2.LITERATURE REVIEW

2.1 LUNG CANCER AND ITS TYPES

Lung cancer (also known as carcinoma of the lung) is a disease characterized by uncontrolled cell growth in tissues of the lung. If left untreated, this growth can spread beyond the lung by process of metastasis into nearby tissue or other parts of the body. Most cancers that start in the lung, known as primary lung cancers, are carcinomas that derive from epithelial cells. The main types of lung cancer are small-cell lung carcinoma (SCLC), also called oat cell cancer and non-small-cell lung carcinoma (NSCLC). The most common symptoms are coughing (including coughing up blood), weight loss, and shortness of breath and chest pain. The majority of lung cancers are classified as either non-small cell lung cancer or small cell lung cancer, which get their names because of the appearance of the cancer cells under a microscope. Non-small cell lung cancer accounts for 80% of lung cancers. There are 3 types of non-small cell lung cancer as described below. Further, cancer can be classified in various stages based on tumor size and location. In Stage I, tumor size is less than 5 cm in localized area with no spreading to lymph nodes. Stage II tumor size is 5-7 cm with spreading to nearby lymph nodes. Stage III cancer is known as locally advanced having tumor size greater than 7 cm with spreading to a major structure within the chest. Stage IV is a metastatic cancer in which tumors have spread to another part of body(1).

Adenocarcinoma of the lung

Up to 50% of non-small cell lung cancers are considered adenocarcinomas. This type of lung cancer is often seen in non-smokers and is the lung cancer type most commonly found in women. Non-small cell lung cancer usually begins in the periphery (outer parts) of the lungs, and it can be present for a long time before it is diagnosed. One form of adenocarcinoma, BAC (bronchioloalveolar carcinoma), appears to be increasing worldwide.BAC is a lung cancer that arises in the small air sacs of the lungs, and it is more likely to affect non-smokers, women, and Asians (2).

Squamous cell carcinoma (Epidermoid carcinoma)

Thirty percent of non-small cell lung cancers are squamous cell carcinomas. This lung cancer type usually starts in the bronchial tubes in the central part of the lungs and can

cause symptoms early on, especially hemoptysis (coughing up blood). Squamous cell carcinoma used to be the most common form of lung cancer, but its incidence appears to have decreased since filtered cigarettes became available and smoke is inhaled more deeply into the lungs (the region where adenocarcinoma begins).

Large cell carcinoma

Large cell carcinoma is the least common form of non-small cell lung cancer, responsible for about 10% of cases. It is named for the appearance of large round cells when examined under the microscope. Large cell carcinoma often occurs in the outer regions of the lungs and tends to grow rapidly (2).

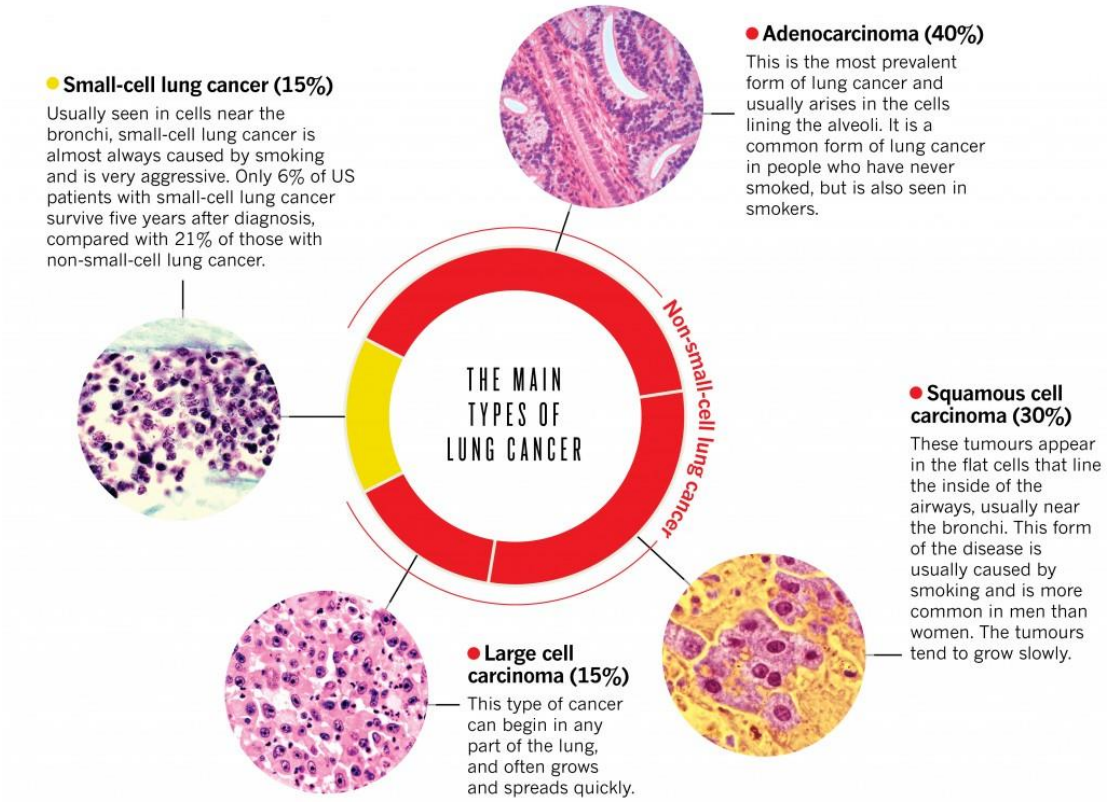


Figure 2-1Lung cancer types and histology

(Ref http://blogs.nature.com/ofschemesandmemes/2014/09/11/the-dominant-malignancy-lung-cancer)

Small-cell lung carcinoma

Small-cell lung carcinoma (SCLC) is less common. It was formerly referred to as "oatcell" carcinoma. Most cases arise in the larger airways (primary and secondary bronchi) and grow rapidly, becoming quite large. The small cells contain dense neurosecretory granules (vesicles containing neuroendocrine hormones), which give

this tumor an endocrine/paraneoplastic syndrome association. While initially more sensitive to chemotherapy and radiation, it is often metastatic at presentation and ultimately carries a worse prognosis. Small-cell lung cancers have long been dichotomously staged into limited and extensive stage disease. This type of lung cancer is strongly associated with smoking (3).

2.1.1 Risk factors

A risk factor is anything that affects a person's chance of getting a disease such as cancer. Different cancers have different risk factors. Some risk factors, like smoking, can be changed. Others, like a person's age or family history, can't be changed. But having a risk factor, or even several, does not mean that you will get the disease. And some people who get the disease may have few or no known risk factors. Several risk factors can make you more likely to develop lung cancer.

- **Risk factors you can change**

- Tobacco smoke
- Secondhand smoke
- Exposure to radon
- Exposure to asbestos
- Exposure to other cancer-causing agents in the workplace: Other carcinogens (cancer-causing agents) found in some workplaces that can increase lung cancer risk include:

- Radioactive ores such as uranium
- Inhaled chemicals such as arsenic, beryllium, cadmium, silica, vinyl chloride, nickel

Compounds, chromium compounds, coal products, mustard gas, and chloromethyl ethers

- Diesel exhaust
- Arsenic in drinking water

- **Risk factors you cannot change**

- Previous radiation therapy to the lungs
- Air pollution
- Personal or family history of lung cancer

If you have had lung cancer, you have a higher risk of developing another lung cancer. Brothers, sisters, and children of people who have had lung cancer may have a slightly higher risk of lung cancer themselves, especially if the relative was diagnosed at a younger age. It's not clear how much of this risk might be due to shared genes among family members and how much might be from shared household exposures (such as tobacco smoke or radon). Researchers have found that genetics seems to play a role in some families with a strong history of lung cancer (3).

