CHAPTER 2:

LITERATURE REVIEW

Chapter II: Literature Review

Genes are biological unit of heredity, building block of life and directs the production of protein that makes up a cell's structure and operates its vital chemistry. A genetic disorder is a disease caused by abnormalities in an individual's genetic material (genome). Human genetic diseases occurs because of the mutation or deletion of genes impairing the normal metabolic pathways, ligand / receptor function, regulation of cell cycle, structure and function of cytoskeletal or extracellular proteins. The diseases that can be suitably treated and managed by gene therapy are either hereditary or acquired diseases. The hereditary diseases as severe combined immunodeficiency, hemophilia, familial hypercholesterolemia, cystic fibrosis, hemoglobinopathies, Gaucher's disease, inherited emphysema are normally caused by a single gene mutation or deletion [I. M. Verma, and N. Somia. (1997)]. But, no single gene can be singly pointed out for development and prognosis of acquired diseases as Cancer, Parkinson's disease, Alzheimer's disease, cardiovascular diseases with VEGF defects, and infectious diseases as suppressed immune response and liver disorders [I. M. Verma, and N. Somia. (1997)]. However, single transgene delivery with desired expression delivered suitably by a gene delivery system to the target cell can significantly improve the disease state.

In lungs, three major diseases observed by alternations in genetic makeup of the body **are cystic fibrosis, lung cancer and α-1- antitrypsin deficiency.** While cystic fibrosis and α-1- antitrypsin deficiency are hereditary diseases present since conception and affects the infants and newborns most; **lung cancer is an acquired diseases caused because of changes in genes acquired during life.** Cancer is one of the most dreadful diseases affecting the mankind and is characterized by uncontrolled growth and spread of cells that may affect almost any tissue of the body. It is a disease of the cells characterized by shift of control mechanisms of cellular growth and differentiation (from DNA instructions) and spread of abnormal cells, resulting in excessive cell proliferation to form local tumors that may invade and spread into other tissues. Most of the cancers are characterized by abnormalities in genetic makeup acquired during life as activation of certain oncogenes and suppression or mutation of growth and division controller genes including **tumor suppressor *p*53 gene** thus suggesting major role of genetic mutations in cancer.

2.1 Lung Cancer and Chemotherapy: Current Status and Issues

Lung cancer is an acquired genetic disorder responsible for excessive cellular growth in the lung tissue as a primary tumor or because of secondary metastatic growth. Lung cancer is the second most common cancer in both men and women, and is responsible for highest death count after

heart diseases [http://www.cancer.gov/cancertopics/types/lung]. It is characterized by uncontrolled cell growth in lung tissues leading to metastasis, invasion of adjacent tissue and infiltration beyond the lungs. It is responsible for over 0.15 million deaths in United States in 2010 with over 0.2 million cases registered annually [http://www.cancer.gov/cancertopics/types/lung]. The main reason for the unfavorable prognosis of these tumors is their propensity to metastasize early and develop resistance to a wide range of anticancer drugs leading to very low survival rate.

There are two main types of lung cancer based on the characteristics of the disease and its response to treatment. **Non-small-cell lung carcinoma (NSCLC)** accounts for 80% of all lung cancers. NSCLC is the very aggressive type of lung cancer and is responsible for significant mortality. Although surgery is a preferred method of cancer removal, it can not remove the tissue completely and is required to be supplemented by multidrug chemotherapy and / or radiation as preferred treatment of choice. NSCLC is further divided into:

1. **Squamous carcinoma**, accounting for 35% of all lung cancer cases. The cells are usually well differentiated and locally spread. Widespread metastases occur relatively late.
2. **Large-cell carcinoma**, which accounts for 10% of all lung cancers. It is less well differentiated than the first type and metastasizes earlier.
3. **Adenocarcinoma**, which accounts for approximately 27% of lung cancers. It arises from mucous glands and from scar tissues. Metastasis is common to the brain and bones. It is the most common type of lung cancer associated with asbestos and is proportionally more common in non-smokers, women and older people.
4. **Alveolar cell carcinoma**, accounting for 1-2% of lung cancers.

The second major type is the **small-cell lung carcinoma (SCLC)**, which accounts for 20% of all lung cancers. Arising from endocrine cells, these tumors secrete many polypeptide hormones. Some of these hormones provide feedback to the cancer cells and cause tumor growth. This type of tumor grows rapidly, taking approximately three years from initial malignant change to presentation. Surgery is the preferred method of treatment of SCLC along with responsive chemotherapy. However, significant success has not been achieved because of fast growth of the SCLC and insufficient drug reaching the target site at required rate and extent.

Treatment of NSCLC generally requires partial surgery along with radiotherapy and chemotherapy. Both, radio and chemotherapy cause painful toxicity to the patient theirby

requiring premature end of the therapy leaving the treatment halfway even though the tumor cells are successfully killed. The reason for this is that all cytotoxic drugs kill normal cells as well as cancerous cells resulting in severe side effects. Furthermore, because of the blood circulation in the body, only a small fraction of the drug actually reaches the target tumor tissue and most of the drug acts on normal tissues or is rapidly eliminated. Therefore, to obtain a therapeutic effect, a relatively high dose of drug must be administered and usual drug formulations are used in a balance between killing the tumor (efficacy) and killing the patient by causing lower toxicity to normal organs. The use of combination chemotherapy, such as: 1-etoposide with cisplatin or carboplatin, 2-doxorubicin and cyclophosphamide with etoposide or vincristine, and 3-cisplatin, 2-doxorubicin, cyclophosphamide and etoposide, has been used in current treatment of NSCLC and is associated with a response rate of over 50% and a median survival of 8-12 months [J. Aisner et al, (1992)]. This chemotherapy comprises only symptomatic management and partial cure. The major problems associated with chemotherapeutic agents are inadequate tumor specificity, narrow therapeutic indices and emergence of resistant cancer cells. Extensive side effects due chemotherapeutic anticancer drugs on normal dividing cells as hair follicles, germ cells and hematopoietic cells are well known resulting in dose limiting toxicity and incomplete therapy.

Recent advances in research using liposomal and nanoparticulate systems in treatment of cancer have lead to development of many products for more efficacious means of treating the lung cancer. Development of liposomal doxorubicin (DOXIL), daunorubicin, nanoparticulate paclitaxel (Abraxane), monoclonal antibody based Herceptin formulation have been the most successful and effective formulations or treatment of solid tumor and metastatic cancer with higher selectivity at site of cancer and lower systemic side effects. However, direct targeting of these drugs to the lung tissue without systemic side-effects has still been a mystery without any suitable treatment solution.

2.2 Aerosolized Local Delivery for Direct Lung Administration: Liposomal Dry Powder Inhalers

As with other respiratory diseases treated locally through the use of inhalation aerosols, there are numerous pharmacokinetic and pharmacodynamic advantages favoring the pulmonary route in cancer therapy. In general, chemotherapy is characterized by a dose dependent response (cell apoptosis) coupled to a high degree of non-specificity, so that despite the introduction of several

newer generations of chemotherapy agents, toxicity remains the principle limitation for effective anti-tumor response. Accordingly, the rationale for pulmonary delivery of these agents primarily focuses on the ability to increase regional targeting and the associated benefits arising from this pharmacokinetic advantage. These advantages are specific to the nature of the tumor microenvironment to which inhalation aerosols are targeted.

For inhalation drug delivery, patients are dependent on inhalation of an aerosol generated either from a liquid drug solution in a nebulizer, a dry powder inhaler (DPI), or a pressurized metered dose inhaler (pMDI) with a specialized device producing a mist containing particles of varying sizes for suitable lung distribution and deposition. The larger particles above 3 micron are observed to be deposited in upper lungs and throat especially used for local lung therapy and upper respiratory track targeting. However, the smaller nanosized particles of 0.1-3 micron have been observed to be deposited in deep lungs and even have shown systemic effects [A. Misra et al (2009)].

Apart from the advantages of direct drug targeting, enhanced pharmacokinetic properties of the drug and reduced side-effects of anticancer drugs, the formulation shows disadvantages of small percentage of the dose actually reaching intended target in the lower respiratory system. The delivered drug quantity depends on the formulation particle size, density and patient's ability to use the inhaler system correctly and/or control of their breathing [A. Misra et al (2009)].

DPIs are the promising devices for delivering a dry powder formulation of an active drug for local or systemic effect by a suitable inhaler device. The development of DPIs has been motivated by the desire for alternatives to pMDIs and nebulizers to overcome their disadvantages and to facilitate the delivery of macromolecules and products of biotechnology. In general DPIs are easier to use, more stable and efficient systems. Since DPIs are typically formulated as one-phase, solid particle blends, they are also preferred from stability and processing standpoint [S. Ashurst et al (2000)].

