CHAPTER I

Review of Literature
Part I

Cancer, Chemotherapy and Unsaturated Fatty Acids
Cancer (medical term: malignant neoplasm) is a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize. Most cancers form a tumor but some, like leukemia, do not. Cancer affects people at all ages with the risk for most types increasing with age. Over 0.7 million new cases and 0.3 million deaths occur annually due to cancer. Data from population-based registries under National Cancer Registry Programme, India, have indicated cancers of oral cavity, lungs, oesophagus and stomach; which account for over 50% of all cancer deaths in India.

Cancer differs widely in its causes and biology. Any organism, even plants, can acquire cancer. Nearly all known cancers arise gradually, as errors build up in the cancer cell. Anything which replicates (living cells) will probably suffer from errors (mutations). Unless error correction and prevention is properly carried out, the errors will survive, and might be passed on to daughter cells. Normally, the body safeguards against cancer via numerous methods, such as: apoptosis, helper molecules (some DNA polymerases), possibly senescence, etc. Cancer is thus a progressive disease, and these progressive errors slowly accumulate until a cell begins to act contrary to its function in the organism. Genetic abnormalities found in cancer typically affect two general classes of genes. Cancer-promoting oncogenes are typically activated in cancer cells, giving those cells new properties, such as hyperactive growth and division, protection against programmed cell death, loss of respect for normal tissue boundaries, and the ability to become established in diverse tissue environments. Tumor suppressor genes are then inactivated in cancer cells, resulting in the loss of normal functions in those cells, such as accurate DNA replication, control over the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system.
During cancer development, the transformation of a normal somatic cell into a malignant tumor cell occurs via a complex multistage process involving various biochemical and genetic changes (Slaga \textit{et al.}, 1995; Greenwald, 1996). As depicted in Figure 1.1, the whole process of cancer development consists of three distinct sequences. In the first step the normal cell gets converted into a neoplastic cell (initiation), which is followed by transformation into an overt neoplasm known as ‘promotion’ phase that finally led to final progression stage (Marks & Furstenberger, 1984; DiGiovanni, 1992).

![Four Basic Stages of Cancer](image)

**Figure 1.1: Stages of cancer.**

**Hallmarks of cancer**

The vast catalog of cancer cell genotypes is a manifestation of at least six essential alterations in cell physiology that collectively dictate malignant growth as shown in Figure 1.2.

1. Self-competent growth signals
2. Tolerance to antigrowth signals
3. Apoptosis evasion
4. Limitless replicative potential
5. Sustained angiogenesis
6. Tissue invasion and metastasis

Figure 1.2: Hallmarks of cancer

Each of these physiologic changes–novel capabilities acquired during tumor development represent the successful breaching of an anticancer defense mechanism imposed by cells and tissues. These properties are shared in common by most and perhaps all types of human tumors.

Causes of cancer

From the ancient times physicians were not able to resolve the issue over induction of cancer. Of late, many causes of cancer have been discovered and documented. In 1911, Peyton Rous described a sarcoma in chickens caused by what later became known as the Rous sarcoma virus. In 1915, cancer was artificially induced in laboratory animals for the first time by coal tar, a chemical applied to rabbit skin at Tokyo University. Today we recognize and avoid many specific substances that cause cancer by inducing abnormalities in the genetic material of the transformed cells (Kinzler and Vogelstein, 2002). These abnormalities may be due to the effects of carcinogens (tobacco smoke), radiation, chemicals (coal tars, benzene, hydrocarbons, aniline and asbestos) or infectious agents. Viruses like Hepatitis virus, Herpes virus, Epstein-Barr virus, Human immunodeficiency virus (HIV) and Human papilloma viruses (HPVs) have also been reported to link with various forms of cancer. Other cancer-promoting genetic
abnormalities may randomly occur through errors in DNA replication, or are inherited, and thus present in all cells from birth. The heritability of cancers is usually affected by complex interactions between carcinogens and the host's genome. The errors/ mutations which cause cancer are often **self-amplifying**, eventually compounding at an exponential rate.

**Types of cancer**

Cancers or malignant tumors are classified by the type of cell that resembles the tumor and, therefore, the tissue presumed to be the origin of the tumor. These are the histology and the location, respectively. About two hundred distinct types of cancers have been recognized. The following are the various types of tumors of general categories:

**Carcinomas:**

Malignant tumors derived from epithelial cells of ectodermal and endodermal origin. The solid tumors in nerve tissue and tissues of body surfaces or their attached glands are examples of carcinomas. This group represents the most common cancers, including the common forms of breast, prostate, cervical, skin, lung, colon and brain carcinomas. About 85% of cancers are carcinomas.

**Sarcomas:**

Malignant tumors derived from connective tissues or mesenchymal cells, which are of mesodermal origin. They are solid tumors growing from connective tissue, cartilage, bone and muscle. Although they account for most of the cancers studied in laboratory animals, they constitute only about 2% of human cancers.

- **Lymphomas:** Malignancies derived from hematopoietic (blood-forming) cells. Lymphomas are cancers in which there is excessive production of lymphocytes by the lymph nodes and spleen. Hodgkin’s disease is an example of lymphoma. Lymphomas constitute about 5% of human cancers.
• **Leukemias:** Leukemias are neoplastic growth of leukocytes (WBC) and are characterized by excessive production of the cells. They constitute about 4% of human cancer.

• **Germ cell tumors:** Tumors derived from totipotent cells. In adults most often found in the testicle and ovary; in fetuses, babies, and young children most often found on the body midline, particularly at the tip of the tailbone; in horses most often found at the poll (base of the skull).

• **Blastic tumors or blastomas:** A tumor (usually malignant) which resembles an immature or embryonic tissue. Many of these tumors are most common in children.

**Classification of tumors**

The tumors can be classified on the basis of their aetiology, anatomy, histology, function or behaviour.

**Group I – Tumors of epithelial tissues**

If benign, the tumors could be papillomas or adenomas. If malignant, such tumors are called as carcinomas.

**Group II – Tumors of non-haemopoietic mesenchymal tissues**

Benign tumors of connective, skeletal, vascular, meningeal, and muscular tissues are described as fibroma, myxoma, lipoma, chondroma, osteoma, benign osteoclastoma, synovinoma, angiomas (haemangioma, tlymphangioma and glomangioma), and meningeinoma, leiomyoma and rhabdomyoma. A suffix sarcoma is used for malignant members of same classes corresponding to the benign counterpart, e.g. fibrosarcoma, osteosarcoma liposarcoma and haemangiosarcoma etc.

**Group III – Tumors of haemopoietic tissues**

Free circulatory or mobile cells are peculiar characteristic feature of haemopoietic tissues and their tumors. These tumors are grouped into:
**Tumors of lymphoid tissue**

(i) Follicular lymphoma  
(ii) Lymphosarcoma and lymphatic leukaemia  
(iii) Hodgkin’s disease  
(iv) Reticulosarcoma

**Group IV – Tumors of neural tissues**

These tumors include:

(A) Gliomas  
(i) Astrocytic  
(ii) Oligodendroglioma  
(iii) Medulloblastoma  
(iv) Ependymal glioma  
(v) Pinealoma  
(B) Papillary tumors of choroids plexus  
(C) Neurileoma  
(D) Neuroblastoma and ganglioneuroma  
(E) Chromaffinoma  
(F) Tumors of carotid body and allied structures  
(G) Retinal and ciliary tumors  
(H) Retinoblastoma  
(I) Dictyoma  
(J) Epithelial tumors of ciliary body

**Group V – sundry special classes of tumors**

(A) Melanoma  
(B) Chondroma  
(C) Embryonic tumors of viscera  
(i) Nephroblastoma  
(ii) Hepatoblastoma  
(iii) Embryonic tumors of other parts  
(D) Teratomas
**Prevention**

Cancer prevention is defined as active measures to decrease the incidence of cancer. Greater than 30% of cancer is preventable via avoiding risk factors including: tobacco, overweight or obesity, low fruit and vegetable intake, physical inactivity, alcohol, sexually transmitted infection, air pollution. This can be accomplished by avoiding carcinogens or altering their metabolism, pursuing a lifestyle or diet that modifies cancer-causing factors and/or medical intervention (chemoprevention, treatment of pre-malignant lesions). The epidemiological concept of "prevention" is usually defined as either primary prevention, for people who have not been diagnosed with a particular disease, or secondary prevention, aimed at reducing recurrence or complications of a previously diagnosed illness. There are two major methods of cancer prevention:

1. **Vaccination**

   Prophylactic vaccines have been developed to prevent infection by oncogenic infectious agents such as viruses, and therapeutic vaccines are in development to stimulate an immune response against cancer-specific epitopes (Bertagnolli *et al.*, 2006). As reported above, a preventive human papilloma virus vaccine exists that targets certain sexually transmitted strains of human papilloma virus that are associated with the development of cervical cancer and genital warts. The only two HPV vaccines on the market are Gardasil and Cervarix. There is also a hepatitis B vaccine, which prevents infection with the hepatitis B virus, an infectious agent that can cause liver cancer (Bertagnolli *et al.*, 2006).