Factors with uncertain or unproven effects on lung cancer risk

- Smoking marijuana
- Talc and talcum powder

- **What causes non-small cell lung cancer?**

We don't know what causes each case of lung cancer. But we do know many of the risk factors for these cancers and how some of them cause cells to become cancerous.

- **Smoking**

Tobacco smoking is by far the leading cause of lung cancer. About 80% of lung cancer deaths are caused by smoking, and many others are caused by exposure to secondhand smoke.

Lung cancer in non-smokers

Not all people who get lung cancer are smokers. Many people with lung cancer are former smokers, but many others never smoked at all. Lung cancer in non-smokers can be caused by exposure to radon, secondhand smoke, air pollution, or other factors. Workplace exposures to asbestos, diesel exhaust, or certain other chemicals can also cause lung cancers in some people who don't smoke.

- **Gene changes that may lead to lung cancer**

Scientists know how some of the risk factors for lung cancer can cause certain changes in the DNA of lung cells. These changes can lead to abnormal cell growth and, sometimes, cancer(1).

· Genes that help cells grow, divide, or stay alive are called oncogenes.

· Genes that help keep cell division under control or cause cells to die at the right time are called tumor suppressor genes.

Cancers can be caused by DNA changes that turn on oncogenes or turn off tumor suppressor genes.

- **Inherited gene changes**

Some people inherit DNA mutations (changes) from their parents that greatly increase their risk for developing certain cancers. But inherited mutations alone are not thought to cause very many lung cancers. Still, genes do seem to play a role in some families with a history of lung cancer. For example, people who inherit certain DNA changes in a particular chromosome (chromosome 6) are more likely to develop lung cancer, even if they don't smoke or only smoke a little. Other people inherit faulty DNA repair mechanisms that make it more likely they will end up with DNA changes. People with DNA repair enzymes that don't work normally might be especially vulnerable to cancer-causing chemicals and radiation. Researchers are developing tests that may help identify such people, but these tests are not yet used routinely. For now, doctors recommend that all people avoid tobacco smoke and other exposures that might increase their cancer risk.

- **Acquired gene changes**

Gene changes related to lung cancer are usually acquired during life rather than inherited. Acquired mutations in lung cells often result from exposure to factors in the environment, such as cancer-causing chemicals in tobacco smoke. But some gene changes may just be random events that sometimes happen inside a cell, without having an outside cause.

Acquired changes in certain genes, such as the TP53 or p16 tumor suppressor genes and the K-RAS or ALK oncogenes, are thought to be important in the development of non-small cell lung cancer. Changes in these and other genes may also make some lung cancers more likely to grow and spread than others. Not all lung cancers share the same gene changes, so there are undoubtedly changes in other genes that have not yet been found (4).

2.1.2 Signs and Symptoms & Pathogenesis of Non-Small cell lung cancer

2.1.2.1 Signs & Symptoms

- Coughing up blood or rust-colored sputum (spit or phlegm)
- Chest pain that is often worse with deep breathing, coughing, or laughing
- Symptoms due to the cancer mass pressing on adjacent structures: chest pain, bone pain, superior vena cava obstruction, or difficulty swallowing
- Hoarseness

- Weight loss and loss of appetite
- Shortness of breath
- Feeling tired or weak
- Infections such as bronchitis and pneumonia that don't go away or keep coming back
- New onset of wheezing

When lung cancer spreads to distant organs, it may cause:

- Bone pain (like pain in the back or hips)
- Nervous system changes (such as headache, weakness or numbness of an arm or leg, dizziness, balance problems, or seizures), from cancer spread to the brain or spinal cord
- Yellowing of the skin and eyes (jaundice), from cancer spread to the liver
- Lumps near the surface of the body, due to cancer spreading to the skin or to lymph

nodes (collections of immune system cells), such as those in the neck or above thecollarbone(3).

2.1.2.2 Pathogenesis of Non-small cell lung cancer

NSCLC comprises a heterogeneous group of histology types, with the most frequent types being adenocarcinoma, squamous cell carcinoma, large cell carcinoma, adeno squamous carcinoma, and sarcomatoid carcinoma. Adenocarcinoma accounts for nearly 40% of all lung cancers. According to the 2004 World Health Organization classification, adenocarcinoma can be subclassified into five major subtypes: acinar, papillary, solid with mucin production, bronchioloalveolar (BAC), and mixed adenocarcinoma (5). There is evidence suggesting that at least two molecular pathways are involved in adenocarcinoma early pathogenesis(6); smoking and *KRAS*-related and nonsmoking and *EGFR*-related which are described in Figure 2-2(7).

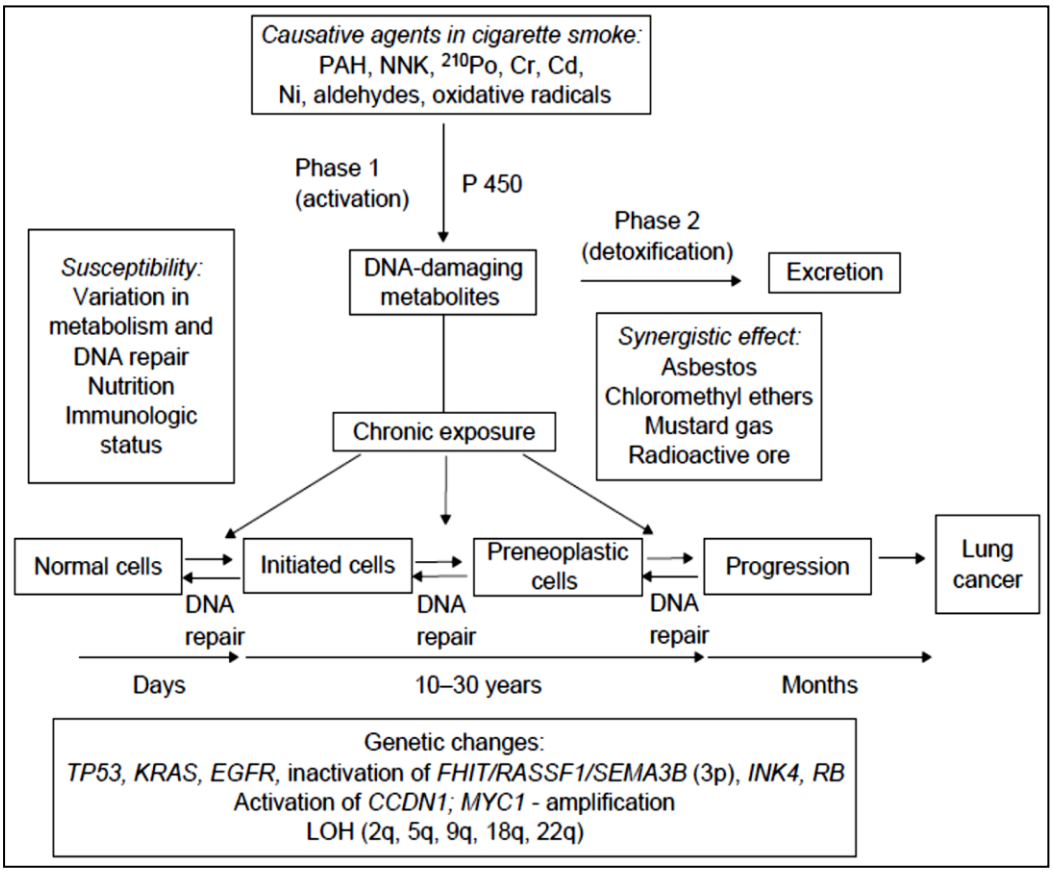


Figure 2-2Lung carcinogenesis(4)

