1. INTRODUCTION

Cancer is a group of diseases involving continual unregulated cell growth with the potential to invade or spread to other parts of the body through metastasis(1). Cancer is one of the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases. Cancer is the second leading cause of death globally, nearly 1 in 6 deaths is due to cancer(2). Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements. Staging is a way of describing the size of a cancer and how far it has grown. Most types of cancer have 4 stages, numbered from 1 to 4(3). Stage 1 usually means that a cancer is relatively small and contained within the organ it started in. Stage 2 usually means that the tumour is larger than in stage 1, but the cancer has not started to spread into the surrounding tissues. Sometimes stage 2 means that cancer cells have spread into lymph nodes close to the tumour. This depends on the particular type of cancer. Stage 3 usually means the cancer is larger. It may have started to spread into surrounding tissues and there are cancer cells in the lymph nodes in the area. Stage 4 means the cancer has spread from where it started to another body organ. This is also called secondary or metastatic cancer (4).

1.1 INTRODUCTION TO LUNG CANCER AND KEY STATISTICS

Lung cancer is the second most common cancer in both men and women. In men, prostate cancer is more common, while in women breast cancer is more common. About 14% of all new cancers are lung cancers and the most common cause of death from cancer with 1.38 million deaths (18.2 % of total). Lung cancer is currently treated with IV administration of chemotherapeutic agents along with radiation and surgery but they are nonselective as it cannot differentiate between host cell and cancer cell leading to normal cell toxicity. Moreover, the diagnostic tools available to detect tumors are inadequate which makes the condition miserable. However, the emergence of resistance to the administered medication and chances of tumor developing refractoriness is a prominent issue. It offers stimulus to pursue research for effectively treating multi drug resistant (MDR) lung cancer (5).

Lung cancer is a malignant lung tumor characterized by uncontrolled cell growth in tissues of the lung. It starts when cells of the lung become abnormal and

begin to grow out of control. As more cancer cells develop, they can form into a tumor and spread to other areas of the body. Most cancers that start in the lung, known as primary lung cancers, are carcinomas (4). There are 2 main types of lung cancer. About 85% to 90% of lung cancers are non-small cell lung cancer (NSCLC) and about 10% to 15% are small cell lung cancer (SCLC).The American Cancer Society's estimates for lung cancer in the United States for 2019 are:

- About 228,150 new cases of lung cancer (116,440 in men and 111,710 in women)
- About 142,670 deaths from lung cancer (76,650 in men and 66,020 in women)

Lung cancer is by far the leading cause of cancer death among both men and women; about 1 out of 4 cancer deaths are from lung cancer. Each year, more people die of lung cancer than of colon, breast, and prostate cancers combined. Lung cancer mainly occurs in older people. About 2 out of 3 people diagnosed with lung cancer are 65 or older, while less than 2% are younger than 45. The average age at the time of diagnosis is about 70 (6). As 80-90 % of lung cancers are NSCLC, it is picked for effectively targeting with special emphasis on multi drug resistance (MDR) in NSCLC.

1.2 DRUG RESISTANCE IN CANCER

Chemotherapy combined with radiotherapy and surgical resection is the current standard approach to cancer treatment. A major obstacle to successfully treating malignant diseases is the emergence of multidrug resistance (MDR) to chemotherapy, whereby cancer cells become resistant to the cytotoxic effects of various structurally and mechanistically unrelated chemotherapeutic agents. This phenomenon contributes to treatment failure in over 90% of patients with metastatic disease. Tumor MDR can be intrinsic or acquired through exposure to chemotherapeutic agents. The selection pressures within a tumor microenvironment and inherent high expression of the ATP-binding cassette (ABC) transporters by tumor cells may result in the development of intrinsic MDR. An acquired resistance in cancers may come from a drug stimulus, which leads to the overexpression of ABC transporters and subsequent efflux of anticancer drugs from the cancer cell cytoplasm (7).