2. **Chemotherapy**

   The concept that medications could be used to prevent cancer is an attractive one, and many high-quality clinical trials support the use of such chemoprevention in defined circumstances. Daily use of tamoxifen, a selective estrogen receptor modulator (SERM), typically for 5 years, has been demonstrated to reduce the risk of developing breast cancer in high-risk women by about 50%. A recent study reported that the selective estrogen receptor modulator raloxifene has similar benefits to tamoxifen in preventing breast cancer in high-risk women, with a more favorable side effect profile (Cole *et al.*,
Finasteride, a 5-alpha-reductase inhibitor, has been shown to lower the risk of prostate cancer, though it seems to mostly prevent low-grade tumors (Vogel et al., 2006). The effect of COX-2 inhibitors such as rofecoxib and celecoxib upon the risk of colon polyps have been studied in familial adenomatous polyposis patients (Thompson et al., 2003) and in the general population (Hallak et al., 2003; Baron et al., 2006). In both groups, there were significant reductions in colon polyp incidence, but this came at the price of increased cardiovascular toxicity.

**CHEMOTHERAPY** utilizes different types of drugs, natural compounds, dietary products etc. to combat the progress of cancer. Depending on the type of agents used chemotherapy can be categorized as following:

- **Chemotherapy using natural compounds**
  
a) **Diallyl sulphide (DAS):** Garlic (*Allium sativum*) has been shown to possess potential health benefits (lipid lowering, antimicrobial, chemo-preventive, and anticarcinogenic properties, for example) since the beginning of recorded history and is probably one of the most widely studied medicinal plants. The chemotherapeutic and anti-tumor activity associated with garlic has been attributed to the presence of various organosulfide-based active compounds including DAS. Laboratory investigations provide sufficient evidence that it reduces the incidence of a multitude of chemically induced lung, skin, colon, esophageal and forestomach neoplasia (Wargovich et al., 1992; Singh & Shukla, 1998, 1999; Yang et al., 2001). It has also been shown to inhibit aflatoxin B1 and NDMA induced liver preneoplastic foci in rats (Haber-Mignard et al., 1996). Several *in vitro* studies have also demonstrated its inhibitory effects on the tumor cells (Hageman et al., 1997, Hong et al., 2000). It has been reported that the topical application of DAS inhibit the development of tumors in both complete and two-stage model of mouse skin carcinogenesis (Singh & Shukla, 1998, 1999. DAS has also shown to possess antiproliferative effects on the growth of transplantable Ehrlich ascetic tumor cells and inhibit angiogenesis in Swiss albino mice (Shukla et al., 2003). A deeper insight revealed that DAS achieves its anticancer properties by modulating phase I and II detoxifying enzymes, scavenging of free radicals and abrogating their mutagenic potential (Guyonnet...
et al., 1999; Smith and Yang, 2000; Yang et al., 2001; Shukla et al., 2003; Prasad et al., 2006).

b) **Perillyl alcohol:** It has been reported to possess strong anticancer properties against several cancer types including breast, pancreatic and liver cancer (Kelloff et al., 1996; Bailey et al., 2008; Lebedeva et al., 2008). The compound is basically a member of monoterpane class of chemicals. It is a constituent of essential oil from number of plants like perilla (*Perilla frutescens*), lavendin, pepper mint, gingergrass, savin, caraway, and celery seeds etc. (Belanger, 1998).

c) **Curcumin:** Turmeric has been used historically as a component of Indian Ayurvedic medicine since 1900 BC to treat a wide variety of ailments (Aggarwal et al., 2007). Research in the latter half of the 20th century has identified curcumin as responsible for most of the biological activity of turmeric (Aggarwal et al., 2007). *In vitro* and animal studies have suggested a wide range of potential therapeutic or preventive effects associated with curcumin. *In vitro* and animal studies have suggested that curcumin may have antitumor (Aggarwal and Shishodia, 2006; Choi et al., 2006), antioxidant, antiarthritic, anti-amyloid, anti-ischemic (Shukla et al., 2008) and anti-inflammatory properties. As of now, numerous clinical trials in humans are underway, studying the effect of curcumin on numerous diseases including multiple myeloma, pancreatic cancer, myelodysplastic syndromes, colon cancer, psoriasis, and Alzheimer's disease (Hatcher et al., 2008). Curcumin acts as a free radical scavenger and antioxidant, inhibiting lipid peroxidation (Shukla et al., 2003) and oxidative DNA damage. Curcuminoids induce glutathione S-transferase and are potent inhibitors of cytochrome P450. Another 2009 study on curcumin effects on cancer states that curcumin "modulates growth of tumor cells through regulation of multiple cell signaling pathways including cell proliferation pathway (cyclin D1, c-myc), cell survival pathway (Bcl-2, Bcl-xL, cFLIP, XIAP, c-IAP1), caspase activation pathway (caspase-8, 3, 9), tumor suppressor pathway (p53, p21) death receptor pathway (DR4, DR5), mitochondrial pathways, and protein kinase pathway (JNK, Akt, and AMPK) (Ravindran et al., 2009).

➤ **Chemotherapy using synthetic drugs**
a) **Doxorubicin:** Doxorubicin (trade name Adriamycin; also known as hydroxydaunorubicin) is a drug used in cancer chemotherapy. It is an anthracycline antibiotic, closely related to the natural product daunomycin, and like all anthracyclines it works by intercalating DNA. Doxorubicin is commonly used to treat some leukemias, Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others. Acute adverse effects of doxorubicin can include nausea, vomiting, and heart arrhythmias. It can also cause neutropenia (a decrease in white blood cells), as well as complete alopecia (hair loss). Reactive oxygen species, generated by the interaction of doxorubicin with iron, can then damage the myocytes (heart cells), causing myofibrillar loss and cytoplasmic vacuolization. Due to these side effects and its red color, doxorubicin has earned the nickname "red devil" or "red death".

b) **Cisplatin:** Cisplatin, cisplatinum, or cis-diamminedichloroplatinum(II) (CDDP) is a platinum-based chemotherapy drug used to treat various types of cancers, including sarcomas, some carcinomas (e.g. small cell lung cancer, and ovarian cancer), lymphomas, and germ cell tumors. It was the first member of a class of anticancer drugs which now also includes carboplatin and oxaliplatin. These platinum complexes react in vivo, binding to and causing crosslinking of DNA which ultimately triggers apoptosis (programmed cell death). Cisplatin combination chemotherapy is the cornerstone of treatment of many cancers. Initial platinum responsiveness is high but the majority of cancer patients will eventually relapse with cisplatin-resistant disease. Many mechanisms of cisplatin resistance have been proposed including changes in cellular uptake and efflux of the drug, increased detoxification of the drug, inhibition of apoptosis and increased DNA repair (Stordal and Davey, 2007). Cisplatin has a number of side-effects like nephrotoxicity, neurotoxicity, electrolyte disturbance, nausea and vomiting that can limit its use.

c) **5-fluorouracil:** Fluorouracil (5-FU or f5U) (sold under the brand names Adrucil, Carac, Efudex and Fluoroplex) is a drug that is a pyrimidine analog which is used in the treatment of cancer since about 40 years. It works through noncompetitive inhibition of thymidylate synthase. Due to its noncompetitive nature and effects on thymidine synthesis, 5-FU is frequently referred to as the "suicide inactivator". It belongs to the
family of drugs called antimetabolites. It is typically administered with leucovorin. Some of its principal uses are in colorectal cancer, and pancreatic cancer, in which it has been the established form of chemotherapy for decades (platinum-containing drug). It is also sometimes used in the treatment of inflammatory breast cancer, an especially aggressive form of breast cancer. Side effects include myelosuppression, CNS damage, mucositis, dermatitis, diarrhea and cardiac toxicity.

d) Paclitaxel: It is a mitotic inhibitor used in cancer chemotherapy. It was discovered in a National Cancer Institute program at the Research Triangle Institute in 1967 when Monroe E. Wall and Mansukh C. Wani isolated it from the bark of the Pacific Yew tree, *Taxus brevifolia* and named it 'taxol'. A newer formulation, in which paclitaxel is bound to albumin, is sold under the trademark Abraxane. Paclitaxel is now used to treat patients with lung, ovarian, breast cancer, head and neck cancer, and advanced forms of Kaposi's sarcoma. Paclitaxel is also used for the prevention of restenosis. Paclitaxel is approved in the UK for ovarian cancer, breast cancer, lung cancer. It is also used in the treatment of Kaposi's sarcoma (Saville et al., 1995). Common side-effects include nausea and vomiting, loss of appetite, and more serious side effects such as unusual bruising or bleeding, pain/redness/swelling at the injection site, facial flushing, female infertility by ovarian damage (Ozcelik et al., 2010) and chest pain can also occur.