Unlike pMDIs, DPIs avoid the problems inherent in the use of propellant gases and the need for coordination of inhalation and actuation [A. J. Hickey et al (1994)]. DPIs are also very portable, patient-friendly, easy to use and do not require spacers. DPIs are compact in nature, breath actuated, easy to use, with high drug dose carrying capacities, high lung deposition (from 50 – 70%) and minimal extra pulmonary loss of drug due to low oropharyngeal deposition, low device retention and low exhaled loss.

Pulmonary disposition of drugs is associated with lung clearance mechanisms operating with high reticulo-endothelial system (RES) uptake, irrespective of the method used for delivery. In consequence, this drawback leads to high drug dosing frequency, causing patient incompliance (4 – 6 times in some diseases) and increased dose with time. Although conventional DPIs are more useful for enhanced pulmonary delivery, more efficient and controlled delivery to the lungs via DPIs is required for enhanced efficacy of the drug by increasing pulmonary residence time and reducing lung clearance.

Liposomes, a phospholipid bilayer system enclosing aqueous compartments, provide an efficient delivery system for the treatment of pulmonary disorders because of their biocompatibility, biodegradability and relative non-toxicity [P.N. Shek & R.F. Barber (1986)]. Liposomes can significantly alter the pharmacokinetics and pharmacodynamics of entrapped drugs [H.K. Kimelberg & E.G. Mayhew (1978), M.J. Poznansky & R.L. Juliano (1984)]. Targeting drug delivery into the lungs has become one of the most important aspects of systemic or local drug delivery systems. To convey a sufficient dose of drug to the lungs, suitable drug carriers are required. Administration of liposomes to the respiratory tract is particularly attractive because of the accessibility of the lung as a local target organ, the compatibility of liposomes and lung surfactant components (85% phospholipid), need for sustained local therapy following inhalation [K.K.G. Taylor & J.M. Newton (1992)], improved therapeutic index of the drug due to enhanced intracellular delivery and slow systemic dilution and clearance, minimizing/eliminating side effects, reducing dose/frequency of dosing and possibly drug resistance, systemic toxicities and the cost of therapy [S.P. Newman (2004)].

Studies have indicated that liposomes can be effectively deposited in the human respiratory tract by aerosolization. Delivery of liposomes in suspension form has been investigated by various researchers using either a nebulizer or pMDIs and is under clinical trials. However, in a dispersed aqueous system, liposomes have problems associated with lipid degradation by hydrolysis or oxidation and sedimentation, aggregation, leakage of drugs or fusion of liposomes during storage [H.A. Chengjiu & D.G. Rhodes (1999)]. The liposomal suspension during storage and liquid based aerosolization may result in inadequate chemical and physical stability. The integrity (physical and chemical stability) of liposome during aerosolized from suspension form is also another concern, which needs to be addressed in the delivery of liposome suspension to lungs. Although instable in suspension form, the liposomes processed using freeze drying, spray

drying, spray freeze drying or supercritical fluid technology into dry powder form help to achieve long-term stability and overcome the issues of degradation by hydrolysis or oxidation and sedimentation, drug leakage, aggregation, or fusion of liposomes associated with liposome in suspension form [R.W. Niven (1995), H.M. Courrier et al (2002)]. The dry liposomes encapsulating therapeutics can be delivered to lungs as a DPI, due to their propellant free nature, high stability, flexibility in formulation development and patient compliance. However, the performance of DPIs relies on many aspects including the design and type of inhaler device, the powder formulation and the airflow generated by the patient [H.K. Chan (2006), S. Ashurst et al (2000)].

The anticancer drugs investigated as liposomal inhalation product have demonstrated enhanced anticancer cytotoxic activity of transferrin attached liposomal doxorubicin formulation. The transferrin-conjugated doxorubicin-containing liposomes were tested on different cell lines as 16HBE14o, A 549, Calu-3 and were found to have higher cell uptake and higher cytotoxicity [S. Anabousi et al (2006)]. Another anticancer drug which has been investigated for possible use as an inhaled liposome preparation is interleukin-2 (IL-2), a lymphokine which stimulates the proliferation of T-lymphocytes and thus amplifies immune response to an antigen. It also acts on B-lymphocytes, and modulates interferon-gamma production and natural killer cell activation. The system has been proven *in vitro* anti-tumor activity but systemic *in vivo* toxicity has been a problem. C. Khanna et al. reviewed the use of IL-2 liposomes inhalation therapy in dogs in two separate studies [C. Khanna, et al (1996), C. Khanna et al (1997)]. The first study confirmed an increased leukocyte cell count after inhalation of IL-2 liposomes versus inhalation of free IL-2. The second study reported that pet dogs with naturally occurring pulmonary metastases and primary lung carcinomas accepted inhalation treatments with IL-2 liposomes easily. It was concluded that nontoxic and effective treatment of pulmonary metastases of osteosarcoma is possible with nebulized IL-2 liposomes [C. Khanna et al (1997)].

2.3 Lung Cancer: Liposomal p53 Mediated Gene Therapy

Clinical results of chemotherapy, radiotherapy, surgery and other therapeutic modalities have improved for several types of cancers over the past decades. Nonetheless, high mortality has been observed for lung cancer, prostate cancer and breast cancer with the need of more efficient and specific cancer therapy [M.P. Coleman et al (1993)]. The advances in understanding the molecular basis for cancer has emerged into new therapeutic strategies specifically targeting the

cancer cells. Genes and / or proteins frequently altered in tumors are promising targets for cancer therapy, since these genetic defects are likely to affect critical mechanisms for control of cell growth and survival. The gene therapy is defined as the transfer of nucleic acids (DNA, siRNA, Antisense Oligonucleotides etc) to the different somatic cells for resulting into a desired therapeutic effect, by either correcting the genetic defect or by over-expressing the required therapeutic proteins that are therapeutically useful. Therapeutically effective gene therapy in clinical practice has been a reality with few molecules as siRNAs, *p*53, interleukins hitting the market and many other gene based products either in clinical trials or in preclinical studies.

Among the genes known to play important roles in tumor development, the *p*53 gene on chromosome 17p13 is of particular interest due to its frequent inactivation and mutation in tumors. These mutations observed in *p*53, a tumor suppressor and apoptosis inducer gene, induction of apoptosis in cancer cells after *p*53 restoration and regulatory role of *p*53 in normal cell functioning, make *p*53 one of the premiere candidates in cancer gene therapy. **Statistically, mutation in *p*53 has been associated in 15-50 % of breast cancer, 25-75 % of lung cancer, 25-70 % prostate and bladder cancer, 33-100 % of head and neck cancer and various lymphomas and leukemias** [K.F. Pirollo et al (1997)].

In a normal cell, both alleles of *p*53 are wild-type (WT). In most human carcinomas with p53 mutations, only one allele of *p*53 is present. Broadly, three types of *p*53 mutations can be identified 1) "loss of function," where the tumor suppressor activities of *p*53 are abolished; 2) "dominant negative," where hetero-oligomeric complex formation between WT and mutant p53 results in the inactivation of WT *p*53 present in the cells; and 3) "gain of function," where mutant *p*53 procures a dominant oncogenic role that does not depend on complex formation with WT *p*53 [http://en.wikipedia.org/wiki/P53].

Loss of *p*53 function results in abrogation of *p*53 dependant apoptosis in response to DNA damage, hypoxia and oncogenes activation and allows cellular immortalization (**Figure 2.1**). In addition, *p*53 status determination of the tumor is also important because it also determines the chemotherapy strategy for the tumor.

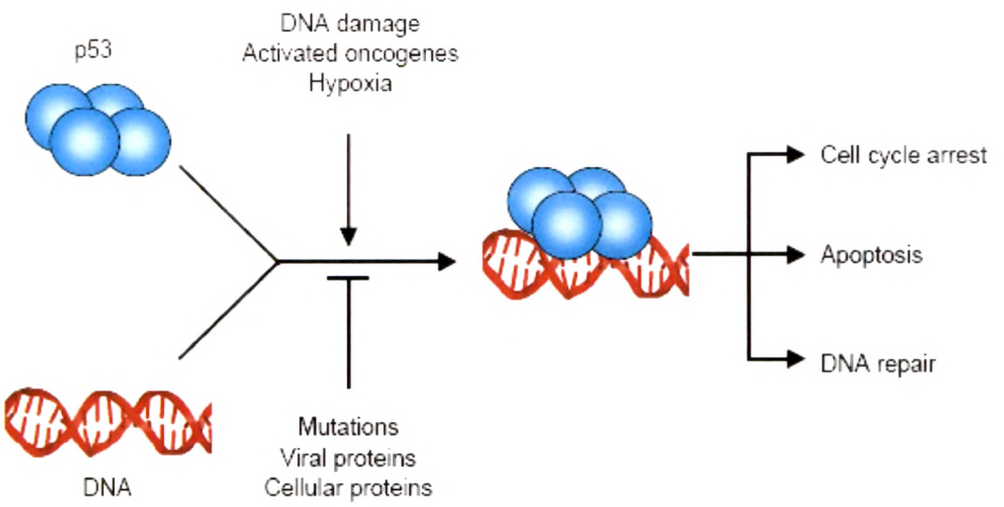


Figure 2.1: The p53 pathway – p53, a tetrameric transcription factor which, on binding to specific DNA – binding elements, stimulates the transcription of various genes involved in apoptosis, cell cycle arrest, DNA repair and other processes. The p53 protein is activated by various signals (e.g. genotox stresses, hypoxia and metabolite changes) and is inhibited by mutations (cancer), viral proteins and cellular proteins (MDM-2)

Presence of mutant *p*53 has also been associated with an unfavorable prognosis for many human cancers including lung, colon and breast [H.E. Ruley (1996)]. In addition, p53 protein transcriptionally regulates genes involved in angiogenesis essential for solid tumor growth [V. Chiarugi et al (1998)]. *p*53 also plays a significant role in diverse cellular pathways activated in response to DNA damage, such as DNA repair, regulation of the cell cycle and programmed cell death (apoptosis) which when malfunctioned results in tumorogenesis. Further, increased chemo and radiation therapy resistance of cancerous cells in absence of *p*53 or presence of mutant *p*53 also supports the regulatory role of active *p*53 in maintaining the normal cell function [L. Xu et al (2001)]. These abnormalities in *p*53 gene in a significant fraction of human cancers and its regulatory role for normal cell functioning make it one of the premiere candidates for cancer gene therapy.