The most extensively mechanism of MDR is the overexpression of cell surface pumps, such as the ATP-binding cassette superfamily. These pumps can successfully purge a wide spectrum of chemotherapeutic agents from cells, thereby decreasing their intracellular accumulation. However, the ABC superfamily is only one of several modalities that cause MDR (7). The major mechanisms of tumor drug resistance can be grouped into at least five categories: decreased drug influx, increased drug efflux, DNA repair activation, detoxification, and inactivation of apoptosis pathways with simultaneous activation of anti-apoptotic cellular defense modalities. The MDR phenotype is usually the synergistic result of a combination of MDR mechanisms. Thus, inhibiting only one contributor to cellular resistance is usually insufficient to overcome all mechanisms of cancer-cell resistance to chemotherapy. Therefore, designing an advanced multifunctional delivery system should be a priority to reverse MDR in cancer chemotherapy. Thus MDR can be treated by gene knock down approach to inhibit expression of these ABC family proteins which are responsible for efflux of oncological therapeutics and reduced therapeutic action. RNA interference, P-gp inhibitors and few peptides are extensively used approaches and gene silencing through RNA interference technology is most impactful tool now-a-days amongst all approaches.

Nanotechnology provides an innovative and promising alternative to conventional small molecule chemotherapeutics, circumventing MDR by encapsulating, attaching, and conjugating drugs or therapeutic biological products to nanocarriers. Nanocarriers can include small molecules such as lipids or polymer nanoparticles that target the therapeutic payload to tumors or tumor cells. Simultaneously, multifunctional drug-loaded nanocarriers can also enhance particle penetration of physiological barriers and protect the labile drugs or therapeutic biological products(8).

1.3 GENE DELIVERY VECTORS

Ideally, gene delivery methods should have specificity for the target diseased cell, should protect the transgene against metabolic degradation by nucleases in intercellular matrices and against immune attack, should transfer the transgene across the plasma membrane and into the nucleus of target cells for appropriately regulated transgene expression and desired safety profile with minimum side effects. In general

terms, gene delivery methods for optimal delivery and expression of exogenous DNA to the desired mammalian cells in vitro and in vivo are subdivided into two categories: (1) the use of biological vectors and (2) techniques employing either chemical or physical approaches. The biological methods utilize viral and bacterial vectors and are the method of choice due to their high transfection efficiency. These vectors target to the cell receptor and the biological entry mechanism for achieving the cell transfection. Retroviruses and adenoviruses are the most commonly used vectors under clinical trials. Some bacterial vectors are delivered through the gastrointestinal (GI) tract by transfecting the cells at mucosal surfaces. However, difficulties with viral vectors in formulation and side effects like immunogenicity, allergic reactions, host rejection, mutagenicity, and oncogenicity restrict them to use in clinical practice. Thus, long-term safety studies of these vectors for each application are necessary before these products can be successfully marketed. Manufacturing quality controls, such as stringent quality control standards, higher price, and proper cold storage conditions are impediments in the use of biological vectors for routine clinical use(9).

The non-viral vectors, that is, the non-biological techniques of gene delivery, involve treatment of cells by chemical and physical means. The chemical methods are comprised of DNA delivery using various chemical agents such as cationic lipids and polymers. Moreover, these agents can be modified to improve cell targeting and nuclear localization. Chemical methods can reliably and reproducibly transfect the mammalian cell lines in vitro, but for systemic administration the transfection becomes more challenging because of the extracellular and intracellular gene delivery barriers as well as the need for a large volume of tissue to be transfected for clinically beneficial therapy. Chemical vectors are easy to scale up and reproduce with minimum host immune interaction, and are suitable for selected organs such as the lung or airway with mucosal tissue as the target site, or for fairly localized tissues, such as intra tumoral inoculation; however, the transfection efficiency is compromised compared to viral vectors. Vectors for gene therapy should have following properties:

- The vector must condense or encapsulate DNA into a smaller size able to enter into the cell.
- The vector must be capable of protecting DNA from the external environment.