➢ **Chemotherapy using mono- or poly-unsaturated fatty acids**

In biochemistry, a fatty acid is a carboxylic acid with a long unbranched aliphatic tail (chain), which is either saturated or unsaturated. Most naturally occurring fatty acids have a chain of 4 to 28 carbons. The number of carbon atoms is usually even, because their biosynthesis involves acetyl-CoA, a coenzyme carrying a two-carbon-atom group. Fatty acids are produced by the hydrolysis of the ester linkages in a fat or biological oil (both of which are triglycerides), with the removal of glycerol. Fatty acids are aliphatic monocarboxylic acids derived from, or contained in esterified form in, an animal or vegetable fat, oil, or wax.
**FATTY ACIDS (FA)** can be saturated and unsaturated, depending on double bonds. They differ in length as well. Unsaturated FAs resemble saturated FAs, except that the chain has one or more double-bonds. The differences in geometry between the various types of unsaturated FAs, as well as between saturated and unsaturated FAs, play an important role in biological processes, and in the construction of biological structures (such as cell membranes). **Essential FAs (EFA)** are polyunsaturated FAs (PUFA) and are the parent compounds of the $\omega-6$ and $\omega-3$ fatty acid series, respectively. They are essential in the human diet because there is no synthetic mechanism for them. Humans can easily make saturated FAs or monounsaturated FAs with a double bond at the $\omega-9$ position, but do not have the enzymes necessary to introduce a double bond at the $\omega-3$ position or $\omega-6$ position. The essential FAs are important in several human body systems, including the immune system and in blood pressure regulation, since they are used to make compounds such as **prostaglandins**. FAs play an important role in the life and death of cardiac cells because they are essential fuels for mechanical and electrical activities of the heart (Honoré *et al.*, 1994; Landmark and Alm, 2006; Reiffel and McDonald, 2006).
POLYUNSATURATED FATTY ACIDS (PUFA) belongs to the class of simple lipids, as are FAs with two or more double bonds in cis position. The location of the first double bond, counted from the methyl end of the FA, is designated by the omega- or n-number. There are two main families of PUFA: ω-3 and n ω-6. These fatty acid families are not convertible and have very different biochemical roles. Linoleic acid (LA) and α-linolenic acid (LNA) are two of the main representative compounds, known as dietary EFA because they prevent deficiency symptoms and cannot be synthesized by humans.

Dietary PUFA have effects on diverse physiological processes impacting normal health and chronic disease, such as the regulation of plasma lipid levels, cardiovascular and immune function, insulin action, and neuronal development and visual function. Ingestion of PUFA will lead to their distribution to virtually every cell in the body with effects on membrane composition and function, eicosanoid synthesis, and signaling as well as the regulation of gene expression. Cell specific lipid metabolism, as well as the
expression of fatty acid-regulated transcription factors likely plays an important role in determining how cells respond to changes in PUFA composition.

**Sources of PUFA:** The predominant sources of ω-3 FAs are vegetable oils and fish. Other sources include nuts and seeds, vegetables and some fruits, and egg yolk, poultry, and meat, all of which collectively contribute minor quantities of n-3 fatty acids to the diet. Fish is the main source of eicosapentanoic acid (EPA) and of docosahexaenoic acid (DHA) (Simopoulos, 1986). Vegetables are the main sources of ω-6 fatty acids. The most important ω-6 fatty acid, LA, is found in large amounts in western diets in corn oil, safflower oil, sunflower oil, and soybean oil (Adam, 1989). It is plentiful in nature and found in practically all plant seeds with the exception of palm, and cocoa.

**PUFA effects on cell proliferation and signal transduction:** Preclinical studies have shown that certain PUFAs may actually enhance the cytotoxicity of several antineoplastic agents and the anticancer effects of radiotherapy. These effects are possibly mediated by incorporation of the PUFAs into cancer cell membranes, thus altering the physical and functional properties.

Many investigators have demonstrated that ω-6 and ω-3 PUFAs including linoleic acid, gamma-linolenic acid, dihomogamma-linolenic acid, arachidonic acid, alphalinolenic acid, eicosapentaenoic acid, and docosahexaenoic acid inhibit growth and are cytotoxic to cancer cells *in vitro* (Begin *et al*., 1985, 1986,1988; Hawkins *et al*., 1998; Das, 1991; Chow *et al*., 1989; Fujiwara *et al*., 1986; Finstad *et al*., 1998); that the effects are associated with the production of lipid peroxides and aldehydes; (Hawkins *et al*., 1998; Das, 1991; Chow *et al*., 1989; Fujiwara *et al*., 1986; Finstad *et al*., 1998) and that the cytotoxicity of the added PUFAs is reduced by the addition of antioxidants (Hawkins *et al*., 1998; Das, 1991; Chow *et al*., 1989; Fujiwara *et al*., 1986; Finstad *et al*., 1998). Studies with laboratory animals have also demonstrated that feeding a diet containing peroxidation products of fish oil reduces tumor growth, and that the effect is reduced by administering antioxidants (Gonzalez *et al*., 1991, 1993).
However, the effects *in vitro* are observed at PUFA concentrations (30μM and above in most studies) exceeding normal plasma free FA levels. PUFAs in culture medium undergo lipid peroxidation more readily than those of plasma or tissues because: (Esterbauer *et al.*, 1991) culture medium, compared to plasma, contains lower levels of albumin that binds free FAs (Rose and Connolly, 1990) and sequesters iron and copper that promote lipid peroxidation; (Dianzani, 1993) culture medium generally contains fewer antioxidants than plasma; (Hampton and Orrenius, 1997). PUFAs in plasma lipoproteins are protected by antioxidants within the lipoproteins; and (Hampton *et al.*, 1998) cellular PUFAs are protected from lipid peroxidation by multiple antioxidants. Additionally, growth inhibition *in vitro* does not necessarily correlate with the degree of lipid peroxidation (Falconer *et al.*, 1994) and antioxidants preventing lipid peroxidation *in vitro* do not completely reverse the effects of certain PUFAs on cell growth (Chow *et al.*, 1989; Fujiwara *et al.*, 1986; Finstad *et al.*, 1998). The impact of PUFAs on the sensitivity to antineoplastic agents has been investigated in several neoplastic cell lines of laboratory animal and human origin.

**Clinical Studies:** Baronzio *et al.*, (1998) reported an improved response to chemotherapy and radiation in patients who received 5-7 g/day of ω-3 PUFAs in combination with 2-3 g/day of unspecified antioxidants. Although these results are encouraging, it is difficult to ascribe the benefits observed to the administration of PUFAs since antioxidants may also enhance the efficacy of chemotherapy (Conklin, 2000). In addition to this interventional study, Boubnoux *et al.*, (1999) investigated the relationship between breast adipose tissue-PUFA content of 56 patients with localized breast carcinoma and the response to three cycles of chemotherapy with mitoxantrone, vindesine, cyclophosphamide, and 5-FU (47 patients), or the same chemotherapy regimen with epirubicin in place of mitoxantrone (9 patients). Twenty-six patients had a complete or partial response to chemotherapy; whereas, the remaining patients exhibited no response or tumor progression. The level of ω-3 PUFAs in adipose tissue was higher in those patients with a complete or partial response to treatment, and DHA content was significantly associated with an improved response.
**PUFAs, Oxidative Stress, and Cancer Therapies:** Supplementing the diet with PUFAs creates oxidative stress, reflected by reduced levels of antioxidants, e.g., vitamin E (Muggli, 1994), if supplementation is not accompanied by the administration of antioxidants. Oxidative stress can impact the proliferation of cancer cells by slowing cell cycle progression (prolonging the G1 phase or causing cells to enter the GO phase) and inducing cell cycle checkpoint arrest. Although these effects may slow cancer growth and progression, they may also reduce the cytotoxicity of chemotherapy and radiation. Oxidative stress has also been shown to alter the mode of chemotherapy-induced cell death, usually occurring by apoptosis following cellular damage by antineoplastic agents. (Haq and Zanke, 1998; Schmitt and Lowe, 1999) Oxidative stress inhibits drug-induced apoptosis and results in cell death by necrosis, an effect that reduces the cytotoxicity of chemotherapeutic agents, including doxorubicin, etoposide, cisplatin, and cytosine arabinoside. (Lee and Shacter, 1999; Schacter et al., 2000) Certain antioxidants have been shown to prevent the oxidative stress-induced inhibition of apoptosis by antineoplastic agents and to enhance the drugs' cytotoxicity (Schacter et al., 2000). Thus, administering antioxidants with PUFAs during chemotherapy may enhance the effectiveness of the treatment.
Part II

Nanoparticles
The past quarter century of outstanding progress in fundamental cancer biology has not translated into even distantly comparable advances in the clinic. This is largely due to the highly toxic and nonspecific nature of most cancer therapeutics, which limits their use and effectiveness \textit{in vivo}. Inadequacies in the ability to administer therapeutic moieties so that they will selectively reach the desired targets with marginal or no collateral damage has largely accounted for this discrepancy (Langer, 1998; Duncan, 2003). Most striking is the recognition that only between 1 and 10 parts per 100,000 of intravenously administered monoclonal antibodies reach their parenchymal targets \textit{in vivo} (Li et al., 2004). There are two general, synergistic goals that should be striven for to increase the efficacy per dose of any therapeutic or imaging contrast formulation: to increase its targeting selectivity (Allen, 2002) and to endow the agent(s) comprising the therapeutic formulation with the means to overcome the biological barriers that prevent it from reaching its target (Jain, 1998). An ideal therapeutic system would be selectively directed against cell clusters that are in the early stages of the transformation towards the malignant phenotype (Srinivas et al., 2002). Nanotherapeutics has the potential to actively target tumors, increasing treatment effectiveness while limiting side effects. This improved therapeutic index is one of the great promises of bionanotechnology. The realization of such a system faces formidable challenges, including the identification of suitable early markers of neoplastic disease, and understanding their evolution over time; the deployment of these markers in screening and early detection protocols; and the development of technology for the biomarker-targeted delivery of multiple therapeutic agents, and for the simultaneous capability of avoiding biological and biophysical barriers.

