# Comparison of Conventional Doxorubicin with Liposome Encapsulated Doxorubicin as a Treatment of Breast cancer-A Review

By

Honufa Akter
19346039

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

School of Pharmacy
Brac University
July 2023

## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing a degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

**Student's Full Name & Signature:**

**Honufa Akter**

19346039

# Approval

The project titled "Comparison of conventional doxorubicin with liposome encapsulated doxorubicin as a treatment of breast cancer-A review" submitted by Honufa Akter (19346039) of Summer, 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of pharmacy on July, 2023.

**Supervised By:**

Supervisor:

Sabrina Sharmin, PhD
Assistant Professor
School of Pharmacy
Brac University

**Approved By:**

Assistant Dean & Program Director:

Professor Dr. Hasina Yasmin
Assistant Dean and Program Director,
School of Pharmacy
Brac University

Dean:

Professor Dr. Eva Rahman Kabir
Dean, School of Pharmacy
Brac University

## Ethics Statement

The project does not involve any clinical trial or human participants, no animals were used or harmed.

## Abstract

Breast cancer continues to be one of the most common reasons for hospitalization and mortality each year. As time goes on, novel anticancer drug formulations are being released on the market, raising the concern of medical professionals due to their superiority, toxicology, and cost-effectiveness compared to the traditional formulation of the same drugs. Doxorubicin is an effective cancer drug that comes in three forms: pegylated liposomal, nonpegylated liposomal, and conventional non-liposomal. Although it is more expensive, liposomal doxorubicin has been shown to have a more favorable toxicological profile than conventional. The cost-effectiveness of liposomal doxorubicin has not been thoroughly investigated due to the small number of research that have been conducted. Apart from that this article highlights the use of traditional doxorubicin has been somewhat restricted due to the adverse effects it can produce, whereas liposomal formulation has favorable safety profile. In the overall treatment of breast cancer, the formation of a liposomal form of doxorubicin, which is less harmful to the heart, better tolerated, and just as effective, expands the therapeutic alternatives available.



**Keywords:** Doxorubicin; breast cancer; liposome; liposomal doxorubicin; cardiotoxicity; metastasis.

# Dedication

*This is dedicated with sincere gratitude to my respected mentors, family, and friends for their constant support and faith in my ambitions.*

## Acknowledgement

To start, I wish to express my gratitude to Almighty Allah for giving me the resilience I needed throughout this entire time; I am grateful and would like to show my sincere gratitude towards Dr. Sabrina Sharmin, Assistant Professor, School of Pharmacy, Brac University for being a constant guide during my studies and for being so helpful, kind, and inspiring along the way.

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## List of Acronyms

| | |
|---|---|
| DOX | Doxorubicin |
| LD | Liposomal Doxorubicin |
| EGF | Epidermal Growth Factor |
| VEGF | Vascular Endothelial Growth Factor |
| MBC | Metastatic Breast Cancer |
| SUV | Small Unilamellar Vesicles |
| LUV | Large Unilamellar Vesicles |
| MLV | Multilamellar Vesicle |
| MVV | Multivesicular Vesicles |
| CHF | Congestive Heart Failure |
| HFS | Hand Foot Syndrome |
| MTP-PE | Muramyl Tripeptide Phosphatidyl Ethanolamine |
| POPC | Palmitoyl Oleoyl Phosphatidylcholine |
| OOPS | Dioleoyl Phosphatidylserine |
| NPLD | Non-pegylated Liposomal Doxorubicin |
| PLD | Pegylated Liposomal Doxorubicin |

# Chapter 1

# Introduction

Breast cancer is now the most common type of cancer in the world, with 2.3 million new cases in both men and women. It has surpassed lung cancer as the most common type of cancer to be identified. It was by far among the most common cancer in women in 2020, accounting for 25% of all female cancer cases, and its burden has been rising globally, especially in transitioning nations (Heer et al., 2020).The World Health Organization (WHO) and its affiliates are working to reduce the death rate associated with breast cancer by promoting early detection of the disease in addition to effective treatment and care of patients. This will be accomplished through involving the assistance of international partners and coordinating long-term initiatives designed to improve results (Arnold et al., 2022). And to treat this fatal disease doxorubicin is considered as the most effective therapeutic agents. Because of the importance of doxorubicin in the treatment of breast cancer, a considerable amount of research has been carried out in an effort to improve the therapeutic index of doxorubicin. In particular, efforts have been focused on reducing the cardiac toxicity that is associated with the drug. Doxorubicin is widely regarded as the gold-standard anthracycline for treatment of breast cancer, whether it is administered as a single agent or as a part of combination regimens (Batist et al., 2002).

## 1.1. Cancer

Cancer termed as “a wound that never heals”, is distinguished by the uncontrolled multiplication of abnormal cells and atypical identification of the immune system. The World Health Organization estimates that 9.6 million deaths in 2018 were caused by cancer (Yin et al., 2021). Generally, the cancer is caused due to a buildup of genetic, epigenetic, and transcriptional changes that give cancer cells important traits, such as the ability to keep growing, invade, angiogenesis also metastasis and apoptosis and these traits are referred as

hallmarks of cancer cells (Hanahan & Weinberg, 2011; Nassar & Blanpain, 2016) . A normal cell's genome will acquire mutations in tumor suppressor genes, proto-oncogenes, and other genes involved in the regulation of cell growth in the process of developing into a cancer cell. These mutations are necessary for the development of cancer. Research on molecular markers is ongoing, with the overarching goal of determining how the presence or absence of molecular markers promotes the progression of cancer. The protein p53 is an example of a tumor suppressor. The p53 tumor suppressor protein, which is one of the most crucial controllers on the cell cycle in both types of cells and which is present in normal human cells as well as in human cancer cells, is altered in practically every occurrence of the latter. This occurs in both normal human cells as well as in human cancer cells. When normal cellular conditions are present, p53 is the factor that is responsible for putting a stop to cell growth in the event that physiological stress or damage is present. And to eradicate this cancer cell there are several treatments has been developed such as chemotherapy, surgery, radiation therapy along with recently developed immunotherapy. Despite the fact that these treatments have prolonged cancer patients' survival, a large proportion of patients experience its again and are unable to achieve long-term survival. Therefore, it is essential to have a comprehensive comprehension of cancer from its onset to its metastasis and relapse.

## 1.2. Different type of cancer

The burden of cancer has consistently increased despite advances in our understanding of the disease. Cancers have surpassed cardiovascular diseases as the primary cause of death in several high-income countries (HICs), causing twice as many deaths as heart disease and stroke (Mattiuzzi & Lippi, 2019; Siegel et al., 2020). Cancer is characterized by the rapid production of abnormal cells that grow beyond their normal boundaries, invading adjacent body regions and spreading to other organs. Metastasis is the leading cause of cancer-related mortality. Environmental factors play a significant role in initiating cancer. Cancer is estimated to account

for one in six fatalities worldwide. There are numerous varieties of cancer, some of which are more prevalent in males and females than others, such as prostate cancer and breast cancer, respectively (Bisoyi, 2022). And there is other several common cancers such as bladder cancer, thyroid cancer, liver, lung, Melanoma, pancreatic cancer etc. (National Institute for Health, 2021).

## 1.3. Doxorubicin in Cancer:

Doxorubicin is an antibiotic produced from the bacteria Streptomyces paucities. The Streptomyces spp. bacteria were altered genetically by researchers so that they might produce a substance formerly known as Adriamycin and now known as doxorubicin. In spite of the fact that doxorubicin had a greater therapeutic index, cardiotoxicity remained a significant obstacle. These molecules served as models for later research, which has resulted in the discovery of approximately more than 2000 different analogs of doxorubicin at this point (Heer et al., 2020). Since the 1960s, it has been widely used as a chemotherapeutic agent and it belongs to the anthracycline class. The malignancies of the breast, ovarian, bladder, and thyroid can all be treated with doxorubicin. It is also a potential treatment for sarcomas of the bone and soft tissue. Acute lymphoblastic leukemia and several other leukemia such as acute myeloblastic leukemia, Hodgkin lymphoma, and small cell lung carcinoma are some of the other forms of cancer that can be treated with this medication (National Cancer Institute, 2018). Adriamycin, cyclophosphamide (AC), Taxotere, AC, Adriamycin, and Taxotere are some examples of the doxorubicin-containing regimens that are commonly used (Rivankar, 2014a). In the treatment of some types of thyroid cancer as well as particular kinds of soft tissue or bone sarcomas (cancer that forms in muscles and bones), doxorubicin may be administered on its own or in conjunction with other types of therapy. In addition, it is utilized in the treatment of neuroblastoma, which is a form of cancer that originates in nerve cells and most commonly affects children, as well as Wilms' tumor, which is a form of kidney cancer that affects children.

## 1.4. Aim of the project

Breast cancer is one of the most frequent types of cancer in women, with one out of every seven women being diagnosed with it at some point in their lives. And doxorubicin is recognized as one of the potential treatments. Here we will focus comparison of conventional doxorubicin with liposomal encapsulated in treatment of breast cancer. Despite the fact that conventional doxorubicin can be curative in certain cases, doxorubicin induces toxicity in most of the main organs, particularly cardiotoxicity, which can be fatal. A significant step forward in the attempt for reducing the cardiotoxicity that is associated with the use of conventional doxorubicin was liposomal formulation of doxorubicin. And aim of the research is to show liposomal formulation reduce toxicities without compromising the effectiveness of the drug.

## 1.5. Objective of the study

- To give an idea of how liposomal formulation is used to treat breast cancer.

- In order to make more informed drug selections during breast cancer treatment.

-To know about comparison of conventional and liposomal formulation of doxorubicin.

# Chapter 2

# Methodology

In the treatment of breast cancer, this article offers a comprehensive summary of a comparison between conventional doxorubicin and liposomal doxorubicin. This review paper's information was obtained from studies that had been published and had been subjected to peer review, as well as from news articles, academic papers that had been published, and websites. In addition, for the purpose of this investigation, articles published in notable journals such as Springer, Nature, Cells, The Lancet, MDPI, Frontiers, Bio pharma etc were analyzed. The pros of liposomal formulation, as well as its potential in the future, was determined with the help of information and data gathered from a large number of studies. The fact that all of the information has been compiled and referenced correctly has led to an increase in comprehension. Efforts were undertaken to find information that was missing from the accessible literature or that had been withheld. Apart from that information was also taken from different clinical studies after thorough studying the all the studies.