2.1.3 Treatment approaches for Non-Small cell lung cancer

- ✓ **Surgery**

Pneumonectomy, Lobectomy, Segmentectomy or wedge resection and Sleeve resection

- ✓ **Radiofrequency ablation (RFA)**

RFA uses high-energy radio waves to heat the tumor. A thin, needle-like probe is put through the skin and moved in until the tip is in the tumor. Placement of the probe is guided by CT scans. Once the tip is in place, an electric current is passed through the probe, which heats the tumor and destroys the cancer cells.

- ✓ **Radiation therapy**

Radiation therapy uses high-energy rays (such as x-rays) or particles to kill cancer cells. There are 2 main types of radiation therapy:

External beam radiation therapy and brachytherapy (internal radiation therapy)

- ✓ **Chemotherapy**

Table 2-1Chemotherapeutics used for NSCLC

Cisplatin	Carboplatin
Paclitaxel (Taxol)	Docetaxel (Taxotere)
Gemcitabine (Gemzar)	Vinorelbine (Navelbine)
Irinotecan (Camptosar)	Vinblastine
Pemetrexed (Alimta)	Etoposide (VP-16)

- ✓ **Targeted therapies**

Drugs that target cells with EGFR changes

Epidermal growth factor receptor (EGFR) is a protein on the surface of cells. It normally helps the cells grow and divide. Some NSCLC cells have too much EGFR, which makes them grow faster. Drugs called *EGFR inhibitors* can block the signal from EGFR that tells the cells to grow. Some of these drugs can be used to treat NSCLC.

EGFR inhibitors used in NSCLC with *EGFR* gene mutations

Erlotinib (Tarceva), Afatinib (Gilotrif), Gefitinib (Iressa)

EGFR inhibitors that also target cells with the T790M mutation

Osimertinib (Tagrisso)

EGFR inhibitors used for squamous cell NSCLC

Necitumumab (Portrazza) (MoAb)

Drugs that target cells with ALK gene changes

Crizotinib (Xalkori), Ceritinib (Zykadia), Alectinib (Alecensa)

Drugs that target tumor blood vessel growth (angiogenesis)

Bevacizumab (Avastin), Ramucirumab (Cyramza)

- ✓ **Immunotherapy**

Immunotherapy is the use of medicines to stimulate a person's own immune system to recognize and destroy cancer cells more effectively. Immunotherapy can be used to treat some forms of non-small cell lung cancer (NSCLC). **Nivolumab (Opdivo)** and **pembrolizumab (Keytruda)** target PD-1, a protein on immune system cells called *T cells* that normally helps keep these cells from attacking other cells in the body. By blocking PD-1, these drugs boost the immune response against cancer cells. This can shrink some tumors or slow their growth(3).

- ✓ **Palliative treatment**

Treating fluid buildup in the area around the lung

Thoracentesis, Pleurodesis and Catheter placement

Treating fluid buildup around the heart

Pericardiocentesis

Treating an airway blocked by a tumor

Photodynamic therapy (PDT) and laser therapy

2.2 MECHANISM OF MULTIDRUG RESISTANCE AND APPROACHES USED FOR REVERSAL OF RESISTANCE IN CANCER

2.2.1 Multi drug resistance mechanism

Multidrug resistance (MDR) is the most frequent phenomenon by which cancer cells elude chemotherapy. Mechanisms responsible for the MDR can be broadly divided into cellular factors and physiological factors. Multidrug resistance (MDR) is the most frequent phenomenon by which cancer cells elude chemotherapy. Mechanisms responsible for the MDR can be broadly divided into cellular factors and physiological factors. Cellular factors include altered molecular targets, increased drug metabolism, genetic defects such as polymorphism and gene deletion, reduced apoptosis, and over-expression of efflux pumps whereas physiological factors include cell–cell interaction, higher interstitial fluid pressure, low pH environment, hypoxic region in the tumor core, irregular tumor vasculature, and the presence of cancer cells in areas difficult to penetrate. Most of these factors lead to the requirement of higher doses of chemotherapeutic agents, which demonstrate systemic toxicity. Based on the type of disease and given treatment, mechanisms responsible for the demonstration of MDR vary (8).

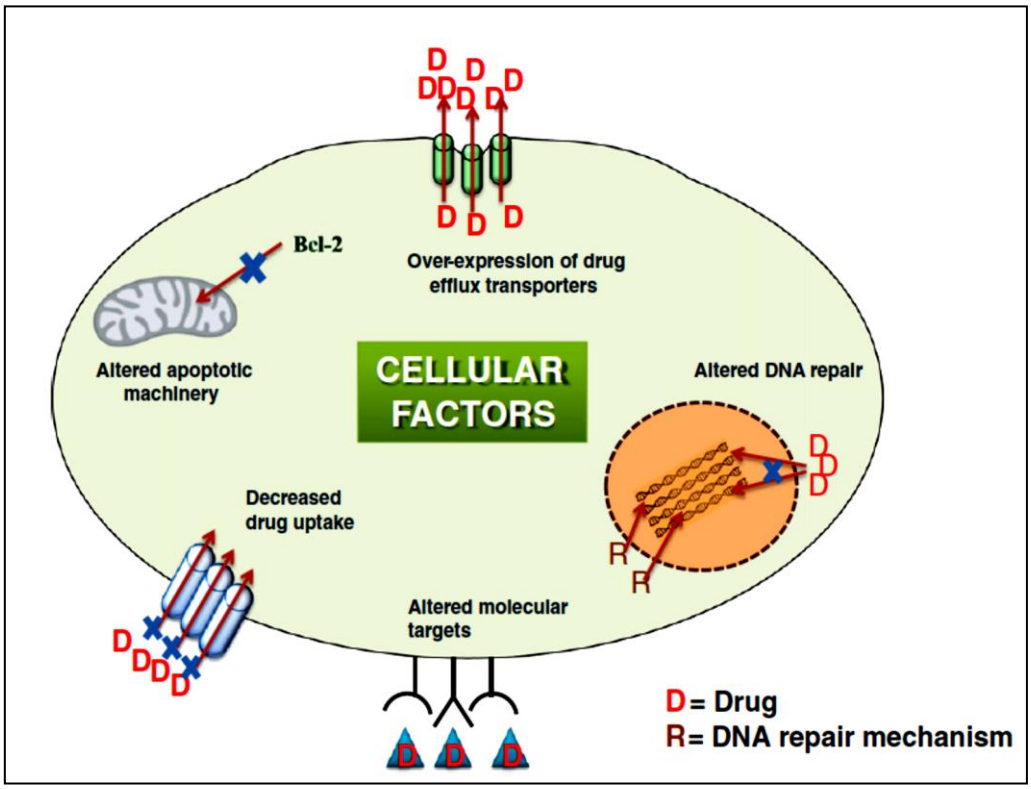


Figure2-3 Cellular factors responsible for drug resistance

These factors are inter-related and considered to affect one another. (Figure2-3; Figure2-4) (9)