\textbf{NANOTECHNOLOGY,} shortened to nanotech, is the study of the control of matter on an atomic and molecular scale. Nanotechnology is the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale or one billionth of a meter. It is a multidisciplinary field, which covers a vast and diverse array of devices derived from engineering, biology, physics and chemistry. These devices include nanovectors that are themselves or have essential
components in the 1–1,000 nm dimensional range (that is, from a few atoms to subcellular size) for the targeted delivery of anticancer drugs. Nanotechnology has the potential to provide novel, paradigm shifting solutions to medical problems (Ferrari, 2005). In oncology, nanomaterials can enable targeted delivery of imaging agents and therapeutics to cancerous tissue; nanoscale devices enable multiplexed sensing for early disease detection and therapeutic monitoring. In recognition of this potential, the National Cancer Institute (NCI) launched its Alliance for Nanotechnology in Cancer in 2004 to fund and to coordinate research that seeks to apply advances in nanotechnology to the detection, diagnosis, and treatment of cancer.

Structures constructed using nanotechnology, given the general term ‘NANOPARTICLES’ are being increasingly used in various applications ranging from industry and diagnostics to medicine. One of the most promising applications of nanotechnology is in the realm of medicine and involves the development of nanoscale tools and machines designed to monitor health, deliver drugs, cure diseases, and repair damaged tissues, at the molecular level in cells and organelles. The National Institutes of Health, USA has coined the term ‘nanomedicine’ to refer to the mushrooming innovations in nanotechnology that find applications in diagnosis, treatment, monitoring, and manipulation of biological systems. Initially nanotechnology was used in the form of passive structures (such as in cosmetics), then as active structures in the form of new, more effective delivery systems. Research in nanomedicine is chiefly focused on understanding the issues related to toxicity, environmental impact of nanoscale materials and the rational delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents with nano-sized particles. Nanomedicine is now within the realm of reality starting with nanodiagnostics and drug delivery. In the last 30 years there has been an explosive growth of nanotechnology in the purview of medicine which has ushered in challenging innovations in pharmacology.

**Emerging nanoparticle therapeutics**

Informative diagnoses of the future will exploit new advances in nanotechnology in order to provide *in vitro* molecular measurements of pathophysiology from body fluids
such as blood. These advanced diagnostic methods will provide information that will allow the design of new intervention strategies, provided appropriate therapeutics are available. Nanotechnology is playing a role in providing new types of therapeutics for cancer. This nanotherapeutics has the potential to provide effective therapies with minimal side effects. Most cancer patients die from drug resistant, metastatic disease. Thus, the ultimate goal for cancer therapies would be the ability to treat this stage as well as any of those leading up to it. It is hoped that as diagnostic methods improve, treatment can be initiated at earlier stages of disease progression. However, in the most general sense, it would be advantageous to develop therapies that could be employed at all stages of cancer because of the enormous resources that are required to bring a new therapeutic to market. Targeted nanoparticles have the potential to provide therapies not achievable with any other drug modalities. By tuning the size and surface properties of the nanoparticle, manipulation of the pharmacokinetics (PK) from a systemic administration is achievable. Nanoparticles should be larger than ~10 nm to avoid single-pass renal clearance and not be positively charged to any great extent (minimizing nonspecific interactions with proteins and cells) in order to allow these PK manipulations. The particles can be tuned to provide long or short circulation times, and with careful control of size and surface properties, they can be directed to specific cell types within target organs (e.g., hepatocytes versus Kupffer cells in the liver). Other types of therapeutics, such as molecular conjugates (e.g., antibody-drug conjugates), can also meet these minimum specifications, but targeted nanoparticles are distinguished from all other therapeutic entities by at least four features:

1. The nanoparticle can carry a very large “payload.” For example, a 70-nm nanoparticle can contain ~2000 siRNA drug molecules (Bartlett and Davis, 2007) whereas antibody conjugates have<10 (Song et al., 2005). The nanoparticle payloads are located within the particle and do not participate in the control over PK and biodistribution. In molecular conjugates, by contrast, the type and number of therapeutic entities conjugated to the targeting ligand (e.g., an antibody) significantly modify the overall properties of the conjugate.

2. Nanoparticles are sufficiently large to contain multiple targeting ligands that can provide multivalent binding to cell surface receptors (Hong et al., 2007). Nanoparticles
have two parameters for tuning the binding to target cells: (a) the affinity of the targeting moiety and (b) the densities of the targeting moiety. Thus, the repertoire of molecules that can be used as targeting agents is greatly expanded, since many low-affinity ligands can be installed on nanoparticles to create higher affinity via multivalent binding to cell surface receptors.

3. Nanoparticles are sufficiently large to accommodate multiple types of drug molecules. Numerous therapeutic interventions can be simultaneously applied with a nanoparticle in a controlled manner.

4. Nanoparticles bypass multidrug resistance mechanisms that involve cell surface protein pumps, e.g., glycoprotein P, because they enter cells via endocytosis. These properties provide the opportunity to create therapeutic strategies not possible with non-nanoparticle drugs. A controlled combination of these features can minimize side effects while enhancing drug efficacy, and offers the potential to treat drug-resistant disease if the resistance is from cell surface pumps. Clinical results are emerging that suggest nanoparticle therapeutics will lead to new methods of treatment for cancer.

**Nanoparticles in drug delivery system offer many advantages over free drugs:**
- protect the drug from premature degradation;
- prevent drugs from prematurely interacting with the biological environment;
- enhance absorption of the drugs into a selected tissue (for example, solid tumour);
- control the pharmacokinetic and drug tissue distribution profile;
- improve intracellular penetration.

**For rapid and effective clinical translation, the nanoparticles should:**
- be made from a material that is biocompatible, well characterized, and easily functionalized;
- exhibit high differential uptake efficiency in the target cells over normal cells (or tissue);
- be either soluble or colloidal under aqueous conditions for increased effectiveness;
- have an extended circulating half-life, a low rate of aggregation, and a long shelf life.
Passive and active targeting using nanoparticles

Nanocarriers encounter numerous barriers en route to their target, such as mucosal barriers and non-specific uptake (Alonso, 2004; Couvreur and Vauthier, 2006). To address the challenges of targeting tumours with nanotechnology, it is necessary to combine the rational design of nanocarriers with the fundamental understanding of tumour biology. General features of tumours include leaky blood vessels and poor lymphatic drainage. Whereas free drugs may diffuse nonspecifically, a nanocarrier can extravasate (escape) into the tumour tissues via the leaky vessels by the EPR effect (Matsumura and Maeda, 1986). The increased permeability of the blood vessels in tumours is characteristic of rapid and defective angiogenesis (formation of new blood vessels from existing ones). Furthermore, the dysfunctional lymphatic drainage in tumours retains the accumulated nanocarriers and allows them to release drugs into the vicinity of the tumour cells. Experiments using liposomes of different mean size suggest that the threshold vesicle size for extravasation into tumours is ~400 nm (Yuan et al., 1995), but other studies have shown that particles with diameters <200 nm are more
effective (Hobbs et al., 1998; Torchilin, 2005). Although passive targeting approaches form the basis of clinical therapy, they suffer from several limitations. Ubiquitously targeting cells within a tumour is not always feasible because some drugs cannot diffuse efficiently and the random nature of the approach makes it difficult to control the process. This lack of control may induce multiple-drug resistance (MDR) - a situation where chemotherapy treatments fail patients owing to resistance of cancer cells towards one or more drugs. One way to overcome these limitations is to programme the nanocarriers so they actively bind to specific cells after extravasation. This binding may be achieved by attaching targeting agents such as ligands — molecules that bind to specific receptors on the cell surface — to the surface of the nanocarrier by a variety of conjugation chemistries (Torchilin, 2005). Nanocarriers will recognize and bind to target cells through ligand–receptor interactions, and bound carriers are internalized before the drug is released inside the cell (Fig 1.2). In general, when using a targeting agent to deliver nanocarriers to cancer cells, it is imperative that the agent binds with high selectivity to molecules that are uniquely expressed on the cell surface.

**Types of nanoparticles**

The age of nanostructural delivery systems began with the development of liposomes by Bangham (1965). Since then a large number of nanoparticulate systems have been developed and as of this day the sheer number and types of nanoparticulate structures that have been already developed or being researched upon is tremendous. The concept of the 'magic bullet' proposed a century ago by Nobel laureate Paul Ehrlich came to reality with the recent appearance of several approved forms of drug-targeting systems for the treatment of certain cancer and serious infectious diseases. A recent example is the launch of the FDA-approved breast-cancer drug Abraxane (American Pharmaceuticals, U.S.A). Furthermore many other nanomedicinal formulations for treatment of skin disorders and infections are in the pipeline.
A vast array of different types of nanoparticles, of different shapes and sizes and composed of an assortment materials, and with various chemical and surface properties, has already been constructed. These nanoparticulate systems comprise a variety of constructs: nanospheres, nanocapsules, lipid nanoparticles, microemulsions, macromolecular complexes, ceramic nanoparticles and vesicular carriers like liposomes, eschreosome and niosomes. Progress in nanotechnology is very dynamic and new systems continue to be developed. Some more common general classes of nanoparticulate delivery systems and their functions are listed below:

Figure 1.6: Types of nanoparticles for biomedical applications in targeting cancer
(i) **Bucky balls and Carbon tubes:** These are carbon based lattice like, potentially porous molecules and are grouped as fullerene class of structures. Bucky balls are spherical while carbon tubes are cylindrical. In nanomedicine, carbon tubes have been used as carriers for vaccines, drugs and other molecules.

