobiles, cooking vapors, or indoor decorations increases lung cancer. Preliminary environmental tests have found that 50% or more of all lung cancers occur in urban areas, probably more likely than in rural areas to have air pollution from industrial sources and vehicles. A series of case-control and comprehensive studies found an essential relationship between lung cancer and air pollution with appropriate adjustments for tobacco use and other potential risk factors [19].

Asbestos

This ordinary stone has been viewed as a potential cancer-causing agent for many years. Shipyard workers, advancement workers, and asbestos diggers are high-hazard occupations. Cellular breakdown in the lungs is a crucial infection related to asbestos, representing 20% of deaths in exposed populations. It is accepted that a wide range of asbestos strands are hazardous factors for all histological categories of lung cancer. The span of induction usually is 20 y. It should be noted that just breathing in asbestos seems to be engaged in a cellular breakdown in the lungs, as there is no absolute requirement for this ailment related to the existence of fibres in drinking water [20].

Radiation

Radiation is one of the causes of lung cancer; most of the time, high doses of radiation can produce lung cancer by exposure to ionizing radiation. There are two types of radiation based on the transfer rate of energy to tissue, i.e., low linear energy transfer (LET) radiation example γ-rays, x-rays and the second is high LET radiation example radon and neutrons. High LET is more responsible for producing lung cancer due to the higher production of ionization radiation than low LET [21].

Current treatment options for lung cancer

There are various treatment options available for managing lung cancer. A few are the frequently employed methods are discussed below:

Surgery

A patient who stages I, II, and III-A NSCLC ordinarily has the activity to clear the tumor if the tumor is viewed as resectable and the patient can persevere through the training. Experts may oust a lung fragment that has cancer. To choose whether a tumor is resectable, imaging studies and biopsies are done similarly to evaluate patient factors to determine operability. Various experts use the video-helped thoracoscopic medical procedure (VATS), where a somewhat cut is made in the chest, and a thoracoscopic is introduced. A flap can be removed utilizing this slight section point enlargement, so a more prominent slice isn't expected to make [22, 23].

Chemotherapy

About 40% of the investigated cellular breakdown in the lung's developments are in stage IV. The target of remunerating these patients decreases the risk of perseverance and disease. For Stage IV NSCLC, cytotoxic mixed chemotherapy is the star treatment, which can be influenced by histology, age versus comorbidity, and execution status. The results show that none of the practices demonstrated a colossal commonness over the other compound. The center's general perseverance for patients in these examinations was around 8–10 mo [23, 24].

Radiotherapy

Radiotherapy utilizes high-energy beams to terminate the deoxyribonucleic acid (DNA) inside malignant cells and obliterate them. This treatment is to control or kill the tumor locally in the body. Patients with NSCLC are localized to the chest and are not likely to resemble caution and can benefit from radiotherapy. Furthermore, radiotherapy can fill in the thought of the considerable advantages of improving patients' satisfaction with NSCLC who fails to respond to chemotherapy [25].

Immunotherapy

Immunotherapy utilizes medications to stimulate an individual's immune system to adequately recognize and crush disease cells. Several drugs that may be used in lung cancer chemotherapy are discussed below:

Immune checkpoint inhibitors

An essential job of the body's resistant system is its capability to prevent itself from invading body cells. The body uses "checkpoints" or proteins on immune cells that should be turned on or off to initiate an immune reaction. Cancer cells use checkpoints a couple of times to keep the immune system from attacking. In any case, the drugs that focus on these checkpoints are beneficial [26].

Nivolumab and pembrolizumab

Target programmed death (PD-1), a protein on T cells [a kind of resistant cell], typically keep these cells safe from offensive s cells inside the body. By blocking PD-1, these medications help in the insusceptible reaction against malignancy cells. This can make a few tumors shrivel or delay down. They are utilized to treat progressed SCLC in people whose cancer keeps on becoming in the wake of getting, in any event, two past medicines, incorporating chemotherapy with cisplatin or carboplatin [27].

Atezolizumab

It targets PD-L1, a protein identified with PD-1 found in some cancer and immune cells. Blocking this protein can help support first-line treatment in contradiction of cancer cells. Such medication can be utilized as a procedure for first-line treatment of SCLC, alongside the chemo drugs carboplatin and etoposide, and afterward proceeded alone as a maintenance treatment. These medications are taken intravenously infusion, typically every 2, 3 w, or a month [26].

Concomitant chemotherapy and radiotherapy

For the study of NSCLC, platinum-based drug complexes or single platinum compounds are used. Cisplatin drugs and carboplatin drugs have been affirmed in clinical examinations based on radiosensitive properties. Practicality examines shown that when taking 6–8 mg/m^2 everyday portion or 30 mg/m2 w after week portion, standard suppositories are compelling as a radio beginning. In a couple of Phase III examinations utilizing comfort platinum mixtures and radiotherapy, just one improved neighborhood control and longer endurance. For this examination directed by the European Organization for the Research and Treatment of Cancer

(EORTC), 331 patients were arbitrarily appointed to one of three governments: confined outspread separation [30 Gy. Followed in 10 sections, with three weeks' rest. Period and past 25 Gy in ten divisions], through radiotherapy (comparable everyday practice) day by day cisplatin (6 mg/m2), or a week after-week cisplatin (30 mg/m2) with radiotherapy [27].

Targeted therapy

The epidermal growth factor receptor (EGFR) is a transmembrane protein that is primarily over-expressed in lung carcinoma. The function of the EGFR receptor involves cell proliferation, regulating apoptosis, cell migration, and adhesion. So, the drug (gefitinib, erlotinib, cetuximab, and panitumumab) that directly binds with this receptor is essential to increase the survival time by reducing uneven toxicity and increasing patient compliance [20].

Clinical limitations of current treatments

Current therapy employed for lung cancer has lots of adverse reactions and other complications, and lope holes of the current treatment are shown below:

Platinum-based chemotherapy

Platinum-based chemotherapy offers symbolic relief and unprecedented improvement in survival for a survival time of approximately 7-10 mo [residual>2 mo]. The current immovable, non-selective choice of NSCL patients with chemotoxic chemotherapy rise to a bit increase in survival at the cost of adverse effects to the patient. Hence, more competent, less toxic compounds are required [28].

Epidermal growth factor receptor inhibitors

Although first-generation, reversible first-generation epidermal growth factors are promising treatment agents for NSCLC patients, most patients eventually resist them. Resistance to EGFR inhibitors may be categorized as initial or acquired [28].

Bevacizumab

A crucial limitation of bevacizumab therapy is that it is of limited usefulness for NSCLC patients in specific populations. Bevacizumab is allied with adverse events associated with bleeding, particularly squamous cell histology in patients. As a result, bevacizumab squamous cell is not indicated for NSCLC treatment [28].

Radiation therapy

Sometimes, radiation directly burns healthy tissues surrounding the tumor during treatment. At that time, radiation produces stress, injury, or death of a cell to lung resident cells and numbers of Damage-Associated Molecular Patterns (DAMPs) released by local immune cells into the extracellular room, end of an immunogenic cell, or both. In-sensitive patients produce sterile inflammation induced by radiation through DAMPs via Pathogen Recognition Receptors (PRR), and it can be harmful to normal tissue inflammation with life-threatening complications [25].

Worldwide regulatory and non-regulatory comments on lung cancer

Lung cancer is the most common reason for cancer death in men worldwide [29]. According to an old report by the World Health Organization (WHO), the fig. of extinction because of lung cancer will continue to rise worldwide, especially in Asia, due to raised tobacco usage. Tobacco use is a chief risk aspect causing lung cancer, and a large proportion of all pulmonary carcinomas are associated with the results of cigarette smoking [30]. Recently, in 2020 American Cancer Society's cancer static center gave data on Lung cancer along with estimated new cases and deaths in the United States of America (USA) [31]. The annual description of cancer by the National Cancer Institute in 2019, observing the mortality rate in adults between the ages of 20 and 9, stated that lung cancer is the subsequent deadliest cancer in men and women [32]. A recent World Cancer Report says that cancer is the next principal source of death worldwide, with around 70% of cancer deaths occurring in low-and middle-income nations. The economic effect of lung cancer is enormous and continuously rising. The complete yearly monetary expense of malignant growth in 2010 was assessed at US$ 1.16 trillion [33]. A new Global Cancer Observatory (GLOBOCAN) Indian fact sheet of 2018 stated the unique measurements and concluded that lung cancer's root of death is in the fourth position among other cancers. Even the United State Food and Drug Administration (USFDA) reported more than 228,000 new cases of both SCLC and NSCLC and almost 160,000 deaths from lung cancer, creating it the principal root of cancer mortality in the country for both men and women [34, 35]. The rest of the regulatory and non-regulatory institutions' discussion on lung cancer is listed in fig. 2 below:

