

# *Chapter-1*

## *Introduction*



## 1.1 Introduction

### Ovarian cancer

Cancer is characterized by abnormal growth of cells which tend to proliferate and spread in an uncontrolled manner. In women, ovarian cancer (OC) is ranked as the second most prominent cancer of gynaecological origin leading behind the endometrial cancer. A worldwide incidence of 2,38,719 cases in 2012 accounting for 1,51,917 deaths per year have been reported for OC (1). OC is diagnosed annually in nearly a quarter of a million women globally, and is responsible for approx. 140,000 deaths each year (1). Almost 75% of OC are detected at last stages III or IV and thus it is also called as “silent killer”, that makes the odds of survival poor (2). The ovarian tumors are categorized for their noticeable heterogeneity of tissues. Accordingly, histogenesis of a normal ovary, the ovarian tumors is categorized depending on their cells of the origin. The three major types of OC include epithelial cell, germ cell and stromal cell type of OCs. Amongst these three, 90% cases are of the epithelial origin (3). Though there is a high frequency and mortality level of OC, the etiology and contribution of molecular markers in this deadly disease is not absolutely known. The standard chemotherapy method every so often depends on combination of extremely toxic taxanes and platinum based anti-cancer drugs. However, development of resistance towards chemotherapy and recurrence are often developed mostly in 60 % of the cases at advanced stages of OC (4). Moreover, non-specific bio-distribution of chemotherapeutic drugs is responsible for severe adverse effects. All the above aspects conclude into a 5-year survival rate of only 20-30% for advanced stage OC patient (5,6).

### Diagnosis, Treatment challenges and drawbacks of current therapy

Various diagnostic methods for OC consist of imaging tests like Magnetic Resonance Imaging (MRI), Computerized tomography scans (CT scan), scans for the pelvic examination, ultrasonography or transvaginal ultrasound, serum biomarkers detection of CA 125 (Cancer Antigen 125) etc. However, none of these techniques is able to provide a reliable diagnosis of OC in its early stage. Pelvic examination and ultrasound imaging lack specificity and sensitivity in the diagnosis of early stage

epithelial OC due to their inability to distinguish between malignant tumor and other benign abnormalities (7). Serum biomarkers such as cancer antigen CA125 is one of the thoroughly assessed biomarkers used to diagnose OC (8). However, elevated levels of CA125 observed in other pathological conditions such as peritonitis, pelvic inflammatory disease, inflammatory bowel disease, pancreatitis, liver disease and endometriosis makes it less specific. Further, the detection of the CA125 is not that much sensitive for early stage screening of the OC with levels elevated in only 50% of patients in stage I OC. Therefore, diagnosis of OC in earlier stage of its development is a formidable challenge and demands applications of biological screening techniques such as antibodies, multiple biomarkers, complementary serum markers and other advanced screening techniques like proteomics and microarray analysis (9). Current, therapeutic approaches for the OC include cyto-reductive surgery and chemotherapy. Cyto-reductive surgery followed by standard chemotherapeutic treatment including platinum compounds (cisplatin and carboplatin) with taxol derivatives (paclitaxel and docetaxel) is currently the first line therapy for the newly diagnosed OC (10). In spite of several advancements made in the treatment therapy for OC, chemotherapy is clinically handicapped due to adverse effects, drug resistance, relapse (risk of recurrence) and poor quality of life of patients. Adverse effects of chemotherapy generally depend on type or mechanism of action of cytotoxic agents. Adverse effects vary from minor though hampering adverse events like nausea and vomiting to major consequences such as nephrotoxicity, neurotoxicity, ototoxicity, myelosuppression etc. The basic reason of adverse effects is non-specific distribution of highly cytotoxic drugs to organs other than tumor. Resistance to chemotherapy is another factor which restricts the treatment of OC. It often originates due to oncogene and tumor suppressor gene mutation, multidrug resistance proteins, p-gp efflux mechanism, altered or mismatched repairing process, failure of apoptosis pathways and mechanisms that inactivate the drug (11). Other major limiting factor for OC treatment is relapse or recurrence which starts with cancer cells those are untouched or not removed in the first treatment. Therefore, current prospects of research in OC treatments are to overcome limitations of both the diagnosis and therapeutic alternatives in OC .