(ii) **Nanoshells:** Nanoshells also called core-shells are few nanometers thick spherical cores composed of a particular compound surrounded by a shell or outer coating of another compound. Their ability to absorb at biologically useful wavelengths, depending on the shell thickness justifies their use in nanomedicine. For example silica is used to form the core and some sticky compound to adhere gold particles as the outer shell. Such nanoshells can be injected into a tumor, followed by application of radiation whereby nanoshells heat up enough to kill the tumor cells.

(iii) **Dendrimers:** Dendrimers are highly branched structures having hook-like structures on their surfaces that can be used to attach cell-identification tags, fluorescent dyes, enzymes and other molecules. Dendrimers are of two basic structural types-

   a) Globular with branches radiating from a central core.

   b) A series of highly branched polymers with no central core.

   Nanomedical applications for dendrimers are many and include nanoscale catalysts and reaction vessels, micelle mimics, imaging agents and chemical sensors, and agents for delivering drugs or genes into cells.

(iv) **Quantum dots:** Also known as nanocrystals, quantum dots behave as semiconductors emitting light in the entire visible light spectrum. These nanostructures confine conduction band electrons, valence band holes, or excitons in all three spatial directions. Examples of quantum dots are semiconductor nanocrystals and core-shell nanocrystals, where there is an interface between different semiconductor materials. They are used for cell labelling and imaging, particularly in cancer imaging studies.

(v) **Superparamagnetic nanoparticles:** These nanoparticles are attracted to a magnetic field but do not retain residual magnetism after the field is removed. Nanoparticles of iron oxide with diameters in the 5-100 nm range have been used for
selective magnetic bioseparations. Typical techniques involve coating the particles with antibodies to cell-specific antigens, for separation from the surrounding matrix. Used in membrane transport studies, superparamagnetic iron oxide nanoparticles (SPION) are applied for drug delivery and gene transfection. Targeted delivery of drugs, bioactive molecules or DNA vectors is dependent on the application of an external magnetic force that accelerates and directs their progress towards the target tissue. They are also useful as MRI contrast agents.

(vi) **Nanorods:** Nanorods are usually 1-100nm in length, and are most often made from semiconducting materials like small cylinders of silicon, gold or inorganic phosphate. They are used as imaging and contrast agents.

(vii) **Cross-linked micelles:** Micelles are self-forming particles that are prepared from individual surfactant molecules that have a water-loving and a water-hating component. The surfactant molecules orientate themselves to form spheres where the water-loving component is on the outside and in contact with the water. The water-hating component remains inside the sphere, preferring to interact with itself rather than with the water. This hydrophobic core makes them ideal carrier systems for water-hating small molecules. Crosslinked micelles are similar. However, they are held in their configuration much more tightly and are therefore more robust. Typically prepared in the size range 5–50nm, they offer more protection than conventional micelle systems do. Size and physicochemical properties can be manipulated by varying the composition or molecular weight of the surfactant.

(viii) **Solid lipid nanospheres:** Prepared by homogenisation of a melted lipid in an aqueous surfactant solution, these colloidal carrier systems have good bioavailability, stability and low toxicity. The inclusion of an active or other small molecule does not affect the particle stability or particle size, which can be between 10nm–2mm. The matrix can release the incorporated active/chemical entity either by heating the matrix or by rubbing the nanospheres on to a material such as human skin or fabric.
(ix) **Nanocapsules:** Liquid-filled capsules can be prepared with an aqueous or organic core to enable solubilization, stability and protection of compounds in a cross-linked polymer matrix. These compounds can be released by the rupture of the nanocapsule membrane in a burst profile.

(x) **Microgels:** Materials that respond to subtle changes in external stimuli may be described as “intelligent” or “smart”, for example, colloidal microgels. Acting like microsponges when held in dispersion, they undergo a conformational change as a function of temperature, pH, ionic strength and solvency. They can exhibit as much as a fourfold change in particle volume as they shrink and swell in response to environmental conditions. These microsponges, with their responsive porous network, can be used for a variety of applications, including drug adsorption (poorly soluble drugs) and drug protection (sensitive to pH extremes), as well as drug delivery. These nano-particulate systems provide a vehicle to deliver a number of actives or volatile components. The range of commercial applications of these materials is expanding, as is this area of research.

(xi) **Vesicular systems:** Vesicular systems are highly ordered assemblies of one or several concentric bilayers that are formed when certain amphiphilic building blocks are dispersed in water. The commonly used vesicular systems include liposomes, niosomes, transferosomes, pharmacosomes, ISCOMS, etc. These vesicles can be formed from a diverse range of amphiphilic building blocks. Vesicular carriers are used extensively in the pharmaceutical and cosmetic industries because of their capacity for entering into and breaking down inside cells. Liposomes have the distinction of being the first engineered nanoparticles used for drug delivery. However their affinity to fuse together in aqueous environments and release entrapped material has lead to devising of measures to stabilize them or replacing them with alternative nanoparticles made from more stable materials.

The basic structure of vesicular systems is exemplified by liposomes and niosomes. These vesicles comprise a bilayered membrane enclosing an aqueous core. The walls of the vesicles consist of amphiphilic molecules in a bilayer conformation. In an excess of water these amphiphilic molecules can form one (unilamellar vesicles) or more
(multilamellar vesicles) concentric bilayers. Hydrophilic drugs can be entrapped into the internal aqueous compartment, whereas amphiphilic, lipophilic and charged hydrophilic drugs can be associated with the vesicle bilayer by hydrophobic and/or electrostatic interactions. The thickness of the membrane (phospholipid bilayer) measures approximately 5 to 6nm. Liposomes and niosomes can be categorized on the basis of their size (shown in Table I) which is also the most widely accepted terminology.

The earliest nanovectors for drug delivery were liposomes, and the first cancer specific nanotherapeutic was DOXIL, a liposomal formulation of doxorubicin approved by the FDA in the mid-1990s for treatment of Kaposi’s sarcoma and now also indicated for the treatment of refractory breast and ovarian cancer. The use of a liposomal cage improved the pharmacokinetic profile of the hydrophobic doxorubicin, promoting accumulation at the tumor site and probably also triggering enhanced permeability and retention in the tumor’s leaky vasculature.

**Table I: Types of vesicular systems in terms of size**

<table>
<thead>
<tr>
<th>Type</th>
<th>Specifications</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLV</td>
<td>Multilamellar large vesicles</td>
<td>&gt;0.5 μm</td>
</tr>
<tr>
<td>MVV</td>
<td>Multivesicular vesicles</td>
<td>0.1-20 μm</td>
</tr>
<tr>
<td>OLV</td>
<td>Oligolamellar vesicles</td>
<td>0.1-1.0 μm</td>
</tr>
<tr>
<td>SUV</td>
<td>Small unilamellar vesicles</td>
<td>20-100 nm</td>
</tr>
<tr>
<td>LUV</td>
<td>Large unilamellar vesicles</td>
<td>&gt; 100 nm</td>
</tr>
<tr>
<td>GUV</td>
<td>Giant unilamellar vesicles</td>
<td>&gt; 1 μm</td>
</tr>
</tbody>
</table>

**Characteristics of Nanoparticles**

Nanoparticles possess different types of features that determine their role in drug delivery system. Underlying Table II shows various structures of nanoparticles and their probable role in drug delivery in cancer therapy.
Table II: Characteristics of nanoparticles used for cancer drug delivery

<table>
<thead>
<tr>
<th>Structure</th>
<th>Size</th>
<th>Role in drug delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon magnetic Nanoparticles</td>
<td>40-50 nm</td>
<td>For drug delivery and targeted cell destruction</td>
</tr>
<tr>
<td>Dendrimers</td>
<td>1-20 nm</td>
<td>Holding therapeutics substances such as DNA in their cavities</td>
</tr>
<tr>
<td>Ceramics Nanoparticles</td>
<td>~35 nm</td>
<td>Accumulate exclusively in the tumor tissue and allow the drug to act as sensitizer for photodynamics therapy without being released</td>
</tr>
<tr>
<td>Chitosan Nanoparticles</td>
<td>110-180 nm</td>
<td>High encapsulation efficiency. <em>In vitro</em> release studies show a burst effect flowed by a slow and continuous release.</td>
</tr>
<tr>
<td>Liposomes</td>
<td>20-25 nm</td>
<td>A new generation of liposomes that incorporate fullerenes to deliver drug that are not water soluble, that tend to have large molecules</td>
</tr>
<tr>
<td>Low Density Lipoprotein</td>
<td>20-25 nm</td>
<td>Drug solublized in the lipid core or attached to the surface</td>
</tr>
<tr>
<td>Nanoemulsions</td>
<td>20-25 nm</td>
<td>Drug in oil/or in liquid phases to improve absorption</td>
</tr>
<tr>
<td>Nanolipospheres</td>
<td>25-50 nm</td>
<td>Carrier incorporation of lipophilic and hydrophilic drugs</td>
</tr>
<tr>
<td>Nanoparticles composites</td>
<td>~40 nm</td>
<td>Attached to guiding molecules such as Mabs for targeted drug delivery</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>25-200 nm</td>
<td>Act as continuous matrices containing dispersed or dissolved drug</td>
</tr>
<tr>
<td>Nanopill/Micelle</td>
<td>20-45 nm</td>
<td>Made for two polymer molecules-one water repellent and the other hydrophobic-that self assemble into a sphere called a micelle that can deliver drugs to specific structures within the cell</td>
</tr>
<tr>
<td>Nanospheres</td>
<td>50-500 nm</td>
<td>Hollow ceramic nanospheres created by ultrasound</td>
</tr>
<tr>
<td>Nanovesicles</td>
<td>25-3000 nm</td>
<td>Single or multilamellar bilayer spheres containing the drugs in lipids</td>
</tr>
<tr>
<td>--------------------</td>
<td>------------</td>
<td>---------------------------------------------------------------------</td>
</tr>
<tr>
<td>Polymer Nanocapsules</td>
<td>50-200 nm</td>
<td>Used for enclosing drugs</td>
</tr>
</tbody>
</table>