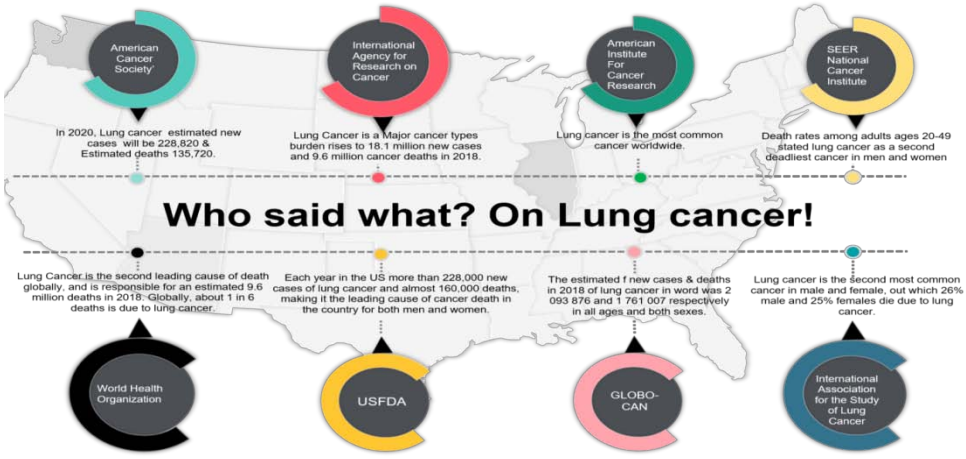

Fig. 2: Worldwide regulatory and non-regulatory comments on lung cancer [Authors Creation] [36, 37]

Liposomal dry powder inhaler-based lung-targeted drug delivery system

The worldwide regulatory and non-regulatory data indicates more cases, major fear, and enormous challenges for this disease and the need for a drug system that is engineered in an unconventional form; in this regard, Liposomes can serve as a better delivery system as they are enclosed with medications having anticancer potential and prove the activity in the lung after pulmonary delivery [38]. Liposomes are vascular systems containing natural phospholipids and cholesterol ranging in size from 30 mm to a few micrometers [fig. 3]. They are biologically compatible, biodegradable, less toxic, and capable of taking lipophilic and hydrophilic drugs [39]. They are capable of ligand conjugation to facilitate the site-related delivery of drugs. For the past two decades, liposomes had been perceived as a potential drug carrier for therapeutic interventions. Liposome

design focuses primarily on the supply of anti-cancer agents and includes anesthetic, anti-inflammatory, anti-parasitic, enzymatic, and vaccine agents [40]. Liposomes are formed naturally when phosphatides are dissolved in water. When the phosphatides are placed in the middle of the fluid, the hydrophilic interaction of the groups of lipid heads with water brings about a multi and uni-lamellar system [41]. The drug's therapeutic index may be improved by incorporating liposomes that should be non-toxic, the biodegradable method for dissolving drugs with water solubility, get rid of the medication quickly, which is why the time for the medicine to stay inside the body is better. And reduces drug toxicity [42]. Inhalation drug delivery is an ancient concept for delivering active pharmaceutical constituents [43]. A liposomal powder for respiratory delivery via aerosol, prepared with phospholipid-like lung endogenous surfactant, provides a unique opportunity in pulmonary nanomedicine while controlling the drug and providing better stability [44]. Delivery of LDPI through the pulmonary route has excellent advantages, like their capability to produce controlled size, sustained release, ability to retain for a more extended period in the lungs, biocompatible, and enhanced bioavailability. Targeting drugs as nanoparticles demonstrate the potential for improved lung cancer treatment, and a blend of respiratory therapy and drug targeting is even more effective for this cancer [45]. Using DPI composed of liposomes, a novel combination of inhalation therapy, may serve as an even more accurate and target-oriented method or site-specific delivery [46].

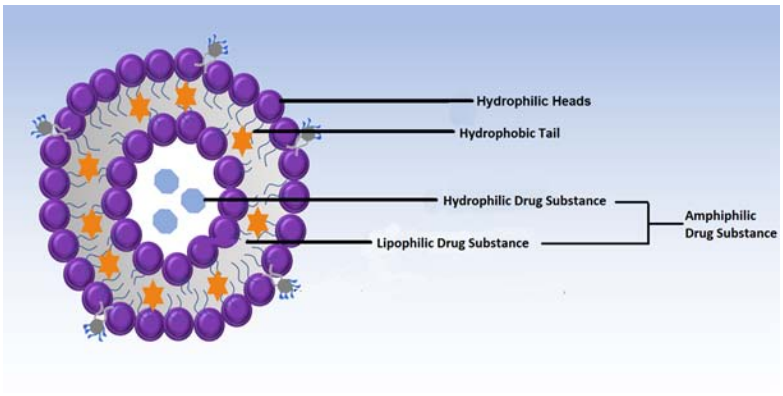

Fig. 3: The basic structure of the liposome [Authors creation]

Dry powder for inhalation is a tool that uses the pulmonary tract to produce the local or systemic effect. Inhalers DPIs offer different benefits from different techniques for supplying lung medicine [47]. A wide choice of liposomal formulations for inhalation has been developed recently to be utilized in nebulizers. That is a fast and straightforward path for researchers to create aqueous solution formulations of liposomes and enter animal and human clinical trials with the same formulation and delivery system. Importantly, nebulizers are easy to use even in infants, the elderly, patients with severely compromised lungs, and even ventilated ones. Structurally, liposomes are viscous bleeder vesicles where the volume of water is wholly enclosed in a membrane-bound lipid bile duct. Those membranes are commonly composed of phospholipids with an aqueous head cluster and a hydrophobic tail cluster.

Recently, Zhang *et al.* [48] proposed an inhalation treatment for primary lung malignant growth in 2018 by using a liposomal curcumin DPI by a freeze-drying technique. The aerodynamic air diameter of liposomal curcumin dry powder inhalers (LCDs) was 5.81 µm, and the fine particle fraction was 46.71%, recommended for pulmonary transmission. The take-up of LCDs through A549 cells was higher as well as faster compared to free curcumin. High and low cytotoxicity on A549 and bronchial BEAS-2B epithelial cells respectively achieved a significant selection index because of cell apoptosis. Curcumin powder, LCD, and drugs were sprayed through the trachea directly to the lungs of mice with induced lung cancer. LCDs demonstrated greater anticoagulants than two other medications associated with the expression of many pathology and cancer markers, including VEGF, malondidide, TNF2, Caspase-3, and BCL2. Another research work on liposomal powder for DPI aerosol delivery has been developed using phospholipids that offer release control and better stability. This study suggests that such systems should be smaller toxic exposures, controlled drug release, and increased stability as an inhaled powder system [22]. Lo, *et al.* investigated disaccharides effects and liposome carrier's activity, solid-state properties, structural preservation, and efficiency of aerosol powders of spray-dried superoxide dismutase (SOD) formulations. Sucrose and lactose as stabilizers were utilized in the spray-drying operation. Dipalmitoyl phosphatidylcholine (DPPC) was a critical lipid constituent in the preparation of liposomes. SOD activity assay was carried out for formulations. They observed that the spray drying outlet/inlet temperature could be raised to 168/122 °C to maintain SOD activity in SOD \ DPPC. Aerosol powder performance tests have shown that the formulation exhibits an excellent emitted dose of 71 %, aerodynamic diameter [2 µm], and a respirable fraction of 72 %. They concluded that spray drying is a practical process to reserve SOD activity in forming DPPC liposomes with sucrose [49].

Both jet and mesh nebulization processes expose the formation of shear and air-liquid interfaces. In jet nebulizers, the building is told several times by the nebulization method because, after each>98%, the drop to drop re-assembled by jets were re-deposited inside the nebulizer after each goes through the jets. This exposure can cause liposomal bilayers to be disrupted [fig. 4], resulting in vesicle fusion with a corresponding increase in vesicle size or fragmentation with its associated reduction in vesicle size. The medicine that is encapsulated within the liposomes, especially during vesicle fragmentation, has the opportunity to be released into the external fluid phase [50]. The present review mainly focuses on the utility of the liposomal dry powder inhaler in treating lung cancer.

Advantages of LDPI over other conventional deliveries

Generally, liposomal dispersion, under storage conditions prone to oxidation and drug leakage behavior due to lipid degradation that causes the instability of formulation or decreases the quality/shelf life of the product. To avoid this problem, liposomal dispersion is converted to dry powder formulation by adding a suitable carrier, anti-adherent, and cryoprotectant by freeze-drying. The liposomal dry powder system is more stable under storage conditions than liposomal dispersion and is preferred over conventional DPI to avoid disadvantages relating to them [50]. Advantages of pulmonary delivery include a Non-invasive administration route for local and systemic delivery of potent therapeutics, Provide rapid onset of action, High pulmonary bioavailability, Thin (0.1–0.2 µm) alveolar epithelium, and large absorptive surface area (up to 100 m 2) for local drug action, Rapid systemic drug absorption and Absence of first-pass metabolism and high blood supply [51].