2.3.1 *p*53 Anticancer Function

*p*53 has many mechanisms of anticancer function, and plays a role in apoptosis, genomic stability, and inhibition of angiogenesis. As shown in **figure 2.2**, *p*53 as a anticancer protein works through several mechanisms:

1. It can activate DNA repair proteins when DNA has sustained damage.
2. It can induce growth arrest by holding the cell cycle at the G1/S regulation point on DNA damage recognition (if it holds the cell here for long enough, the DNA repair proteins will have time to fix the damage and the cell will be allowed to continue the cell cycle).
3. It can initiate apoptosis, the programmed cell death, if DNA damage proves to be irreparable.

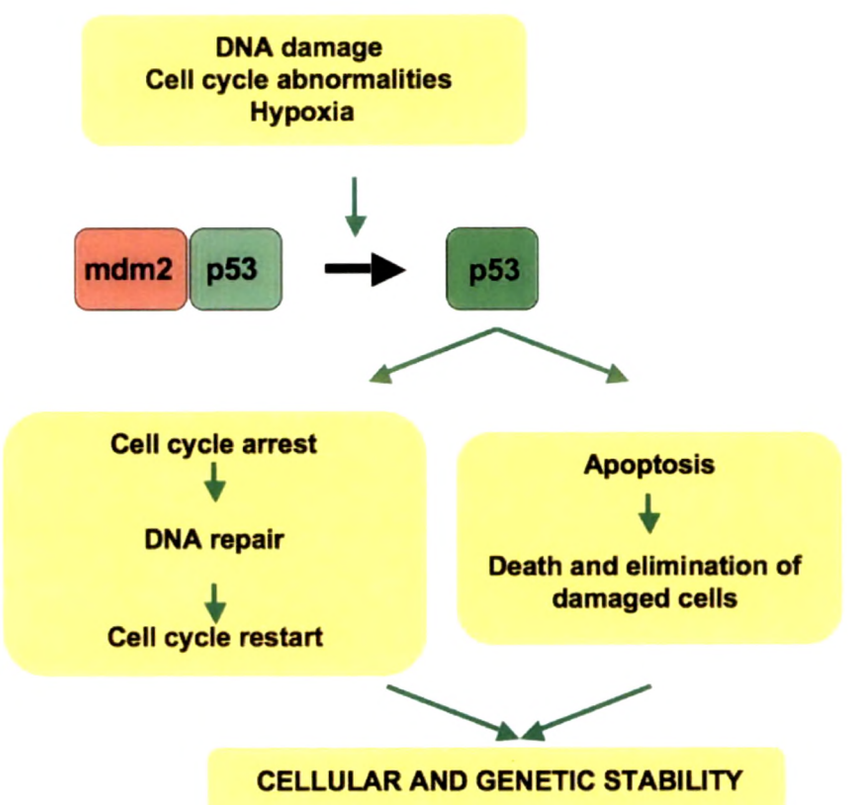


Figure 2.2: Normal p53 pathway and its function in preventing cancer- In a normal cell *p*53 is inactivated by its negative regulator, MDM2. Upon DNA damage or other stresses, various pathways will lead to the dissociation of the *p*53 and MDM2 complex. Once activated, *p*53 will induce a cell cycle arrest to allow either repair or survival of the cell or apoptosis to discard the damaged cell.

In healthy humans, the p53 protein is continually produced and degraded in the cell. The degradation of the p53 protein is, as mentioned, associated with MDM2 binding. In a negative feedback loop, MDM2 is itself induced by the p53 protein. However, mutant p53 proteins often do not induce MDM2, and are thus able to accumulate at very high concentrations. Worse, mutant p53 protein itself can inhibit normal p53 protein levels. This presence of mutant p53 results ultimately in development of tumor with high cell division index.

Cancer treatment using p53 gene as a target delivery molecule has been widely studied with great success. The reintroduction of p53 gene into tumor by a suitable delivery system has shown reduction in tumor mass by means of apoptosis of tumor cells. Both viral and nonviral delivery vectors as well as physical methods using external force have been studied or delivering p53 gene into tumor.

2.3.2 *p*53 delivery vectors

Gene delivery, especially as a plasmid has been delivered to the tumor cells in vitro and in vivo by using viral vectors as well as nonviral vectors. The viral vectors involve use of retrovirus, adenovirus, adeno-associated virus etc. for successful transmission of *p*53 at required site. Whereas nonviral vectors involve use of cationic liposomes (lipoplexes), cationic polymeric (PLGA, PEI, chitosan etc) nanoparticles (polyplexes) and lipid based nanoparticles, and combination of lipid and polymeric particles (lipopolxplexes) for successful *p*53 transfection. The viral vectors have shown greater transfection and access because of viral properties of the carrier along with higher intracellular delivery. However, because of possible immunogenicity the viral vectors are less used. In comparison to viral vectors, nonviral vectors have shown lower transfection, but very high safety, low toxicity and immunogenicity. Further, physical gene delivery methods as electroporation, iontophoresis, etc. have also been used for clinically significant gene transfer especially in liver and skeletal muscles. Examples of various vectors used in the delivery of *p*53 for treatment of lung cancer have been depicted in the table 2.1 below:

Table 2.1: *p*53 gene delivery vectors

Vector Used	Cancer Type Treated	Remarks	Reference
Adeno virus	Li-Fraumeni syndrome	Advexin (Introgen Therapeutics Inc., TX, USA), an adenoviral-based experimental therapeutic delivery system providing a delivery of wild-type *p*53 to cancer cells with adequate *p*53 expression and anticancer activity was developed	J. M. Nemunaitis & J. Nemunaitis (2008)
	Cancer Therapy	Multi-functional helper-dependent adenovirus based system for cancer gene therapy specialized for every individual cancer patient was developed.	N. Long et al (2009)
	Non small cell lung cancer.	Repeated intratumoral injections of Ad-*p*53 showed transgene expression of p53, and mediated antitumor activity in NSCLC patients (86 % patients showed reduced antitumor activity)	S.G. Swisher et al (1999)
	Non small cell lung cancer.	Higher survival rate in Ad-*p*53 treated group as compared to control group was observed.	Y.S. Guan et al (2009)
	Non small cell lung cancer.	Adenoviral CMV promoted *p*53 transfer to tumor tissue was found to efficiently radio-sensitize H1299 cells to subclinical-dose C-beam irradiation through the restoration of *p*53 function.	B. Liu et al (2009)
Retro virus	Orthotopic human lung cancer model in nu/nu mice.	Administration of a retroviral vector expressing wt-*p*53 was found to inhibit in vivo local growth of human lung cancer cells with mutated *p*53 expression	T. Fujiwara et al (1994)
	Human non small cell lung cancer	Direct injection of Rt-*p*53 into NSCLC tissue resulted in tumor regression in 33 % patients.	J.A. Roth et al (1996)
	Human non small cell lung cancers	Restoration of *p*53 resulted into reduction of tumor mass in vivo.	D.P. Carbone & J.D. Minna (1994)