# Chapter 3

# Liposome as a drug delivery system

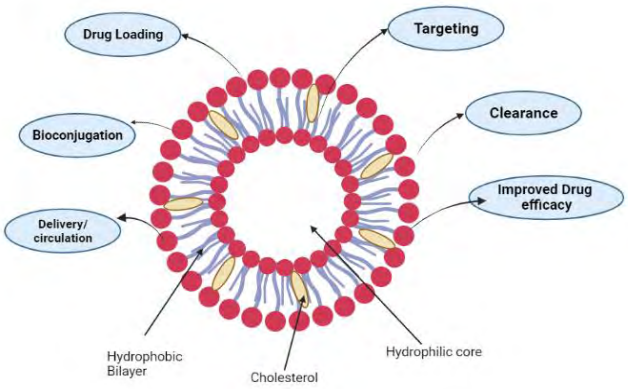


*Figure 1. Liposome as a vehicle in drug delivery*

The self-assembling phospholipid-based drug vesicle known as a liposome can either form a single bilayer (known as a uni-lamellar liposome) or a concentric array of multiple bilayers (known as a multi-lamellar liposome), both of which enclose an aqueous compartment in the middle (Liu et al., 2022). Liposomes range in size from 30 nm to micrometers, having a phospholipid bilayer that is 4-5 nm thick (Mazur et al., 2017). Numerous studies have been conducted on the use of liposomes as delivery systems for small-molecule medications, proteins, nucleic acids, and imaging agents (Man et al., 2019a, 2019b; Mirzavi et al., 2021; G. Wang et al., 2021). Liposomes are effective drug delivery systems because they protect the encapsulated compounds from physiological deterioration (Niu et al., 2012), increase the drug's half-life, regulate the release of drug molecules (N. Wang et al., 2009), and have good biocompatibility and safety. Also, liposomes can deliver the medicine directly to the diseased site through passive and/or active targeting, which reduces systemic side effects, raises the maximum dose that can be taken, and improves the therapeutic benefits (Zeng et al., 2021). In

contrast to normal tissues, abnormal tissues, such as solid tumors or an inflammatory patch, have very permeable capillaries (ranging from 100 nm to 2 m depending on the size and kind of tumor tissue). Normal tissues have tight intracellular connections between endothelial cells, which measure between 2 and 6 nm. Abnormal tissues, on the other hand, have very porous capillaries. Normal tissues also contain tight intracellular junctions between endothelial cells. The term "increased permeability and retention" (EPR) refers to the mechanism by which liposomes are able to flow across discontinuous neo vasculature and then be passively gathered and maintained at aberrant tissues. This process is known as the "EPR effect." When active targeting is performed, the chemicals and receptors that are present on the surface of the liposomes and the tumor cells interact in a very particular manner. There is a possibility that tumor cells have an increased level of expression of certain receptors. These receptors include those for the vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), folic acid (FA), integrin, and CD44. Liposomes are an efficient mode of drug administration that can be used in conjunction with a variety of treatments (Figure1).

## 3.1. Structure of liposome

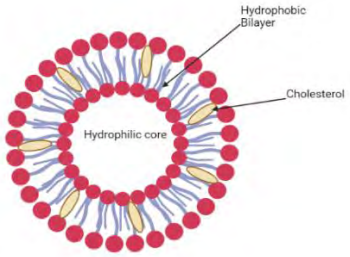


*Figure 2. Structure of Liposome*

Liposomes have been divided into four groups based on their size and number of bilayers: small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), multilamellar vesicle

(MLV), and multivesicular vesicles (MVV)(Nsairat et al., 2022). In their unilamellar structure, liposomes have a monophospholipid bilayer, whereas in their multilamellar structure, they have an onion-like structure as many unilamellar vesicles are made inside bigger liposomes, MVV form a multilamellar structure with concentric phospholipid spheres (Maja et al., 2020). Unlike MLVs, MVLs have a structure resembling a honeycomb and are made up of numerous non-concentric aqueous compartments that are separated from one another by a single bilayer lipid membrane (Kim et al., 1993). ULVs are classified into three types based on particle size: small unilamellar vesicles range from (30-100 nm), large unilamellar nm vesicles range is greater than 100 nm, and giant unilamellar vesicles is greater than 1000 (Fan et al., 2021). Only for hydrophilic compounds does the efficacy of liposome encapsulation increase with increasing liposome size, while it decreases with the number of bilayers (Ong et al., 2016). The half-life of liposomes is affected by many things, but one of the most important is the size of the vesicles. The amount of drug inside depends on both the size and the number of bilayers. When liposomes are used to deliver drugs, the desired vesicles are generally between 50 nm and 150 nm in length (Figure2).

## 3.2. Basics composition of liposome

The basics composition of liposomes is lipid (natural lipid, synthetic lipid), phospholipids and surfactant.

### 3.2.1. Lipid and phospholipids

Liposomes are made of spherical or multilayered spherical vesicles that form when diacyl-chain phospholipids (lipid bilayers) in aqueous liquids self-assemble (Wu et al., 2021).A hydrophilic head and a hydrophobic tail make up the phospholipid bilayer membrane (Akbarzadeh et al., 2013), which makes it an amphiphilic shape. Phospholipids, which can be found in nature or made in a lab, can be used to make liposomes (Sahoo & Labhasetwar, 2003). A liposome's size, rigidity, fluidity, stability, and electrical charge are all greatly affected by its

lipid composition (Calvagno et al., 2007). Lipid hydrophilic groups can be negatively, positively, or zwitterionic charged. Through electrostatic repelling, the hydrophilic group's charge promotes stability. The length, symmetry, and saturation of the acyl chains in the hydrophobic group of lipids vary (Large et al., 2021).

### 3.2.2. Surfactant

In liposome formulations, surfactants were used to change the way liposomes enclose and release substances by lowering the surface tension between different phases that don't mix phase (Cipolla et al., 2014)s. The lipid bilayer of liposomal nanoparticles is destabilized by surfactants, which increases the deformability of nano vessels (Lee et al., 2005). Sodium cholate, Span 60 and several other surfactants like Span 80, Tween 60, and Tween 80 are frequent surfactants found in liposomes formulations (Bozzuto & Molinari, 2015). In order to improve the skin penetration of encapsulated medicinal agents, numerous types of liposomes including surfactants have been utilized extensively as carriers in drug delivery studies. Ultra-deformable liposomes, which are also known as transferosomes, are nanovesicles that are based on surfactants and have shown promising results in transdermal medication delivery. Edge activator is the most important factor in liposomes' ability to be deformed. The edge activator has the ability to change the lipid bilayers of the vesicles, which increases the vesicles' deformability. In contrast to traditional liposomes, these nanovesicles have the ability to respond to changes in osmotic pressure through the process of fast shape alterations that require only a small amount of energy. Furthermore, ultra-deformable liposomes increased the drug's trans epidermal flow, making them a better nanovesicle to use for topical application of antihypertensive medicines (Paolino et al., 2012).

### 3.2.3. Synthetic lipid

The non-polar and polar sections of natural phospholipids are subjected to particular chemical alterations in order to facilitate the production of synthetic phospholipids. Because of the

alteration, it is now possible to produce an infinite variety of phospholipids that are clearly defined and organized. Stearic or palmitic fatty acids are used as the starting point for the bulk of saturated synthetic phospholipids (van Hoogevest & Wendel, 2014).

## 3.3. Available liposomal product in the market

There are 14 types of liposomal product that have been authorized by FDA and EMA and FDA had approved the first liposomal product (Table1), called Doxil (doxorubicin HCl liposome injection), in 1995. 43% of these products that are now on the market had their permission granted prior to the year 2000, while 57% of these items had their approval granted prior to the year 2010. The therapeutic field focuses the majority of its attention on the management of cancer, yet it also includes a wide range of other subjects, such as the treatment of infections and anesthesia, the development of vaccines, the treatment of lung diseases, and photodynamic therapy. The lyophilization powder and the sterile suspension make up the majority of the available dosage forms (Liu et al., 2022).

*Table 1. List of Liposome-based products with clinical application.*

| **Reference** | **Available product name** | **Active ingredients** | **Clinical Application** | **Name of the company** |
|---|---|---|---|---|
| (Working & Dayan, 1996) | Doxil® | Doxorubicin | Breast cancer | Sequus Pharmaceuticals |
| (Bulbake et al., 2017) | Myocet® | Doxorubicin | Metastatic breast cancer combined with cyclophosphamide | Elan Pharmaceuticals |
| (Balazsovits et al., 1989; | Mepact® | Mifamurtide | Osteosarcoma | Takeda Pharmaceutical Limited |

| | | | | |
|---|---|---|---|---|
| Bulbake et al., 2017) | | | | |
| (Alphandéry et al., 2015) | Marqibo® | Vincristine | Acute lymphoblastic leukemia | Talon Therapeutics, Inc. |
| (Bulbake et al., 2017) | Onivyde™ | Irinotecan | Adenocarcinoma of the pancreas | Merrimack Pharmaceuticals Inc. |
| (Bulbake et al., 2017) | Exparel® | Bupivacaine | In treatment of pain management | Pacira Pharmaceuticals, Inc. |
| (Bulbake et al., 2017) | Depocyt® | Cytarabine | Neoplastic meningitis | SkyPharma Inc. |

## 3.4. Method to prepare a liposome

The ethanol injection, double emulsion, thin-film hydration and in situ techniques are among the frequently employed manufacturing processes (Liu et al., 2022).

### 3.4.1. Solvent injection Technique

In this technique, lipid materials and lipophilic substances are dispersed in an organic solvent that is miscible with water, and then they are injected into a substantial amount of an aqueous buffer. This causes the formation of small unilamellar liposomes in a spontaneous manner (Sala et al., 2017). Depending on the preparation conditions, the particle size between 80 and 300 nm can be produced (Schubert & Müller-Goymann, 2003) and no additional energy input is required for particulate size reduction techniques such as sonication and extrusion.The liposome suspensions can be concentrated to the required volume after the organic solvent has been eliminated using various method such evaporation, lyophilization and diafiltration.