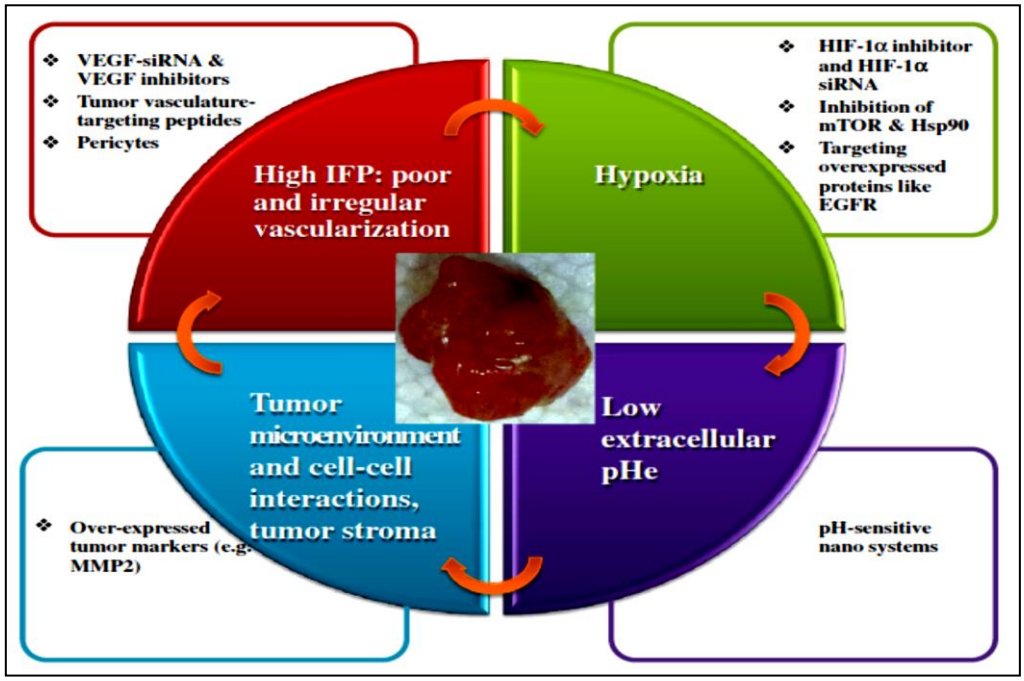


Figure2-4Physiological factors responsible for drug resistance

2.2.2 Various approaches used in reversal of resistance in cancer

Extensive studies have been conducted during the last several decades to enhance the efficacy of chemotherapy by suppressing or evading these MDR mechanisms including the use of new anticancer drugs that could escape from the efflux reaction, MDR modulators or chemo sensitizers, multifunctional nanocarriers, and RNA interference (RNAi) therapy.

Table 2-2Approaches used in reversal of resistance in cancer

Vector	Mode of reversal of resistance	Cytotoxic agent	Tumor type	Remarks
Nano constructs (10)	Anti-sense ODNs targeted to BCl 2 &MRP1 mRNA	Doxorubicin	***Lung cancer***	The experimental results show high antitumor activity and low adverse side effects of proposed complex inhalatory treatment that cannot be achieved by individual components applied separately. The present work potentially contributes to the treatment of lung cancer by describing a unique combinatorial local inhalation delivery of drugs and suppressors of pump and non-pump cellular resistance.
Magnetic Fe3O4 nanoparticles (11)	Combination therapy with MDR1 shRNA	Daunorubicin	Leukemia	Combination of DNR with either MNP (Fe(3)O(4)) or PGY1-2 exerted a potent cytotoxic effect on K562/A02 cells, while combination of MNP (Fe(3)O(4)) and

				PGY1-2 could synergistically reverse multidrug resistance.
Poly(n-butylcyanoacrylate)Nanoparticles (12)	Inhibition of P-gp function	Paclitaxel	Ovarian cancer	Paclitaxel-loaded PBCA nanoparticles can enhance cytotoxicity and overcome MDR through a mechanism of the inhibition of P-gp function caused by the nanoparticles system.
Multi-functional nano-carrier (13)	siRNA targeted to BCl 2 & MRP1 mRNA	Doxorubicin	***Lung cancer***	It enhanced efficiency of chemotherapy to a level that cannot be achieved by applying its components separately
PLGA nanoparticle (14)	siRNA-Stat3	Paclitaxel	***Lung cancer***	These nanoparticles suppressed Stat3 expression and induced cellular apoptosis in human lung cancer cell lines (A549)

2.3 GENE THERAPY & RNA INTERFERENCE

A number of common human diseases have underlying genetic causes, and pharmacological approaches often fall short of curing many of these diseases. Gene therapy is defined as the correction of dysfunctional or deleted genes by supplying the lacking component as a means to permanently treat or reverse such diseases (15). Gene therapy provides a unique approach that can be used in the treatment of both inherited and acquired diseases. Researchers in gene therapy have used one of the ways for correcting defective genes such as replacement of a nonfunctional gene with a normal gene, or an abnormal gene with a normal gene, through homologous recombination; or for repairing an abnormal gene through selective reverse mutation or selectively controlling expression of a defective gene (1). In another type of

application, the genes may also be delivered as genetic vaccines to induce both cell-mediated and humoral immune responses.

RNAi interference is a biological mechanism by which a small dsRNA directs the degradation of complementary mRNA and execute sequence-specific inhibition of a particular gene(16). Many diseases are targeted by post-transcriptional silencing specific gene expression inhibition. These mechanisms were discovered and published officially in *Nature* in 1998,but it gained momentum when Andrew Fire and Craig Mello received the Nobel prize in physiology or medicine in 2006 (17). They investigated the RNAi phenomenon during their experiments of gene expression regulation in thenematode worm *Caenorhabditis elegans*which is explained in Figure 2-6.The simple synthesis of siRNA makes RNAi technology fast and an efficient tool. This is because there isno requirement of cellular expression system, upstream and downstream protein purification related steps andsequences can be designed from sense and antisense strand analysis of target-related specific knockdown of the geneof interest. The delivery of siRNA is an easy and efficient process compared with shRNA plasmid or pDNA delivery. This is because the site of action for siRNA is cytosol, whereas shRNA plasmids and pDNA have to enterthe nucleus for its action.

2.3.1 Fundamental principles of gene therapy

The fundamental principle underlining gene therapy is theoretically straightforward, but difficult to achieve in practice satisfactorily. The two essential components of gene medicine are therapeutic gene coding for a protein along with its expression regulatory component and the gene delivery system (18). Many genes capable of correcting diseased phenotypes have been identified, and it is now possible to produce engineered DNA that carries a therapeutic gene in sufficient quantities for clinical trials. The simplified schematic general process of gene therapy is shown in Figure 2-5. Initially, the required desired gene (or DNA-containing gene) is complexed, compacted, and packaged into a suitable vector, either viral or non-viral, to form the DNA–vector system. This system or the DNA alone is introduced into the target cells, using a physical, chemical, or biological method, where it gets dissociated to release the DNA. The released DNA, with or without integrating to the host cell DNA, expresses itself to produce protein, using the host cell machinery; this is followed by the therapeutic effect.