Inhalation therapy effectively delivers relatively small amounts of an active ingredient straight into the respiratory system. Although the process begins very quickly, its duration is shorter because the drug

is rapidly removed from the lungs. Liposomal drug dry powder formulations have shown many promising features for pulmonary drug administration, such as selective localization of drug within the lung, controlled drug release, reduced local and systemic toxicities, propellant-free nature, patient compliance, high dose carrying capacity, and increased therapeutic efficacy [fig. 5], stability, and patent protection [52]. These systems may be divided into pressurized metered-dose inhalers (pMDIs), nebulizers, and DPI. These frameworks are carefully crafted and provide an accurate and reproductive dose of aerosolized drugs to the lungs. However, they experience the adverse effects of chlorofluorocarbon-based propellants, low lung fatigue, and problems with the rapid oropharyngeal nature. Many patient-related factors of nebulizers and pMDI can be minimized by LDPI [53]. In contrast to the nebulizer and pMDI, Liposomal DPIs have various benefits for lung delivery explicitly regarding controlled delivery, more potency, reduced toxicity, force-free, understanding consistency, high dose conveying capacity, steadiness, and patent security [53]. This delivery minimizes drug exposure to systemic circulation and thereby reduces adverse effects. Lower dosage regimens might significantly cost savings, especially with costly drugs [54]. DPI offers methods for enhancing protein-drug stability during aerosolization by utilizing a DPI that would not expose the protein formulation to the pressure regularly seen with nebulization. Proteins are progressively steady in dry powder forms; thus, dry powder product allows for long-haul stockpiling [55].

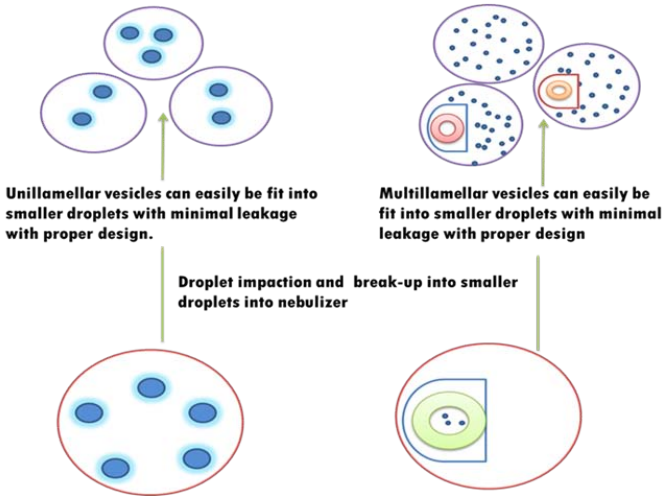

Fig. 4: Mechanism of drug release from LDPI [Authors creation]

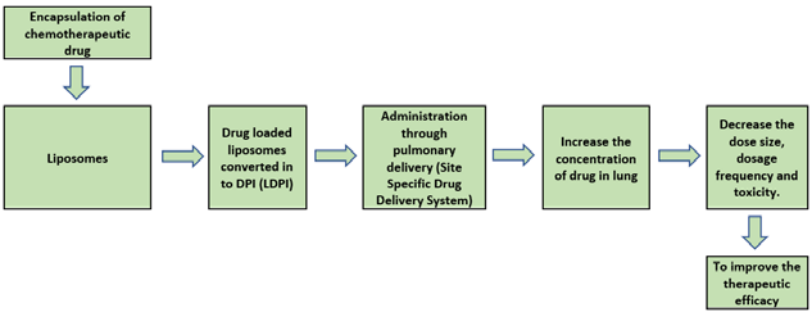

Fig. 5: Advantages of LDPI over the conventional deliveries [Authors creation]

Ideal characteristics of inhaler device for the delivery of LDPI

An inhaler gadget should be viable with the elements of LDPI; further, its physical and chemical stability should be unbent. It ought to have the least maintenance necessities, just as simplicity for refilling. An inhaler gadget should deliver a dynamic fixing dose, and the deposition of the active ingredient in the lungs should be adequately high and reproducible. The inhaler gadget ought to have control systems that will guarantee an ideal respiratory stream when the dose is set off, a proper inward breath moves, and permit the patient to check the fruitful finish of the inward inhalation maneuver.

Formulation techniques for the preparation of liposomes and LDPI

Formulation techniques for the preparation of liposomes

Several techniques based on passive and active loading principles have been used to produce liposomes. Thin-film hydration, ethanol and ether injection, reverse phase evaporation method, and double/multiple emulsification method are based on passive loading techniques, and the gradient method is based on active loading technique. There are four primary stages involved in the preparation of liposomes [56], and these are

1. Drying lipids from organic phase solvent.

2. Dispersing the lipid in aqueous phase media.

3. Purifying the obtained liposomes.

4. Characterization of purified product.

Thin film hydration method

A mixture of lipids, i.e., phospholipid and cholesterol, were dispersed in an organic solvent. The organic solvent was removed by an evaporation process mainly using a rotary evaporator at reduced pressure. A dry, thin lipid layer or film formed on the round bottom flask wall was hydrated by adding an aqueous buffer solution under agitation above the lipid transition temperature. This method is very commonly used for the production of liposomes, but the drawback

of this method is to produce large-size and multilamellar liposomes (MLVs). Thus, liposome size reduction techniques, such as sonication or extrusion through polycarbonate filters produce a smaller and more uniformly sized population of liposomes [57].

Ethanol injection/Ether injection

This method is also called solvent injection met, in which dissolve the lipid into an organic phase (ethanol or ether), and injected into the aqueous phase media (Heat the media just above the phase transition temperature of the lipid) by injection forming liposomes. The main advantages of the ethanol injection method are forming small-sized liposomes with narrow distribution and no need to use further processes like sonication or extrusion. The ether injection method differs from the ethanol injection method since the ether is immiscible in the aqueous phase, which is also heated to remove the solvent from the liposomal product. The method involves the injection of ether-lipid solutions into warmed aqueous phases above the boiling point of the ether. The ether vaporizes upon contacting the aqueous phase, and the dispersed lipid forms primarily unilamellar liposomes. An advantage of the ether injection method compared to the ethanol injection method is the removal of the solvent from the product, enabling the process to be run for extended periods forming a concentrated liposomal product with high entrapment efficiencies [57].

Reverse-phase evaporation method

A lipidic film is prepared by evaporating the organic solvent under reduced pressure. The system is purged with nitrogen, and the lipids are re-dissolved in a second organic phase, usually constituted by diethyl ether and isopropyl ether. Large unilamellar and oligolamellar vesicles are formed when an aqueous buffer is introduced into this mixture. The organic solvent is subsequently removed, and the system is maintained under continuous nitrogen. These vesicles have aqueous volume-to-lipid ratios 30 times higher than sonicated preparations and four times higher than multilamellar vesicles. Most importantly, a substantial fraction of the aqueous phase (up to 62% at low salt concentrations) is entrapped within the vesicles, encapsulating even large macromolecular assemblies with high efficiency [57].

Double/multiple emulsification methods

The lipid is dissolved in an organic solvent, and then this solution is mixed with a drug solution of aqueous phase (W1) to form a primary emulsion (W/O); then this direct emulsion is dispersed into another aqueous phase solution (W2), resulting in the formation of Double emulsion (W1/O/W2). The solvent is evaporated and homogenized and sonicated and liposomes are obtained [57].

Gradient method using active loading technique

This method is also called as remote loading method and, in this method, first prepare blank liposomes using a suitable conventional method, then add drug solution to the external medium that creates pH difference/imbalance inside and outside of liposomes that drive drug to inside liposomes. Mostly alkaline or weakly acidic drug molecules are loaded into the blank or placebo liposomes by active loading, and the process is driven by an electrochemical potential created by the pH or ion gradients established across the lipid bilayer of the liposomes. The pH or ion gradients are made during the preparation of liposomes by using the specified pH of the buffer and the concentration of the ion. The external pH of liposomes is then replaced with another buffer of different pH or ion concentrations through size exclusion or dialysis, or chromatography. The drug is loaded by mixing with liposomes, typically at a temperature above the phase transition temperature of the lipids after creating the pH gradient across the liposome's membranes to ensure fluidity and efficient transport across the bilayer. The drug molecules get charged after interacting with the ions within liposomes, and charged drug molecules cannot come out and remain entrapped within liposome vesicles [58, 59].

Formulation techniques for the preparation of LDPI

The basic conversion method of liposomes to DPI is the elimination of water from the liposomal dispersion after the addition of small quantities of cryoprotectants (trehalose, mannitol, etc.), carriers (sucrose, lactose, etc.), and anti-adherents [fig. 6]. LDPI is then sieved successively by various sieves. LDPI is prepared by mixing the liposomal dry powder with a suitable carrier, i.e., coarse and finer grade of lactose-containing blend in a specific sequence [60-62]. Moreover, the addition of anti-adherents and lubricants is also required for improving the flow property of the powder, thus increasing lung deposition.