### 3.4.2. Film-Hydration Technique

This method has been used for a long time and is suitable for adding lipophilic drugs. By allowing the lipid-solvent solution to evaporate while the flask is rotating under vacuum, a thin layer is produced. The lipid film can be hydrated with the aqueous solution, and MLVs can be suspended as a result. The drug material may be passively or actively injected prior to or following the formation of liposomes, and SUVs may be created by further reducing the particle size. AmBisome, Visudyne, and Shingrix commercial products use this production process (Liu et al., 2022).

### 3.4.3. Double-Emulsification Technique

This method is additionally referred to as the DepoFoamplatform, and it has been used to make products like epoCyte, DepoDur, and Expel for sale. The whole show is made up of the four steps below, which happen in order:1) Making a "water-in-oil" emulsion, 2) Making a "water-in-oil-in-water" emulsion, 3) Implementing stripped gas or vacuum pressure to extract the solvent, and 4) Using microfiltration to get rid of free drug, concentrate, and swap external solution (Mantripragada, 2002; Ye et al., 2000).

### 3.4.4. In situ technique

"In situ" refers to liposomes that are formed prior to clinical application (Utsugi et al., 1991). This production method was employed by Mepacthas for its commercial product. After the medication and phospholipids are combined to form a bulk solution, they are filtered for sterilization before being sealed and lyophilized. Mepact only has three pharmaceutical ingredients: palmitoyl-oleoyl-phosphatidylcholine (POPC), dioleoyl-phosphatidylserine (OOPS), and the active ingredient muramyl tripeptide phosphatidyl ethanolamine (MTP-PE) and the ration of this active agent is (POPC: OOPS=7:3, MTP-PE: phospholipids = 1:250) (Frost, 1992).

## 3.5. Quality attribution of liposomal drug delivery

When compared to the administration of conventional doxorubicin, the use of Doxil results in a marked reduction in the amount of cardiac toxicity that is caused by the drug. This is because the circulating liposome particles are unable to pass through the continuous endothelial junctions that are found in the heart's blood channels. When compared to traditional drugs, DaunoXome increases the delivery of daunorubicin to tumors by roughly 10 times and delivers a sustained release in vivo (Forssen, 1997). The quality attributes (QAs) of a product are any features or characteristics that have an effect on the pharmacokinetic and pharmacodynamic effects of the product. These properties and characteristics might be physical, chemical, biological, or microbiological. And based on the mechanism of pharmacokinetics size of distribution, surface modification and phase transition are the critical quality attributes. An essential tool for liposome modification, extremely flexible PEG chains coated on liposomes to generate a hydration layer reduce MPS clearance, extend circulation lifetime, and inhibit liposome aggregation.

# Chapter 4

# Conventional doxorubicin in breast cancer

Breast cancer is one of the most common malignancies in women, accounting for 20 to 25 percent of all female cancers. Patients with metastatic breast cancer are generally considered incurable; the median survival time after the appearance of metastases is approximately two years. However, a portion of patients who receive systemic chemotherapy regimens based on doxorubicin continue to live and have no further disease development for an extended length of time. Doxorubicin is regarded as one of the most active chemotherapeutic medicines in the treatment of metastatic breast cancer, either as a single agent or in combination regimens. In the treatment of breast cancer, doxorubicin is frequently administered alongside other chemotherapy medications as part of a multidrug regimen. This strategy, known as combination chemotherapy, seeks to target cancer cells via multiple mechanisms and improve treatment efficacy (Hu et al., 2022).Doxorubicin may be administered intravenously alone or in conjunction with other medications. The dosage and duration of treatment may vary based on the specific characteristics of the breast cancer and the oncologist-recommended overall treatment plan (X. Wang et al., 2021).

## 4.1. Mechanism of action

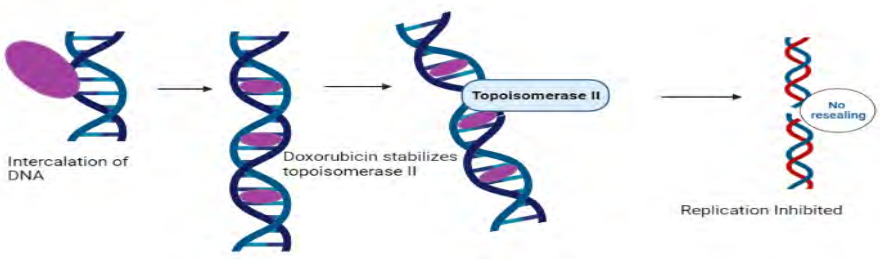


*Figure 3. Mechanisms of Doxorubicin*

Doxorubicin and other anthracyclines exert it cytotoxic effects through various mechanism

- Firstly, doxorubicin inhibits DNA and RNA synthesis by intercalating between DNA/RNA base pairs.
- Doxorubicin prevents DNA replication by inhibiting the activity of the enzyme topoisomerase II. At the replication fork during DNA replication, topoisomerase II is in charge of preventing DNA supercoiling. It accomplishes this by making nicks in both DNA strands, allowing them to relax, and then resealing them. After the strands have been nicked, dox stabilizes the DNA-topoisomerase II complex, further slowing the replication process (Renu et al., 2018).
- Free radicals produced by doxorubicin, including superoxide, hydroxyl, and hydrogen peroxide, can cause cell membrane lipid peroxidation as well as the immediate oxidation of thiols, amines, and purine or pyrimidine bases causing the DNA strand to split or break. Guanine oxidation by ROS, for instance, happens frequently (Koleini et al., 2019; Thorn et al., 2011).

## 4.2. Administration and dosing frequency of doxorubicin

Doxorubicin is often given intravenously at intervals of 21 days. The drug's highly pigmented, reddish color in liquid form makes it simple to identify. Heparin and fluorouracil are incompatible with doxorubicin, which when combined with them can precipitate. To lessen the danger of infusion responses, gradual administration of the liposomal formulation is advised even if doxorubicin can be given fast (over 15 to 20 minutes). Prior to administration, doxorubicin should be kept in a refrigerator and away from light. Doxorubicin enters tissues quickly and has an up to 48-hour half-life before it leaves the body. Doxorubicin goes through enzymatic reduction and needs to be protected before being excreted by the bile (Sritharan & Sivalingam, 2021).

**Dosing Indication:**

As an adjuvant for axillary node-positive breast cancer:

- On the first day of a 21-day cycle, administer 60 mg/m2 IV once. Utilize alongside cyclophosphamide. Four cycles should be given to patients(Oikonomou et al., 2018a).

**Monotherapy for leukemia:**

- On the first day of a 21-day cycle, provide 60 to 75 mg/m2 IV. The cumulative maximum dosage is 550 mg/m2(Oikonomou et al., 2018a).

**Combination therapy for leukemia:**

- 40–75 mg/m2 IV on the first day of a 21-day or 29-day cycle. The cumulative maximum dosage is 550 mg/m2.

## 4.3. Toxicity associated with conventional doxorubicin

Doxorubicin, an anthracycline anticancer medication, is a popular and efficient chemotherapeutic treatment for treating a variety of cancers. Cardiotoxicity is its main side effect, which may restrict its use (Chatterjee et al., 2010). Doxorubicin can cause acute cardiotoxicity, which manifests itself within two to three days of being administered. In roughly 11 percent of cases, patients have acute cardiotoxicity. The most prevalent signs are chest pain caused by myopericarditis and/or palpitations caused by sinus tachycardia, paroxysmal no persistent supraventricular tachycardia, and early atrium and ventricular pulses. Doxorubicin's anticancer mechanism is distinct from its cardiac toxicity mechanism. Doxorubicin induces cardiac myocyte apoptosis via increased oxidative stress, down-regulation of heart-specific genes, and cell death. With an estimated frequency of about 1.7%, chronic doxorubicin cardiotoxicity is substantially less common. It normally manifests within 30 days of the last dose being administered, but it can even happen 6 to 10 years afterwards. The incidence of doxorubicin cardiomyopathy is largely proportional to the drug's dosage (Takemura & Fujiwara, 2007). The incidence is approximately 4% when the doxorubicin dose is between

500 and 550 mg/m2, 18% when the dose is between 551 and 600 mg/m2, and 36% when the dose exceeds 600 mg/m2 (Luu et al., 2018).Besides this, there are several side effects that are frequent and include oral sores, baldness, exhaustion, and nausea and vomiting. It's possible to experience bone marrow suppression and an elevated chance of subsequent cancer diagnosis. Extravasation of doxorubicin following intravenous delivery can cause severe tissue necrosis and ulceration, which gets worse with time. It has been reported that doxorubicin-induced irreversible cardiomyopathy can occur anywhere from a few months to twenty years after the completion of treatment. However, the majority of cases occur within a few months after treatment's conclusion. In addition to that, congestive heart failure is a possibility (Singal et al., 1987; Swain et al., 2003). Higher cumulative drug dose, extreme age, chemotherapy combined with additional cardiotoxic drugs, and left ventricular dysfunction prior to treatment are risk factors for doxorubicin-induced congestive heart failure. Apart from that, high blood pressure, and previous radiation to the mediastinum are also the risk factors (Oikonomou et al., 2018b; Pipicz et al., 2018). When doxorubicin is given, about half of the people who get chronic heart failure die within a year.

## 4.4. Novel formulation of doxorubicin

The reason behind the novel formulation of doxorubicin is its toxicity. The toxicity of conventional anthracyclines has been lessened in a number of ways, such as by making new anthracycline analogs, using low-dose, long-term, continuous infusion schedules, combining the drug with the heart-protective agent dexrazoxane, and using liposomal encapsulation technology. The side effect of this drug that stops it from being used is cardiomyopathy, which can cause CHF and death. One way to reduce the harmful effects of doxorubicin is to use drug carriers, which change the way the drug is distributed in the body and lead to lower drug amounts in the heart (Birtle, 2000; Waterhouse et al., 2001).