	Human lung cancer cell lines H358a (deleted p53) and H322a (mutant p53).	Reduced growth of the cell lines after *p*53 transfection and restoration with Rt-p53.	D.W. Cai et al (1993
Cationic Micelles and Liposomes	Endobronchial lung cancer -	Different liposomal and miceller formulations with varying lipids and ratios were formed and lipoplexes at different N:P ratios were formed to check p53 transfection. The optimized liposomal formulation DP3 was developed composed of 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine (DPEP) and dioleoyl 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine (DOPE) at a weight ratio of 3 : 1 (particle size, 60–110 nm) . The formulation was effective against H 358 cells *in vitro* and endobronchial tumors after *in vivo* intratracheal administration.	Z. Yiyu (2000)
Cationic Liposomes	Endobronchial lung cancer	An optimized liposomal formulation (DP-53) composed of 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine (DPEP) and dioleoyl 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine (DOPE) at a weight ratio of 3 : 1 (particle size, 60–110 nm) (DP3) was able to transfect *p*53 after forming lipoplex in H 358 cell line. DP3–*p*53 was able to effectively introduce and express the *p*53 gene and induce G1 arrest and apoptosis in H358 cells *in vitro* and to introduce and transcribe the *p*53 gene in the bronchial epithelium of transgenic mice that lack the *p*53 gene *in vivo*. The formulation after intratracheal administration significantly inhibited lung tumor formation and prolonged by	Z. Yiyu et al (1998)

		approximately twofold the survival of mice bearing H358 or H322 endobronchial tumor cells in contrast to the survival among untreated mice and mice treated with the DP3–empty vector	
	Localized human primary lung cancers and to experimental disseminated metastases	The *p*53 null nude animals when treated with DOTAP: Chol–*p53 co*mplex, showed significant suppression in primary and metastatic lung cancer. Further, repeated multiple treatments revealed a 2.5-fold increase in *p*53 gene expression and increased therapeutic efficacy compared to single treatment with prolonged survival period after treatment with liposome–*p53* DNA complex	R.Ramesh (2001)
	NSCLC cell line A 549 injected lung cancer	Stearylamine/DOPE (SA liposome) formulated liposomes, complexed with *p*53 showed significantly lower tumor burden as compared to the control nude mice after intratumoral injection.	J. Zhonghua et al (2000)
Solid Lipid Nanop articles	Non Small Cell Lung Carcinoma – H 1299 cell line.	A novel optimized formulation of cationic solid lipid nanoparticles (SLNs) of tricaprin (TC) as a core, 3b[N-(N0, N0-dimethylaminoethane) carbamoyl] cholesterol (DC-Chol), dioleoylphosphatidyl ethanolamine (DOPE) and Tween 80 in 0.3:0.3:0.3:1 ratio was produced by the melt homogenization technique for pp53-EGFP complexation. The formulation showed significantly higher *p*53 expression than lipofectamine in H 1299 cells and showed higher apoptosis in the cell line.	H.C. Sung et al (2008)
PEI based Nanop articles	B16-F10 mediated lung metastases	A formulation regimen consisting of sequential aerosol delivery of PEI-*p*53 polyplex and 9-Camphothesin –DLPC complex showed added growth inhibitory growth of established B16-F10	A. Gautam et al (2002)

		tumor metastases in the lungs.	
	B16-F10 mediated lung metastases	An aerosol system utilizing a cationic polymer, polyethyleneimine (PEI), for topical gene delivery directly to the lungs for treatment of lung cancer and metastasis was developed. Using a B16-F10 murine melanoma model in C57BL/6 mice, aerosol delivery of PEI-*p*53 DNA resulted in highly significant reductions in the tumor burden ($P < 0.001$) in treated animals, and also lead to about 50% increase in the mean length of survival of the mice-bearing B16-F10 lung tumors.	A. Gautam et al (2002)

The list of viral and nonviral vectors listed above is continuously increasing with many different lipid based and polymer based vectors being synthesized and used for *p*53 transfection for lung cancer. The use of PLGA-PEI block copolymers, Human serum albumins and its block co-polymers, chitosan and its salts, chitosan block copolymers etc. are some of the most widely studied polymers for gene delivery including *p*53. Use of protamine sulphate as transfection enhancing nuclear localization signal sequence along with liposomes have also been well documented in the literature. However, enhanced *p*53 transfection with higher cell uptake and targeting to the cancer tissue requires use of active drug targeting approach using antibodies and surface over-expressed receptor targeting ligands attached to the vectors. The various targeting ligands which are used or have the potential to target lung cancer cells after attaching to the nanosized carriers have been depicted in table 2.2:

Table 2.2: Various Potential Ligands for Targeting the Nano-constructs to Lung Cancer:

Ligand	Remarks	Reference
Folate	The expression of folic acid receptors have been observed on some of the malignant cancer cells as Oral Head and Neck cancer, Metastatic lung carcinoma and many more. Association of folate synthesized lipids as a part of liposomes have shown enhanced liposomal intracellular uptake with higher therapeutic action and gene expression of the cDNA.	A. Yamada et al (2008), P. Zhao et al (2010), S. Duarte et al (2009).

Transferrin	The expression of surface transferrin receptors is particularly high on proliferating malignant lung cancer cells (10,000–100,000 molecules per tumor cell) as compared with the corresponding normal ones (low or undetectable levels), making this receptor an attractive target for cancer gene therapy. Transferrin (Tf) is an 80 kDa serum glycoprotein that traffics ferric ions into cells. Upon binding to its receptor, Tf is internalized into the cell through receptor-mediated endocytosis The association of transferrin (Tf) with cationic liposomes followed by complexation with DNA has been shown to generate vectors that are far more efficient in gene transfer than conventional lipoplexes	C. Tros de Ilarduya , et al (2002), L. Xu et al (2005), S. Anabousi et al (2006).
Hyaluronic Acid	Hyaluronic acid is a poly-anionic polysaccharide, providing multivalent charges for enhancing the particle condensation with pDNA. HA is also a biogenic component, distributed widely in the extracellular matrix and found in the viscous fluid of the mammalian joints. HA has been observed to tightly bound with CD 44 receptors over-expressed on the lung and breast cancer cells. This over-expression has been the basis for HA attached liposomes for cancer targeting. The HA attached liposomes containing encapsulated drugs and complexed with pDNAs have shown enhanced gene transfection and cytotoxicity.	S.Chono, et al (2008), S. Taetz et al (2009).
Haloperidol and Anisamide	Sigma receptors have shown to be over-expressed on many tumor cells including NSCLC. Haloperidol and Anisamide have shown affinity towards sigma receptors as neuroleptic drugs and have been previously researched as a tool to enhance gene transfection of a liposomal system towards in vivo breast cancer model.	A. Barbara et al (2004), A.Mukherjee et al (2005), R. Banerjee et al 2004.
Wheat Germ Agglutinin	Previously, the propensity of malignant cells to exhibit high lectin agglutination, associated with the loss of contact inhibition and tumorigenicity has led to the development of lectins as tumor diagnostic tools. The lectin mediated membrane glycosylation process i.e. the biochemical process of modifying membrane associated proteins or other molecules with sugar residues, is essential to many of the	Y.Mo & L.Y. Lim (2005)

	signaling pathways involved in the process that turns a normal cell to a malignant one. This property of lectin inspired the idea of using lectin-modified carrier systems for the selective delivery of chemotherapeutic drugs. The WGA has been preferentially found to agglutinates cancerous lung cells relative to normal lung fibroblasts and the WGA-cell interaction led to an enhanced cellular uptake of WGA conjugated Nanoparticles (NP) and liposomes via receptor mediated endocytosis. These findings formulate the basis for surface conjugation of PLGA nanoparticles and liposomes with WGA for potentiating the extent and selectivity of the anticancer activity.	
Peptides	RGD peptides have shown high specific ainity to preferentially bind $\alpha_v\beta_3$ integrin receptors, expressed in endothelial cells of tumor vasculature. RGD (Arg-Gly-Asp) as a targeting ligand has been used for targeting PEGylated nanoparticles, microspheres and liposomes with significantly improved receptor mediated endocytosis. Other peptides as epidermal growth factor, leutinizing hormone releasing hormone, HIV-TAT etc. has also been used for targeting carriers to their respective over-expressed receptors.	D. Bibby et al (2005), S. Ko et al (2009), T. Frohlich, et al (2010)

2.3.3 Liposomes as Gene Delivery Vectors

Since Bangham's original description of bilayered phospholipid (PL) vesicles in 1965, liposomes have received much attention as transporters of pharmacological agents. These vesicles, ranging in size from 0.025 μm to greater than 20 μm, are composed of single or multiple Phospholipid membranes surrounding an aqueous compartment. According to their hydrophilic or hydrophobic tendencies, drugs can be entrapped in the aqueous or membrane phases, respectively. Molecules with both hydrophilic and lipophilic character are arranged in stable conformations within the liposome as shown in **figure 2.3.**

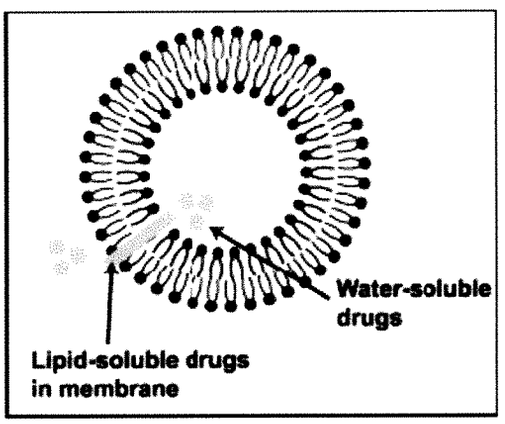


Figure 2.3: Diagrammatic representation of a liposome in which the bilayers of polar phospholipids alternate with aqueous compartments