Various techniques have been utilized to formulate LDPI like freeze drying, spray drying, spray freeze drying, and supercritical fluid technology. Each method presently has some possible challenges listed in fig. 7.

Lyophilization or freeze drying

Lyophilization or freeze-drying is the dehydration technique in which removal of moisture from the product and converted into powder form with the help of a cryoprotectant. There are three steps involved in the lyophilization or freeze-drying process, 1. Freezing: Frozen the product below its eutectic temperature and the product obtained in the form of crystalline. 2. Primary Drying: It is also called as sublimation phase. The partial pressure of vapor is reduced below the equilibrium vapor pressure of ice, and energy supplied in the form of heat must remain lower than the product's eutectic temperature. This step requires the longest time of the three stages in the lyophilization process and needs a heavy economical cost. 3. Secondary Drying: This is the elimination process of remaining traces of moisture from the product by increasing the product temperature to the primary drying. A diffusion and desorption of remaining moisture occur in the product, and the objective of secondary drying is to reduce the final residual water content to an acceptable level [63].

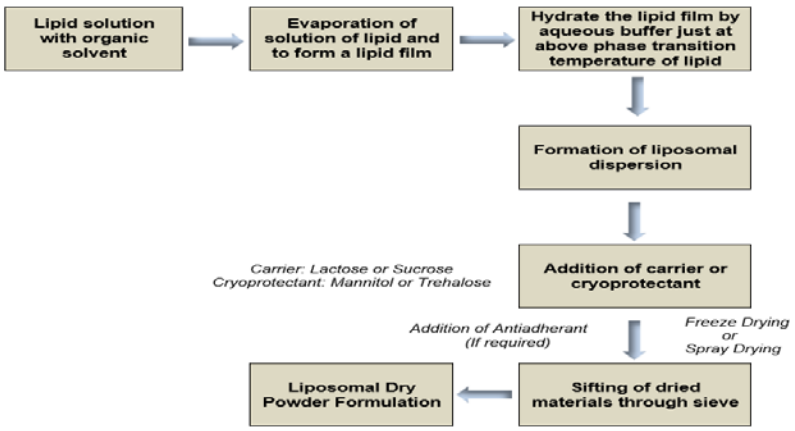

Fig. 6: Process flow for liposomal dry powder formulation [Authors creation]

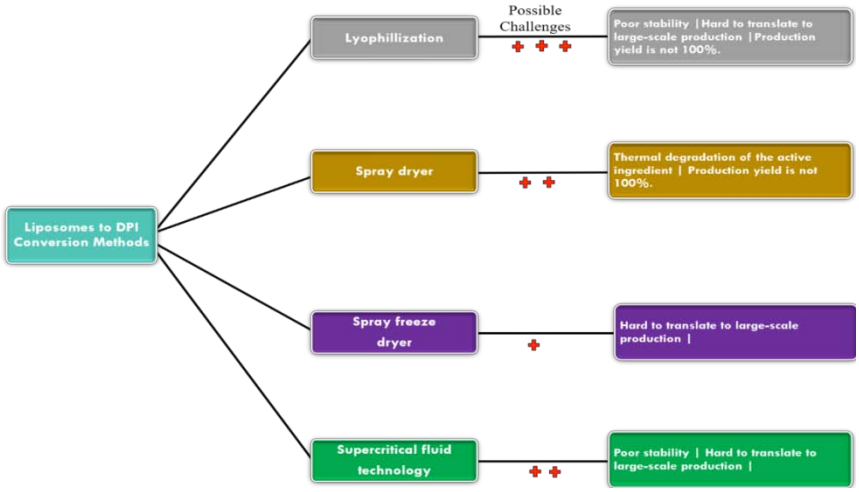

Fig. 7: Various techniques utilized to formulate LDPI [Authors creation]

Spray-dryer

Spray drying is the continuous transformation of feed from a fluid state into dried particulate form by spraying the feed into a hot drying medium. The feed may be liquid, dispersion, or semisolid, provided it is pumpable and capable of being atomized. There are three basic principles and stages involved in spray drying. 1. Preparation of fine droplets by applying atomization to the liquid feed. 2. Drying prepared droplets by mixing spray droplets with a heated gas phase. 3. Separation of dried droplets from the gas phase. This technique is the most commonly used because of particle formation and drying simultaneously [64].

Spray freeze-dryer

The main advantages of this technique are helpful for thermally sensitive materials, obtaining uniform particle size, and increased product stability. In this technique, a three-step operation involves 1. Preparation of droplets by applying atomization with the help of nozzles to a liquid solution or suspension. 2. Rapid freezing of droplets in cryogenic gas or liquid, and 3. Drying of frozen moisture to obtain the final dry particles or product [65].

Supercritical fluid technology

In which thermodynamic condition of any chemical substance, when temperature and pressure increased their critical values respectively, the substance remains neither in liquid nor in gas state and behaves as both a liquid and a gas. The density of the supercritical fluid is like that of liquids with intermediate viscosity, diffusivity, and high compressibility, and these are the main driving properties that help to precipitate solid mass from liquid solution in the medium. A supercritical fluid is a new technology for drying to prepare micro-sized particles for pulmonary delivery [66].

Inhalation device used for delivery of liposomes

As with any outer particles that arrive in the body, liposomes face multiple defense systems intended to recognize, neutralize, and eliminate foreign substances. Such types of hindrances must be avoided for optimal liposome function. Additionally, the enhanced permeability and retention (EPR) effect can be utilized to enhance drug delivery. Indeed, liposomes have some drawbacks as every formulation has an active ingredient that may release towards the external phase, potential lipid oxidation, and hydrolysis of the ester moiety. Further, the stability of liposomes is explicitly limited in the case of an aqueous environment. Before nebulization, such parameters should be considered. Apart from these, some other drawbacks are the manufacturing cost is high, leakage, and fusion of encapsulated drugs/molecules. Furthermore, sometimes phospholipid undergoes oxidation and hydrolysis-like reactions and liposomes have a Short half-life and low solubility [67, 68]. Hence, the maximum liposomal formulations are nebulized to generate an inhalable aerosol cloud. The ideal properties of Inhaler devices are that they can be used at any age for any disease severity [69]. There are several other advantages to Inhaler devices, like, as some drugs for inhalation are available only in solution form that requires Nebulizer (NBs). Second, some patients cannot master the correct use of conventional methods like metered-dose inhalers or dry powder inhalers. Further, some patients prefer the nebulizer over other aerosol-generating devices [70].

Four types of inhalation devices are used for the delivery of liposomes to the lung: pressurized metered-dose inhalers (pMDIs), dry powder inhalers (DPIs), soft mist inhalers (SMIs), and medical nebulizers [38].

Pressurized metered-dose inhalers

pMDIs are good devices with drugs dissolved or dispersed in liquefied propellants. The main advantage of this device is the release of drugs in a precise dose. Due to the high vapor pressure property of this device, the fuel rapidly evaporates and delivers an accurate amount of the aerosolized drug particles to be inhaled by the patient [38].

Dry powder inhalers

DPIs have been used to deliver liposomes with drying technologies such as spray drying, freeze drying, spray freeze drying, or air jet micronization. It was presumed that the rehydration of liposomes might take place following the deposition of the powder in the aqueous environment of the lung using a technique of spray drying of drugs in liposome formulations, and it is appropriate for manufacturing particles with a small aerodynamic size (i.e., high fine particle fraction) [38].

Soft mist inhalers

It is a hand-held propellant-free metered-dose inhalation device that creates slow-moving aqueous aerosols for the deposition of deep-lung. The main advantage of SMI is that it delivers liposome-DNA complexes in repairable aerosols. High doses are needed for the treatment of many diseases in the lung (e. g., cancers) [38].

Nebulizers

Large volumes of 'respirable' aerosol can be produced by this type of device compared to other devices. The main advantages of this device are not required a drying process like DPIs, and no involvement of propellants like pMDIs. It is the most common inhalation device used to deliver liposomes [38]. There are numerous categories of nebulizers: air-jet, ultrasonic and vibrating-mesh devices, which due to their different technology, might affect liposomal stability to a varying degree [71]. Inhaler devices like NBs are classic pulmonary drug delivery devices [72]. They have been used to treat respiratory diseases for many years [73] and are the oldest form of aerosol generation. They have been available since the beginning of the twentieth century.

Air-jet nebulizer's

In this system, compressed air is constrained through an orifice over the capillary tube's open end, making a region of low pressure. The droplet size is impacted by the pressure of the compacted air and the architecture of the nebulizer [74].

Ultrasonic nebulizer's

This system generates aerosols using high-frequency ultrasound waves [1–3 MHz]. These devices produce vibrations y using piezoelectric crystals and form a fountain of fluid in the nebulization cavity. The more significant will be the frequency, the smaller droplets will be produced [74].