### 4.4.1. Liposomal Doxorubicin

It was necessary to develop a liposomal formulation of doxorubicin that was just as effective as the conventional treatment but had less adverse effects. This was made possible because to the liposomal delivery system. Because the phospholipids that are utilized in the production of these vesicles are sourced from natural sources such as egg yolks or soybeans, the use of liposomes as a delivery mechanism is completely risk-free. This is one of the key advantages of employing liposomes as a delivery method. Furthermore, in addition, the extent of the saturation of phospholipid bilayer can be changed in order to modify the rate at which the drug is released. After the reticuloendothelial system (RES) gets opsonin's from the plasma, it takes a few minutes to a few hours for Liposomes, which are made of natural phospholipids combined with different quantities of cholesterol, to leave the bloodstream. Because of this short half-life, standard liposomes are not very useful in clinical settings (Rivankar, 2014b).

### 4.4.2. Pegylated liposomal doxorubicin

Research in liposomal drug-delivery methods has been revived by the capacity of many polymers, including polyethylene glycol (PEG), ganglioside, and cerebroside sulfate, to prevent liposomal opsonization by proteins in the plasma and enhance the duration of half-life of liposomal pharmaceuticals. There may be a connection between the greater accumulation of drug-loaded liposomes in tumor tissue and the improved therapeutic efficacy of liposomal anthracyclines, which has been connected to prolonged circulation of liposomes. The development of this formulation is a unique kind of liposomal doxorubicin that results when the liposomes are coated with PEG by a process known as pegylation, such as Doxil®/Caelyx®,, represented a subsequent step in the progression of this kind of treatment (Hilger et al., 2005; Twelves et al., 1991). This blocks the uptake of the drug by the RES, which in turn lengthens the circulation period beyond that conventional formulation and enables the drug to remain encapsulated until it reaches the site of the tumor (Gabizon et al., 1994; Gabizon

& Martin, 1997). On the other hand, some evidence raises questions about the effectiveness of utilizing pegylation to treat cancer. Because PEG is a rather big molecule, the presence of this molecule on the surface of liposomes may reduce the interactions of liposomes with cells and make it more difficult for liposomes to enter tumor tissue (Hong et al., 1999). This could stop liposomal drugs from building up in the tumor cells. A study that looked at how this formulation accumulated in murine Lewis lung carcinoma indicate the benefits of PEG liposomal doxorubicin, like higher blood levels and longer time in circulation, may not be as important as maximizing drug buildup in tumors (Parr et al., 1997). Pegylated liposomal doxorubicin's, on the other hand, have been linked to a condition known as dose-limiting hand-foot syndrome (HFS), And it is also referred as palmar-plantar erythrodysesthesia and is distinguished by eruptions of the skin on both palms of the hand and/or soles of the feet. This condition causes treatment to be halted for at least two weeks and subsequent dosage to be reduced by 25 percent (Rivankar, 2014b).

### 4.4.3. Nonpegylated liposomal doxorubicin

There is still a requirement for stable liposomes that have a long half-life in circulation and do not produce detrimental side effects like the HFS. Nonpegylated liposomal doxorubicin (NPLD) is a new type of liposomal doxorubicin that offers the benefits of pegylated-liposomal doxorubicin without its severe side-effects such as HFS. This new formulation of liposomal doxorubicin features a novel drug-delivery method and represents a significant advancement in the treatment of cancer. Not only does nonpegylated liposomal doxorubicin injection offer a superior safety profile to traditional doxorubicin, but it also outperforms both Doxil® and Caelyx® in this regard. NPLD decreases the risk of cardiac toxicity caused by doxorubicin, but it also lessens the risk of toxicity like dose-limiting toxicity related to the utilization of Doxil®/Caelyx®, such as HFS. This is made possible by combining (1) a specific composition with (2) a one-of-a-kind manufacturing technique of the NPLD's liposome, whereby provides

it with the suitable physicochemical features. This results in the liposome having the required physicochemical characteristics. Both of these aspects of the liposome have been granted patents. In comparison to the traditional doxorubicin, the NPLDs have a longer circulation duration and reduced cardiotoxicity. As this formulation do not have a PEG coating, they are not linked to the excruciating HFS that is associated with PEG-doxorubicin, which is an adverse event that limits the dose of the drug. Enzon Pharmaceuticals is the company responsible for producing the NPLD known as Myocet® on behalf of Cephalon in Europe and Sopherion Therapeutics in the United States of America and Canada. In conjunction with cyclophosphamide, Myocet® is licensed for the treatment of breast cancer in Europe and Canada (Gabizon & Martin, 1997; Rivankar, 2014b).

### 4.4.4. Other formulation of doxorubicin

Stealth liposomes have earned a significant role in the field of cancer treatment; nonetheless, the attachment of ligands to these liposomes continues to be the primary method for modifying the selectivity of these liposomes for cancers (Immordino et al., 2006). Antibody coated liposomes, also known as immunoliposomes, have been the subject of substantial research. In this type of liposome, an antibody either connects with the phospholipid head group of the liposome or it connects the end of a PEG polymer (Schnyder & Huwyler, 2005). For the purpose of tumor targeting, temperature-sensitive liposomes can also be manufactured. In a similar vein, studies are being conducted on the release of doxorubicin using acid triggered, enzyme prompted, and light induced mechanisms. Sulfatide-mediated liposome targeting (Shao et al., 2006) and (Saul et al., 2003)folate receptor targeting are two further examples of active targeting. For doxorubicin, block co-polymers comprising poly (ethylene oxide) and poly (propylene oxide) block copolymers, sometimes known as Pluronic's, are also being researched. For the treatment of osteogenic sarcoma, hydroxyapatite implants that contain doxorubicin have been produced and evaluated using an in vivo model (Itokazu et al., 1996).

There has also been research conducted on thermosensitive poly (organophosphazenes) hydrogels that contain doxorubicin. Over the course of 20 days, there was a discernible continuation of the loaded doxorubicin's release from the polymer hydrogel. It was discovered that Caco-2 cells might be inhibited through the use of microgels made of Pluronic's that were administered orally (Bromberg & Alakhov, 2003).

Just the liposomal forms of doxorubicin have been subjected to extensive study and evaluation in clinical trials with cancer patients at various stages.

# Chapter 5

# Liposome encapsulated doxorubicin in breast cancer

It is generally agreed upon that doxorubicin serves as one of the chemotherapy medications that is most successful at treating breast cancer that has progressed to other areas of the body. It can be used alone or with other drugs. When compared to traditional doxorubicin, the therapeutic index of a novel liposomal version of doxorubicin (MyocetTM, Elan Pharmaceuticals) is much higher (Batist et al., 2002). The discovery of Myocet, a variant of doxorubicin that is less harmful to the heart, better tolerated, and just as effective, broadens the treatment choices available for the management of breast cancer in its entirety. One of the most prevalent types of disease among women is breast cancer, and it accounts for 20–25 percent of all malignancies found in women. Patients with breast cancer who have developed secondary tumors of the body are largely considered incurable; the median survival time after the appearance of metastases is about 2 years (Clark et al., 1987; Mick et al., 1989). However, after the initial course of treatment with doxorubicin-based systemic chemotherapy regimens, a certain percentage of patients continue to show no signs of disease development and remain alive for extended periods of time. It is possible that the major therapeutic goals of raising this fraction of long-term disease-free survivors and improving overall survival rates can be achieved with the help of improved medicines that have a convincing tumor response and little toxicity standard of living (Greenberg et al., 1996).

## 5.1. Formulation of liposomal encapsulated doxorubicin

One way to lessen the adverse effects of doxorubicin is to use drug carriers. These carriers change the way the drug works in the body, which lowers the amount of drug in the heart. Several of those carrier systems are based on lipids (liposomes), whereas others are based on proteins (virions) (Abraham et al., 2005).

There are two approved versions that change the way doxorubicin is distributed in the body in an effective manner. Liposomes are effective drug delivery vehicles because the microvasculature in tumors is often discontinuous. The pore sizes in tumor microvasculature range from 100 to 780 nanometers, which allows liposomes to pass from the blood compartment into the extravascular area surrounding the tumor cells. Liposomes that are between 100 and 200 nm in size easily leak out into the area where a tumor is growing. This is one of the main purposes of liposomal formulation, which is to give drugs in a highly concentrated form near the tumor. The major goal of encapsulating doxorubicin in liposomes has been to reduce nonspecific organ damage. The doxorubicin can be directed away from regions with tight capillary connections, such as the heart muscle, using liposomes. Instead, they are found in regions of the body that have fenestrations or holes in their vasculature, such as the liver, the spleen, and the bone marrow, as well as locations of inflammation and tumors. These particle carrier systems can be recognized as "foreign" by the phagocytic cells that make up the mononuclear phagocyte system (MPS). Since distribution of these cells that phagocyte depends on the physical (size) and chemical (charge) characteristics of the liposomes utilized, it should be highlighted that the MPS cells are negatively impacted when liposomes include doxorubicin. In particular, subsequent uptake by phagocytic cells, the release of doxorubicin leads these cells dying, thereby diminishing the MPS's ability to build up the injected liposomes(Allen et al., 2002). This, in turn, is reflected by significantly longer liposome circulation lifetimes. Following intravenous injection, liposomal formulations that contain poly (ethylene glycol) (PEG)-modified lipids have been shown to have a decreased ability to assemble, which results in increased circulation time (Papahadjopoulos et al., 1991). Nevertheless, these types of formulations also have MPS toxicity (Parr et al., 1993). Doxil (in the United States) or Caelyx (in Canada and Europe) and Myocet, which was approved by the

European Commission in August 2000 to treat metastatic breast cancer, are two liposomal doxorubicin forms that have passed clinical trials (Allen et al., 2002).