The net charge of a liposome is varied by incorporation of lipids with negative or positive charges. Long-chain amines as sterylamine, DOTAP, etc. have been used to prepare positively charged vesicles, and diacetyl phosphate, glycerols as DSPG have been used to form negatively charged vesicles. By altering their physical parameters such as size, electrostatic charge, phospholipid profile, and membrane characteristics, liposomes can be engineered to efficiently encapsulate different types of drugs and effectively transport them within the circulation. Each drug encapsulated in a liposome must be regarded as a unique pharmacologic entity dependent upon the components of the liposome and the conditions under which drug encapsulation takes place. Small changes in liposomal preparation or composition can have profound effects on drug bioavailability, activity, and toxicity. Since liposomes produced by standard methods are sequestered primarily by the RES, they can be used to target therapy directly to malignant disease of the liver and spleen or activate macrophages for immunomodulation. By manipulating the physical properties of vesicles, for example, using small unilamellar vesicles composed of uncharged lipids, the RES can be avoided, circulation time increased, and tumor-targeting augmented. Various types of liposomes reported in the literature are shown in **figure 2.4** as below:

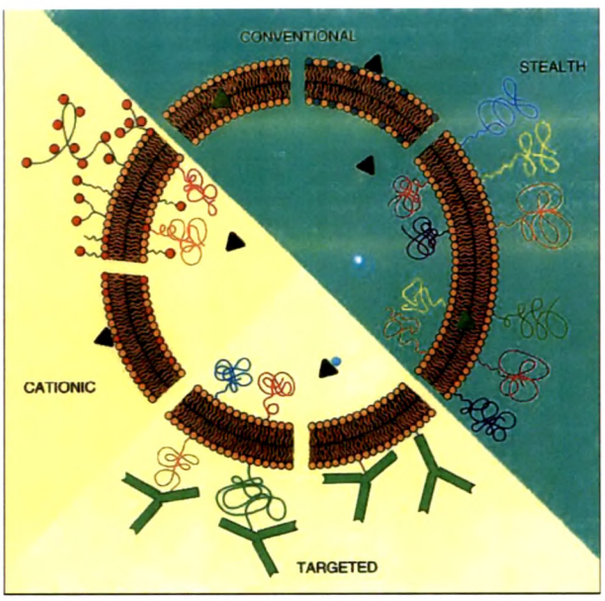


Figure 2.4: Types of liposomes -A) Conventional Liposomes B) Cationic liposomes for DNA delivery by complexation C) Stealth liposomes for intravenous delivery for prolonged circulation and D) Targeted liposomes for active targeting to a particular group of cells.

There are two types of commonly used liposomes:

1. Multilamellar vesicles (MLVs; l-5 μm)
2. Unilamellar vesicles (UVs; 0.05-0.2 μm).

Because MLVs are composed of concentric layers of PLs, the aqueous compartment is reduced, allowing for better encapsulation of lipophilic drugs. These drugs associate with the inner and outer PL membranes. When MLVs are subjected to sonication or extrusion through filters, UVs are formed. Small UVs (SUVs) measure less than 0.1 μm, whereas large UVs (LUVs) measure between 0.1 and 0.25 μm. Generally, UVs are better suited for delivery of hydrophilic drugs. Since large liposomes are rapidly sequestered by the RES, SUVs are employed to increase liposome circulation time. In general, the smaller the vesicle, the longer the circulation time. Small vesicles are utilized for slow drug release in the circulation and for non-RES targeting Liposomes have also been useful in pulmonary drug delivery systems from a delivery, targeting, prolonging the release and toxicity-limiting point of view. Administration to the lung directly can deliver the drug to the site of action and therefore avoid stability problems of IV delivery, in which liposomes may release their drug content in the blood before reaching the target site. Free

drug in solution is generally absorbed rapidly from the airways to the circulation. By retaining the drug in liposomes, it may be possible to increase the drugs contact time at the active site and retard its absorption into the bloodstream. This would mean a lower dosage requirement and a reduction in systemic toxicity associated with high levels of the drug in systemic circulation.

Liposomes are prepared by several methods as reported in the literature. The methods with promise and frequent use are summarized in the section below:

1. Thin film hydration:

The method involves formation of a thin film of lipids by evaporation of solvents under vacuum followed by hydration of film by aqueous solvent. MLVs are formed after hydration and are required to be sonicated or extruded for size reduction as per requirement. The method used versatility of lipids and drugs to be encapsulated and has been most widely cited in the literature. For delivery of DNA, the cationic liposomes are formed by incorporating a cationic lipid along with other lipids in the organic solvents followed by film formation and hydration.

2. Dehydration Rehydration:

The method is used for the hydrophilic drugs and DNA which are difficult to encapsulate within liposomes. The method involves forming the SUVs as a suspension in water by thin film hydration followed by sonication of the MLVs and mixing with the DNA. This mixture is the freeze dried and then rehydrated above glass transition temperature of lipids to form the MLVs ready to use with entrapment above 90 %. (**Figure 2.5**)

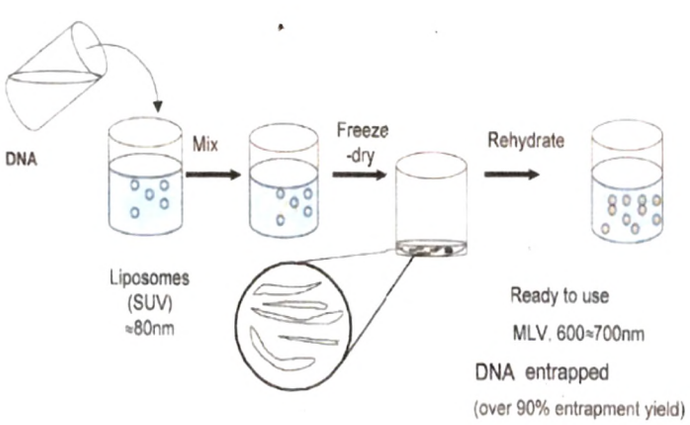


Figure 2.5: Dehydration-Rehydration method for liposome formation with superior entrapment.

3. Ethanol Injection Method:

The method involves dissolving the lipids in ethanol by slight warming and injecting the lipids at fast rate into stirring aqueous solution with temperature above glass transition temperature of lipids. The ethanol is removed under stirring or by vacuum and the liposomal concentrate is used as desired. The method generally forms SUVs with size below 200 nm and is useful for lipophillic drugs.

4. **Supercritical Fluid Technology:**

The conventional liposome development methods requires use of large quantity of organic solvents which are harmful to the environment as well as to human body and are required to be removed completely from the delivery system. Also these methods require large amount of time, energy and requires multiple instruments making it a costly affair for industrial use. Supercritical fluid (SCF) technology has been in commercial use for the past 30 years as an environmentally benign, energy- and cost- saving tool in various industries including pharmaceutical industry. SCF technology has been a promising technology for pharmaceutical industrial operations including crystallization, particle size reduction, preparation of drug delivery systems, coating, and product sterilization. It has also been shown to be a viable option in the formulation of particulate drug delivery systems, such as microparticles and nanoparticles, liposomes, and inclusion complexes, which control drug delivery and/or enhance the drug stability [U. Compella & K. Koushik (2001)]. The advantages of SCF technology include use of mild conditions for pharmaceutical processing (which is advantageous for labile proteins and peptides), use of environmentally benign nontoxic materials (such as CO_2), minimization of organic solvent use, and production of particles with controllable morphology, narrow size distribution, and low static charge. Currently, patents are pending or issued for using SCF technology to obtain particles in the size range of 5 to 2,000 nm and to improve the formulation of poorly water-soluble compounds. SCF have liquid-like densities with gas-like transport properties and moderate solvent power, which moreover can be adjusted with changes in pressure and temperature. Several precipitation methods take advantage of the possibility of tuning the properties of the SCF, or suddenly change these properties during the process with a change in pressure and temperature, for the achievement of very homogeneous supersaturation conditions which lead to the production of fine powders with a narrow particle size distribution.

SCF precipitation processes can be classified according to the role of the supercritical fluid in the process:

1. As a solvent, as in the Rapid Expansion of Supercritical Solutions (RESS) process.
2. As an anti-solvent, as in the Supercritical Anti Solvent processes (SAS) (**Figure 2.6**).
3. As a solute, as in the precipitation from Gas Saturated Solution process (PGSS).
4. As a propeller, as in the Supercritical Assisted Atomization (SAA) process.

In some SCF precipitation methods the necessity of organic solvents is completely eliminated, while in others a reduced amount of organic solvent is used, which can be completely removed from the product due to the high solubility of these solvents in SCF, therefore avoiding the contamination of the product.