- The vector should maintain integrity and stability after *in vivo administration.*
- The vector must possess some functional moieties to facilitate its escape from the endosomes into the cytoplasm.
- The vector should release DNA from the complex inside the cell in its active form.
- The vector should have some targeting moiety to direct the delivery to a particular type of cell for gene transfection and nuclear localization.

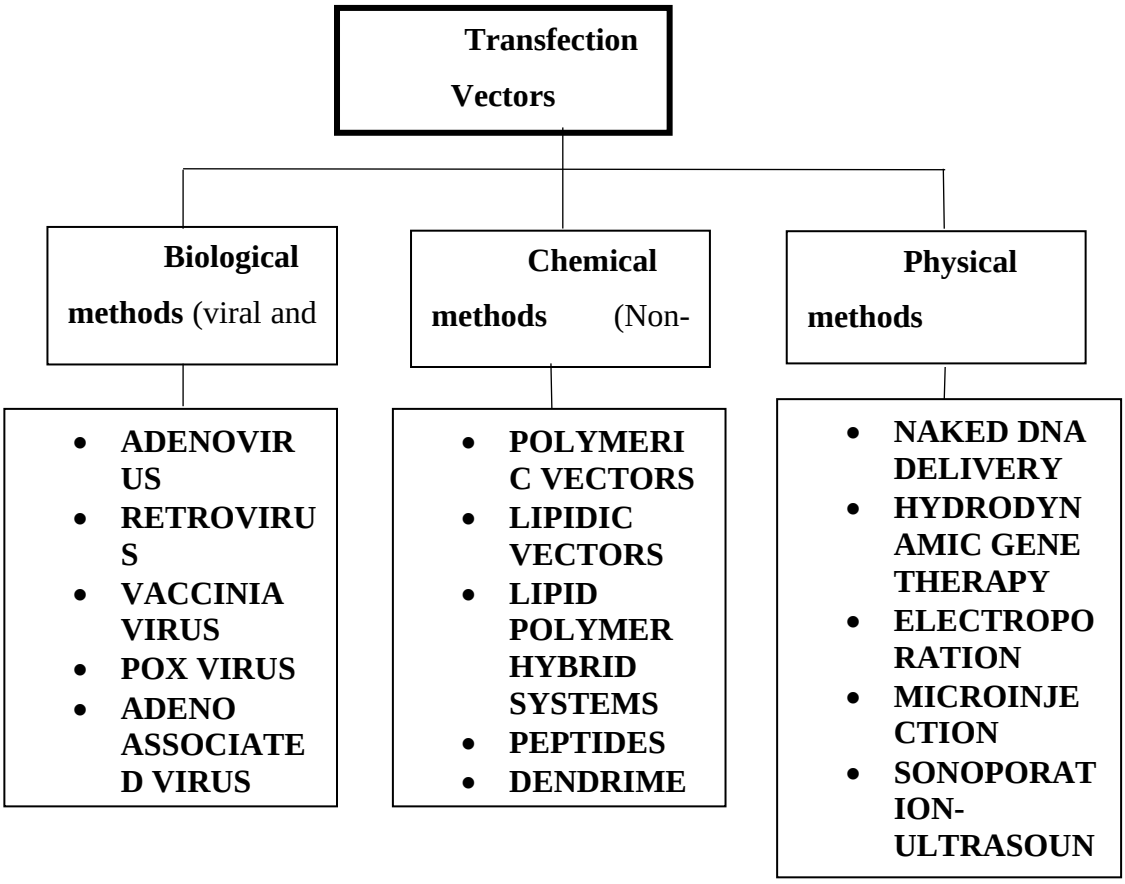

Figure 1-1Classification of transfection vectors

1.4 CHEMOTHERAPEUTICS AND COMBINATION THERAPY IN LUNG CANCER

Non-small cell lung cancer is often treated with Cisplatin or carboplatin, in combination with gemcitabine, paclitaxel, Docetaxel, Etoposide or Vinorelbine. In small cell lung cancer, Cisplatin and etoposide are most commonly used. Combinations with carboplatin, gemcitabine, paclitaxel, Vinorelbine, topotecan and Irinotecan are also used. In extensive-stage small cell lung celecoxib may be