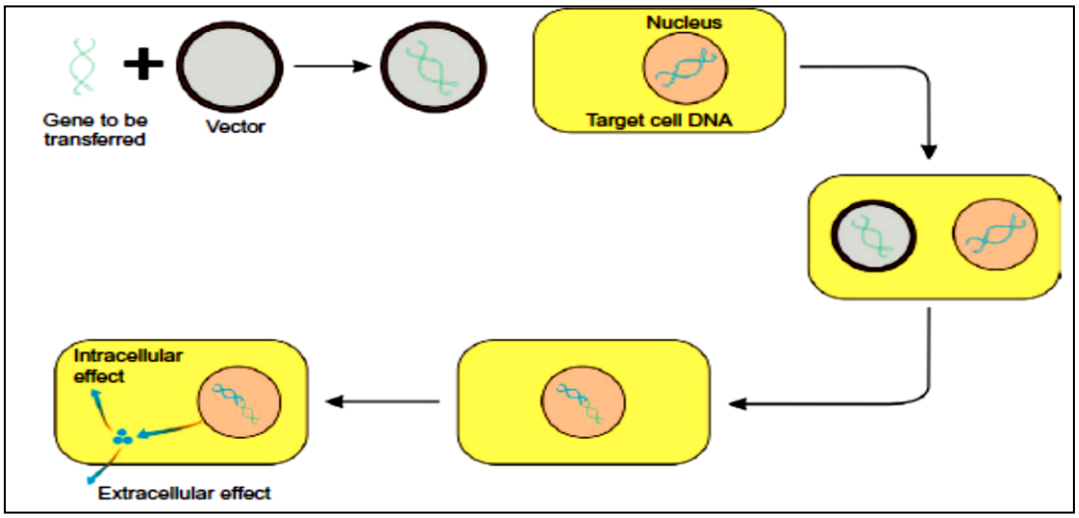


Figure 2-5Simplified schematic diagram on the basis of gene therapy (23)

First, the foreign genetic material to be delivered has to be packaged in a suitable vector.

(A) Introduction of the vector containing therapeutic genetic material into the cell cytoplasm.

(B) Transfer of therapeutic genetic material into the nucleus of the recipient cell, followed by integration to cellular DNA.

(C) Expression of the foreign therapeutic gene, resulting in synthesis of the desired protein product.

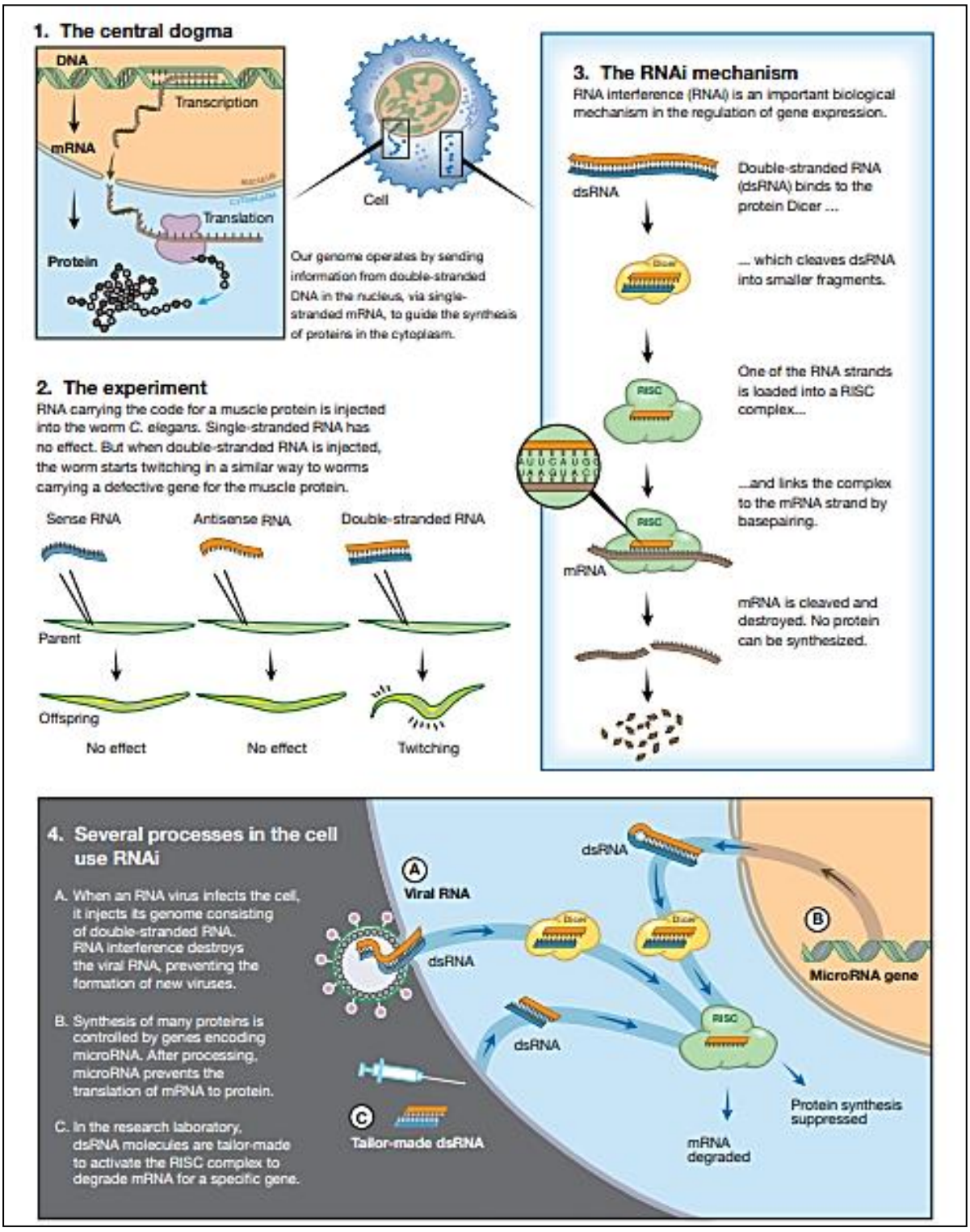


Figure 2-6Gene silencing mechanism through RNA interference technology (RNAi)

ONPATTRO is the only therapeutic product in market based on RNA interference technology till date developed by Alnylam, USA. *ONPATTRO*

(patisiran) is lipid complex injection, for intravenous use approved by USFDA in 2018(19).

It contains contains a transthyretin-directed small interfering RNA and is indicated for the treatment of the polyneuropathy of hereditary transthyretin-mediated amyloidosis in adults. The molecular formula of patisiran sodium is $C_{412}H_{480}N_{148}Na_{40}O_{290}P_{40}$ and the molecular weight is 14304 Da and it is supplied as a sterile, preservative-free, white to off-white, opalescent, homogeneous solution for intravenous infusion in a single-dose glass vial. It exerts its action through knock-down of TTR protein made in liver which in turn helps to decrease the amount of amyloid deposits.

2.3.2 Approaches for gene therapy

Gene therapy is a relatively new technology that holds great promise for treating a myriad of diseases in a unique manner. In genetic diseases, delivering the functional gene can potentially cure the disease, which would be a significant improvement over lifelong protein replacement therapy or no therapy at all. For acquired diseases like cancer, gene therapy allows local production or cancer-cell targeted expression of a therapeutic gene product. Basically, there are four protocols or strategies that are used to achieve the goals of gene therapy namely *In vitro* approach, *Ex vivo* approach, *In situ* approach and *In vivo* approach(20).

