CHAPTER 1
INTRODUCTION

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Cancer can be defined as a disease in which uncontrolled manipulation and spread of abnormal forms of the body's own cells. It is one of the leading causes of death in advanced countries. Cancer chemotherapy is limited by adverse side effects resulting from toxicities to normal tissues (Theresa et al., 1992; www.NewsRx.com).

Despite huge advances in prevention and treatment, experts opinion that cancer is poised to become the leading cause of death worldwide as people refuse to ditch bad habits and the population ages, experts said. In the United States medical advances and education campaigns have helped slash the death rate from cancer by nearly 16 per cent in 20 years. Cancer still struck 1.5 million people and killed 560,000 in the United States in 2009 and experts predict it will this year edge out heart disease to become the most deadly disease worldwide. Cancer usually strikes people later in life, from age 55 upwards, so as people live longer and the population ages, the risk of being diagnosed with cancer has risen: nearly half of men and a third of women will be diagnosed during their lifetime with cancer. Half the men and women who are diagnosed with cancer in their lifetime will die of the disease or related complications. "Current estimates say around two-thirds of US adults are overweight or obese and obesity and overweight are now known to cause many types of cancer, "Avoiding overweight and obesity is going to be critical for keeping our progress going forward in this battle against cancer (http://www.who.int/en/).

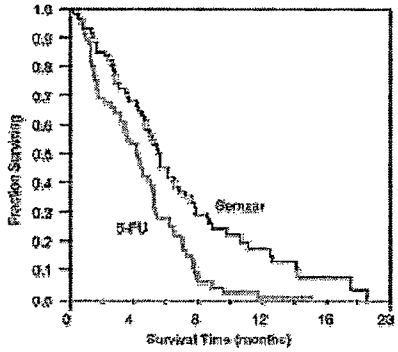
Cancer being one of the disastrous diseases to mankind from centuries even after a lot of research work carried out in this field. Solid tumors have historically provided many challenges to systemic therapy. Theoretical barriers to drug penetration with in solid tumors interstitium results from high interstitial pressures and large interstitial space compared with normal tissue, particularly in necrotic core. Vascular permeability in tumors is heterogeneous with respect to tumor type, location of the vessel within the tumor, and the tumor microenvironment (Jain, 1989; Yuan et al., 1994) will help engineer liposomes for more effective delivery of their contents to the tumor.

Chemotherapy, whether given systemically or by regional perfusion of a particular organ or tissue is impended by the lack of specificity of the drugs for cancer cells. Therefore, therapy is often limited by systemic toxicity before truly therapeutic drug levels in the tumor can be achieved. None of these traditional therapies alone or in combination have achieved complete cure for all the cancer types in all patients. Therefore, many researchers have been exploring targeted-delivery options like liposomes and nanocapsules for the treatment of cancer. The delivery vehicle itself can act to target the active ingredient to the tumor and limit its effects to the tissue in closest proximity. Targeted delivery of drugs to diseased tissues *in vivo* would help to reduce these side effects (www.NewsRx.com; Yuan et al., 1994). Novel drug delivery system (NDDS) has gained a considerable attention from the last few decades. The reason for this paradigm shift is the low developmental cost and time required for introducing a NDDS, as compared to a new chemical entity.

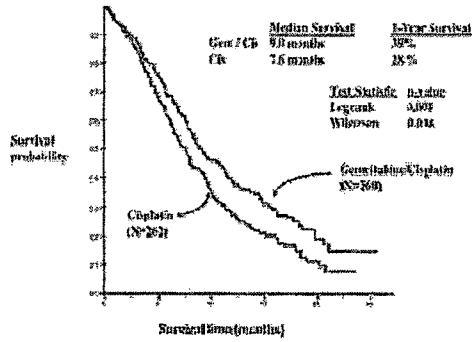
The colloidal Drug delivery systems offer the potential to enhance the therapeutic index of these anticancer agents, either by increasing the drug concentration in tumor cells and or by decreasing the exposure to normal tissues. Association of drugs with carriers such as liposomes has marked effects on both the pharmacokinetic profile of the carrier and of the carrier-associated drug. In general, incorporation of drugs in liposomes delays drug absorption, alters and restricts drug biodistribution, decreases the volume of distribution, delays clearance and retards drug metabolism (Imran Ahmad et al., 1993).

Liposomes are being increasingly utilized to deliver drugs, enzymes, antisense oligonucleotides and genes to various therapeutic targets. Presently a number of drugs are available in the market in liposomal preparation such as Doxil® (Alza Corp.; Palo Alto, CA), Evacet® (Liposome Co.; New Brunswick, NJ), Daunoxome® (Nexstar Pharm.; Boulder, CO) and conventional liposomal vincristine VincaXome®. Significant advances have been made in overcoming many of the barriers associated with liposomal drug delivery; an elusive problem has been the ability to selectively increase the bioavailability of the drug at the target tissue, while maintaining stability in the circulation.