combined with etoposide, this combination shoed improve outcomes.Radiotherapy is often together with chemotherapy, and may be used with curative intent in patients with non-small cell lung cancer who are not eligible for surgery. For both non-small cell lung cancer and small cell lung cancer patients, smaller doses of radiation to the chest may be used for symptoms control (palliative radiotherapy).In recent year, various molecular targeted therapies have been developed for the treatment of advanced lung cancer (10). Currently campothecin, carboplatin, gemcitabine, paclitaxel, Docetaxel, topotecan are most widely used anticancer agents in treatment of lung cancer with their known reported toxicities. The medications are available as injections for systemic use results in hazardous side effects due to their non-specificity on the dividing cells in the body (11).

Chemotherapy, which is thought to be powerful strategy for cancer treatment,has been widely used in clinical therapy in recent years. However, due to thecomplexity of cancer, a single therapeutic approach is insufficient for the suppressionof cancer growth and migration. Therefore, there has been increasing attention to theuse of smart multifunctional carriers to combinatorial deliver chemotherapeutic drugsand functional genes to maximize therapeutic efficiency. Combination therapy usingselected drugs and genes can not only overcome multidrug-resistance and inhibit thecellular anti-apoptotic process but also can achieve synergistic therapeutic effect.Because multifunctional nanocarriers are important for achieving these goals, thisreview will illustrate and discuss some advanced biomaterial nanocarriers forco-delivering therapeutic genes and drugs including multifunctional micelles,liposomes, polymeric conjugates and inorganic Nanoparticles.

1.5 TARGETED DELIVERY OF SIRNA THERAPEUTICS IN LUNG CANCER

Recent research on targeted drug nanoparticles,liposomes, micellarformulations encapsulating these anticancer drugs after attaching with cancer cell over expressed receptors specific ligandis gaining high impetus owing to its very high selectivity and sensitivity towards cancer cells. Use of apoptotic genes like p53, mdm inhibitor genes and the siRNA is also a topic of current research and yielding good outcomes. However the realities of marketing these targeted products is still a mile away. The recent success of CFTR gene delivery using liposomes has been a

great impetus to the nanocarriers based gene delivery and it further improves the chances for viral and non-viral p53 gene delivery entering into the market.

1.5.1 Gene targeting:

The following genes are mainly responsible for lung cancer that may be targeted through RNA interference technology via gene knock-down approach or plasmid (p53 DNA) gene insertion approach.

ABCC3 gene (MRP3)

Recently role of ABCC3 as a marker for multidrug resistance and highly upregulated in resistant groups has been identified. (12)ABCC3 is a member of ATP binding cassette (ABC) transporter family. They are highly expressed in tumor cells where they actively efflux a broad spectrum of anti-cancer drugs and thus contribute to MDR. Targeting siRNA for silencing gene imparting multidrug resistance (MRP3 /ABCC3) can impart synergistic therapeutic activity by decreasing the resistance of cell to chemotherapeutic agents Cisplatin, etoposide, Docetaxel. However, these agents have limited use in patients who have relapsed and have metastatic disease. Therefore, novel strategies are required to improve the clinical outcome.

KRAS gene

Three different human RAS genes have been identified: KRAS (homologous to the oncogene from the Kirsten rat sarcoma virus), HRAS (homologous to the oncogene from the Harvey rat sarcoma virus), and NRAS (first isolated from a human neuroblastoma). RAS has been implicated in the pathogenesis of several cancers. Activating mutations within the RAS gene result in constitutive activation of the RAS GTPase, even in the absence of growth factor signaling. The result is a sustained proliferation signal within the cell. Specific RAS genes are recurrently mutated in different malignancies. KRAS mutations are particularly common in colon cancer, lung cancer, and pancreatic cancer(9).