2.4 DELIVERY VECTOR: HYBRID NANOCARRIERS

Nanomedicine drug delivery systems for cancer therapy are designed to protect the drug from inactivation due to the biological environment, protect non-pathological tissues from non-specific toxic actions of the drug, and to change or control drug pharmacokinetics (21). Lipid-polymer hybrid nanoparticles are a nanomedicine formulation platform that can be used for cancer treatment. The anatomy of a hybrid nanoparticle consists of a hydrophobic poly (lactic-co-glycolic acid) (PLGA) polymer core or PCL etc. , a lipid monolayer surrounding the core, and a lipid-PEG (for example: 1, 2- Distearoyl-sn-glycero-3-phosphoethanolamine-N-carboxy (poly (ethylene glycol)) 2000 (DSPE-PEG-COOH)), which is distributed within the lipid monolayer to form a polyethylene glycol (PEG) corona. The polymeric core affects drug encapsulation and release. Drug release from the nanoparticles begins with diffusion processes, followed by erosion, then swelling of

the matrix(22). The polymer degrades due to hydrolysis and the degradation rate depends on the polymer composition and molecular weight. The lipid shell serves the purpose as a biocompatible shield, a template for surface modifications, and a barrier for preventing water-soluble drugs from leaking out of the core (23).The corona affects biodistribution and circulation half-life. The PEG corona provides electrostatic and steric stabilization as well as a protective layer from adsorptive recognition proteins in the bloodstream.

With all the functionality that is available to lipid-polymer hybrid nanoparticles, another opportunity can be used with this system for combinatorial delivery. In this system a hydrophobic drug can be encapsulated in the PLGA core while a lipophilic drug can be incorporated within the lipid shell. Co-formulation of multiple drugs in a single system has the advantage of delivering the correct drug ratio to the target of interest as well as synergistic therapeutic effects, suppressed drug resistance, and a timed drug exposure control (24). One proof of concept is the encapsulation of docetaxel with a Cisplatin prodrug conjugated to the polymer to treat prostate cancer cells (28). Similarly, Aryal et. Al., have demonstrated the combinatorial drug delivery system in which paclitaxel (a hydrophobic drug) and gemcitabine (a hydrophilic drug) were conjugated by a hydrolysable linker, followed by the encapsulation of the drug conjugate into a hybrid nanoparticle (29,30). Nanoparticle formulation platforms have several advantages in delivering cancer therapeutics. They provide a system that improves drug solubility, increases half-life circulation due to evasion of the mononuclear phagocytic system, enhances the drug accumulation in target cells, provides a stable drug release, and reduces efflux pump mediated drug resistance (25). In this work, the next steps were taken with the nanoparticle platform to optimize surface charge in order to take advantage of cellular uptake mechanisms and to encapsulate multiple drugs within a single system in order to improve the cytotoxicity and drug release kinetics (26).

Table 2-3Types of HNCs

Type	**Description**	**Synonyms**
Polymer core–lipid shell	Colloidal supramolecular assemblies consisting of polymer particles coated with lipid layer (s)	➢ Lipo-particles ➢ Lipid–polymer particle assemblies ➢ Lipid-coated

		NPs ➢ Nanocell ➢ Polymer-supported lipid shells
Core–shell-type hollowlipid–polymer–lipid NPs	Hollow inner core surrounded by concentric lipid layer, followed by polymeric layer, again followed by lipid layer along with lipid–PEG.	
Erythrocyte membrane-camouflagedpolymeric NPs	Sub-100-nm polymeric particles are coated with RBC membrane derived vesicles to mimic complex surface chemistry of erythrocyte membrane	Biomimetic NPs
Monolithic LPHNs	Lipid molecules are dispersed in a polymeric matrix	Mixed lipid–polymer Particles
Polymer-caged liposomes	These systems are composed of polymers, anchored or grafted at the surfaces of the liposomes to provide stability	

Advantages of HNCs:

➢ The solid polymeric core acts as a cytoskeleton that provides mechanical stability, controlled morphology, biodegradability, narrow size distribution, and high available specific surface area.

➢ The lipid shell enveloping the core is biocompatible and exhibits behavior similar to that of cell membranes. The shell has the ability to interact with a wide variety of molecules, either within the membrane or on the surface.

➢ Improved encapsulation of hydrophobic drugs with therapeutically effective drug entrapment efficiency and drug loading has been reported for a number of drugs compared to liposomes or polymeric nanoparticles.

➢ Amphiphilic character of lipids facilitates the adsorption of hydrophilic compounds on the bilayer surface and insertion of hydrophobic molecules into the hydrophobic lamellar region. This feature allows HNCs to entrap and deliver multiple hydrophilic and hydrophobic therapeutic agents simultaneously.

➢ Optimization of the core and shell can result in tunable and sustained drug release profiles.

- HNCs exhibit storage and serum stability over prolonged periods.
- Besides passive targeting of HNCs based on particle size, they can be conjugated with appropriate targeting ligands such as aptamers, folic acid, transferrin, anti-carcinoembryonic antigen half-antibody, or single chain single chain tumor necrosis factor to deliver NPs at the target tissues for treating cancers (27).
- Particles smaller than 100 nm (similar to virus-like architecture) are promising for intracellular drug targeting and vaccine adjuvants.

2.4.1 Methods of preparation of HNCs

The Methods used to prepare HNCs broadly fall into two categories; the two-step method and the single-step method.

A. Two-Step method (22, 23)

B. Single-Step method(28)

B1 Modified Solvent extraction (29, 30)

B2 Modified nanoprecipitation (30)

B3 Thin lipo-polymeric film formation followed by hydration, and extrusion (31)

2.4.2 HNCs Reported in Literature with their applications

Table 2-4 HNCs Reported in Literature

Encapsulant	Polymer	Lipid	Particle size	EE/ DL	Application
Doxorubicin and combretastatin	PLGA	PC/Chol/DSPE-PEG	180-200 nm	NR	Melanoma , Lewis lung carcinoma
Doxorubicin	PLGA	DPPC	195 nm	DL 0.52 %	MDR breast cancer
Paclitaxel	PLGA	Lecithin	83-95 nm	NR	Pancreatic cancer
Verapamil HCI	Dextran	Decanoic acid	342.5 nm	90% - 99%	NR
Paclitaxel	PLGA	DLPC	200-300 nm	43% - 56%	Cancer
Paclitaxel	PLGA	OQLCS	184-194 nm	84% - 88%	Cancer

Docetaxel, indium II I and yttrium 90	PLGA	DMPE-DTPA/lecithin	65 nm	60%	Prostate cancer
AChE	PMOXA—PDMS—PMOXA	EPC/DPPC	75 nm	NR	Protein delivery
Plasmid DNA	PEI	Triolein/EPC/ DSPE-PEG	128 nm	NR	Gene delivery
Plasmid DNA	PLGA	DOTAP/DC-Chol	100-400 nm	NR	Gene delivery
Plasmid DNA	PLA	DPPC/ DPTAP	325 - 340 nm	NR	Gene delivery
mRNA	PBAE	DOPC/DOTAP	230-300 nm	NR	mRNA based vaccine delivery
siRNA	PLGA	EPC/Lecithin/DSPE-PEG	225 nm	78-82%	Tumor suppression