In an SAS process, the organic solution is sprayed into a precipitation chamber where a supercritical fluid (anti-solvent) already exists, which causes rapid contact between the two media. This generates a higher super-saturation ratio of the solution, which results in fast nucleation and growth, and consequently creates smaller particles. An active substance and a carrier (often a polymer) dissolved in an organic solvent are sprayed together or separately in an anti-solvent. The Antisolvent expands the solvent that leads to the formation of active-substance-loaded micro-/nanoparticles.

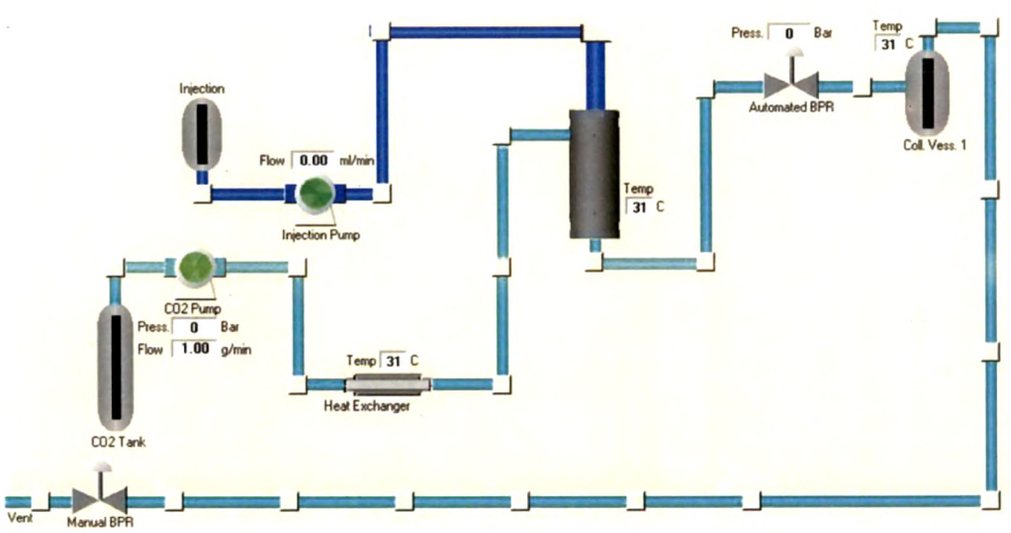


Figure 2.6: Supercritical Antisolvent Technique for Particle Formation

The liposomes with varying charge and size may be produced according to desired application by varying lipid composition, solvents used, solvent low rate, temperature of evaporating chamber, CO2 back pressure and CO2 automization pressure. These liposomes may be directly administered in vivo after reconstitution or may be complexed with DNA and used further. The liposomes have been developed or Amphotericin B [U. Kadimi et al (2007)], Docetaxel [S. Naik et al (2010)] and many more. Further, delivery of plasmids and proteins through SCF has also been researched and successfully developed [M. Tservistas et al (2001), N. Jovanovic et al (2004)].

The liposomes, especially cationic liposomes have been established as one of the most prevalent synthetic vectors for *in vitro* and *in vivo* transfection of DNA and siRNA mainly in mammalian cells in research applications as well as *in vivo* administration. These liposomes after complexing with anionic DNA base pair forms the complex termed as lipoplex [**Figure 2.7**]. These lipoplexes stabilizes the DNA by base pair condensation and protects it against extracellular stressful condition and enzymes *in vivo*. Even though, the lipoplexes are very safe as compared

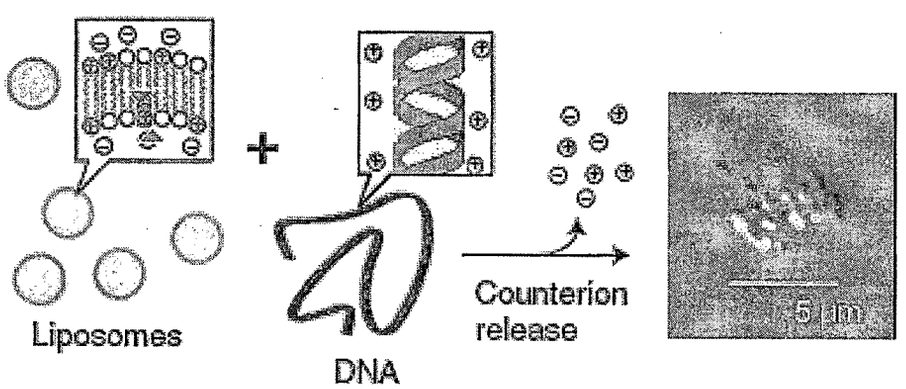


Figure 2.7: Schematic Representation of Cationic Lipid-DNA (Lipoplex) formation. [The lipid and DNA because of their opposite charge are attracted and interacts with each other. Both release their counterions to form the lipoplex]

to viral vectors, they suffer from the disadvantages of low transfection efficiency and physical instability in solution. The lower transfection occurs because of interaction of lipoplexes with anionic cell membrane, anionic serum proteins, the events leading to improper release of DNA

inside cell for expression and endosomal degradation of DNA. However, recent developments in cationic lipids as DOTAP, DC-Cholesterol, DOTMA, Cationic Gemini lipids and helper lipids as DOPE, DOPC have helped to overcome the issues to some extent and enhance the DNA expression *in vitro*. Further, use of targeting ligands and formulation approaches also helps to reach the delivery system at target site and enhance the DNA expression *in vivo*.

2.4 Chemosensitization

Recent advances in non viral gene delivery vectors have shown efficient gene transfection in tumor cells *in vitro* and *in vivo*. Recently, delivery of genes i.e. *p*DNA, siRNA and AS ODN have been attempted with nanoparticulate and liposomal vectors encapsulating the chemotherapeutic agent, thus demonstrating co-administration of drug and gene [M. Saad et al (2008), Y. Wang et al (2006)]. Co-administration of drug and gene in the same vehicle not only can improve patient compliance due to the reduced number of injections, but can also achieve synergistic therapeutic effect because both drug and gene can be delivered to the same cancer cells or tissues. The same has been true, especially in case of multiple drug resistant tumors which show incomplete cell death after chemotherapy and consequently require higher drug dose leading to side-effects [**Figure 2.8**].

These vectors have shown dual advantage of cytotoxic behavior of drug along with expression of gene for producing or blocking the desired protein for improving the cellular entry of chemotherapeutic agent and its anticancer activity though multiple mechanisms of action, thus helping to overcome the resistance. AS ODNs inhibiting PGP and BCL-2 mRNAs along with the chemotherapeutic drug doxorubicin have been delivered using cationic liposomes for effective anticancer therapy of multidrug resistant tumors [L. Xua et al (2001)]. Further, better anticancer activity after complexing siRNA to the cationic liposomal doxorubicin formulation was achieved by blocking the PGP and BCL-2 proteins [M. Saad et al (2008)]. Cationic core–shell nanoparticles, self-assembled from biodegradable amphiphilic cholesterol based copolymer encapsulating paclitaxel and coated with GFP *p*DNA has also been reported to show cell cytotoxicity and efficient GFP expression [Y.Wang et al (2006)]. A novel amphiphillic block peptide based miceller system encapsulating doxorubicin has also been reported for delivering *p*53 for synergistic anticancer activity in HepG2 cells *in vitro* [N. Wiradharma et al (2009)]. These systems have shown significant efficiency of the treatment because of simultaneous

intracellular delivery of the therapeutic agent and the biomolecules responsible for suppression of drug resistance and antiapoptotic signals.

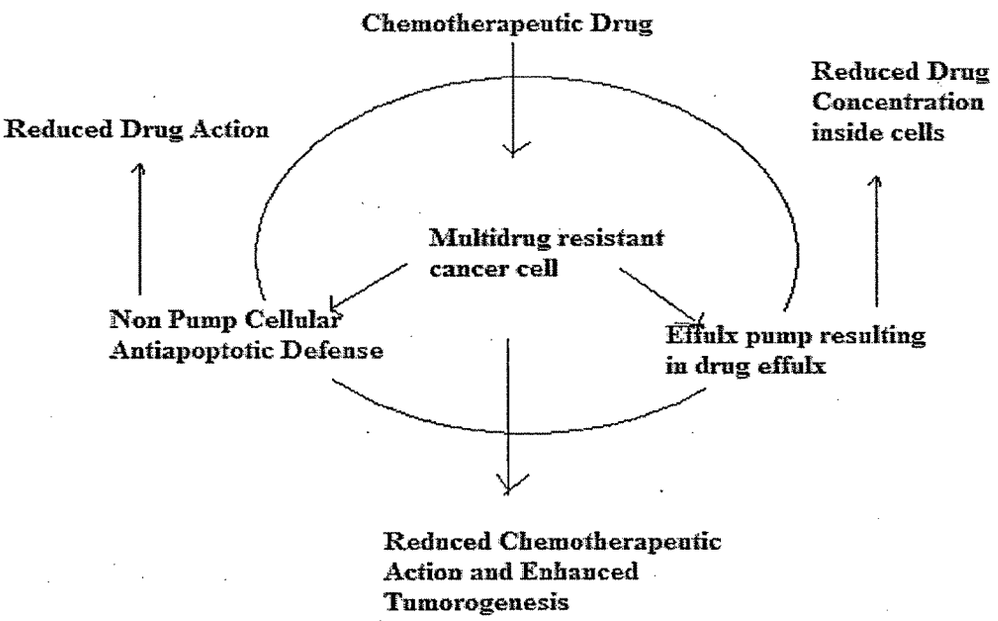


Figure 2.8: Multiple drug resistant tumor cells demonstrating poor drug action because of Pump and nonpump resistance theirby enhancing the tumorogenesis.