Paclitaxel: The first line anti-cancer drug for ovarian cancer

Anticancer drugs such as paclitaxel, docetaxel, cisplatin, carboplatin, melphalan are used commonly for the chemotherapeutic treatment of ovarian cancer. In present research work, we have selected Paclitaxel (PTX) which has gained increased importance as a first line anti-neoplastic drug indicated for treatment of ovarian cancer (12). Paclitaxel (Taxol<sup>®</sup>), a diterpenoid ester derivative is derived from and bark of the Pacific Yew tree (*Taxus brevifolia*). It is a mitotic spindle inhibitor that enhances polymerization of tubulin to stabilize microtubules at metaphase stage and also interacts with microtubule to prevent their depolymerization leading to cell cycle arrest in anaphase stage (13). It is a potent inhibitor of cell replication in epithelial ovarian carcinoma, breast cancer, colon, head and neck cancers and non-small cell lung cancer. In the clinical application, PTX is usually administered as a 3-hour and 24-hour infusion representing a total dose of 135–175 mg/m<sup>2</sup> of the body every 3 weeks (14).

Current challenges for paclitaxel chemotherapy

- PTX has limited aqueous solubility and thus, currently supplied with Cremophor EL<sup>®</sup> (CrEL)/ (polyethoxylated castor oil) and dehydrated alcohol to improve its solubility for intravenous administration (Taxol<sup>®</sup>). The vehicle has been observed to cause serious, life-threatening anaphylactoid reactions. Therefore, Taxol<sup>®</sup> treatment includes a prophylactic regimen of corticosteroids. With premedication, the severity and incidence of serious hypersensitivity reactions is reduced, but not eliminated. CrEL also has been associated with bronchospasm, hypotension, neuro- and nephrotoxicity.
- The short half-lives, large volumes of distribution and rapid elimination of Taxol<sup>®</sup> from the body is responsible for partial drug accumulation in the tumor areas with comparatively high drug exposure in normal organs of the body. Non-specific bio-distribution of PTX leads to high toxicity and low therapeutic indices of drugs. The major adverse effects linked with Taxol<sup>®</sup> and its non-specific bio-distribution are fluid retention, myalgias, neutropenia, peripheral neuropathy, gastrointestinal toxicity and myelosuppression. Further, CrEL is responsible for

non-linear pharmacokinetic profile of PTX that complicates the co-administration of other anti-tumor drugs.

- Taxol<sup>®</sup> has short-term physical stability, as out of the aqueous media, some particles slowly tend to precipitate. It is physically incompatible with the compounds of some intravenous (I.V.) infusion sets, as evidenced by the extraction of plasticizers, such as diethylhexylphthalate (DEHP), which is hepatotoxic (15)
- Another clinically approved alternative is Abraxane<sup>®</sup>, albumin bound PTX nanoparticles. The newer formulation thus can eliminate the use of toxic solubilizers and their serious hypersensitivity related side effects. However, relative to Taxol<sup>®</sup> the antitumor effectiveness enhancement of Abraxane<sup>®</sup> is only minimal with significantly higher cost. Additionally, the short half-lives, large volumes of distribution and rapid elimination of Abraxane<sup>®</sup> from the body is responsible for insufficient accumulation of drug at tumor sites.

Though, PTX is a first line drug for chemotherapy for OC (16), The lack of any efficient delivery vehicle limits and delays the extensive clinical use of PTX. It is imperative to deliver PTX only to the targeted tumor sites to minimize systemic toxicity. Hence, PTX was selected to improve its delivery aspects by incorporating it into targeted liposomal system.