EGFR gene

Epidermal growth factor receptor (EGFR) belongs to a family of receptor tyrosine kinases (RTKs) that include EGFR/ERBB1, HER2/ERBB2/NEU, HER3/ERBB3, and HER4/ERBB4. The binding of ligands, such as epidermal growth factor (EGF), induces a conformational change that facilitates receptor homo- or heterodimer formation, thereby resulting in activation of EGFR tyrosine kinase

activity. Activated EGFR then phosphorylates its substrates, resulting in activation of multiple downstream pathways within the cell, including the PI3K-AKT-mTOR pathway, which is involved in cell survival, and the RAS-RAF-MEK-ERK pathway, which is involved in cell proliferation. Activation of the epidermal growth factor receptor (EGFR) protein stimulates protein tyrosine kinase, which leads to activation of signaling pathways associated with cell growth and survival. Both EGFR overexpression and activating mutations in the tyrosine kinase domain of the EGFR gene lead to tumor growth and progression (12).

p53

p53 Is a "tumor suppressor" protein that is critical to maintaining the integrity of the genome as cells traverse the cell cycle. The majority of p53 mutations results in the loss of its capacity for DNA binding, and as a result, these mutations disable the protein's ability to regulate transcription. The p53 protein monitors cellular stress and DNA damage, either causing growth arrest to facilitate DNA repair or inducing apoptosis if DNA damage is extensive. When a cell is stressed by oncogene activation, hypoxia, or DNA damage, an intact p53 pathway may determine whether the cell will receive a signal to arrest at the G1 stage of the cell cycle, whether DNA repair will be attempted, or whether the cell will self-destruct via apoptosis (programmed cell death). Apoptosis plays a key role in numerous normal cellular mechanisms, from embryogenesis to destruction of irreparable DNA damage due to random mutations, ionizing radiation, and DNA damaging chemicals including chemotherapeutic agents. The observation that expression of a wild-type *p53* gene in a cancer cell triggers apoptosis provided the rationale for gene therapy approaches .

VEGF2/integrin

VEGF is a homodimeric heparin-binding glycoprotein. The VEGF family of angiogenic factors includes six secreted glycoproteins based on the number of amino acids (2). VEGF, unlike other angiogenic factors, is selectively mitogenic for endothelial cells and increases vessel permeability. Some VEGF family members are involved in lymphangiogenesis (VEGF-C and -D). VEGF expression can be seen in the vast majority of tumors and is usually elevated above normal tissue levels. As tumors grow, the size outstrips the blood supply, and there is impaired supply of oxygen and nutrients, with resultant accumulation of metabolites within the tumor.

This is accompanied by changes in the tumor microenvironment, for example, hypoxia, hypoglycemia, and acidosis, which stimulate the upregulation and release of hypoxia-inducible factor-1α (HIF), VEGF, and other angiogenic factors by the tumor cells (13).

The non-viral vector with siRNA can be constructed that is PEGylated with an Arg-Gly-Asp (RGD) peptide ligand attached at the distal end of the polyethylene glycol (PEG), as a means to target tumor neo-vasculature expressing integrins and used to deliver siRNA inhibiting vascular endothelial growth factor receptor-2 (VEGF R2) expression and thereby tumor angiogenesis (14).

1.6 AIM

The aim of present work is to evaluate effectiveness of silencing a multi drug resistant gene ABCC3 that confers resistance to chemotherapeutic agents in treatment of non-small cell lung cancer (NSCLC) by formulating combinatorial hybrid nanocarriers (HNCs) as a novel non-viral vector comprising of lipids and polymers and silencing RNA.