**LIPOSOMES:** A liposome is a tiny bubble (vesicle), made out of the same material as a cell membrane (lipid bilayer). Liposomes or phospholipids vesicles are self-assembled colloidal particles that occur naturally and can be prepared artificially as shown by Bangham et al. in mid 1960’s. Liposomes are microscopic spherical particles in which membranes, consisting of one or more lipid bilayers, encapsulate a fraction of the solvent in which they are suspended into their interior. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. The size of liposomes ranges from 50nm to several µm. They are uni-multilamellar depending on the method of preparation.

![Figure 1.7: Unilamellar liposome showing entrapped drug for delivery](image)

Liposomes can be composed of naturally derived phospholipids with mixed lipid chains (like egg phosphatidylethanolamine), or of pure surfactant components like DOPE (dioleoylphosphatidylethanolamine). Liposomes usually, but not by definition, contain a core of aqueous solution (Stryer, 1981); lipid spheres that contain no aqueous material are called micelles, however, reverse micelles can be made to encompass an aqueous environment.
The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. A liposome can be formed at a variety of sizes as uni-lamellar or multi-lamellar construction, and its name relates to its structural building blocks, phospholipids, and not to its size. In contrast, the term Nanosome does relate to size and was coined in the early 1990s to denote special liposomes in the low nanometer range; liposome and Nanosome are not synonyms. A liposome does not necessarily have lipophobic contents, such as water, although it usually does. Liposomes were first described by British haematologist Dr. Alec D. Bangham (1965), at the Babraham Institute, Cambridge. They were discovered when Bangham and R. W. Horne were testing the Institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the plasmalemma was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure.

Liposomes are used for drug delivery due to their unique properties. A liposome encapsulates a region on aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be (indiscriminately) delivered past the lipid bilayer. There are three types of liposomes - **MLV** (multilamellar vesicles) **SUV** (Small Unilamellar Vesicles) and **LUV** (Large Unilamellar Vesicles). These are used to deliver different types of drugs.

Another interesting property of liposomes is their natural ability to target cancer. The endothelial wall of all healthy human blood vessels is encapsulated by endothelial cells that are bound together by tight junctions. These tight junctions stop any large particle in the blood from leaking out of the vessel. Tumour vessels do not contain the same level of seal between cells and are diagnostically *leaky*. This ability is known as the Enhanced Permeability and Retention effect. Liposomes of certain sizes, typically less
than 400 nm, can rapidly enter tumour sites from the blood, but are kept in the bloodstream by the endothelial wall in healthy tissue vasculature. Anti-cancer drugs such as Doxorubicin (Doxil), Camptothecin and Daunorubicin (Daunoxome) are currently being marketed in liposome delivery systems.

**Different types of liposome from different sources**

*a) Fusogenic liposomes*

Since the inception of the concept of using liposomes as antigen carriers, numerous attempts have been made to develop liposome based particulate delivery systems. Among various strategies employed to improve liposome mediated antigen delivery, however, the fusogenic-liposome based vaccines remained more convincing approach to deliver antigens to the proteasome machinery through membrane-membrane fusion with the phagolysosome membrane of the target cells (Reddy *et al.*, 1991; Owais *et al.*, 2001; Owais and Gupta, 2000), that eventually leads to MHC class I mediated presentation of the antigen ensuing specific CTLs generation. One method for imparting fusogenicity to liposomes is to incorporate charged phospholipids in their preparation; thereby positively charged liposomes are capable of delivering encapsulated soluble antigens to the cytosol for class I MHC presentation unlike neutral lipid liposomes (Nakanishi *et al.*, 1997). Moreover, apart from intrinsic fusogenic property of the lipids, various glycoproteins such as hemagglutinin (HA) of influenza virus and fusion protein (F-protein) from Sendai virus, which are responsible for the entry of virus into the host cells, have also been used for imparting fusogenicity to the conventional liposomes (Kunisawa *et al.*, 2001).

**Escheriosomes**

They are liposomes prepared from polar lipids extracted from *Escherichia coli*. Such kinds of delivery vehicles have been shown to elicit high cytotoxic T lymphocyte (CTL) responses. Escheriosomes have shown to deliver their entrapped molecules right in to the cytosol of the APCs (Antigen Presenting Cells) that leads to the processing of entrapped antigen via endocytic pathway leading to the antigen presentation by MHC
Class I mode. Expression via MHC class I molecules results in CD8⁺ T cell activation (Faisal et al., 2003; Sharma et al., 2006a and 2006b).

**Virosomes**

The interest to analyze the adjuvant effect of liposomes on one hand, and curiosity to know morphological and immunological aspects of influenza virus, on the other, led to the creation of the first so-called virosomes (Almeida et al., 1975). The name itself reflects the structural similarities between the viral liposomes and the actual influenza virus particles. The fusion potential of influenza virosoome is based on the major viral envelop glycoprotein hemagglutinin (HA) and neuraminidase (NA) in which HA acts as a targeting device as it binds to sialic acid residues present on APCs and directs a passage for viral entry into the host cells (Matlin et al., 1981; Skehel and Wiley, 2000). After endocytic uptake, the acidic environment in the endosome induces a conformational change in the HA component of the virosomes that leads to the fusion of the virosomes with the endocytic membrane. Keeping in view the significance of HA conformation in the fusion mechanism, the protocols for influenza virosoome preparation have been modified to conserve the properties of HA (Stegmann et al., 1987). The virosomes thus produced retain the receptor binding and membrane fusion activity of the native virus, by preserving the conformational integrity of the viral HA. These functionally reconstituted influenza virosomes have the capacity to deliver encapsulated macromolecules to the cytosol of the target cells (Schoen et al., 1993; Bron et al., 1994). An additional advantage of influenza derived virosomes is that HA may also activate immune system of the host thereby acting as a potential adjuvant (Watts, 1997). Beside their use as antigen delivery systems, Sendai virosoome mediated delivery of anti-tumor drugs facilitated total disappearance of S-180 tumors from the abdominal cavity without any side effects (Mizuguchi et al., 1996a & 1996b).

**Archaeosomes**

Liposomes prepared from polar archaebacterial glycerolipids (archaeosomes) have been shown to induce strong adjuvant action in mammals. Membranes of archaebacteria are reported to contain lipids that are chemically distinct from that of
eukaryotic or other prokaryotic organisms. The saturated, branched C-20, C-25 and C-40 phytanyl chains form liposomes with unique properties in context to physical and chemical stability and uptake by APCs (Patel and Sprott, 1999) and it was speculated that such properties of lipids are the possible factor for observed adjuvanticity to occur (Sprott et al., 2004). Immune responses comparable to immunization with CFA and superior to conventional liposomes have been reported after immunization with archaeosomes (Krishnan et al., 2000b; Conlan et al., 2001). Authors have shown the potential of liposomes composed of archaeabacterial lipids of various archaeabacteria in evoking CTL as well as antibody responses to their entrapped antigens (Krishnan et al., 2000a; 2001 & 2003). In these studies, ether glycerolipids extracted from various archaeabacteria were formulated into liposomes and mice of varying genetic backgrounds, immunized via various parenteral routes with archaeosomes containing BSA demonstrated markedly enhanced serum anti-BSA antibody titers. These titers were often comparable to those achieved with CFA and considerably more than those with alum or conventional liposomes (PC/PG/chol, 1.8:0.2:1.5 molar ratios). Furthermore, antigen-specific IgG1, IgG2a, and IgG2b isotype antibodies were all induced. Apart from BSA, encapsulation of OVA or hen egg lysozyme within archaeosomes showed similar immune responses (Krishnan et al., 2000b). Moreover, antigen-archaeosome immunizations induced strong cell-mediated immune response as evident from antigen-dependent proliferation and substantial production of both Th1 (IFN-γ) as well as Th2 (IL-4) cytokine responses. In contrast, conventional liposomes induced little cell-mediated immunity, whereas alum stimulated IL-4 response only. Furthermore, archaeosome-entrapped listeria antigen elicited rapid and prolonged specific immunity against L. monocytogenes in the mice model (Conlan et al., 2001). In this regard, superiority of tested archaeosomes to conventional liposomes (made up of PC/PG/chol) further emphasizes significant contribution of unique features of archaeosomes in antigen delivery.