## 5.2. Pharmacokinetics of liposomal encapsulated doxorubicin

After administration of Myocet (liposomal doxorubicin), the pharmacokinetics of doxorubicin differ significantly from those following administration of standard doxorubicin. The liver is responsible for a significant portion of the metabolism of doxorubicin, which results in the production of doxorubicinol as the primary, functional metabolite. When compared to the pharmacokinetics of traditional doxorubicin, the pharmacokinetics of Myocet resulted in a mean clearance of total doxorubicin that was about 9 times lower, a volume of distribution that was approximately 25 times lower, and an area under the curve (AUC) that was approximately 20 times greater (Batist et al., 2002). Doxorubicin had a terminal half-life of 16 hours after being given Myocet, but it had a half-life of 43 hours when given as conventional doxorubicin (Stewart et al., 1993). It has been estimated that at least 85% of the doxorubicin that is circulating in patients is in the form of liposome-encapsulated particles. Doxorubicin and doxorubicinol were excreted at comparable rates in the urine following treatment with either Myocet or conventional doxorubicin. It has been proposed that doxorubicinol might cause doxorubicin-mediated cardiotoxicity; therefore, it is preferable to avoid or reduce high peak plasma concentrations of both doxorubicin and doxorubicinol. Tissue distribution studies have shown that the drug's therapeutic index is raised when there is more doxorubicin in the bloodstream and less at its highest point in the plasma. The amount of radiation (representing doxorubicin and/or doxorubicinol) in the myocardium of dogs given Myocet was less than 67% of what was seen in the myocardium of animals given conventional doxorubicin. According to the findings of two different sets of comparative preclinical research, Myocet is significantly less harmful to the heart than the same cumulative dose of conventional doxorubicin. As demonstrated in murine tumor models, the antitumor activity of Myocet at equivalent

concentrations is at least equivalent to that of conventional doxorubicin. Additional investigations in human breast tumor models have demonstrated that doxorubicin is more enduring and widespread than conventional doxorubicin, with the same effectiveness in inhibiting tumor growth after administration of Myocet (Rossi et al., 1987; Symon et al., 1999).

## 5.3. Toxicological profile of liposomal encapsulated doxorubicin

The goal of the liposome-encapsulated anthracyclines was to change the tissue distribution and pharmacokinetics of doxorubicin in order to lessen its toxicity while maintaining its anticancer activity. The cardiac muscle and gastrointestinal tract are examples of areas with tight capillary connections where intravenously administered liposomes cannot exit the circulatory space. Most often, tissues and organs coated with loosely linked cells are where liposomes exit the bloodstream as well as in regions where capillaries are disturbed as a result of inflammation or tumor growth. Therefore, it is important for liposomes to divert doxorubicin out of potentially dangerous areas, but they must nevertheless leave the tumor unprotected. Liposomal doxorubicin had much lower cardiac and gastrointestinal toxicity, although anticancer activity was at least comparable to the parent molecule. The pharmacokinetics and biodistribution of liposomal encapsulated doxorubicin are significantly different from those of conventional doxorubicin, which contributes to the drug's distinctive toxicological profile. The liposomal formulation makes it possible to manage how much of the medicine is released into the body while also allowing for preferential accumulation of the drug in tumor tissues (Rafiyath et al., 2012). This results in increased therapeutic efficacy and maybe less toxicity. The toxicological profile of liposomal encapsulated doxorubicin has some key aspects-

**Cardiotoxicity:** The primary problem with traditional doxorubicin is cardiotoxicity. The risk of cardiotoxicity can be decreased via liposomal encapsulation, which can lessen the drug's buildup in the heart. According to studies, liposomal encapsulated doxorubicin is less likely to cause cardiotoxicity than conventional doxorubicin (Rivera, 2003).

**Hematological Toxicity:** It is still possible for liposomal encapsulated doxorubicin to have hematological side effects, such as myelosuppression (the suppression of bone marrow activity), which can result in lower blood cell counts. On the other hand, in comparison to traditional doxorubicin, it typically has a fewer effect (O'Shaughnessy, 2003).

**Infusion related toxicities:** Fever, chills, and allergic responses are potentially possible side effects of liposomal encapsulated doxorubicin infusions. However, the frequency and severity of these reactions are typically lower than those seen with traditional doxorubicin(Rivera, 2003).

**Other toxicities:** Even though liposomal encapsulated doxorubicin is thought to have a better toxicity profile, it can still cause stomach problems (like sickness, vomiting, and diarrhea), mucositis, and damage to the liver. But compared to regular doxorubicin, these side effects happen less often and are often less severe (Rafiyath et al., 2012).

## 5.4. Clinical exposure of liposomal encapsulated doxorubicin in breast cancer

A form of chemotherapy known as liposomal encapsulated doxorubicin is one of the drugs that is utilized in the process of treating breast cancer. It is crafted to enhance the efficacy of the medication while simultaneously reducing the severity of its adverse effects. Utilization of liposomal encapsulated doxorubicin in specific patient cases, followed by an analysis of the drug's effectiveness and safety (Rafiyath et al., 2012; Rivera, 2003). Liposomal encapsulated doxorubicin has been the subject of substantial research and is currently being utilized as a method of treatment for breast cancer in clinical settings. There is evidence that liposomal doxorubicin is an effective treatment for metastatic breast cancer (O'Shaughnessy, 2003). As a component of a treatment plan, it is frequently used in conjunction with a number of other chemotherapy medications. Studies conducted in clinical settings have shown that it has the ability to reduce the size of tumors and increase patients' chances of surviving metastatic breast

cancer. Additionally, liposomal encapsulated doxorubicin has been investigated as a potential adjuvant therapy for breast cancer in its early stages. Adjuvant therapy is a type of treatment that is administered after the primary treatment, such as surgery, in order to assist lower the likelihood of the cancer coming back (Rosati et al., 2011). In clinical trials, the utilization of liposomal formulation in combination with other chemotherapeutic drugs or in sequential treatment regimens has been investigated with the goal of improving the prognosis of patients whose breast cancer was diagnosed at an early stage (Chan et al., 2004).

# Chapter 6

# Comparison of conventional doxorubicin and liposome encapsulated doxorubicin.

In patients with MBC, it has been demonstrated that liposomal doxorubicin has the same level of efficacy as conventional doxorubicin while having a lower level of toxicity. In terms of managing metastatic breast cancer (MBC), liposomal formulations have demonstrated efficacy as monotherapy or in conjunction with other drugs for the treatment of patients who have received anthracycline therapy and experienced a progression-free interval of greater than 6-12 months (Lao et al., 2013; Rosati et al., 2011).

A review of the clinical tests that have evaluated safety and efficacy of conventional doxorubicin with liposomal formulation both as monotherapy and in combination therapy. We are going to look at both the efficacy and the toxicity of the drug, with a particular focus on the data relating to its effect on the heart. There have been two Phase III studies that have been published in which the researchers directly compared the efficacy and toxicity of liposomal doxorubicin to that of conventional doxorubicin. Regarding the response rate, progression-free survival (PFS), and overall survival, no statistically significant disparities in efficacy were seen between the two treatment modalities (Lao et al., 2013; O'Brien et al., 2004).

*Table 2. Comparison of liposomal doxorubicin with conventional doxorubicin either in combination therapy or monotherapy*

| **Reference** | **Trial Phase** | **No of patients** | **Dosage's regimen** | **Disease stages** | **Progression free survival** | **Overall survival** | **Response rate** | **Toxicity** |
|---|---|---|---|---|---|---|---|---|
| (Harris et al., 2002) | III | 224 | Liposomal doxorubicin (75 mg) Vs Conventional doxorubicin ADR (75 mg) | IV (17% ADR prior) | 3.8 months vs 4.3 months | 16 months vs 20 months | 26% | Cardiac: 13% Vs 29% CHF:5.9% vs 15% |
| (Chan et al., 2004) | III | 160 | Liposomal doxorubicin (75 mg+ CTX 600 mg) Vs conventional EPI (75 mg + CTX 600 mg) | IV (no prior ADR) | 7.7 month Vs 5.6 month | 18.3 month Vs 16 months | 46 % Vs 39 % | Cardiac:11% Vs 10% CRF: No |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (Batist et al., 2001; Chan et al., 2004) | III | 297 | Liposomal doxorubicin (60 mg+ CTX 600 mg) Vs Conventional doxorubicin ADR (60 mg+ CTX 600 mg) | IV (10%prior ADR) | 5.1-month Vs 5.5 month | 19-month Vs 16 month | | Cardiac:6% Vs 21 vs CRF: 0% Vs 3.2% |

In a study with 224 patients who had metastatic breast cancer, Harris (Harris et al., 2002) aimed to evaluate the effectiveness and safety of LD (75 mg/m2 every 3 weeks) in comparison to the conventional doxorubicin regimen (75 mg/m2 every 3 weeks). From table 2, a total of 14.3% of the individuals had received prior treatment with anthracyclines, either in the form of adjuvant therapy or neoadjuvant therapy. The response rate observed in both forms of doxorubicin was 26%. The duration of free survival in patients treated with liposomal doxorubicin was found to be 3.8 months, whereas those treated with conventional doxorubicin saw a slightly longer duration of 4.3 months. Additionally, the overall survival rate for patients receiving liposomal doxorubicin was 16 months, compared to 20 months for those receiving conventional doxorubicin. The incidence of cardiac toxicity was shown to be 13% in liposomal doxorubicin, but conventional doxorubicin exhibited a higher rate of 29%.

In a subsequent Phase III study ( Chan et al., 2004), 160 patients were assigned at random to receive cyclophosphamide at a dose of 600 mg/m2 in combination with either epirubicin 75 mg/m2 or liposomal doxorubicin 75 mg/m2. The rate of asymptomatic decline in LVEF was observed to be the same in both groups (11 versus 10%), indicating that there was no significant difference. During the course of this research, not a single patient experienced clinical symptoms of heart failure. It is important to note that the dosage of epirubicin was less than that of the equivalent amount of doxorubicin.

In the study conducted by Batist (Batist et al., 2001), thirty percent of patients exhibited any cardiotoxicity risk factor, and ten percent of patients had previously been treated with anthracyclines (adjuvant) at a mean cumulative dose of two hundred forty milligrams per square meter. Within the scope of this investigation, it was seen that a proportion of 21% of individuals who underwent treatment with conventional doxorubicin encountered varying degrees of cardiotoxicity. Conversely, a mere 6% of patients belonging to the liposomal doxorubicin group displayed similar adverse effects. In the case of conventional doxorubicin, a clinical heart failure occurred in 3.2% of patients, but in the case of liposomal doxorubicin, this incidence was seen to be 0%.

# Chapter 7

## Future perspective of doxorubicin in cancer

In the treatment of breast and ovarian cancers, the traditional drug doxorubicin has been an essential component. Recent efforts have significantly improved the drug's safety and tolerability, which is significant given that the usage of conventional doxorubicin has been somewhat restricted due to the adverse reactions it can cause. To this day, liposomal encapsulation has proven to be the most effective method for increasing the therapeutic index of conventional formulations of doxorubicin (Rivankar, 2014b). This method leads to preferential accumulation of the medication within the tumor site, which in turn maximizes efficacy while simultaneously minimizing toxicity. Overall, it appears that there will be an extended future for the ongoing application of doxorubicin in clinical settings for the treatment of cancer, provided that certain enhancements are made (Greenberg et al., 1996). In the future, after the efficacy and safety profiles of novel liposomal doxorubicin formulations have been established, ideally the focus of research will shift to assessing the costs of therapy with these innovative formulations in order to evaluate their potential for widespread use and robustness in the treatment of cancer patients.