1.7 OBJECTIVE OF PROPOSED WORK

- The current project aims to develop a novel combinatorial approach for treatment of lung cancer combining the use of chemotherapeutic agents cisplatin and silencing RNA (siRNA) for combating multi drug resistance type NSCLC
- To evaluate the feasibility of different methods of production Hybrid nanocarriers (HNCs) found in literature and find out the best method of formulation which yields nanocarriers of uniform lipid coating, desired surface zeta potential , uniform and controllable particle size, low polydispersity index, high drug loading and entrapment efficacy of cisplatin
- To silent multi drug resistant gene ABCC3 (MRP3) through siRNA (RNA interference technology) can impart synergistic therapeutic activity by decreasing the resistance of cell to chemotherapeutic agent such as cisplatin
- To achieve sufficient pulmonary drug concentration by Dry powder Inhaler of the prepared HNCs
- Development of folate receptor ligand conjugated non-viral vectors HLPs for targeting to cancer cells

- To compare combinatorial nanocarriers containing cisplatin and ABCC3 siRNA (HNCs Aqueous dispersion for IV route and DPI for pulmonary route) their performance through in vitro and in vivo evaluation using appropriate cell lines and animal models, respectively, by administering them through IV and pulmonaryroute.

1.8 RATIONALE

Use of nanocarriers has by far been evaluated in a number of ways to battle the unending necessity of availing an efficacious treatment alternative to the current chemotherapeutic agents. These carrier systems having the properties like low-toxicity, biocompatibility, ability to load range of therapeutic molecules, increasing selectivity to cancerous cells by conjugation with targeting ligand and selective extravasation in tumor cells due to size characteristics are gaining increasing importance in the medical sciences. Two of the major classes of nano drug delivery systems that has largely captured the area of investigation with few of the blockbuster products in market belong to class of liposomes and nanoparticles. Till date they are used for carrying only drug molecules, however they are also being evaluated for delivering gene/siRNA therapeutics as well. On one hand liposomal drug delivery provide superior pharmacokinetic profiles, higher drug delivery efficiency and ease of surface modification, while nanoparticulate drug delivery provide high drug encapsulation efficiency, sustained release profiles and higher cellular internalization potential. A prospective formulation approach to put together these properties to design a combined delivery system has been sought wherein some of disadvantages like limited drug loading, instability on shelf storage would be avoided.

The development of drug resistance marked by a decreased effectiveness of the drug on the cancer cells is a major hurdle in effective cancer therapy(8). Cancerous cells due to its dynamic cellular machinery has the capability to adapt, maneuver, survive and to continue proliferation if the regimen and course of treatment has not been designed efficiently to eradicate the last of the surviving cancerous cell mass. An in-depth understanding of complex signalling pathway and a heterogeneous micro-environment that tumor cells display is thus required for effective therapy. The use of a single agent possesses significant risk of tumor turning refractory and failure of chemotherapy. Such resistance may sometimes grow to an extent that some tumors becomes resistant to not only single drug but multiple drugs(15). Two of the major

determinants limiting the therapeutic potential of anticancer agents are presence of drug efflux pumps and activation of anti-apoptotic pathway in the cancer cells. Several studies have been conducted during the last decades to enhance the efficacy of chemotherapy by suppressing multi drug resistance (MDR) mechanisms including the use of new anticancer drugs that could escape from the efflux mechanism, MDR modulators or chemosensitizers, multifunctional nanocarriers, and RNA interference (RNAi) therapy.MRP2 has entirely different expression pattern in the apical plasma membrane of hepatocytes, the brush-border membrane of renal proximal tubules and small intestine, where it is situated to play an essential role in the elimination and bioavailability of a wide variety of drugs including endogenous glucuronides, sulphates and GSH conjugates from the cells. It was suggested that use of a combination of agents (co-treatment) that act on cancer cells via different pathway would be more effective in decreasing the chances of resistance and improve therapeutic outcome.Further, to this co-delivery of drugs (simultaneous delivery of two drugs or gene and drug in a single carrier system) is being investigated for the plausible advantage of maximizing delivery cum therapeutic aspects. A combination of therapeutic approach involving chemotherapy and gene/siRNA therapy may prove fruitful in comparison to their individual application in the treatment of MDR-type cancers. This approach is also referred to as chemo-sensitization, wherein the efficiency of therapeutic agent is improved by knock down of multi drug resistant gene by the siRNA. Herein, delivery using nanocarriers also protects gene/siRNA from catalytic/enzyme-based degradation in the blood stream and prevents their rapid clearance leading to longer half-life in circulation.