Vibrating-mesh

This innovation is viewed as more qualified for nebulizing sensitive medication, for example, liposomal protein formulations, as the temperature rise is reduced in contrast to ultrasonic nebulizers. The low working recurrence and the vitality required for nebulization are applied to the vibration part rather than the drug solution [71].

Characterization of liposomes dispersion and liposomal dry powder inhaler

The character of particulate systems is central to the performance of DPI s. The LDPI for pulmonary delivery may be evaluated at two levels i) liposome and ii) LPDI, and those are listed in table 1 below.

Clinical trial on lung cancer

Many Clinical studies are performed and still going on for promising drug candidates that can be suitable for human use. Detail description of various clinical studies and their phases and study titles is mentioned below in table 2.

Future applications of dry powder inhalers

The DPI of liposomes may be used for various therapeutic purposes. A few of the essential future implications of LDPI are discussed below in table 3.

Table 1: Characterization of liposomal dispersion and liposomal dry powder inhaler

Characterization		References
Liposomes	**Liposomal dry powder inhaler**	
i. Percentage of drug entrapment	i. Flow behavior	[53]
ii. Particle Size	ii. Moisture content determination	[53]
iii. Zeta potential	iii. Reconstitution time and volume	[53]
iv. Morphology	iv. Drug retention and stability study	[53]
v. Entrapped volume	v. *In vitro* and *In vivo* lung deposition and Ex vivo study	[53]
vi. Oxidative index	vi. Scanning electron microscopy photomicrographs	[53]

Table 2: Clinical trials were performed on promising drug candidates against lung cancer

Study title, type, and phase	Summary and references
Title: Niraparib in Combination with Osimertinib in EGFR-Mutated Advanced Lung Cancer. **Type:** Interventional **Phase:** 1	This investigation aims to assess the combination of medications as a potential treatment for EGFR-Mutated lung disease. In this exploratory examination, investigators are trusting that the blend of Niraparib and Osimertinib is well tolerated and that is the ideal dose of Niraparib in EGFR-mutated lung cancer volunteers [75].
Title: A Study of EGF816 and Gefitinib in TKI-naïve EGFR-mutant Non-Small Cell Lung Cancer. **Type:** Interventional **Phase:** 2	In this examination, specialists concentrate on the well-being and viability of combining the new products EGF816 and gefitinib. Both EGF-816 and gefitinib are inhibitors that focus on a particular malignant growth change and can keep tumor development from seizures [76].
Title: Lung Cancer Vaccine Plus Oral Dietary Supplement. **Type:** Interventional **Phase:** 1	This investigation looks at the Allogeneic Cellular Vaccine 1650-G immunization's capability to enhance resistant distinguishing proof of cancer cells in patients with malignant lung growth. The main reason for this investigation was to inspect changes in the number of resistant cells that may help diminish the chances of cancer recurrence [77].
Title: Stepped Palliative Care Versus Early Integrated Palliative Care in Patients with Advanced Lung Cancer (STEP PC). **Type:** Interventional **Phase:** NA	The principal motivation behind this investigation was to inspect patients having serious cancers, for example, propelled lung disease, who frequently experience physical side effects, like shortness of breath. Examination reveals that once the palliative group performs personally with the oncology group to consider propelled disease patients, they m have better indication control, personal satisfaction, and temperament, and their loved ones feel less anxious [78].
Title: Early Integrated Telehealth Versus In-Person Palliative Care for Patients with Lung Cancer (REACH PC). **Type:** Interventional **Phase:** NA	The fundamental objective behind this investigation was to look at patients with critical malignant growths, for example, propelled lung disease, like physical manifestations, instance. Examination shows that when the Alzheimer's Meditation Group works intimately with the Oncology Group to consider propelled malignancy patients, they may experience better manifestation control, personal satisfaction, and state of mind [79].
Title: Safety and Efficiency of γδ T Cell Against Lung Cancer. **Type:** Interventional **Phase:** 1 and 2	In this examination, the impacts of γδT cells on human Lung Cancer in blend with tumor-diminishing medical procedures, for instance, cryosurgery, was investigated [80].
Title: AZD1775 Plus Carboplatin-Paclitaxel in Squamous Cell Lung Cancer. **Type:** Interventional **Phase:** 2	The main intention of this trial was to evaluate what effects AZD1775 utilized as a combination of carboplatin and paclitaxel [81].
Title: Stereotactic Body Radiation for Consolidation After Standard Chemoradiation for Stage 3 Lung Cancer. **Type:** Interventional **Phase:** 2	The primary reason for the trial was to decide the adequacy and toxicity of stereotactic physical radiation [SBRT] as an adjustment after standard chemotherapy for patients in Phase III (NSCLC) [82].
Title: Radiotherapy and Durvalumab/Durvalumab Combo (Tremelimumab/Olaparid) for Small Cell Lung Cancer. **Type:** Interventional **Phase:** 1	This was a randomized multi-arm trial to assess the safety or efficacy of monotherapy of durvalumab or to consolidate it with tremelimumab or olaparib in individuals from the broadened (SCLC). Prime platinum-depended chemotherapy process, total continuous effect, stable disease, and partial response [83].

Table 3: Nebulized liposomes-therapeutic efficacy, drugs, and outcome of pre-clinical assessments

Pathology	Animal species used for studies	Drug	Method for preparation	Therapeutic outcomes and Refs
Cancer (Breast, Lung, Colon)	Nude Mice	9-nitrocamptothecin	Modified Solvent Evaporation and Freeze Drying (Lyophilization)	9-NC liposome aerosol was strikingly effective in treating three human cancer xenografts growing subcutaneously over the thorax in nude mice at doses much smaller than those traditionally used in mice administered by other routes [84].
Melanoma and Osteosarcoma Lung Metastases	Mice	9-nitrocamptothecin	Modified Solvent Evaporation and Freeze Drying (Lyophilization)	The dosages used in this study were 3–20 times lower than the dosages of 9NC previously found to be effective when given by the i. m., i. v., or intragastric routes in mice [85].
Pulmonary Metastases	Murine Renal Carcinoma Model	Paclitaxel	Modified Solvent Evaporation and Freeze Drying (Lyophilization)	Reduction of tumor growth in the lung [86].
Pulmonary Metastases	Pet Dogs	Interleukin-2	Modified Solvent Evaporation	Inhalation formulation with liposomes is a very effective and non-toxic treatment for pulmonary metastases [87].
Pulmonary Aspergillosis	Rats	Amphotericin B	Modified Solvent Evaporation	Increased survival rate and lower lung weights [88].
Fungal infections (Candidosis and Aspergillosis)	Mice, Rat, Rabbits and Dogs	Amphotericin B	Modified Solvent Evaporation	It is an effective treatment for both intracellular (leishmaniasis and histoplasmosis) and extracellular (candidosis and aspergillosis) systemic infections due to its low toxicity at the organ level [89].
Intracellular Francisella tularensis infection	Mice	Ciprofloxacin	Remote/Active Loading (Ammonium sulfate gradient)	Aerosol delivery of ciprofloxacin encapsulated liposomes enhanced the delivery and drug retention in the lower respiratory region [90].
Asthma and Diabetes	Mice	Insulin	Thin-film hydration method	Animal studies were showed an effective reduction of glucose levels after inhalable administration of insulin when liposomes used as a drug carrier [91].
Diabetes	Rat	Insulin	Thin-film hydration method	Inhalable liposomal delivery of insulin provided rapid glucose drop (Short time) followed by long-acting (~20 h) sustaining glucose level [92].
Cystic fibrosis	Sheep	Plasmid DNA	Complexation	Nebulized/Aerosol delivery of plasmid DNA improved distribution and the absence of pooling effects [93].
Cystic fibrosis	Sheep	Adeno-associated virus serotype 2.	Complexation	The prolonged-expression after aerosol administration versus i. v. Administration of cationic liposomes [94].