# Chapter 8

## Discussion and findings

Doxorubicin is widely recognized for its ability to elicit a diverse range of cytotoxic effects through its interactions with DNA-associated enzymes, intercalation inside DNA base pairs, and selective targeting of many molecular targets. For example, it triggers the activation of a variety of molecular signals that are sent by AMPK (AMP-activated protein kinase triggering apoptosis), which then have an effect on the Bcl-2/Bax apoptotic pathway. Apoptosis can be induced by changing the proportion of Bcl-2 to Bax, which leads to the activation of many caspases in the cell's downstream pathway. Doxorubicin is known to cause toxicity in the brain, liver, kidneys, and heart. It does this by inducing apoptosis and necrosis in healthy tissue and among all this toxicity cardiac toxicity is the main. The primary cause of cardiotoxicity is the development of cardiomyopathy as a result of myocyte damage from free radicals. High peak plasma anthracycline levels cause more harm. Cumulative cardiomyopathy is thought to be caused by free radicals repeatedly damaging the mitochondria of myocytes (Birtle, 2000; Waterhouse et al., 2001). Numerous experiments, including those using liposomes, hydrogels, and nanoparticulate systems, have been carried out over time to develop a drug delivery system that will eradicate these adverse effects and we try to highlight pros of liposomal encapsulated doxorubicin compared to conventional doxorubicin. As the goal of the liposome-encapsulated doxorubicin was to change distribution and pharmacokinetics of doxorubicin in order to lessen its toxicity while maintaining its anticancer activity (Rafiyath et al., 2012).

# Chapter 9

# Conclusion

It is possible to alter the pharmacokinetics and pharmacodynamics of cytostatic medicines using drug delivery systems that are based on liposomes. This gives us the ability to raise the concentration of the medication that is delivered into the neoplastic tissue while simultaneously lowering the amount of the drug that is exposed to the normal tissue. Although doxorubicin are essential drugs for treating metastatic breast cancer, cardiotoxicity continues to be one of the most significant obstacles in the way of their application. Encapsulation of the drug in liposomes is one of the ways used to reduce the severity of this adverse effect. There are multiple doxorubicin formulations available, each of which demonstrates distinctive pharmacological properties. Liposomal doxorubicin (Myocet) and pegylated liposomal doxorubicin (Caelyx) are the formulations that are being used most of the time. In patients who has metastatic breast cancer liposomal doxorubicin has been shown to be just as effective as conventional doxorubicin while causing less side effects. This makes it possible for patients to undergo therapy for a longer period of time and receive a larger cumulative dose of the doxorubicin. Moreover, liposomal doxorubicin has demonstrated both efficacy and safety when combined with other cytotoxic agents in the treatment of advanced as well as early breast cancer.

## Reference


Abraham, S. A., Waterhouse, D. N., Mayer, L. D., Cullis, P. R., Madden, T. D., & Bally, M. B. (2005). The liposomal formulation of doxorubicin. *Methods in Enzymology*, *391*, 71–97. https://doi.org/10.1016/S0076-6879(05)91004-5

Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S. W., Zarghami, N., Hanifehpour, Y., Samiei, M., Kouhi, M., & Nejati-Koshki, K. (2013). Liposome: classification, preparation, and applications. *Nanoscale Research Letters*, *8*(1), 102. https://doi.org/10.1186/1556-276X-8-102

Allen, C., Dos Santos, N., Gallagher, R., Chiu, G. N. C., Shu, Y., Li, W. M., Johnstone, S. A., Janoff, A. S., Mayer, L. D., Webb, M. S., & Bally, M. B. (2002). Controlling the physical behavior and biological performance of liposome formulations through use of surface grafted poly(ethylene glycol). *Bioscience Reports*, *22*(2), 225–250. https://doi.org/10.1023/a:1020186505848

Alphandéry, E., Grand-Dewyse, P., Lefèvre, R., Mandawala, C., & Durand-Dubief, M. (2015). Cancer therapy using nanoformulated substances: scientific, regulatory and financial aspects. *Expert Review of Anticancer Therapy*, *15*(10), 1233–1255. https://doi.org/10.1586/14737140.2015.1086647

Arnold, M., Morgan, E., Rumgay, H., Mafra, A., Singh, D., Laversanne, M., Vignat, J., Gralow, J. R., Cardoso, F., Siesling, S., & Soerjomataram, I. (2022). Current and future burden of breast cancer: Global statistics for 2020 and 2040. *Breast*, *66*. https://doi.org/10.1016/j.breast.2022.08.010

Balazsovits, J. A., Mayer, L. D., Bally, M. B., Cullis, P. R., McDonell, M., Ginsberg, R. S., & Falk, R. E. (1989). Analysis of the effect of liposome encapsulation on the vesicant properties, acute and cardiac toxicities, and antitumor efficacy of doxorubicin. *Cancer Chemotherapy and Pharmacology*, *23*(2), 81–86. https://doi.org/10.1007/BF00273522

Batist, G., Barton, J., Chaikin, P., Swenson, C., & Welles, L. (2002). Myocet (liposome-encapsulated doxorubicin citrate): a new approach in breast cancer therapy. *Expert Opinion on Pharmacotherapy*, *3*(12), 1739–1751. https://doi.org/10.1517/14656566.3.12.1739

Batist, G., Ramakrishnan, G., Rao, C. S., Chandrasekharan, A., Gutheil, J., Guthrie, T., Shah, P., Khojasteh, A., Nair, M. K., Hoelzer, K., Tkaczuk, K., Park, Y. C., & Lee, L. W. (2001). Reduced cardiotoxicity and preserved antitumor efficacy of liposome-encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology*, *19*(5), 1444–1454. https://doi.org/10.1200/JCO.2001.19.5.1444

Birtle, A. J. (2000). Anthracyclines and cardiotoxicity. *Clinical Oncology (Royal College of Radiologists (Great Britain))*, *12*(3), 146–152. https://doi.org/10.1053/clon.2000.9141

Bisoyi, P. (2022). Malignant tumors - as cancer. In *Understanding Cancer: From Basics to Therapeutics*. https://doi.org/10.1016/B978-0-323-99883-3.00011-1

Bozzuto, G., & Molinari, A. (2015). Liposomes as nanomedical devices. *International Journal of Nanomedicine*, *10*, 975–999. https://doi.org/10.2147/IJN.S68861

Bromberg, L., & Alakhov, V. (2003). Effects of polyether-modified poly(acrylic acid) microgels on doxorubicin transport in human intestinal epithelial Caco-2 cell layers. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *88*(1), 11–22. https://doi.org/10.1016/s0168-3659(02)00419-4

Bulbake, U., Doppalapudi, S., Kommineni, N., & Khan, W. (2017). Liposomal Formulations in Clinical Use: An Updated Review. *Pharmaceutics*, *9*(2). https://doi.org/10.3390/pharmaceutics9020012

Calvagno, M. G., Celia, C., Paolino, D., Cosco, D., Iannone, M., Castelli, F., Doldo, P., & Frest, M. (2007). Effects of lipid composition and preparation conditions on physical-chemical

properties, technological parameters and in vitro biological activity of gemcitabine-loaded liposomes. *Current Drug Delivery*, *4*(1), 89–101. https://doi.org/10.2174/156720107779314749

Chan, S., Davidson, N., Juozaityte, E., Erdkamp, F., Pluzanska, A., Azarnia, N., & Lee, L. W. (2004). Phase III trial of liposomal doxorubicin and cyclophosphamide compared with epirubicin and cyclophosphamide as first-line therapy for metastatic breast cancer. *Annals of Oncology : Official Journal of the European Society for Medical Oncology*, *15*(10), 1527–1534. https://doi.org/10.1093/annonc/mdh393

Chatterjee, K., Zhang, J., Honbo, N., & Karliner, J. S. (2010). Doxorubicin cardiomyopathy. *Cardiology*, *115*(2), 155–162. https://doi.org/10.1159/000265166

Cipolla, D., Wu, H., Gonda, I., Eastman, S., Redelmeier, T., & Chan, H.-K. (2014). Modifying the release properties of liposomes toward personalized medicine. *Journal of Pharmaceutical Sciences*, *103*(6), 1851–1862. https://doi.org/10.1002/jps.23969

Clark, G. M., Sledge, G. W., Osborne, C. K., & McGuire, W. L. (1987). Survival from first recurrence: relative importance of prognostic factors in 1,015 breast cancer patients. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology*, *5*(1), 55–61. https://doi.org/10.1200/JCO.1987.5.1.55

Fan, Y., Marioli, M., & Zhang, K. (2021). Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. *Journal of Pharmaceutical and Biomedical Analysis*, *192*, 113642. https://doi.org/10.1016/j.jpba.2020.113642

Forssen, E. A. (1997). The design and development of DaunoXome® for solid tumor targeting in vivo. *Advanced Drug Delivery Reviews*, *24*(2–3), 133–150. https://doi.org/10.1016/S0169-409X(96)00453-X

Frost, H. (1992). MTP-PE in liposomes as a biological response modifier in the treatment of cancer: current status. *Biotherapy (Dordrecht, Netherlands)*, *4*(3), 199–204. https://doi.org/10.1007/BF02174206

Gabizon, A., Catane, R., Uziely, B., Kaufman, B., Safra, T., Cohen, R., Martin, F., Huang, A., & Barenholz, Y. (1994). Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Research*, *54*(4), 987–992.