Another approach of enhancing the chemotherapeutic action uses the pretreatment approach. It involves pretreatment of *p*DNA and SiRNA as lipoplex to the cell line followed by drug treatment after a specified period. The pretreatment of genetic material helps to enhance the apoptosis as well as reduced the resistance developing cellular proteins theirby enhancing and sensitizing the cells towards chemotherapy. Successful sensitization of Cisplatin with reduced IC 50 values was observed after cell sensitization with As-Oligonucleotides and SiRNA blocking the human surviving protein. [S.D. Li and L.Huang (2006)]

2.5 Drug and pDNA profile:

2.5.1. pCB6+ ARG *p*53 – Tumor Suppressor Plasmid

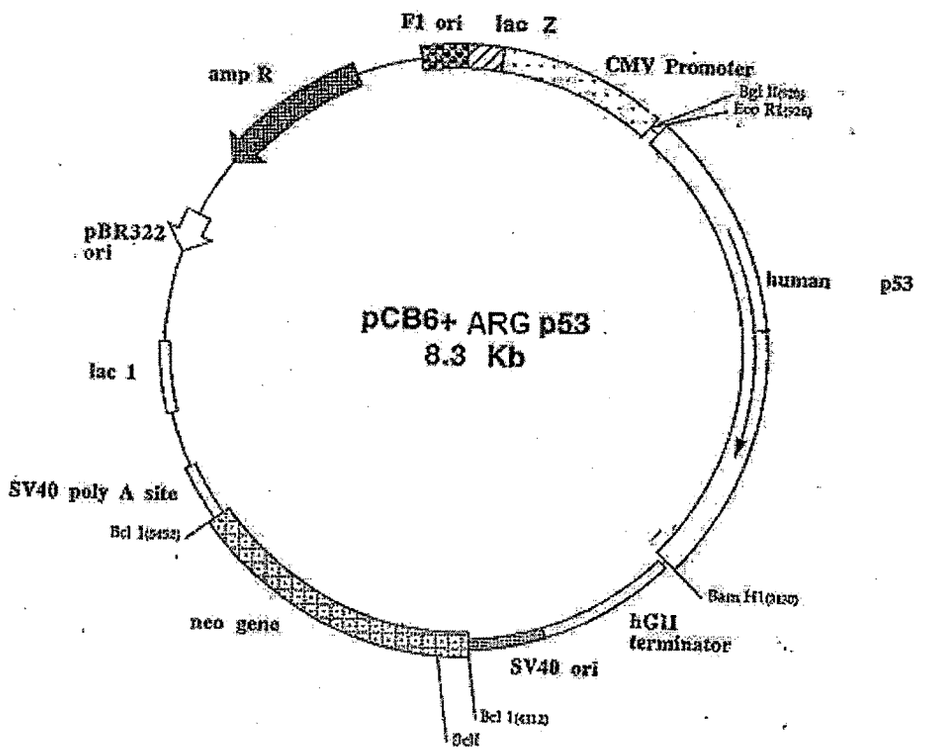


Figure 2.9 *p*53 Plasmid Map

Description:

Plasmid p53 is used as a tumor suppressor gene. The plasmid contains p53 taken from Sp53 from Mattasheski 2017 bp Eco RI Bam HI. The plasmid includes 125bp 5'UTR and 712bp 3'UTR. The plasmid contains the reporter gene p53 of human origin and a pCB6+ vector. The vector consists of Ampicillin resistance and CMV promoter for p53 expression. The Ampicillin resistance is a selection marker for transformed cells after growing in the Ampicillin containing LB plates. The plasmid has a SV40 origin of replication and is responsible for replication of the plasmid inside the bacteria.

2.5.2. pCMV-SPORT-βgal – β galactosidase Plasmid: [A marker plasmid used in Transfection studies]

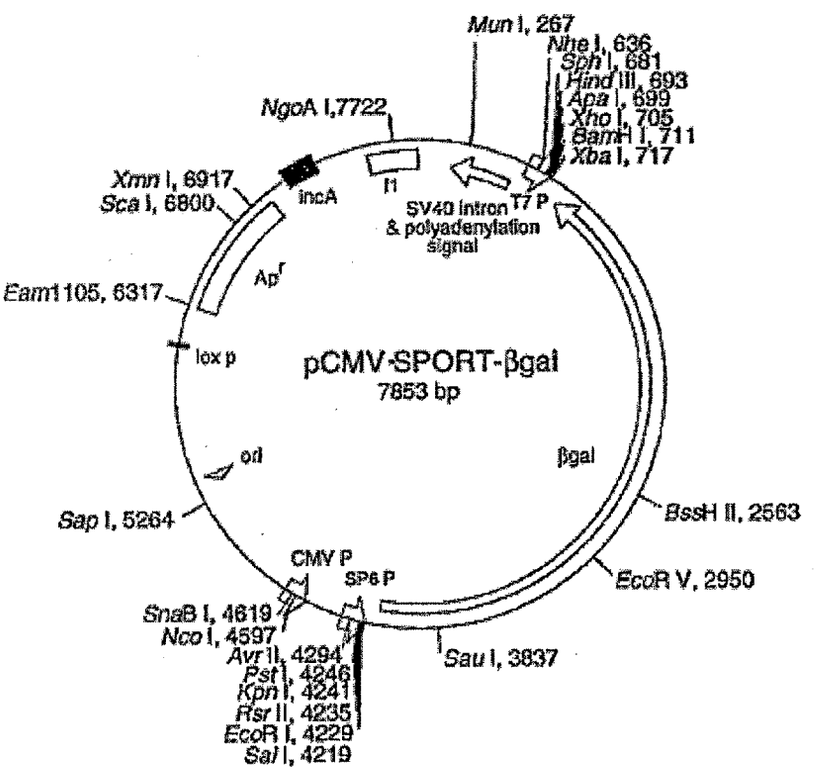


Figure 2.10 β –galactosidase Map

Description:

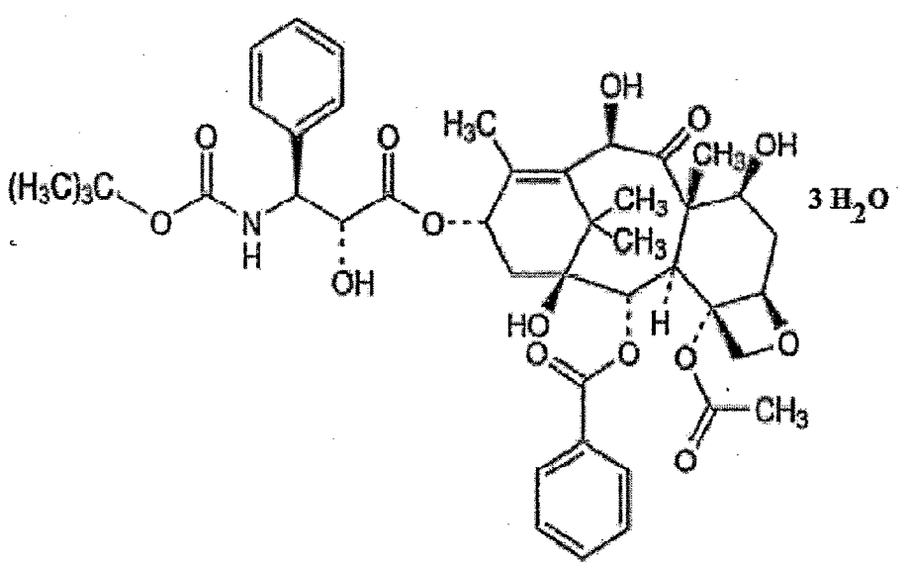
Plasmid pCMV·SPORT-bgal is used as a positive control for monitoring expression in eukaryotic cells. The plasmid contains the reporter gene b-galactosidase (b-gal) from *E. coli* cloned as a *Not* I fragment into plasmid pCMV·SPORT1. The plasmid also contains a CMV promoter upstream of the b-gal gene, followed by the SV40 t-intron and polyadenylation signal. The b-lactamase gene allows selection for ampicillin resistance in *E. coli.*

2.5.3. Docetaxel Trihydrate [Drug used for Chemosensitization]

Docetaxel (Taxotere®) is an analogue of Paclitaxel, obtained by semisynthesis from 10-deacetylbaccatin III, extracted from the needles of Eurpean Yew Tree Taxus baccata. Docetaxel exerts its cytotoxic properties by inhibiting microtubule depolymerization and promoting tubulin assembly. Docetaxel has shown excellent antitumor activity, in both *in vitro* and *in vivo* models and is more active than Paclitaxel.