## 1.2 Need for the study

New drug formulations and targeting approaches have been developed to better control the bio-distribution of drugs, to increase the therapeutic effectiveness and lessen the side effects at the same time (17). Many researchers have formulated various formulations of PTX such as emulsions, nanoparticles, micelles, liposomes and dendrimers to eliminate side effects and to improve its pharmacokinetic profile as well as efficiency as anti-tumor drug in cancer (18). Amongst the various carrier systems for drug delivery, liposomes because of their biological inert nature, freedom from antigenic, pyrogenic or allergic reaction and enhanced stability have gained immense importance (19). For cancer therapy, the first nanotechnology based drug

delivery formulation approved by FDA was liposomes loaded with anti-cancer drug. As a carrier system they comprise of aqueous volume trapped by one or more bilayers of natural or synthetic lipids. Liposomes can encapsulate hydrophilic as well as hydrophobic drug in its aqueous core and lipid bilayer respectively, can be formulated to achieve predetermined release in biological environment (18). The entrapment of drug in bilayer or core of the liposomes, loading efficiency of drug molecules in the liposomes and release of drug from the liposomes depends on drug to lipid ratio, solubility of drug and its partitioning between aqueous and lipidic layer. Liposomes can augment the therapeutic effectiveness of chemotherapeutic drugs, either by increasing the accumulation of drug in tumor cells, improving pharmacokinetic profile, reducing the adverse effects associated with normal tissues damage by following enhanced permeability and retention (EPR) phenomenon or by utilizing the targeting approaches (20). It can encapsulate and retain drug molecule to provide higher stability, has long circulation time when PEGylated and can be designed to achieve site specific delivery. But, it is observed that the amount of drug loaded in the bilayer is limited and further, lipophilic drugs such as PTX, incorporated in higher amount in bilayer may lead to crystallization during preparation and in bilayer that destabilize the membrane leading to premature release of the encapsulate (21) (22-24). Conventional PTX liposomes have previously been investigated and found to have low carrier mediated toxicity compared to Taxol® and tested successfully on various *in vivo* experimental models. PTX concentrations of 1, 1.5 and 2 mg PTX per ml liposome suspension, were prepared. In all samples fine, needle-shaped crystals, often lying in groups forming stars, immediately were seen (25, 26). Therefore, the amount of PTX was decreased and liposomes with 0.4, 0.5, 0.6, 0.75 and 1 mg/mL PTX were prepared. Only in the liposome samples with less than 0.5 mg PTX/ml, no crystals were found, even after days of storage. Therefore, the crystal-free liposome formulation with the highest amount of PTX was 0.5 mg/ml. In other words, PTX loading of 3-3.5 mole % (paclitaxel to phospholipids) has been found to be stable for weeks to months (27) (28).

Thus the loading efficiency of a hydrophobic drug such as PTX in the lipid bilayer usually relies on the drug:lipid molar ratio. It is hard to get high loading efficiency in the lipid bilayers because the space offered by lipid bilayer is limited,

and a large amount of hydrophobic drug molecules can destabilize the structural integrity of liposomal bilayers. When liposomes are used as drug delivery systems, it is important that the therapeutic agent is efficiently and adequately encapsulated. Though two of the liposomal PTX formulations have entered in clinical trial, the low loading efficiency of PTX in liposomes, leakage of drug in the circulation before reaching the target, stability of liposomes, formation of PTX crystals at higher concentration, high drug:lipid ratio and nonspecific bio-distribution are major limitations.

### **1.3 Proposed research strategy**

#### ➤ **Double loading of paclitaxel in liposomes**

Cyclodextrin (CD), cyclic amylose-derived oligomers (6-8 units linked), have been widely researched as a distinct class of molecules for improving the aqueous solubility of lipophilic drugs by forming inclusion complexes (ICs) via non-covalent interaction. 2,6-di-O-methyl beta cyclodextrin (DM $\beta$ CD), a derivative of  $\beta$ CD has higher solubility in water and can accommodate more payload of an apolar drug molecule in the cavity, due to more hydrophobic core than native  $\beta$ CD (29). Attempts have been made to formulate Inclusion Complexes (ICs) of PTX with various types of CDs (30). However, poor dilution stability, rapid clearance from circulation, replacement of drug molecule by other molecules having greater affinity for the cavity in biological milieu are the limiting factors for ICs of PTX (31). “Drug in cyclodextrin in liposomes” was reported in 1994 in the area of drug delivery which takes benefits of definite characteristics of CDs and liposomes and combines them in a single system to evade limitations associated with both the systems (21). The concept, allows entrapment of water soluble cyclodextrin ICs of water insoluble drugs such as PTX in aqueous core of liposomes. However, the volume of aqueous core is very low compared to the volume of bilayer which limits the entrapment beyond a certain theoretical limit. In addition, the extent of loading efficiency also depends on the entrapment of drug into CD cavity and the method of preparation of liposomes. This strategy seems to be promising in improving loading efficiency (32, 33). Therefore, in order to maximize the drug load, encapsulating drug in