An important aspect of the higher efficacy of the nanocarriers is their ability to selectively accumulate at the tumor site due to EPR effect which is also pertinent to their escape from detection by the immune system due to their small size (typically <200 nm)(16). However, this also leads to their accumulation in several non-target metabolizing organs leading to severe chronic or acute generalized toxicity. To increase the specificity of the systems towards cancer cells and minimize off targeting effects, targeting approaches are used. An active targeting approach make use of specifically targeting receptors that are overexpressed on the tumor cells by surface conjugation of nanocarrier surface with ligand molecules of desired choice.

Additionally, Cisplatin is given through Intravenous route and the various drawbacks of conventional cisplatin injection include development of resistance, only 10 % dose reaches at the site of action, off targeted effects due to lesser tumor targeting and tissue damage and Nephrotoxicity, hepatotoxicity, myelo-suppression.Therefore,formulating Dry Powder Inhaler of HNCs of Cisplatin would have the following advantages like higher lung tissue concentration due to DPI, structural integrity and high stability through polymeric core, biocompatibility and high internalization due to lipid shell, high drug loading and sustained release etc.This will reduce the exposure of other cells to gene & chemotherapeutic agent.

The reaction of Cisplatin's hydrolysed species formed at acidic pH with negatively charged lipids has been used here to form ionic complex of Cisplatin with negatively charged lipids i.e. caprylic acid and stearic acid (all lipids used as sodium salts); that will make the neutral complex with Cisplatin and due to lipophilic carbon chain of these lipids will enhance the interaction of complex with lipophilic compartment of lipid-bilayer of liposome causing the complex to be inserted into the lipid bilayer and thus enhancing entrapment efficiency of Cisplatin in liposomes. It will reduce toxicity and protein binding and enhance drug targeting to tumor sites.

1.9 HYPOTHESIS

It is hypothesized that masking drug resistance will result in increased therapeutic index of chemotherapeutic agent through maximization of efficacy of drug, reduction in dose and possibility of prolongation of therapy due to reduced toxicity. This would result in a complete remission of the disease and in a reduced possibility of metastasis, with consequent increased survival rate of patients. The current formulated combinatorial HNCs comprising cisplatin caprylate and ABCC3 siRNA will exert its effect by silencing multi drug resistant gene ABCC3(MRP3) through siRNA (RNA interference technology) to enhance efficiency of chemotherapy via chemo-sensitization by decreasing the resistance of cancer cells.

1.10 RESEARCH PLAN

In this context, we have proposed silencing multi drug resistant gene ABCC3(MRP3) through siRNA (RNA interference technology) to enhance efficiency of chemotherapy by decreasing the resistance of cancer cells.Among the co-delivery

model, cisplatin is selected as it shows non-cycle specific antitumor effects. As cisplatin display low loading efficiency, its caprylate conjugates are prepared to improve loading. A simple thin film hydration method was employed to obtain hybrid nanocarriers (HNCs) with desired physicochemical and pharmaceutical properties. We have used PEG-PLA block copolymer which serves multiple purpose, first as an amphiphilic block to entrap drug in its matrix and secondly it serves as a bilayer component of the formed HNCs to provide structural rigidity. DOTAP was used as cationic lipid and to also serve for complex formation with siRNA. DSPE-PEG folatewas used for PEGylation of HNCs to provide stealth nature and serves a ligandas well to target folate overexpressed cancerous cell types.