Table 4: Patents on dry powder liposomal inhalers

Inventor	Patent number	Summary of the invention and references	Date of patent
Wang Z *et al.*	US2006/0280691A1	In the current investigation, researchers developed the DPI for inhalation purposes, the powder having: spray freeze-dried liposome substances with a biologically active ingredient, for example, an anti-toxin, entrapped inside a phospholipid, and an active ingredient with a phospholipid for formulation a liquid liposome Suspension; and spray technique for manufacturing a powder for inhalation aerosol delivery, the method including the means of blending a biologically freeze-drying the liposome Suspension to prepare particles of powder [95].	Dec. 14, 2006
Stuart SB *et al.*	US2013/0330274A1	The investigators developed MAN-LIPs liposomes and successfully demonstrated this in TAM in an animal model of lung adenocarcinoma. These liposomes were occupied with Cu to allow PET imaging and contain fluorescent dyes in the lipid bilayer, which is followed by fluorescence microscopy. MN-LIP is a potential novel vehicle for delivering imaging agents to TAM of the lungs [96].	Dec. 12, 2013
Rolf M *et al.*	US2007/0148220A1	The present innovation narrates with liposomes and structures, including liposomes, their manufacturing, and their use for counteractive action for the management of proliferative disease, vascular diseases, rheumatoid diseases, infectious diseases, inflammatory diseases, immune diseases, and allergies [97].	Jun. 28, 2007
Susan N *et al.*	US2002/0119188A1	The present innovation narrates the method of making a liposome, the technique involving the means of [a] blending a lipophilic stage and a hydrophilic stage, the lipophilic stage containing an amphiphilic bilayer-forming Substance; and [b] applying a shear force to the blend to frame the liposome, wherein the Shear force is made by passing the mix by a part at a velocity Sufficient to make turbulence in the mixture [98].	Aug. 29, 2002
Gregory G *et al.*	US 9675554 B1	The invention highlights compositions of reagents shaped by framing empty liposomes, blending them with these formed liposomes with a Sugar Solution, and drying the blend. The procedure is valuable in the manufacturing of pharmaceuticals [99].	Jun. 13, 2017
Ian M *et al.*	US9005654B2	The current investigation deals with an apparatus and processes for formulating liposomes. By giving a buffer solution in a first repository, and a lipid solution in two reservoirs, constantly diluting the lipid solution with the buffer solution in a blending chamber produces a liposome [100].	Apr. 14, 2015
Paul R M *et al.*	US 7491409 B1	This invention gives an approach to formulate liposome-embedded bioactive agents, for example, nucleic acids, involving complexation of the bioactive agents in reverse mi micelles before producing liposomes, and the strategies for utilizing the liposomes so shaped and detailed to convey nucleic acids to cells [101].	Feb. 17, 2009

Patents on liposomes dry powder inhalers

Various patents had been granted to the researcher for their innovation in DPI and liposomes. Patents offer an owner an exclusive right to an invention and prevent others from misusing it. Some patents granted to the researcher for their novel work are discussed below in table 4.

CONCLUSION

Dry powder liposomal inhalers have gotten more consideration because of their unique properties such as such delivery of drugs at the targeted site—improved bioavailability, the better release of medicines, and so forth. Further examination is moreover going on LDPI at the industrial level to further exploit the potential of this dosage form in overcoming the loopholes of current drug delivery systems. This paper suggests the capacity of LDPI as an alternate delivery system to an existing delivery system. Ongoing progressions and the presentation of novel procedures for assembling LDPI brought a better formulation of dosage forms and better reproducibility. Recent investigations recommended that encapsulated anti-cancer drugs in inhalers showed promising efficacy in the treatment of cancer when contrasted with traditional delivery systems. Results are promising, which energizes the utilization of this system as an alternative to managing lung cancer. However, further examination is probably going to redesign the clinical utility of this dosage in cancer illnesses. The upcoming potential of these products is linked to solid product acceptance and the availability of new technologies with patient demand. Numerous drug delivery methodologies may be engaged to improve drug therapy with these dosage forms. To accomplish the operative drug amount in the lung for a prolonged time is an obligatory desire for various formulations. Recent formulations coming into the market take very little time for inhalation and can be carried in pockets, thus helping to increase patient compliance. The review also concludes and predicts the future that it is the duty of Pharmacists, Nurses, Clinical scientists, and Doctors to educate patients to use a particular formulation/inhaler device for a specific condition.

CONSENT FOR PUBLICATION

Not applicable.

ACKNOWLEDGMENT

The authors are grateful to the Parul Institute of Pharmacy and Research, Parul University Vadodara, Gujarat, India, for providing the necessary facilities for this review work. Special thanks to Dr. Pinkal Patel got great support in motivation to write a publication.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

1. Potter J, Higginson IJJ. Pain experienced by lung cancer patients: a review of prevalence, causes and pathophysiology. Lung Cancer. 2004;43(3):247-57. doi: 10.1016/j.lungcan.2003.08.030, PMID 15165082.
2. Kovacevic T, Kovacevic SV, Stanetic M, Kovacevic P, Miljkovic B. Impact of pharmacist's intervention on decreasing erlotinib interactions in the treatment of lung cancer patients in low resource settings. J Oncol Pharm Pract. 2021;27(2):350-8. doi: 10.1177/1078155220921545, PMID 32349642.
3. Subramanian J. Govindan RJJoco. Lung Cancer Never Smokers Rev. 2007;25(5):561-70.
4. Shivapurkar N, Reddy J, Chaudhary PM, Gazdar AFJ. Jocb. Apoptosis Lung Cancer Rev. 2003;88(5):885-98.
5. Zarogoulidis P, Chatzaki E, Porpodis K, Domvri K, Hohenforst Schmidt W, Goldberg EP. Inhaled chemotherapy in lung cancer: future concept of nanomedicine. Int J Nanomedicine. 2012;7:1551-72. doi: 10.2147/IJN.S29997, PMID 22619512.
6. Lee PN, Forey BA, Coombs KJJ. Systematic review with meta-analysis of the epidemiological evidence in the 1900s relating smoking to lung cancer. BMC Cancer. 2012;12(1):385. doi: 10.1186/1471-2407-12-385, PMID 22943444.
7. Haasbeek CJ, Senan S, Smit EF, Paul MA, Slotman BJ, Lagerwaard FJJ. Critical review of nonsurgical treatment options for stage I non-small cell lung cancer. Oncologist. 2008;13(3):309-19. doi: 10.1634/the oncologist.2007-0195, PMID 18378542.
8. Fathi Karkan S, Mohammadhosseini M, Panahi Y, Milani M, Zarghami N, Akbarzadeh A. Magnetic nanoparticles in cancer diagnosis and treatment: a review. Artif Cells Nanomed Biotechnol. 2017;45(1):1-5. doi: 10.3109/21691401.2016.1153483, PMID 27015806.
9. Charumathy A, Ubaidulla U, Sinha P, Rathnam G. Recent update on liposome-based drug delivery system. Int J Curr Pharm Sci. 2022;14(3):22-7. doi: 10.22159/ijcpr.2022v14i3.1991.
10. Bhattacharyya S, S Sogali B. Inhalation therapy–approaches and challenges. Asian J Pharm Clin Res. 2018;11(4):9-16. doi: 10.22159/ajpcr.2018.v11i4.24117.
11. Shetty A, Gjajpcr S. Advancements in dry powder inhaler. Asian J Pharm Clin Res. 2017;10(2):8-12.
12. Rajendran R, Balan R, Ganesan N, Djijpps T. Recent modalities in drug delivery via inhalation therapy–an advanced treatment strategy for pulmonary. Carcinoma. 2015;7:8-21.
13. Bonde S, Nair S. Advances in liposomal drug delivery system: fascinating types and potential applications. Int J App Pharm. 2017;9(3):1-7. doi: 10.22159/ijap.2017v9i3.17984.
14. Gauch R. It's great! Oops, no it isn't: why clinical research can't guarantee the right medical answers. Springer; 2008.
15. Parsons A, Daley A, Begh R, Aveyard PJB. Influence of smoking cessation after diagnosis of early-stage lung cancer on prognosis: systematic review of observational studies with meta-analysis. BMJ. 2010;340:b5569. doi: 10.1136/bmj.b5569, PMID 20093278.
16. Takkouche B, Gestal Otero JJJ. Ejoe. The Epidemiol Lung Cancer Rev Risk Factors Span Data. 1996;12(4):341-9.
17. Malhotra J, Malvezzi M, Negri E, La Vecchia C, Boffetta P. Risk factors for lung cancer worldwide. Eur Respir J. 2016;48(3):889-902. doi: 10.1183/13993003.00359-2016, PMID 27174888.
18. Mao Y, Yang D, He J, Krasna MJ. Epidemiology of lung cancer. Surgical Oncology Clinics of North America. 2016;25(3):439-45. doi: 10.1016/j.soc.2016.02.001.
19. Wong MCS, Lao XQ, Ho KF, Goggins WB, Tse SLA Sr. Incidence and mortality of lung cancer: global trends and association with socioeconomic status. Sci Rep. 2017;7(1):14300. doi: 10.1038/s41598-017-14513-7, PMID 29085026.
20. Zappa C, Mousa SAJ. Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res. 2016;5(3):288-300. doi: 10.21037/tlcr.2016.06.07, PMID 27413711.
21. Muhas C, Kumar P, Raja DJI. JoP, sciences P. Etiological Factors Dev Lung Cancer Non-Smokers Overview. 2019;11:10-6.
22. Howington JA, Blum MG, Chang AC, Balekian AA, Murthy SCJC. Treatment of stage I and II non-small cell lung cancer: diagnosis and management of lung cancer: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. 2013;143(5):e278S-313S.
23. Ramalingam S, Belani CJTo. Systemic chemotherapy for advanced non-small cell lung cancer: recent advances and future directions. 2008;13Suppl 1:5-13.
24. Kelly K, Crowley J, Bunn Jr PA, Presant CA, Grevstad PK, Moinpour CM. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non–small-cell lung cancer: a Southwest oncology group trial. J Clin Oncol. 2001;19(13):3210-8. doi: 10.1200/JCO.2001.19.13.3210, PMID 11432888.
25. Amini A, Yeh N, Gaspar LE, Kavanagh B, Karam SDJ. Stereotactic body radiation therapy (SBRT) for lung cancer patients previously treated with conventional radiotherapy: a review. Radiat Oncol. 2014;9(1):210. doi: 10.1186/1748-717X-9-210, PMID 25239200.
26. McLachlan JJP. Immune checkpoint inhibitors and their side effects. Pathology. 2019;51Suppl 17. doi: 10.1016/j.pathol.2018.12.036.