Gabizon, A., & Martin, F. (1997). Polyethylene glycol-coated (pegylated) liposomal doxorubicin. Rationale for use in solid tumours. *Drugs*, *54 Suppl 4*, 15–21. https://doi.org/10.2165/00003495-199700544-00005

Greenberg, P. A., Hortobagyi, G. N., Smith, T. L., Ziegler, L. D., Frye, D. K., & Buzdar, A. U. (1996). Long-term follow-up of patients with complete remission following combination chemotherapy for metastatic breast cancer. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology*, *14*(8), 2197–2205. https://doi.org/10.1200/JCO.1996.14.8.2197

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. In *Cell* (Vol. 144, Issue 5). https://doi.org/10.1016/j.cell.2011.02.013

Harris, L., Batist, G., Belt, R., Rovira, D., Navari, R., Azarnia, N., Welles, L., Winer, E., & TLC D-99 Study Group. (2002). Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma. *Cancer*, *94*(1), 25–36. https://doi.org/10.1002/cncr.10201

Heer, E., Harper, A., Escandor, N., Sung, H., McCormack, V., & Fidler-Benaoudia, M. M. (2020). Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study. *The Lancet. Global Health*, *8*(8), e1027–e1037. https://doi.org/10.1016/S2214-109X(20)30215-1

Hilger, R. A., Richly, H., Grubert, M., Oberhoff, C., Strumberg, D., Scheulen, M. E., & Seeber, S. (2005). Pharmacokinetics (PK) of a liposomal encapsulated fraction containing doxorubicin and of doxorubicin released from the liposomal capsule after intravenous infusion of Caelyx/Doxil. *International Journal of Clinical Pharmacology and Therapeutics*, *43*(12), 588–589. https://doi.org/10.5414/cpp43588

Hong, R. L., Huang, C. J., Tseng, Y. L., Pang, V. F., Chen, S. T., Liu, J. J., & Chang, F. H. (1999). Direct comparison of liposomal doxorubicin with or without polyethylene glycol coating in C-26 tumor-bearing mice: is surface coating with polyethylene glycol beneficial? *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *5*(11), 3645–3652.

Hu, Q., Yao, J., Wang, X., Wang, Y., Fu, X., Ma, J., Lin, H., Xu, J., Shen, L., & Yu, X. (2022). Combinational Chemoimmunotherapy for Breast Cancer by Codelivery of Doxorubicin and PD-L1 siRNA Using a PAMAM-Incorporated Liposomal Nanoplatform. *ACS Applied Materials and Interfaces*, *14*(7). https://doi.org/10.1021/acsami.1c21775

Immordino, M. L., Dosio, F., & Cattel, L. (2006). Stealth liposomes: Review of the basic science, rationale, and clinical applications, existing and potential. In *International Journal of Nanomedicine* (Vol. 1, Issue 3).

Itokazu, M., Kumazawa, S., Wada, E., & Wenyi, Y. (1996). Sustained release of adriamycin from implanted hydroxyapatite blocks for the treatment of experimental osteogenic sarcoma in mice. *Cancer Letters*, *107*(1), 11–18. https://doi.org/10.1016/0304-3835(96)04337-6

Kim, T., Kim, J., & Kim, S. (1993). Extended-release formulation of morphine for subcutaneous administration. *Cancer Chemotherapy and Pharmacology*, *33*(3), 187–190. https://doi.org/10.1007/BF00686214

Koleini, N., Nickel, B. E., Edel, A. L., Fandrich, R. R., Ravandi, A., & Kardami, E. (2019). Oxidized phospholipids in Doxorubicin-induced cardiotoxicity. *Chemico-Biological Interactions*, *303*, 35–39. https://doi.org/10.1016/j.cbi.2019.01.032

Lao, J., Madani, J., Puértolas, T., Alvarez, M., Hernández, A., Pazo-Cid, R., Artal, A., & Antón Torres, A. (2013). Liposomal Doxorubicin in the treatment of breast cancer patients: a review. *Journal of Drug Delivery*, *2013*, 456409. https://doi.org/10.1155/2013/456409

Large, D. E., Abdelmessih, R. G., Fink, E. A., & Auguste, D. T. (2021). Liposome composition in drug delivery design, synthesis, characterization, and clinical application. *Advanced Drug Delivery Reviews*, *176*, 113851. https://doi.org/10.1016/j.addr.2021.113851

Lee, E. H., Kim, A., Oh, Y.-K., & Kim, C.-K. (2005). Effect of edge activators on the formation and transfection efficiency of ultradeformable liposomes. *Biomaterials*, *26*(2), 205–210. https://doi.org/10.1016/j.biomaterials.2004.02.020

Liu, P., Chen, G., & Zhang, J. (2022). A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives. *Molecules (Basel, Switzerland)*, *27*(4). https://doi.org/10.3390/molecules27041372

Luu, A. Z., Chowdhury, B., Al-Omran, M., Teoh, H., Hess, D. A., & Verma, S. (2018). Role of Endothelium in Doxorubicin-Induced Cardiomyopathy. *JACC. Basic to Translational Science*, *3*(6), 861–870. https://doi.org/10.1016/j.jacbts.2018.06.005

Maja, L., Željko, K., & Mateja, P. (2020). Sustainable technologies for liposome preparation. In *Journal of Supercritical Fluids* (Vol. 165). https://doi.org/10.1016/j.supflu.2020.104984

Man, F., Gawne, P. J., & T M de Rosales, R. (2019a). Nuclear imaging of liposomal drug delivery systems: A critical review of radiolabelling methods and applications in nanomedicine. *Advanced Drug Delivery Reviews*, *143*, 134–160. https://doi.org/10.1016/j.addr.2019.05.012

Man, F., Gawne, P. J., & T M de Rosales, R. (2019b). Nuclear imaging of liposomal drug delivery systems: A critical review of radiolabelling methods and applications in nanomedicine. *Advanced Drug Delivery Reviews*, *143*, 134–160. https://doi.org/10.1016/j.addr.2019.05.012

Mantripragada, S. (2002). A lipid based depot (DepoFoam technology) for sustained release drug delivery. *Progress in Lipid Research*, *41*(5), 392–406. https://doi.org/10.1016/s0163-7827(02)00004-8

Mattiuzzi, C., & Lippi, G. (2019). Current Cancer Epidemiology. *Journal of Epidemiology and Global Health*, *9*(4), 217–222. https://doi.org/10.2991/jegh.k.191008.001

Mazur, F., Bally, M., Städler, B., & Chandrawati, R. (2017). Liposomes and lipid bilayers in biosensors. *Advances in Colloid and Interface Science*, *249*, 88–99. https://doi.org/10.1016/j.cis.2017.05.020

Mick, R., Begg, C. B., Antman, K. H., Korzun, A. H., & Frei, E. (1989). Diverse prognosis in metastatic breast cancer: who should be offered alternative initial therapies? *Breast Cancer Research and Treatment*, *13*(1), 33–38. https://doi.org/10.1007/BF01806548

Mirzavi, F., Barati, M., Soleimani, A., Vakili-Ghartavol, R., Jaafari, M. R., & Soukhtanloo, M. (2021). A review on liposome-based therapeutic approaches against malignant melanoma. *International Journal of Pharmaceutics*, *599*, 120413. https://doi.org/10.1016/j.ijpharm.2021.120413

Nassar, D., & Blanpain, C. (2016). Cancer Stem Cells: Basic Concepts and Therapeutic Implications. *Annual Review of Pathology*, *11*, 47–76. https://doi.org/10.1146/annurev-pathol-012615-044438

National Cancer Institute. (2018). *Doxorubicin Hydrochloride - National Cancer Institute*. NIH National Cancer Institute.

National Institute for Health. (2021). *Common Cancer Types - National Cancer Institute*. August 22. https://www.cancer.gov/types/common-cancers

Niu, M., Lu, Y., Hovgaard, L., Guan, P., Tan, Y., Lian, R., Qi, J., & Wu, W. (2012). Hypoglycemic activity and oral bioavailability of insulin-loaded liposomes containing bile salts in rats: the effect of cholate type, particle size and administered dose. *European Journal of Pharmaceutics and Biopharmaceutics : Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V*, *81*(2), 265–272. https://doi.org/10.1016/j.ejpb.2012.02.009

Nsairat, H., Khater, D., Sayed, U., Odeh, F., Al Bawab, A., & Alshaer, W. (2022). Liposomes: structure, composition, types, and clinical applications. *Heliyon*, *8*(5), e09394. https://doi.org/10.1016/j.heliyon.2022.e09394

O'Brien, M. E. R., Wigler, N., Inbar, M., Rosso, R., Grischke, E., Santoro, A., Catane, R., Kieback, D. G., Tomczak, P., Ackland, S. P., Orlandi, F., Mellars, L., Alland, L., Tendler, C., & CAELYX Breast Cancer Study Group. (2004). Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Annals of Oncology : Official Journal of the European Society for Medical Oncology*, *15*(3), 440–449. https://doi.org/10.1093/annonc/mdh097

Oikonomou, E., Anastasiou, M., Siasos, G., Androulakis, E., Psyrri, A., Toutouzas, K., & Tousoulis, D. (2018a). Cancer Therapeutics-Related Cardiovascular Complications. Mechanisms, Diagnosis and Treatment. *Current Pharmaceutical Design*, *24*(37), 4424–4435. https://doi.org/10.2174/1381612825666190111101459

Oikonomou, E., Anastasiou, M., Siasos, G., Androulakis, E., Psyrri, A., Toutouzas, K., & Tousoulis, D. (2018b). Cancer Therapeutics-Related Cardiovascular Complications. Mechanisms, Diagnosis and Treatment. *Current Pharmaceutical Design*, *24*(37), 4424–4435. https://doi.org/10.2174/1381612825666190111101459

Ong, S. G. M., Ming, L. C., Lee, K. S., & Yuen, K. H. (2016). Influence of the Encapsulation Efficiency and Size of Liposome on the Oral Bioavailability of Griseofulvin-Loaded Liposomes. *Pharmaceutics*, *8*(3). https://doi.org/10.3390/pharmaceutics8030025

O'Shaughnessy, J. (2003). Liposomal anthracyclines for breast cancer: overview. *The Oncologist*, *8 Suppl 2*, 1–2. https://doi.org/10.1634/theoncologist.8-suppl_2-1

Paolino, D., Cosco, D., Cilurzo, F., Trapasso, E., Morittu, V. M., Celia, C., & Fresta, M. (2012). Improved in vitro and in vivo collagen biosynthesis by asiaticoside-loaded ultradeformable vesicles. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *162*(1), 143–151. https://doi.org/10.1016/j.jconrel.2012.05.050

Papahadjopoulos, D., Allen, T. M., Gabizon, A., Mayhew, E., Matthay, K., Huang, S. K., Lee, K. D., Woodle, M. C., Lasic, D. D., & Redemann, C. (1991). Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(24), 11460–11464. https://doi.org/10.1073/pnas.88.24.11460

Parr, M. J., Bally, M. B., & Cullis, P. R. (1993). The presence of GM1 in liposomes with entrapped doxorubicin does not prevent RES blockade. *Biochimica et Biophysica Acta*, *1168*(2), 249–252. https://doi.org/10.1016/0005-2760(93)90132-s

Parr, M. J., Masin, D., Cullis, P. R., & Bally, M. B. (1997). Accumulation of liposomal lipid and encapsulated doxorubicin in murine Lewis lung carcinoma: the lack of beneficial effects by coating liposomes with poly(ethylene glycol). *The Journal of Pharmacology and Experimental Therapeutics*, *280*(3), 1319–1327.