Chemical name: (2R, 3S)-N-carboxy-3-phenylisoserine,N-*tert*-butyl ester, 13-este with 5(beta)-20-epoxy-1,2(alpha),4,7(beta),10(beta),13(alpha)-hexahydroxytax-11-en-9-one 4-acetate-2-benzoate, trihydrate.

Structural Formula:



Category: Antimitotic Agent – Antimicrotubular drug.

Molecular Formula: Docetaxel: $C_{43}H_{53}NO_{14}$

Docetaxel Trihydrate: $C_{43}H_{59}NO_{17}$

Molecular Weight: Docetaxel: 807.9

Docetaxel Trihydrate: 861.9

Melting Point: Docetaxel: 232

Docetaxel Trihydrate: 168.5

Appearance and Color: White to almost white powder

Solubility: Soluble in ethanol, methanol, chloroform, insoluble in water.

Mode of Action: Docetaxel acts by disrupting the microtubular network that is essential or mitotic and interphase cellular functions. It promotes the assembly of tubulin into stable microtubules and inhibits their disassembly, causing inhibition of cell division and eventual cell death. Docetaxel is a radiation sensitizing agent. It is cell cycle specific (G2/M phase). Because microtubules do not disassemble in the presence of docetaxel, they accumulate inside the cell and cause initiation of apoptosis. Apoptosis is also encouraged by the blocking of apoptosis-blocking bcl-2 oncoprotein. Both *in vitro* and *in vivo* analysis show the anti-neoplastic activity of docetaxel to be effective against a wide range of known cancer cells, cooperate with other anti-neoplastic agents activity, and have greater cytotoxicity than paclitaxel, possibly due to its more rapid intracellular uptake.

Pharmacokinetics:

1. **Disposition in the body:** Docetaxel after intravenous injection is rapidly distributed throughout the body into boy tissue and is extensively metabolized by the hepatic cytochromes of CYP3A group. Excretion is mainly in faces (75%) as one major and three minor inactive metabolites and a very low amount of unchanged drug.
2. **Protein binding :**>95 %
3. **Half life:** Half-lives or α, β, and γ phases are 4 min, 36 min and 11.1 hr respectively.
4. **Volume of distribution**: 95 to 15 L/m2 (from various studies), also reported as 113 L.
5. **Clearance**: 17-22 L/h/m2
6. **Distribution in blood**: Little interaction with red blood cells.
7. **Therapeutic concentration:** Four patients with solid tumors, both male and female, were administered with an intravenous dose of 100 mg/m2 docetaxel over 1 to 2 hrs. A peak plasma concentration of 2.41 mg/L was reached by the end of infusion. In another study, 7 patients administered with a 100 mg/m2 dose reached peak plasma concentrations of 3.67mg/L.

Toxicity: Most important dose dependant acute toxicities involved with Docetaxel are myelosupression, peripheral neurotoxicity, moderate immune suppression, febrile neutropenia, hypersensititvity reactions, fluid retentions, nausea, mouth sores and alopecia.

Indications and Usage:

1. Breast Cancer: TAXOTERE® (Docetaxel) for injection concentrate is indicated for the treatment of patients with locally advanced or metastatic breast cancer after failure of prior chemotherapy.

2. Non-Small Cell Lung Cancer: ® (Docetaxel) for injection concentrate is indicated for the treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of prior platinum based chemotherapy.

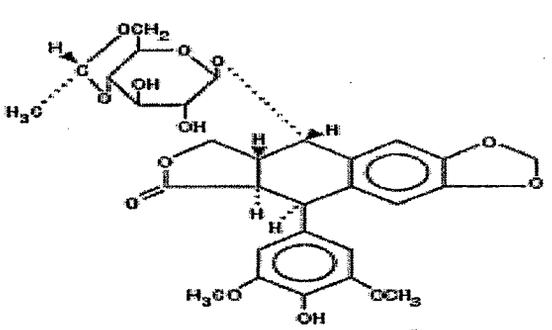
Dosage and Administration: Doses between 55 and 100 mg/m2 body surface are administered or 1 h every 3 weeks; the greater dose is the usual dose. Lower doses are given if adverse reactions are observed during treatment. Patients with hepatic impairment: 75 mg/m^2

2.5.4 Etoposide [Drug used for Chemosensitization]

Etoposide is in a class of drugs known as podophyllotoxin derivatives; and is used in treatment o neoplastic diseases. Etoposide inhibits the enzyme topoisomerase II, which aids in DNA unwinding, and by doing so causes DNA strands to break. As cancerous cells are rapidly breaking, they require higher quantity of enzyme topoisomerase.

Chemical name: 4'-demethyl-epipodophyllotoxin 9-[4,6-O-(*R*)-ethylidene-beta-D-glucopyranoside], 4' -(dihydrogen phosphate)

Structural Formula:



Category: Nucleic Acid Synthesis Inhibitors, Antineoplastic Agents, Phytogenic

Molecular Formula: $C_{29}H_{32}O_{13}$

Molecular Weight: 588.56

Melting Point 236-251^{0}C

Appearance and Color: White to almost white powder

Solubility: It is very soluble in methanol and chloroform, slightly soluble in ethanol, and sparingly soluble in water and ether. It is made more miscible with water by means of organic solvents.

Mode of Action: Etoposide inhibits DNA topoisomerase II, thereby inhibiting DNA re-ligation. This causes critical errors in DNA synthesis at the premitotic stage of cell division and can lead to apoptosis of the cancer cell. Etoposide is cell cycle dependent and phase specific, affecting mainly the S and G2 phases of cell division. With Etoposide, two different dose-dependent responses are seen. At high concentrations (10 µg/mL or more), lysis of cells entering mitosis is observed. At low concentrations (0.3 to 10 µg/mL), cells are inhibited from entering prophase. It does not interfere with microtubular assembly. The predominant macromolecular effect of etoposide appears to be the induction of DNA strand breaks by an interaction with DNA-topoisomerase II or the formation of free radicals.

Pharmacokinetics:

1. **Absorption : (Etoposide is available as IV injection as well as Oral capsules)** - Absorbed well, time to peak plasma concentration is 1-1.5 hrs. Mean bioavailability is 50%.
2. **Metabolism:** Primarily hepatic (through O-demethylation via the CYP450 3A4 isoenzyme pathway) with 40% excreted unchanged in the urine.

Enzyme	Metabolite	Reaction	K_m	V_{max}
Cytochrome P450 3A4	3'-demethyletoposide	3'-demethylation	53.9	0.25

3. **Protein binding :**>95 %
4. **Half life:** Oral: 6 h., IV: 6-12 h., IV in children: 3 h.
5. **Volume of distribution**: The mean volumes of distribution at steady state fall in the range of 18 to 29 liters or 7 to 17 L/m2.
6. **Clearance**: The mean renal clearance of etoposide is 7 to 10 mL/min/m2 or about 35% of the total body clearance over a dose range of 80 to 600 mg/m2.

7. **Excretion:** 42 to 67%, excetion through urine and 0-16 % excretion through feces. Etoposide is cleared by both renal and nonrenal processes, i.e., metabolism and biliary excretion. The effect of renal disease on plasma etoposide clearance is not known. Biliary excretion appears to be a minor route of etoposide elimination. Only 6% or less of an intravenous dose is recovered in the bile as etoposide.
8. **Distribution in blood**: Little interaction with red blood cells.

Toxicity: The most important dose dependant acute hematological toxicities involved with Etoposide administration are leucopenia, thrombocytopenia and anemia. Comparatively lower intensity of GIT disorders as nausea, vomiting, abdominal pain, anorexia, diarrhea, peripheral neurotoxicity, hypotention and allergic reactions are also observed.

Indications and Usage: Etoposide Injection is indicated in the management of the following neoplasms:

1. Refractory Testicular Tumors: Etoposide Injection in combination therapy with other approved chemotherapeutic agents in patients with refractory testicular tumors who have already received appropriate surgical, chemotherapeutic, and radiotherapeutic therapy.
2. Non Small Cell Lung Cancer: Etoposide Injection and/or capsules in combination with other approved chemotherapeutic agents as first line treatment in patients with Non small cell lung cancer.

Dosage and Administration:

Etoposide Injection: The usual dose of Etoposide Injection in testicular cancer in combination with other approved chemotherapeutic agents ranges from 50 to 100 mg/m2/day, on days 1 through 5 to 100 mg/m2/day, on days 1, 3, and 5. In non small cell lung cancer, the Etoposide Injection dose in combination with other approved chemotherapeutic drugs ranges from 35 mg/m2/day for 4 days to 50 mg/m2/day for 5 days. Dosage should be modified to take into account the myelosuppressive effects of other drugs in the combination or the effects of prior x-ray therapy or chemotherapy which may have compromised bone marrow reserve.

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