**Yeast lipid liposomes**

An interesting correlation between the plasma membrane lipid composition of the living organisms and their generation time can be made. For example, bacteria such as *Escherichia coli*, *Bacillus megaterium* and *Bacillus subtilis* have preponderance of
anionic lipids viz. PG and DPG (in combination of PE) in their plasma membranes and have very short generation time of the order of 20-25 minutes (Jain, 1988; Rattray, 1988). On the other hand, membranes of relatively more evolved *Saccharomyces cerevisiae* or *Candida albicans* have greater variety of phospholipids with lower percentage of anionic lipids (e.g. PG, PI, PS, DPG) and the organisms have a generation time of approximately two hours. Since both the classes of organisms multiply by binary fission, it can be presumed that the presence of anionic lipids facilitates the fusion of the membranes essential for high duplication rates (Owais and Gupta, 2000). Unlike the lower organisms, the more evolved eukaryotes have neutral phospholipids as major membrane components and have generation times of the order of days (cf. ~22 hrs). The plasma membrane of lower organisms is mainly composed of amino phospholipids along with cardiolipin and PG. The eukaryotic plasma membrane lipid composition is different from that of lower organisms and earlier work revealed the composition and distribution of phospholipids in eukaryotic cells (Kumar and Gupta, 1983). They contain all classes of phospholipids, distributed in a set fashion in the two leaflets of the bilayer. The amino-phospholipids are mainly confined to the inner leaflet and play a major role in exocytosis, which involves membrane-membrane fusion.

Apart from these fusogenic liposomes used as antigen delivery vehicles, few reports are available on fusogenic vesicles that belong to other classes of vesicle family (e.g. niosomes, transferosomes, proteosomes etc.).

**NIOSOMES** are small unilamellar vesicles made from non-ionic surfactants. Hence, they are also called non-ionic surfactant vesicles (NISV) (Brewer and Alexander, 1994) or Novasomes (Gupta et al., 1996). The chemical stability of niosomes is higher than the stability of conventional phospholipid liposomes. These are small unilamellar vesicles made from non-ionic surfactants. They are also called non-ionic surfactant vesicles (NISV) or Novasomes (Brewer and Alexander, 1994; Gupta et al., 1996). Niosomes are lamellar structures that are microscopic in size. They constitute of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. The surfactant molecules tend to orient themselves in such a way that the hydrophilic ends of the non-ionic surfactant point outwards, while the hydrophobic ends
face each other to form the bilayer. Niosomes are novel drug delivery systems that are
finding application in: 1) Drug Targeting 2) Antineoplastic Treatment 3) Leishmaniasis
Treatment 4) Delivery of Peptide Drugs 5) Studying immune response 6) Carriers for
haemoglobin 7) Transdermal drug delivery systems. BSA in niosomes was shown to be
as immunogenic as BSA with Complete Freund’s adjuvant (Brewer and Alexander,
1992). The chemical stability of niosomes is higher than the stability of phospholipid
liposomes. Non-ionic surfactant vesicles have clearly demonstrated their ability to
function as adjuvants following parenteral administration with a number of different
antigens and peptides such as OVA (Brewer et al. 1996), BSA (Brewer and Alexander
1992), a synthetic measles peptide (Roberts et al. 1996b). Results from these studies have
suggested that the immune responses preferentially activated by NISV are of the Th1-
type, and this is particularly useful for vaccines directed against intracellular pathogens,
which generally require cell mediated immune responses. NISV have been reported to
possess extremely low toxicity and enhanced stability hence they appear to be excellent
candidates for further development as vaccine adjuvants.

Doxorubicin, the anthracyclic antibiotic with broad spectrum anti tumor activity,
shows a dose dependent irreversible cardio toxic effect. Niosomal delivery
of this drug to mice bearing S-180 tumor increased their life span and decreased
the rate of proliferation of sarcoma. Niosomal entrapment increased the half-life of the
drug, prolonged its circulation and altered its metabolism. Intravenous administration of
methotrexate entrapped in niosomes to S-180 tumor bearing mice resulted in total
regression of tumor and also higher plasma level and slower elimination (Chandraprakash
et al., 1992).

**Transferosomes** are ‘ultra deformable’ liposomes with enhanced skin penetrating
properties. These vesicles consist of PC/cholate (9:2 molar ratio). Studies with model
antigen have shown that transfersomes have an upper hand than conventional liposomes
when administered epicutaneously (Paul and Cevc, 1995).

**Cochleates** are another exception in the liposome group because they are non-vesicular
bilayer sheets consisting of PE/PS/chol. Calcium ions are added which intercalate with
the bilayers. This results in a rolled-up bilayer sheet without internal volume. Results
obtained with protein- and DNA-cochleates have been reviewed elsewhere (Gould-Fogerite et al., 1998).

**Proteosomes** are also considered as outsiders in the liposome group as they are mainly comprised of protein. These vesicles are of bacterial origin (outer membrane) and are prepared by solubilization of bacterial membranes, followed by ammonium sulphate precipitation and dialysis against detergent containing buffer (Lowell et al., 1988). Electron micrographs revealed that these vesicles have a size of about 100 nm, but the protein: lipid ratio is higher than can be achieved with purified protein incorporated in liposomes. Proteins and peptides are non-covalently complexed to the proteosomes, making them highly immunogenic (Lowell et al., 1997). Moreover, fusogenic liposomes have been prepared from artificial lipid membranes consisting of synthetic arenavirus ‘fusion peptide’ (Glushakova et al., 1992). In addition, it has been demonstrated that non-phospholipid liposomes composed primarily of dioxyethylene acyl ether and cholesterol have capacity to fuse with membranes composed primarily of phospholipids (Baraka et al., 1996).

**MICROSPHERES:** Among various polymeric systems developed as pharmaceutical dosage forms, poly-lactide co-glycolide (PLGA) microspheres have been widely explored in several immunological studies as a controlled delivery system of peptides, native and synthetic proteins and lately, nucleic acids (Eldridge et al., 1991; O’Hagan et al., 1993; Partidos et al., 1994). PLGA microspheres are composed of a spherical shaped polymeric matrix ranging in diameter from 1 to 250µm. The biodegradable and biocompatible polyesters, the polylactide-co-glycolides (PLG) are the primary candidates for the development of microparticles as adjuvants, since they have been used in humans for many years as suture material and as controlled release drug delivery systems. However, the adjuvant effect achieved through the encapsulation of antigens into PLG microparticles has been demonstrated only relatively recently (Partidos et al., 1994). Microparticles with entrapped antigens (ovalbumin and staphylococcal B enterotoxoid) had comparable immunogenicity to these antigens dispersed in Freund’s adjuvant, the most potent adjuvant available (Eldridge et al., 1991; Maloy et al., 1994). Recent studies have shown that microparticles also exert an adjuvant effect for the induction of cell-
mediated immunity (Moore \textit{et al.}, 1995). Microparticles also appear to have significant potential as an adjuvant for DNA vaccines (Jones \textit{et al.}, 1997). The adjuvant effect of microparticles appears to be largely a consequence of their uptake into DC, macrophages and local lymph nodes following intramuscular injection. A particularly attractive feature of microparticles is their ability to control the rate of release of entrapped antigens. Controlled release of antigen may allow the development of single dose vaccines, which would result in improved vaccine compliance, particularly in the developing world. Although microparticles have significant potential for the development of single dose vaccines, much work is needed to ensure the stability of antigens entrapped in microparticles.

One useful discovery made from the research of microspheres is a way to fight cancer on a molecular level. According to Wake Oncologists, "SIR-Spheres microspheres are radioactive polymer spheres that emit beta radiation. Physicians insert a catheter through the groin into the hepatic artery and deliver millions of microspheres directly to the tumor site. The SIR-Spheres microspheres target the liver tumors and spare healthy liver tissue. Approximately 55 physicians in the United States use Sirtex’s SIR-Spheres microspheres in more than 60 medical centers.

\textbf{Immune Stimulating Complex (ISCOMs)}

ISCOMs are spherical, micellar assemblies of about 40nm. They are made of the saponin mixture Quil A, cholesterol and phospholipids (Mowat and Donachie, 1991). They contain amphiphilic antigens like membrane proteins. Quil A is a potent adjuvant that has been used as such in veterinary vaccines since the early 1970s. Results obtained with oral vaccination using ISCOM have been variable, showing partial protection/clearance in some systems (Behbaudi \textit{et al.}, 1996; Dotsika \textit{et al.}, 1997). ISCOM based vaccines also have the ability to induce CTL which may be important in the protection/recovery from viral as well as other intracellular pathogens. The induction of CTL against specific antigens has been demonstrated for a number of ISCOM based vaccines in both mice and non-human primates (Maloy \textit{et al.}, 1994; Takahashi \textit{et al.}, 1990)). Apart from pre-natal immunization, ISCOMs have been used for mucosal delivery of antigens (Mowat and Donachie, 1991). They are able to boost both humoral and cellular
responses. Antigens from viral, bacterial and parasitic sources have been studied. ISCOMs are able to generate a broad range of immune responses. These include APC activation (Behbaudi et al., 1996), increased MHC II expression on APCs, cytokine induction, especially IL-2 and IFN-γ (Dotsika et al., 1997), CD4+ T-cell responses (Maloy et al., 1994) and CD8+ CTL responses (Takahashi et al., 1990). At the moment, one ISCOM vaccine is licensed. It is an anti-influenza vaccine to be used for horses. More than 1 million doses have been sold in Sweden. A human phase I study with ISCOM-matrix caused mild pain at the injection site (Barr et al., 1998).