Pipicz, M., Demján, V., Sárközy, M., & Csont, T. (2018). Effects of Cardiovascular Risk Factors on Cardiac STAT3. *International Journal of Molecular Sciences*, *19*(11). https://doi.org/10.3390/ijms19113572

Rafiyath, S. M., Rasul, M., Lee, B., Wei, G., Lamba, G., & Liu, D. (2012). Comparison of safety and toxicity of liposomal doxorubicin vs. conventional anthracyclines: a meta-analysis. *Experimental Hematology & Oncology*, *1*(1), 10. https://doi.org/10.1186/2162-3619-1-10

Renu, K., V.G., A., Tirupathi, T. P., & Arunachalam, S. (2018). Molecular mechanism of doxorubicin-induced cardiomyopathy – An update. In *European Journal of Pharmacology* (Vol. 818). https://doi.org/10.1016/j.ejphar.2017.10.043

Rivankar, S. (2014a). An overview of doxorubicin formulations in cancer therapy. In *Journal of Cancer Research and Therapeutics* (Vol. 10, Issue 4). https://doi.org/10.4103/0973-1482.139267

Rivankar, S. (2014b). An overview of doxorubicin formulations in cancer therapy. *Journal of Cancer Research and Therapeutics*, *10*(4), 853–858. https://doi.org/10.4103/0973-1482.139267

Rivera, E. (2003). Liposomal anthracyclines in metastatic breast cancer: clinical update. *The Oncologist*, *8 Suppl 2*, 3–9. https://doi.org/10.1634/theoncologist.8-suppl_2-3

Rosati, M. S., Raimondi, C., Baciarello, G., Grassi, P., Giovannoni, S., Petrelli, E., Basile, M. L., Girolami, M., Di Seri, M., & Frati, L. (2011). Weekly combination of non-pegylated liposomal doxorubicin and taxane in first-line breast cancer: wALT trial (phase I-II). *Annals of Oncology : Official Journal of the European Society for Medical Oncology*, *22*(2), 315–320. https://doi.org/10.1093/annonc/mdq392

Rossi, C., Gasparini, G., Canobbio, L., Galligioni, E., Volpe, R., Candiani, E., Toffoli, G., & D'Incalci, M. (1987). Doxorubicin distribution in human breast cancer. *Cancer Treatment Reports*, *71*(12), 1221–1226.

Sahoo, S. K., & Labhasetwar, V. (2003). Nanotech approaches to drug delivery and imaging. *Drug Discovery Today*, *8*(24), 1112–1120. https://doi.org/10.1016/s1359-6446(03)02903-9

Sala, M., Miladi, K., Agusti, G., Elaissari, A., & Fessi, H. (2017). Preparation of liposomes: A comparative study between the double solvent displacement and the conventional ethanol injection—From laboratory scale to large scale. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *524*. https://doi.org/10.1016/j.colsurfa.2017.02.084

Saul, J. M., Annapragada, A., Natarajan, J. V, & Bellamkonda, R. V. (2003). Controlled targeting of liposomal doxorubicin via the folate receptor in vitro. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *92*(1–2), 49–67. https://doi.org/10.1016/s0168-3659(03)00295-5

Schnyder, A., & Huwyler, J. (2005). Drug transport to brain with targeted liposomes. *NeuroRx*, *2*(1). https://doi.org/10.1602/neurorx.2.1.99

Schubert, M. A., & Müller-Goymann, C. C. (2003). Solvent injection as a new approach for manufacturing lipid nanoparticles--evaluation of the method and process parameters. *European Journal of Pharmaceutics and Biopharmaceutics : Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V*, *55*(1), 125–131. https://doi.org/10.1016/s0939-6411(02)00130-3

Shao, K., Hou, Q., Duan, W., Go, M. L., Wong, K. P., & Li, Q.-T. (2006). Intracellular drug delivery by sulfatide-mediated liposomes to gliomas. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *115*(2), 150–157. https://doi.org/10.1016/j.jconrel.2006.07.024

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians*, *70*(1), 7–30. https://doi.org/10.3322/caac.21590

Singal, P. K., Deally, C. M., & Weinberg, L. E. (1987). Subcellular effects of adriamycin in the heart: a concise review. *Journal of Molecular and Cellular Cardiology*, *19*(8), 817–828. https://doi.org/10.1016/s0022-2828(87)80392-9

Sritharan, S., & Sivalingam, N. (2021). A comprehensive review on time-tested anticancer drug doxorubicin. *Life Sciences*, *278*, 119527. https://doi.org/10.1016/j.lfs.2021.119527

Stewart, D. J., Grewaal, D., Green, R. M., Mikhael, N., Goel, R., Montpetit, V. A., & Redmond, M. D. (1993). Concentrations of doxorubicin and its metabolites in human autopsy heart and other tissues. *Anticancer Research*, *13*(6A), 1945–1952.

Swain, S. M., Whaley, F. S., & Ewer, M. S. (2003). Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer*, *97*(11), 2869–2879. https://doi.org/10.1002/cncr.11407

Symon, Z., Peyser, A., Tzemach, D., Lyass, O., Sucher, E., Shezen, E., & Gabizon, A. (1999). Selective delivery of doxorubicin to patients with breast carcinoma metastases by stealth liposomes. *Cancer*, *86*(1), 72–78.

Takemura, G., & Fujiwara, H. (2007). Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management. *Progress in Cardiovascular Diseases*, *49*(5), 330–352. https://doi.org/10.1016/j.pcad.2006.10.002

Thorn, C. F., Oshiro, C., Marsh, S., Hernandez-Boussard, T., McLeod, H., Klein, T. E., & Altman, R. B. (2011). Doxorubicin pathways: pharmacodynamics and adverse effects. *Pharmacogenetics and Genomics*, *21*(7), 440–446. https://doi.org/10.1097/FPC.0b013e32833ffb56

Twelves, C. J., Dobbs, N. A., Aldhous, M., Harper, P. G., Rubens, R. D., & Richards, M. A. (1991). Comparative pharmacokinetics of doxorubicin given by three different schedules with equal dose intensity in patients with breast cancer. *Cancer Chemotherapy and Pharmacology*, *28*(4), 302–307. https://doi.org/10.1007/BF00685539

Utsugi, T., Nii, A., Fan, D., Pak, C. C., Denkins, Y., van Hoogevest, P., & Fidler, I. J. (1991). Comparative efficacy of liposomes containing synthetic bacterial cell wall analogues for

tumoricidal activation of monocytes and macrophages. *Cancer Immunology, Immunotherapy : CII*, *33*(5), 285–292. https://doi.org/10.1007/BF01756592

van Hoogevest, P., & Wendel, A. (2014). The use of natural and synthetic phospholipids as pharmaceutical excipients. *European Journal of Lipid Science and Technology : EJLST*, *116*(9), 1088–1107. https://doi.org/10.1002/ejlt.201400219

Wang, G., Li, R., Parseh, B., & Du, G. (2021). Prospects and challenges of anticancer agents' delivery via chitosan-based drug carriers to combat breast cancer: a review. *Carbohydrate Polymers*, *268*, 118192. https://doi.org/10.1016/j.carbpol.2021.118192

Wang, N., Wang, T., Li, T., & Deng, Y. (2009). Modulation of the physicochemical state of interior agents to prepare controlled release liposomes. *Colloids and Surfaces. B, Biointerfaces*, *69*(2), 232–238. https://doi.org/10.1016/j.colsurfb.2008.11.033

Wang, X., Yan, J., Shen, B., & Wei, G. (2021). Integrated Chromatin Accessibility and Transcriptome Landscapes of Doxorubicin-Resistant Breast Cancer Cells. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.708066

Waterhouse, D. N., Tardi, P. G., Mayer, L. D., & Bally, M. B. (2001). A comparison of liposomal formulations of doxorubicin with drug administered in free form: changing toxicity profiles. *Drug Safety*, *24*(12), 903–920. https://doi.org/10.2165/00002018-200124120-00004

Working, P. K., & Dayan, A. D. (1996). Pharmacological-toxicological expert report. CAELYX. (Stealth liposomal doxorubicin HCl). *Human & Experimental Toxicology*, *15*(9), 751–785.

Wu, X., Dai, X., Liao, Y., Sheng, M., & Shi, X. (2021). Investigation on drug entrapment location in liposomes and transfersomes based on molecular dynamics simulation. *Journal of Molecular Modeling*, *27*(4), 111. https://doi.org/10.1007/s00894-021-04722-3

Ye, Q., Asherman, J., Stevenson, M., Brownson, E., & Katre, N. V. (2000). DepoFoam technology: a vehicle for controlled delivery of protein and peptide drugs. *Journal of Controlled Release :*

*Official Journal of the Controlled Release Society*, *64*(1–3), 155–166. https://doi.org/10.1016/s0168-3659(99)00146-7

Yin, W., Wang, J., Jiang, L., & James Kang, Y. (2021). Cancer and stem cells. *Experimental Biology and Medicine (Maywood, N.J.)*, *246*(16), 1791–1801. https://doi.org/10.1177/15353702211005390

Zeng, H., Qi, Y., Zhang, Z., Liu, C., Peng, W., & Zhang, Y. (2021). Nanomaterials toward the treatment of Alzheimer's disease: Recent advances and future trends. *Chinese Chemical Letters*, *32*(6). https://doi.org/10.1016/j.cclet.2021.01.014