Abbreviations: EE, entrapment efficiency; DL, drug loading; NR, not reported; HPESO, hydrolyzed polymer of epoxidized soybean oil; MDR, multi-drug resistant; PLGA, poly(lactic-co-glycolic acid); DLPC, dilinoleoylphosphatidylcholine; DMPE-DTPA, 1,2-ditetradecanoyl-sn-glycero-3-phosphoethanolamine-N-diethylenetriaminepentaacetic acid;DSPE-PEG,1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N [amino(polyethylene glycol); PMOXA-PDMSPMOXA,poly(2-methyloxazoline)-block-poly(dimethylsiloxan)-block-poly(2-methyloxazoline);DPPC, dipalmitoylphosphatidylcholine; PEI, polyethyleneimine; EPC, 1,2-dimyristoleoyl-sn-glycero-3-ethylphosphocholine; PGA, poly(glutamic acid); DPTAP, 1,2-dipalmitoyl-3-trimethylammonium-propane; PLA, poly(lactic acid); OQLCS, octadecyl-quaternized lysine-modified chitosan; DHA, cis-4,7,10,13,16,19-docosahexanoic acid; PBAE, poly-(β-amino ester).

2.4.3 Combinatorial delivery of siRNA and anti-cancer drug therapeutics

Various types of nanocarriers have been investigated for the co-administration of siRNA and anticancer drugs in an effort to enhance anticancer effects by overcoming MDR or inducing different apoptosis pathways(32). Judiciously engineered multifunctional drug/siRNA co-delivery nanocarriers can significantly

increase their in vivo tumor accumulation via both passive (i.e. the EPR effect) and active (i.e. via proper conjugation of active tumortargeting ligands such as peptides, antibodies, aptamers, and certain small molecules) tumor-targeting abilities(33). Thus, multifunctional nanomedicines offer great promise in overcoming the drawbacks of current treatment modalities, including chemotherapy. Nevertheless, a deeper understanding of several factors, including the optimal ratio of each therapeutic agent (e.g. siRNA versus anticancer drug) as well as their pharmacological fate at the tumor site, and the nature of cancer heterogeneity, is needed to achieve the maximum synergistic effect of co-administering siRNA with anticancer drugs. Additionally, further studies are needed to avoid unexpected immune stimulation with the simultaneous administration of siRNA and anticancer drugs(25).

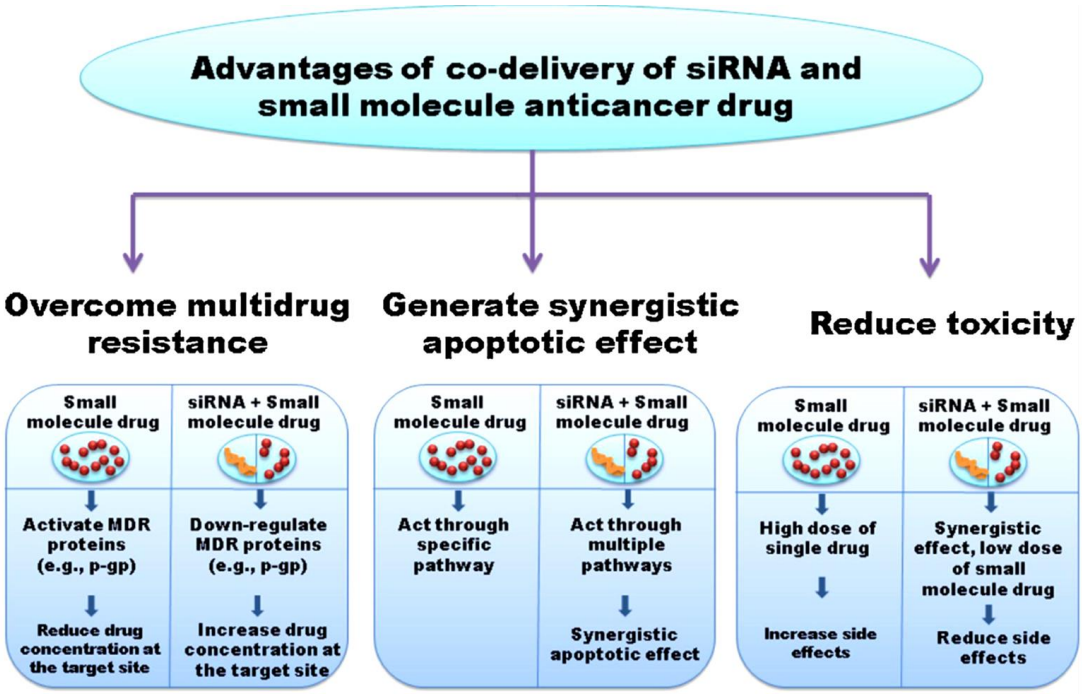


Figure 2-7Major advantages offered by combined chemotherapy and siRNA therapy

2.5 PULMONARY DELIVERY OF NANOCARRIERS

Inhalation is any *drug* or *solution of drug* administered by nasal or oral respiratory route. Inhalation therapy refers to various methods of treatment that work when you breathe in (inhale). Non-invasiveness, lower systemic exposure, rapid deposition in the organ and avoidance of first pass effect are the main advantages associated with pulmonary/inhalational delivery. Pressurized metered-dose inhaler

(MDI), nebulizer and dry powder inhaler (DPI) are main delivery systems in pulmonary delivery. Among these, DPI appears to be the most promising for future use. They are propellant-free, portable, easy to operate and low-cost devices with improved stability of the formulation as a result of the dry state. Example- Spinhaler®. DPIs have to overcome various physical difficulties for effective drug delivery either local or systemic purposes (34). Further discussion of Pulmonary delivery and dry powder inhaler is depicted in chapter 7.

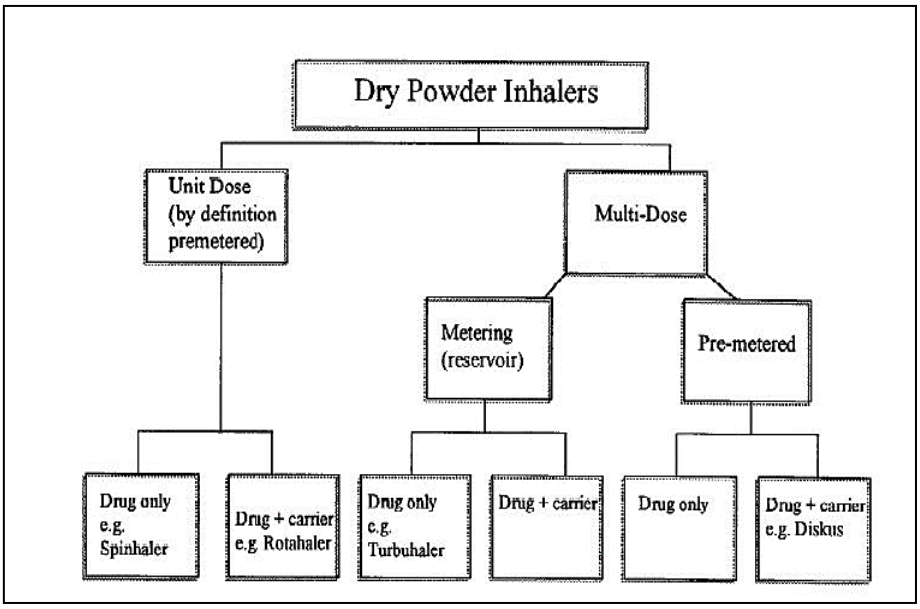
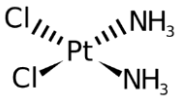


Figure2-8Types of Dry Powder Inhalers

2.6 DRUG PROFILE: CISPLATIN

Name and structure of drug	Cisplatin (IP,BP,USP) 
IUPAC name	(SP-4-2)-diamminedichloroplatinum(II)
Synonyms	CDDP, Cis-DDP,Cisplatinum, diaminedichloride, Peyrone's salt, platamin, neoplatin, cismaplat.
Molecular Formula	$[Pt(NH_3)_2Cl_2]$
Molecular Weight	300.046g/mol
Category	Antineoplastic agent
Appearance	Yellow to orange crystalline powder.