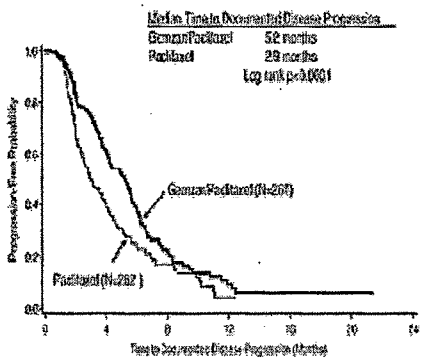
In the recent years, gemcitabine (trade name Gemzar) has emerged as a very potent anti-tumor drug and it is currently used alone or in combination in the treatment of patients with different malignancies including ovarian, pancreatic, colon, non-small cell lung and other cancers (Theresa et al., 1995; Abratt, 1995; Haller, 2003, Fowler and Van, 2003). Gemcitabine is a novel deoxycytidine analogue, a pyrimidine antimetabolite related to cytarabine. Gemcitabine exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and also blocking the progression of cells through the G1/S-phase boundary. Gemcitabine is metabolised intracellularly by nucleoside kinase to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. The cytotoxic effects of gemcitabine are exerted through dFdCDP-assisted incorporation of dFdCTP into DNA, resulting in inhibition of DNA synthesis. Inhibition of this enzyme by the diphosphate nucleoside causes a reduction in the concentration of deoxynucleotides, including dCTP. Secondly, gemcitabine competes with dCTP for incorporation into DNA. Gemcitabine undergoes intracellular metabolism to the active moieties and is rapidly deaminated in the blood, liver, kidneys and other tissues. In the plasma, it is metabolised to its inactive metabolite. Patients treated with gemcitabine (free drug) had statistically significant increases in clinical benefit response, survival and time to disease progression compared to 5-FU in the treatment of pancreatic cancer and combination therapy in the treatment of non small cell lung cancer (NSCLC), breast and ovarian cancers. The Kaplan-Meier curve for survival is shown in Figures 1.1 (Harper, 2003; www.Rx list.com,; Larry et al., 1990; BCCA, 2007; www.FDA.com,; Volker et al., 1992; David et al., 1993; Peter et al., 1978).



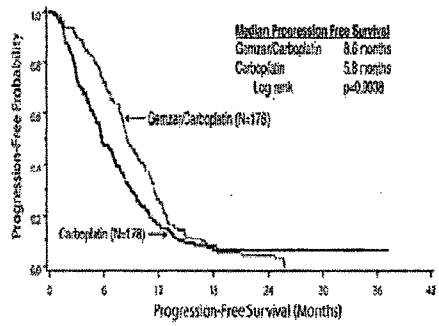
Kaplan-Meier Survival Curve in the treatment of pancreatic cancer



Kaplan-Meier Survival Curve in the treatment of NSCLC cancer



Kaplan-Meier Survival Curve in the treatment of Breast cancer



Kaplan-Meier Survival Curve in the treatment of ovarian cancer

Fig.1.1 Kaplan-Meier Survival Curve

Gemcitabine is rapidly deaminated to the inactive metabolite 2`, 2`-difluro deoxyuridine; it must therefore be administered at very high dose. For this reason gemcitabine has a very short half life. Metabolic stability can be improved by synthesizing a series of increasingly lipophilic prodrugs of gemcitabine by linking the 4-amino group with valeroyl, heptanoyl, lauroyl and stearoyl linear acyl derivatives. Cytotoxicity of gemcitabine prodrugs, encapsulated in liposomes was between two and seven fold more that of free gemcitabine (Maria et., 2004; Barbara et al., 2007; Sandler et al., 1999).

Surface modification of liposomes by the inclusion of hydrophilic components (e.g., carbohydrates, glycolipids or polymers) to form long-circulating liposomes causes

changes in the pharmacokinetic pattern seen for conventional liposomes. While conventional liposomes have non-linear, saturable kinetics. Long-circulating liposomes possess dose-independent, non-saturable, log-linear kinetics. Gemcitabine incorporation within liposomes enhances the drug cytotoxic effect with respect to free gemcitabine, thus suggesting a more effective drug uptake inside the cancer cells.

Liposomes containing lipid derivatives of polyethylene glycol have circulation times sufficiently long to allow for effective *in vivo* drug delivery (Yuan et., 1994; Marilena et al., 2004; Alberto., 1990; Murray et al., 1998). Incorporation of polyethylene glycol-derivatized phospholipids into liposomes results in carriers that can enhance the therapeutic efficacy of encapsulated drugs by imparting the ability to evade the reticuloendothelial system and remain in the circulation for prolonged periods.