- Literature review covering various aspects of cancer, Cisplatin, Lipid Polymer Hybrid Nanoparticle delivery System, Dry Powder Inhalers.
- Procurement of Drug (Docetaxel) and excipients (PEG-PLA, DPPC, DOTAP, DOPE, DSPE-PEG 2000 folate and siRNA etc.)
- Preformulation study including DSC and FTIR analysis.
- Analytical Method development of Cisplatin.
- Preparation of Cisplatincapryalte HNCs by various methods available in literature
- Optimization of Cisplatin caprylate HNCs by Quality by design – design of experiment (QbD-DoE) approach through Box-behnken design Formulation of cisplatin caprylate entrapped HNCs loaded with siRNA by optimizing N/P ratio
- Characterization of optimized formulation by size, surface potential, SEM, TEM and AFM and SAXS analysis.
- In vitro Drug r**e**lease study of HNCs
- Formulation and optimization of Dry Powder Inhaler of Optimized HNCs by lyophilisation technique.
- Solid State Characterization and Powder Performance study of DPI
- Stability study of final DPI Formulation.
- *In-vitro* cytotoxicity study by MTT assay using suitable lung cancer cell line and cellular uptake studies of formulation on lung adenocarcinoma A549 and H1299 cell lines through Flow cytometry and confocal microscopy

- Evaluation of *In-vivo* efficacy of the formulation in suitable animal model.

1.11 REFERENCES

1. Cooper GM, Hausman R. A molecular approach. The Cell 2nd ed Sunderland, MA: Sinauer Associates. 2000.
2. Musumeci T, Ventura CA, Giannone I, Ruozi B, Montenegro L, Pignatello R, et al. PLA/PLGA nanoparticles for sustained release of docetaxel. International journal of pharmaceutics. 2006;325(1-2):172-9.
3. Lan P, Jia L. Thermal Properties of Copoly (L-lactic acid/glycolic acid) by Direct Melt Polycondensation. Journal of Macromolecular Science, Part A: Pure and Applied Chemistry. 2006;43(11):1887-94.
4. . Available from: http://www.cancerresearchuk.org/about-cancer/what-is-cancer/stages-of-cancer.
5. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2018. CA: a cancer journal for clinicians. 2018;59(4):225-49.
6. .Available from: http://lungcancer.org
7. Gao Z, Zhang L, Sun Y. Nanotechnology applied to overcome tumor drug resistance. Journal of controlled release. 2012;162(1):45-55.
8. Peters GJ. Cancer drug resistance: a new perspective. Cancer Drug Resist. 2018;1:1-5.
9. Misra A. Challenges in delivery of therapeutic genomics and proteomics: Elsevier; 2010.
10. Moore MA, Ariyaratne Y, Badar F, Bhurgri Y, Datta K, Mathew A, et al. Cancer epidemiology in South Asia-past, present and future. Asian Pac J Cancer Prev. 2010;11(Suppl 2):49-66.
11. Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. Harrison's principles of internal medicine: McGraw-Hill Professional Publishing; 2015.
12. Albeda SM. Gene therapy in lung disease: CRC Press; 2002.
13. Gautschi O, Mack PC, Heighway J, Gumerlock PH, Gandara DR. Molecular Biology of Lung Cancer as the Basis for Targeted Therapy. Lung Cancer: CRC Press; 2016. p. 11-34.
14. Schiffelers RM, Ansari A, Xu J, Zhou Q, Tang Q, Storm G, et al. Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. Nucleic acids research. 2004;32(19):e149-e.
15. Raghunand N, He X, Van Sluis R, Mahoney B, Baggett B, Taylor C, et al. Enhancement of chemotherapy by manipulation of tumour pH. British journal of cancer. 1999;80(7):1005.
16. Koziara JM, Whisman TR, Tseng MT, Mumper RJ. In-vivo efficacy of novel paclitaxel nanoparticles in paclitaxel-resistant human colorectal tumors. Journal of Controlled Release. 2006;112(3):312-9.