Solubility in different Solvents(at 25°C)	Water : 2.53 mg/ml (25°C) : 8 mg/ml (60°C) Dimethylformamide(DMF) : 16 mg/ml Dimethylacetamide(DMA) : 15 mg/ml Dimethylsulphoxide(DMSO)25 mg/ml Ethanol : (<1 mg/ml)
Melting Point	270-290°C
Routes of administration	Intravenous
Bioavailability	100% (IV)
Log P	0.041
pk_a	5.06(Strongest Basic)
Dose	75 to 120 mg/m2 intravenously once every 3 to 6 weeks. Also 50 mg/m2 intravenously on days 1 and 8 of a four- week course.
Absorption	Following cisplatin doses of 20 to 120 mg/m^2, the concentrations of platinum are highest in liver, prostate, and kidney; somewhat lower in bladder, muscle, testicle, pancreas, and spleen; and lowest in bowel, adrenal, heart, lung, cerebrum, and cerebellum. Platinum is present in tissues for as long as 180 days after the last administration.
Volume of Distribution	Volume of distribution at steady state = 11-12 L/m^2
Protein Binding	Cisplatin does not undergo instantaneous and reversible binding to plasma protein that is characteristic of normal drug-protein binding. However, the platinum itself is capable of binding to plasma proteins, including albumin, transferrin, and gamma globulin. Three hours after a bolus injection and two hours after the end of a three-hour infusion, 90% of the plasma platinum is protein bound.
Route of Elimination	The parent compound, cisplatin, is excreted in the urine. Although small amounts of platinum are present in the bile and large intestine after administration of cisplatin, the fecal excretion of platinum appears to be insignificant.
Half Life	Cisplatin decays monoexponentially with a half life of 20 to 30 minutes following administrations of 50 or 100 mg/m^2. Cisplatin has a plasma half-life of 30 minutes. The complexes between albumin and the platinum from cisplatin do not dissociate to a significant extent and are slowly eliminated with a minimum half-life of five days or more.

Clearance	50 mL/min/m^2 [renal clearance, 6- to 7-hour infusion of 100 mg/m^2] The renal clearance of free (ultrafilterable) platinum also exceeds the glomerular filtration rate indicating that cisplatin or other platinum-containing molecules are actively secreted by the kidneys. The renal clearance of free platinum is nonlinear and variable and is dependent on dose, urine flow rate, and individual variability in the extent of active secretion and possible tubular reabsorption.

Mechanism of action

The mechanism of cisplatin is widely believed to include an interaction with DNA leading to apoptosis, or programmed cell death. Cisplatin is directly administered into the bloodstream of the patient, whereupon the drug interacts with a high concentration (about 100 mM) of blood plasma chloride that prevents aquation of the drug molecule; the chloride concentration is high enough to hinder cisplatin's chloride ligands from being replaced by water molecules. But the drug molecule undergoes attack from thiol-containing blood plasma proteins, including cysteine (amino acid) and human serum albumin. Once the drug is administered, 65-98% of the platinum is protein bound in the blood plasma;this protein binding has been blamed for deactivation of the drugs and some of the severe side effects of cisplatin treatment. The remaining part of the cisplatin molecule may either diffuse through the cell membrane of the tumor cells or may be actively transported across the tumor cell membrane via copper transporting proteins. Inside the tumor cell, the chloride concentration is considerably lower than in the blood plasma (at most 20 mM); thus at this point one of the chloro ligands on the drug molecule is aquated, that is, one chloro ligand is replaced by a water molecule. This monoaquation forms a positively-charged and highly reactive species that is unable to leave the tumor cell; in vitro studies show that this charged species causes 98% of platinum binding to cellular DNA in the nucleus [28] by reacting with one of the DNA bases **.**

The cytotoxicity of cisplatin is primarily ascribed to its interaction with nucleophilic N7-sites of purine bases in DNA to form DNA–protein and DNA–DNA interstrand and intrastrand crosslinks. However, evidence strongly favors intrastrand adducts as lesions largely responsible for the cytotoxic action. This is consistent with the knowledge that 1,2-intrastrand ApG (Adenine-phosphate-Guanine) and GpG

(Guanine-phosphate-Guanine) crosslinks are the major forms of DNA adducts, accounting for 85–90% of total lesions. These adducts distort the DNA in a way that it can be recognized by one or more binding proteins, which then either begin DNA damage repair or trigger the process of apoptosis.

Side effects

Despite the positive effects of cisplatin, patients receiving this drug experience severe side effects including nephrotoxicitydue to the renal excretion of cisplatin, the kidney accumulates a higher effective concentration of cisplatin than any other organ. This accumulation preferentially affects the terminal proximal tubule and the distal nephron and can cause either apoptosis or necrosis, depending on exposure time and concentration.

Other Side effects including nausea, vomiting, decreased blood cell and platelet production in bone marrow (myelosuppresion) and decreased response to infection (immunosuppression). Diarrhoea, pancreatitis, seizures and respiratory failure have also been reported.Serum Electrolyte disturbances such as Hypomagnesemia, hypocalcemia, hyponatremia, hypokalemia, and hypophosphatemia have been reported to occur in patients. More specific side effects include damage of neurons (neurotoxicity) and hearing loss (ototoxicity).

2.7 EXCIPIENTS PROFILE

Table 2-5 HNCs excipients profile

Name	**Molecular formula and molecular weight**	**Tg (Glass transition temp.)**	**Storage temp.**	**CAS number**
DPPC (16:0 PC) **1,2-dipalmitoyl-sn-glycero-3-phosphocholine**	$C_{40}H_{80}NO_8P$ 734 Da	41 C	-20 C	63-89-8
DOPE- 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine	$C_{41}H_{78}NO_8P$ 744 Da	- 16 C	-20 C	4004-05-1
DOTAP (TAP 18:1) **1,2-dioleoyl-3-trimethylammonium-propane (chloride salt)**	$C_{42}H_{80}NO_4Cl$ 698 Da	-	-20 C	132172-61-3
DSPE-PEG(2000) Folate ➢ **1,2-distearoyl-sn-glycero-3-**	$C_{151}H_{286}N_{11}O_{59}P$ 3230 Da	-	-20 C	1236288-25-7

phosphoethanolamine -N- [folate(polyethylene glycol)-2000] (ammonium salt)				
DSPE-PEG(2000) Amine **1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt)**	$C_{132}H_{266}N_3O_{54}P$	-	-20 C	474922-26-4
PEG-PLA **Methoxy poly(ethylene glycol-b-(poly(l-lactide)-2k-5k**	HO[CH(CH3)COO]m[CH2CH2O]nCH3	155-160 C	2-8 C	

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