27. Jassem JJTlo. Comb chemother radiat locally adv nonsmall-cell lung cancer. Semin Oncol. 2001;2(6):335-42.
28. Burris HA. Shortcomings of current therapies for non-small-cell lung cancer: unmet medical needs. Oncogene. 2009;28(1)Suppl 1:S4-S13. doi: 10.1038/onc.2009.196, PMID 19680296.
29. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69-90. doi: 10.3322/caac.20107, PMID 21296855.
30. Cruz CSD, Tanoue LT, Matthay RC. Lung cancer: epidemiology, etiology, and prevention. Clin Chest Med. 2011;32(4):605-44. doi: 10.1016/j.ccm.2011.09.001
31. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC. Colorectal cancer statistics, 2020. CA Cancer J Clin. 2020;70(3):145-64. doi: 10.3322/caac.21601, PMID 32133645.
32. Ward EM, Sherman RL, Henley SJ, Jemal A, Siegel DA, Feuer EJ. Annual report to the Nation on the status of cancer, featuring cancer in men and women age 20–49 Y. JNCI. 2019;111(12):1279-97. doi: 10.1093/jnci/djz106.
33. Stewart B, Wild C. IARC publications Website-world cancer report. Vol. 2014; 2014.
34. Malik PS, Raina VJT. Ijomr. Lung Cancer Prevalent Trends Emerg Concepts. 2015;141(1):5.
35. Mullin TMJFCfDE, Research. Patient-Focused Drug Development Public Meeting; 2012.
36. Miranda Filho A, Pineros M, Bray. F Jspdm. The Descript Epidemiol Lung Cancer Tob Control Glob Overview. 2019;61(3):219-29.
37. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. doi: 10.3322/caac.21492, PMID 30207593.
38. Rudokas M, Najlah M, Alhnan MA. Elhissi AJMp, practice. Liposome delivery systems for inhalation: a critical review is highlighting formulation issues and anticancer applications. 2016;25Suppl 2:60-72.
39. Vanza J, Jani P, Pandya N, Tandel H. Formulation and statistical optimization of intravenous temozolomide-loaded pegylated liposomes to treat glioblastoma multiforme by three-level factorial design. Drug Dev Ind Pharm. 2018;44(6):923-33. doi: 10.1080/03639045.2017.1421661, PMID 29280385.
40. Kong G, Dewhirst MJI. JoH. Rev Hyperthermia Liposomes. 1999;15(5):345-70.
41. Shurtleff W, Aoyagi A. History of lecithin and phospholipids 1850-2016. Extensively annotated bibliography and sourcebook, including phosphatides and liposomes. Soyinfo Center; 2016.
42. Meure LA, Foster NR, Dehghani FJAP. Conventional and dense gas techniques for the production of liposomes: a review. AAPS PharmSciTech. 2008;9(3):798-809. doi: 10.1208/s12249-008-9097-x, PMID 18597175.
43. Prime D, Atkins PJ, Slater A. Sumby BJAddr. Rev Dry Powder Inhalers. 1997;26(1):51-8.
44. Willis L, Hayes D, Mansour HMJL. Therapeutic liposomal dry powder inhalation aerosols for targeted lung delivery. Lung. 2012;190(3):251-62. doi: 10.1007/s00408-011-9360-x, PMID 22274758.
45. Gaspar MM, Radomska A, Gobbo OL, Bakowsky U, Radomski MW, 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. doi: 10.1089/jamp.2011.0928, PMID 22857016.
46. Vanza JD, Shah DM, Patel RB, Patel MRJ. Afatinib liposomal dry powder inhaler: targeted pulmonary delivery of EGFR inhibitor for the management of lung cancer. Journal of Drug Delivery Science and Technology. 2022;74:103506.
47. Islam N, Gladki E. Dry powder inhalers (DPIs)-a review of device reliability and innovation. Int J Pharm. 2008;360(1-2):1-11. doi: 10.1016/j.ijpharm.2008.04.044. PMID 18583072.
48. Zhang T, Chen Y, Ge Y, Hu Y, Li M, Jin Y. Inhalation treatment of primary lung cancer using liposomal curcumin dry powder inhalers. Acta Pharm Sin B. 2018;8(3):440-8. doi: 10.1016/j.apsb.2018.03.004, PMID 29881683.
49. Lo Yl, Tsai JC, Kuo J-hJJocr. Liposomes disaccharides carriers spray-dried powder formulations superoxide dismutase. J Control Release. 2004;94(2-3):259-72.
50. Franze S, Selmin F, Samaritani E, Minghetti P, Cilurzo FJP. Lyophilization of liposomal formulations: still necessary, still challenging. Pharmaceutics. 2018;10(3):139. doi: 10.3390/pharmaceutics10030139, PMID 30154315.
51. Shah N, Shah V. Pulm drug deliv a promising approach. Journal of Applied Pharmaceutical Science 2012;2(6):33-7.
52. Cipolla D, Gonda I, Chan HK. Liposomal formulations for inhalation. Ther Deliv. 2013;4(8):1047-72. doi: 10.4155/tde.13.71, PMID 23919478.
53. Misra A, Jinturkar K, Patel D, Lalani J, Chougule M. Recent advances in liposomal dry powder formulations: preparation and evaluation. Expert Opin Drug Deliv. 2009;6(1):71-89. doi: 10.1517/17425240802652309, PMID 19236209.
54. Chougule M, Padhi B, Misra AJAP. Development of spray-dried liposomal dry powder inhaler of dapsone. AAPS PharmSciTech. 2008;9(1):47-53. doi: 10.1208/s12249-007-9024-6, PMID 18446460.
55. Joshi M, Misra AJC. Pharmacology e, physiology. Dispos Kinet Ketotifen Liposomal Dry Powder Inhal Rat Lung. 2003;30(3):153-6.
56. Akbarzadeh A, Rezaei Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013;8(1):102. doi: 10.1186/1556-276X-8-102, PMID 23432972.
57. Laouini A, Jaafar Maalej C, Limayem Blouza I, Sfar S, Charcosset C, Fessi H. Preparation, characterization and applications of liposomes: state of the art. J Coll Sci Biotechnol. 2012;1(2):147-68. doi: 10.1166/jcsb.2012.1020.
58. Gubernator J. Active methods of drug loading into liposomes: recent strategies for stable drug entrapment and increased *in vivo* activity. Expert Opin Drug Deliv. 2011;8(5):565-80. doi: 10.1517/17425247.2011.566552, PMID 21492058.
59. Wei H, Song J, Li H, Li Y, Zhu S, Zhou X. Active loading liposomal irinotecan hydrochloride: preparation, *in vitro* and *in vivo* evaluation. Asian Journal of Pharmaceutical Sciences. 2013;8(5):303-11. doi: 10.1016/j.ajps.2013.10.006.
60. Chougule MB, Padhi BK, Jinturkar KA, Misra AJR. Podd, formulation. Dev Dry Powder Inhalers. 2007;1(1):11-21.
61. Shah SP, Misra AJDD. Development of liposomal amphotericin B dry powder inhaler formulation. Drug Deliv. 2004;11(4):247-53. doi: 10.1080/10717540490467375, PMID 15371106.
62. Shah SP, Misra AJAP. Liposomal amikacin dry powder inhaler: effect of fines on *in vitro* performance. AAPS PharmSciTech. 2004;5(4):e65. doi: 10.1208/pt050465, PMID 15760062.
63. Kawasaki H, Shimanouchi T, Kimura YJJoc. Recent dev optim lyophilization process. Journal of Chemistry 2019(1):1-14. doi:10.1155/2019/9502856
64. Santos D, Mauricio AC, Sencadas V, Santos JD, Fernandes MH, Gomes PSJB-P. Spray drying: an overview; 2018. p. 9-35.
65. Adali MB, Barresi AA, Boccardo G, Pisano RJP. Spray freeze-drying as a solution to continuous manufacturing of pharmaceutical products in bulk. Processes. 2020;8(6):709. doi: 10.3390/pr8060709.
66. Chaurasiya B, Zhao YY. Dry powder for pulmonary delivery: A comprehensive review. Pharmaceutics. 2020;13(1):31. doi: 10.3390/pharmaceutics13010031, PMID 33379136.
67. Vanza JD, Patel RB, Patel MRJ. Nanocarrier centered therapeutic approaches: recent developments with insight towards the future in the management of lung cancer. Journal of Drug Delivery Science and Technology. 2020;60:102070. https://doi.org/10.1016/j.jddst.2020.102070
68. He Z, Ranganathan N, Li PJN. Evaluating nanomedicine with microfluidics. Nanotechnology. 2018;29(49):492001. doi: 10.1088/1361-6528/aae18a, PMID 30215611.
69. Dolovich MB, Dhand RJTL. Aerosol drug delivery: developments in device design and clinical use. Lancet. 2011;377(9770):1032-45. doi: 10.1016/S0140-6736(10)60926-9, PMID 21036392.
70. Chellappan DK, Sze Ning QL, Su Min SK, Bin SY, Chern PJ, Shi TP. Interactions between microbiome and lungs: paving new paths for microbiome-based bio-engineered drug delivery systems in chronic respiratory diseases. Chem Biol Interact. 2019;310:108732. doi: 10.1016/j.cbi.2019.108732, PMID 31276660.