In slowly growing tumors, small differences in the rate of liposomes accumulatation in tumors, or the rate at which the drug becomes bioavilable will have less impact on efficacy than in rapidly growing tumors, where the overall flux of bioavailable drug through the tumor is more likely to determine treatment success. The move from animals to humans should favor sterically stabilized liposomes (SSLs), where liposomes continue to accumulate in the tumors for days after administration (Daryl et al., 1999).

Liposomes targeted to internalizing receptors have shown considerably greater tumor cell cytotoxicity both *in vitro* and *in vivo* (Lee and Low., 1995; Lopes and Allen., 1998). The most relevant aspect of targeted liposomes is that it potentially increase the bioavailability of the drug. The choice of ligand is important when designing targeted liposomes. The ligand should be specific for cancer cells, especially in contrast to cells readily accessible in the general circulation, where many passes may occur before extravasation into tumor (Daryl et al., 1999).

The receptor for the vitamin, folic acid is overexpressed on a number of human tumors, including cancers of the ovary, kidney, uterus, testis, brain, colon, lung and myelocytic blood cells. Folate-mediated targeting of protein toxins, imaging agents, antisense oligodeoxynucleotides, genes and liposomes specifically to cancer cells *in*

vitro and *in vivo* (Susan Wang and Low., 1998). Folic acid uptake in L1210-B73 cells was found to proceed for more than 98% via the membrane associated folate binding protein (mFBP). The putative role of the mFBP in the uptake of CB3717 and ICI-198,583 in L1210-B73 cells was further supported by the fact that protection from growth inhibition could be achieved by folic acid (Robbin et al., 1991). Folate-targeting is fully compatible with PEG-coating of the liposomes, since incorporation of 4 mol% PEG2000-DSPE does not reduce the uptake or cytotoxicity of folate-PEG-liposomal doxorubicin (DOX). Folate targeting constitutes a possible mechanism for improving the specificity of PEG-coated liposomes for cancer cells (Lee and Low., 1995).

Liposome entrapment can protect rapidly degrading drugs from breakdown *in vivo*, with release of the drugs in a therapeutically active form over periods of up to several days. The dose-independent pharmacokinetics and reduced mononuclear phagocyte system uptake of stealth liposomes gives them distinct advantages over non-stealth liposomes (Theresa et al., 1992).

1.2 Objective of the proposed research work

The present study has been planned to develop liposomal drug delivery system for gemcitabine for achieving the following objectives

- To design and formulate PEGylated liposomal formulation of Gemcitabine. By thin film hydration by pH gradient loading.
- Surface engineering with folic acid for targeting delivery.
- Characterization of the drug loading, particle size, percentage drug entrapment, morphology and chemical interaction between drug and lipid.
- Stability Studies for the optimized formulations.
- Cell cytotoxicity studies in suitable cell lines.
- *In vivo* biodistribution and tumor uptake studies in suitable animal model.

1.3 Advantages

- Decreasing the non-specific delivery of the drug to non-target tissues.
- Increasing the drug concentration at its site of action (be it intracellular or extracellular).
- Surface functionalized liposomes exhibiting both target specificity and circulation longevity thereby prolonging the residence time of the drug at its site of action by reducing clearance.
- Reducing systemic side-effects of drug (myelosuppression & hematological toxicity).
- Decreasing toxicity due to high initial doses of the drug.
- Improving the stability of the drug *in vivo*.
- Decreasing irritation caused by the drug.
- Improving shelf life of the product.

1.4 Advancements and addition to the existing Knowledge

Presently, only free drug dosage form of gemcitabine is available and popular in the market for the treatment of various types of cancer. It is more effective compared to other anticancer drugs. By developing surface engineered liposomes (long circulating and reducing dose) will have major impact on the health care of the population by preventing problems existing with drug like

- Gemcitabine undergoes intracellular metabolism to the active moieties but it is rapidly deaminated in the blood (inactive metabolite), liver, kidneys and other tissues.
- Gemcitabine can suppress bone marrow function as manifested by leukopenia, thrombocytopenia and anemia; myelosuppression is usually the dose-limiting toxicity.
- Adverse reactions reported in the single-agent safety database resulted in discontinuation of gemcitabine therapy in about 10% of patients and 14.3% in pancreatic cancer treatment (4.8% for the 5-FU arm), 15% of patients on combination.

- Red blood cells transfusion is required for about 19% of patients (Single-agent) and for 39% of patients in combination (gemcitabine and cisplatin), platelet transfusions were required in 21% of patients.
- Nausea and vomiting were very commonly reported (69%) but were usually mild to moderate severity.
- In some cases serious hepatotoxicity, including liver failure and death were reported in patients receiving gemcitabine alone or in combination with other potentially hepatotoxic drugs.

By targeting active ingredients to tumor the toxicity to the surrounding tissue can be minimized. This would be of considerable improvement over existing therapies because of putative advantage in dose, dosing schedule, patient compliance and reducing toxicity.

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