**siRNA NANOPARTICLES:** Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, 20-25 nucleotides in length, that play a variety of roles in biology. Most notably, siRNA is involved in the RNA interference (RNAi) pathway (see Figure 1.7), where they are intracellularly generated from long endogenous or exogenous double-stranded RNA molecules (dsRNAs) through the cleavage activity of a ribonuclease III-type protein (Bernstein et al., 2001; Elbashir et al., 2001). siRNA molecules can knockdown their cognate targets specifically and effectively based on direct homology dependent post-transcriptional gene silencing.
Careful selection of siRNA sequences to avoid off-target effects is an important issue and can be minimized or eliminated by avoiding certain sequence motifs, and validation of the siRNA sequences. The most common strategy for increasing the serum half-life of siRNAs is to increase the molecular weight by either complexing the siRNA into certain lipids or encapsulation into polymer based particles. Unmodified siRNA has a half-life of less than 1 hour in human plasma and siRNA is rapidly excreted by the kidneys. The generation of nanosized particles is being investigated to enhance the delivery of siRNA-based drugs. Liposomes and nanoparticles can act as envelopes to protect the siRNA from metabolism and excretion, but can also carry specific molecules designed to target the siRNA to specific tissue types. Liposomes such as Lipofectamine,
cationic DOTAP, and neutral DOPC have been used to carry siRNA into cells. Nanoparticles such as the cationic polymer, polyethyleneimine (PEI) have also been used to successfully deliver siRNA to target cells.

![Figure 1.9: Liposome bearing siRNA](image)

One study using siRNA lipoplexes generated from the commercially available cationic lipid Dharma FECT reported that ~95% of the lipoplexes enter cells through endocytosis and ~50% of endocytosis was clathrin-mediated (Lu et al., 2009). Xu and Szoka (1996) proposed that the release of nucleic acids from cationic lipid complexes may be facilitated by association of cellular anionic lipids with a carrier’s cationic lipids, to form neutral ion pairs which ‘free’ the nucleic acid from the delivery system (Xu and Szoka, 1996; Wolff and Rozema, 2008). Early siRNA therapeutics for the treatment of age-related macular degeneration (AMD) and respiratory syncytial virus (RSV) (Alvarez et al., 2009) were administered locally using unmodified or chemically modified siRNA (in saline). More recently, formulations for systemic administration of siRNA packaged using polymers (Davis, 2009) or lipids have begun to be evaluated in the clinic. For example, a study conducted by Silence Therapeutics is testing a siRNA-liposomal formulation aimed at targeting protein kinase N3. This approach has proven to significantly inhibit tumour growth in prostate and pancreatic cancer models in mice (Aleku et al., 2008), and is being tested in humans with advanced solid tumours. Alnylam Pharmaceuticals is investigating a lipid-based nanoformulation containing two different siRNA molecules aimed at targeting the kinesin spindle protein (KSP) and the vascular endothelial growth factor (VEGF) for their potential antiliver tumour activity. The results
of these trials with lipid and formulated materials will provide important information regarding the translatability of delivery systems developed in rodents and primates.

**Application of Nanoparticulate delivery systems**

- **Tumor targeting using nanoparticulate delivery systems**

  The rationale of using nanoparticles for tumor targeting is based on: 1) nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles; 2) nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ.

  Verdun *et al.* (1990) demonstrated in mice treated with doxorubicin incorporated into poly (isohexylcyanoacrylate) nanospheres that higher concentrations of doxorubicin manifested in the liver, spleen and lungs than in mice treated with free doxorubicin (Verdun *et al.*, 1990). Studies show that the polymeric composition of nanoparticles such as type, hydrophobicity and biodegradation profile of the polymer along with the associated drug’s molecular weight, its localization in the nanospheres and mode of incorporation technique, adsorption or incorporation, have a great influence on the drug distribution pattern in vivo. The exact underlying mechanism is not fully understood but the biodistribution of nanoparticles is rapid, within ½ hour to 3 hours, and it likely involves MPS and endocytosis/ phagocytosis process (Couvreur *et al.*, 1980).

  Propensity of MPS for endocytosis/phagocytosis of nanoparticles provides an opportunity to effectively deliver therapeutic agents to these cells. This biodistribution can be of benefit for the chemotherapeutic treatment of MPS- rich organs/tissues localized tumors like hepatocarcinoma, hepatic metastasis arising from digestive tract or gynaecological cancers, brochopulmonary tumors, primitive tumors and metastasis, small cell tumors, myeloma and leukemia. It has been proved that using doxorubicin loaded conventional nanoparticles was effective against hepatic metastasis model in mice. It was found there was greater reduction in the degree of metastasis than when free drug was used. The underlying mechanism responsible for the increased therapeutic efficacy of the
formulation was transfer of doxorubicin from healthy tissue, acting as a drug reservoir to the malignant tissues (Chiannilkulchai et al., 1990). Histological examination showed a considerable accumulation of nanoparticles in the lysosomal vesicles of Kupffer cells, whereas nanoparticles could not be clearly identified in tumoral cells. Thus Kupffer cells, after a massive uptake of nanoparticles by phagocytosis, were able to induce the release of doxorubicin, leading to a gradient of drug concentration, favorable for a prolonged diffusion of the free and still active drug towards the neighboring metastatic cells.

Several approaches for targeting siRNA have been utilized. *In vivo* administration of folate-conjugated nanoparticles that were composed of cholesteryl-3-beta-carboxyamidoethylene-N-hydroxyethylamine, and PEG-distearoylphosphatidyl ethanolamine (DSPE) resulted in reduction in KB xenograft tumour size after delivery of Her-2 siRNA using folate-linked nanoparticles (Yoshizawa et al., 2008). These strategies have been successfully applied *in vitro* for receptor-specific delivery of chemotherapy agents, radiopharmaceuticals, imaging contrast agents, peptides, and siRNA, suggesting that they can enhance the efficacy of siRNA by increasing the concentration of siRNA in tumours at relatively lower doses than those of nontargeted nanocarriers (Sanguino et al., 2008). Using different cancer targets in a variety of preclinical tumour models in mice, including pancreatic cancer (Pan et al., 2008), melanoma (Villares et al., 2008), liver (Gray et al., 2008), colorectal (Yang et al., 2008), and breast cancers (Ozpolat et al., 2008), we have further demonstrated the efficacy of the neutral DOPC-nanoliposomal delivery system. Overall, data support our hypothesis that neutral DOPC-nanoliposomes effectively deliver siRNA into tumour cells and can be combined with other conventional anti-cancer therapies, such as chemotherapy, to enhance the efficacy of conventional drugs.

➢ *Nanoparticles for oral delivery of peptides and proteins*

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and
their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration (Damge et al., 1990).

- **Targeting of nanoparticles to epithelial cells in the GI tract using ligands**

  Targeting strategies to improve the interaction of nanoparticles with adsorptive enterocytes and M-cells of Peyer’s patches in the GI tract can be classified into those utilizing specific binding to ligands or receptors and those based on nonspecific adsorptive mechanism. The surface of enterocytes and M cells display cell-specific carbohydrates, which may serve as binding sites to colloidal drug carriers containing appropriate ligands. Certain glycoproteins and lectins bind selectively to this type of surface structure by specific receptor-mediated mechanism.

- **Nanoparticles for gene delivery**

  Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Polynucleotide vaccines are set to supersede many conventional vaccines particularly for immunotherapy. However, there are several issues related to the delivery of polynucleotides which limit their application. These issues include efficient delivery of the polynucleotide to the target cell population and its localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotide is maintained during delivery to the target site. Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment (Panyam et al., 2002). Hedley et al. (1998) reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression. This gene delivery strategy could be applied to facilitate bone healing by
using PLGA nanoparticles containing therapeutic genes such as bone morphogenetic protein.

**CONCLUSION**

Efforts in medicinal and combinatorial chemistry continue to give rise to a wide range of anti-cancer agents with great therapeutic potential. However, many of these agents have solubility, stability or toxicity issues that retard or prevent their development into viable treatment strategies. In many cases, delivery or formulation technologies are investigated as a means to exploit the therapeutic potential of these agents. To date, many nano-sized systems such as liposomes and micelles have been explored for systemic delivery of anticancer agents. Since clinical applications of nanoparticles as drug delivery systems began in cancer treatment, a series of strategies have been developed and modified to enhance the therapeutic efficacy of nanoparticles drugs.

The concept of incorporating the drug into different types of nanoparticles for a better targeting of the drug at appropriate tissue destination is widely accepted by researchers and academicians. Moreover nanoparticles represent a promising drug delivery module and hence they can represent alternative vesicular systems with respect to other delivery systems due to their ability to encapsulate different type of drugs within their multi-environmental structure.