71. Gaspar MM, Bakowsky U. Ehrhardt CJJoBN. Inhaled liposomes–curr strateg future chall. Journal of Biomedical Nanotechnology. 2008;4(3):245-57. doi: 10.1166/jbn.2008.334.
72. Reychler G, Leal T, Roeseler J, Thys F, Delvau N. Effect of continuous positive airway pressure combined to nebulization on lung deposition measured by urinary excretion of amikacin. Respir Med. 2007;101(10):2051-5. doi: 10.1016/j.rmed.2007.06.003.
73. Respaud R, Vecellio L, Diot P, Heuze Vourc'h N. Nebulization as a delivery method for mAbs in respiratory diseases. Expert Opin Drug Deliv. 2015;12(6):1027-39. doi: 10.1517/17425247.2015.999039, PMID 25557066.
74. Steckel H, Eskandar F. Factors affecting aerosol performance during nebulization with jet and ultrasonic nebulizers. Eur J Pharm Sci. 2003;19(5):443-55. doi: 10.1016/s0928-0987(03)00148-9, PMID 12907295.
75. ClinicalTrials.gov. United States national library of medicine. Niraparib in Combination with Osimertinib in EGFR-Mutated Advanced Lung Cancer; 2020. Available from: https://www.clinicaltrials.gov/ct2/show/NCT03891615. [Last accessed on 08 Nov 2022]
76. ClinicalTrials.gov. A study of EGF816 and gefitinib in TKI-naïve EGFR-mutant non-small cell lung cancer. United States National Library of Medicine; 2022. Available from: https://clinicaltrials.gov/ct2/show/NCT03292133. [Last accessed on 08 Nov 2022]
77. ClinicalTrials.gov. Lung cancer vaccine plus oral dietary supplement. United States National Library of Medicine; 2013. Available from: https://clinicaltrials.gov/ct2/show/NCT01829373. [Last accessed on 08 Nov 2022]
78. ClinicalTrials.gov. Stepped palliative care versus early integrated palliative care in patients with advanced lung cancer (STEP PC). United States National Library of Medicine; 2022. Available from: https://www.clinicaltrials.gov/ct2/show/NCT03337399. [Last accessed on 08 Nov 2022]
79. ClinicalTrials.gov. Early integrated telehealth versus in-person palliative care for patients with lung cancer (REACH PC). United States National Library of Medicine; 2022. Available from: https://clinicaltrials.gov/ct2/show/NCT03375489. [Last accessed on 08 Nov 2022]
80. ClinicalTrials.gov. Safety and efficiency of γδ T cell against lung cancer. United States National Library of Medicine; 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT03183232. [Last accessed on 08 Nov 2022]
81. ClinicalTrials.gov. Plus carboplatin-paclitaxel in squamous cell lung cancer. United States National Library of Medicine; 2022. Available from: https://clinicaltrials.gov/ct2/show/NCT02513563. [Last accessed on 08 Nov 2022]
82. ClinicalTrials.gov. Stereotactic body radiation for consolidation after standard chemoradiation for stage 3 lung cancer. United States National Library of Medicine; 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT01656460. [Last accessed on 08 Nov 2022]
83. ClinicalTrials.gov. Radiotherapy and durvalumab/durvalumab combo (tremelimumab/olaparid) for small cell lung cancer. United States National Library of Medicine; 2022. Available from: https://clinicaltrials.gov/ct2/show/NCT03923270. [Last accessed on 08 Nov 2022]
84. Knight V, Koshkina NV, Waldrep JC, Giovanella BC, Gilbert BEJ. Cc, pharmacology. Anticancer exffect of 9-nitrocamptothecin liposome aerosol on human cancer xenografts in nude mice. Cancer Chemother Pharmacol. 1999;44(3):177-86. doi: 10.1007/s002800050965.
85. Koshkina NV, Kleinerman ES, Walidrep C, Jia SF, Worth LL, Gilbert BE. 9-Nnitrocamptothecin liposome aerosol treatment of melanoma and osteosarcoma lung metastases in mice. Clin Cancer Res. 2000;6(7):2876-80. PMID 10914737.
86. Koshkina NV, Waldrep JC, Roberts LE, Golunski E, Melton S, Knight V, Knight VJ. Paclitaxel liposome aerosol treatment induces inhibition of pulmonary metastases in murine renal carcinoma model. Clin Cancer Res. 2001;7(10):3258-62. PMID 11595722.
87. Khanna C, Anderson PM, Hasz DE, Katsanis E, Neville M, Klausner JS. Interleukin-2 liposome inhalation therapy is safe and effective for dogs with spontaneous pulmonary metastases. Cancer. 1997;79(7):1409-21. doi: 10.1002/(sici)1097-0142(19970401)79:7<1409::aid-cncr19>3.0.co;2-3. PMID 9083164.
88. Gavalda J, Martin MT, Lopez P, Gomis X, Ramirez JL, Rodriguez D. Efficacy of nebulized liposomal amphotericin B in the treatment of experimental pulmonary aspergillosis. Antimicrob Agents Chemother. 2005;49(7):3028-30. doi: 10.1128/AAC.49.7.3028-3030.2005, PMID 15980392.
89. Adler Moore J, Proffitt RTJ. AmBisome liposomal formulation struct mech action pre-clin experience. J Antimicrob Chemother. 2002;49(Suppl_1):21-30. doi: 10.1093/jac/49.suppl_1.21.
90. Wong JP, Yang H, Blasetti KL, Schnell G, Conley J, Schofield LNJ. Liposome deliv ciprofloxacin intracellular francisella tularensis infect. J Control Release. 2003 Oct 30;92(3):265-73. doi: 10.1016/s0168-3659(03)00358-4.
91. Huang YY, Wang CHJ. Pulm deliv insulin liposomal carriers. Journal of Orthopaedic Case Reports. 2006;113(1):9-14.
92. Karathanasis E, Bhavane R, Annapragada AVJ. Triggered release inhaled insulin agglomerated vesicles pharmacodyn stud rats. Journal of Orthopaedic Case Reports. 2006;113(2):117-27.
93. McLachlan G, Baker A, Tennant P, Gordon C, Vrettou C, Renwick L. Optimizing aerosol gene delivery and expression in the ovine lung. Mol Ther. 2007;15(2):348-54. doi: 10.1038/sj.mt.6300058, PMID 17235313.
94. Griesenbach U, Geddes DM, Alton EW. Gene therapy progress and prospects: cystic fibrosis. Gene Ther. 2006;13(14):1061-7. doi: 10.1038/sj.gt.3302809. PMID 16819538.
95. Wang Z, Orszanska H, Finlay W, Inventor. University of Alberta, Assignee; Spray freeze-dried liposomal ciprofloxacin powder aerosol drug delivery. United States patent US20060280691; 2006.
96. Stuart SB, Landon WL, Inventor. University of virginia, assignee; compositions and methods for detecting and treating cancer. United States patent US20130330274; 2013.
97. Rolf M. Inventor Vectron Therapeutics AG Affitech AS, assignee; liposomes and liposomal compositions for vaccination and drug delivery. United States patent US20070148220; 2007.
98. Susan N. Inventor; Johnson and Johnson Consumer Inc, Assignee. Method of manufacturing liposomes. United States patent US20020119188; 2002.
99. Gregory G. Inventor; UK secretary of state for defence, assignee. Method of forming liposomes. United States patent US9675554B1; 2017.
100. Ian M. Inventor; Arbutus biopharma corp, assignee. Systems and methods for manufacturing liposomes. United States patent US9005654B2; 2015.
101. Paul RM. Inventor; Elan Pharmaceuticals LLC Transave LLC, Assignee. Encapsulation of bioactive complexes in liposomes. United States patent US7491409B1; 2009.