both bilayer and core of liposome, double loading of liposomes was proposed. Such double loading approach is also proposed to control *in vivo* fate of hydrophobic drugs, increase vesicle stability and prolonging the release profile. (34) The idea of combined liposomes and CD complexes of lipophilic drugs by forming **drug-in-CD-in-liposome** can increase the loading efficiency and modify release of the drug *in vivo*, thus participating in improving the pharmacokinetic profile (35), (34).

In this study, we have loaded PTX in both the compartments of liposome i.e. lipidic bilayer and hydrophilic core. PTX as such could be loaded in lipidic bilayer and as the pre-encapsulation of PTX improved water solubility of PTX, the inclusion complex of PTX could be loaded in aqueous core. Thus, we report a novel strategy for simultaneous entrapment of PTX into the bilayer as well as the aqueous compartment of liposomes. This treatment effectively will improve loading of PTX than those obtained by entrapping only the drug in the liposomal bilayer.

➤ **Site specific drug delivery**

The idea of drug carrier with targeted specificity has fascinated scientists for number of years and in the last decade successful efforts have been made to achieve this goal (36). The ultimate form of targeted drug delivery system should be realization of Paul Ehrlich's "magic bullet concept" which documents the delivery of drug exclusively to a preselected targeted cell type. Active targeting of liposomes to tumor cells is generally attempted by conjugating ligands to the liposomal surface which allow a specific interaction with the tumor cells. A number of proteins are exclusively expressed or greatly over expressed by tumor cells as compared to normal healthy cells. The surface of the liposomes can be decorated with targeting ligands to augment the cellular uptake by receptor mediated endocytosis. Till today various types of ligands have been explored for site specific delivery of liposomes, including antibody fragments or whole antibody, peptides, glycoproteins, vitamins, and oligonucleotide aptamers. If an anticancer drug could be delivered only to the correct site in the accurate concentration, cancer could be treated without experiencing fatal side effects.

Among the different approaches of active targeting, **immunoliposomes** using antibody or antibody fragment as a targeting ligand and a lipid vesicle as a carrier for both hydrophilic and hydrophobic drugs, is a fascinating prospect in cancer therapy (Figure 2). Immunoliposomes have the potential to transfer large numbers of drug molecules to tumor cells, and drugs delivered via immunoliposomes have antitumor activities similar to or greater than those of the drug alone. The ability of immunoliposomes to target tumor cells overcomes many limitations of conventional liposomes and provides a novel strategy for tumor-targeted drug delivery.

#### Antibodies as targeting ligands to target tumor cells

Several antibodies can be used as targeting ligands for liposomes to specifically target proteins or receptors overexpressed on cancer cell surfaces. The antibodies can be covalently coupled with the reactive phospholipids in the membrane or simply by hydrophobic bonding wherein the hydrophobic Fc portion of the antibody penetrates the lipid bilayer and immunologically active Fab portions extend into the aqueous phase and become available for interaction with receptors on the cell surface. The liposomes with antibodies attached to the surface of the liposomes are cleared rapidly by the RES. Coating of the liposome surface with hydrophilic polymers can help overcome the problems with clearance and improve circulation time. PEG coating of such liposomes with long PEG chains not only prevent the liposomes being recognized by the opsonins from plasma, but also hamper the interaction of the surface attached ligand with the target receptors. An interesting strategy reported to overcome this issue is attaching the targeting ligand to the distal end of the PEG chains (37). Targeting ability with antibody depends on density of the antibody on the surface of the liposomes. Use of only Fab' fragment instead of whole antibody would minimize the RES uptake by avoiding Fc receptor mediated clearance mechanism. To improve targeting ability without compromising circulation time, Fab' fragments of anti-FSHR monoclonal antibody (mAb) has been coupled to the distal end of the PEG lipid with maleimide functional group in present work.

Follicle Stimulating Hormone Receptor (FSHR) as a target

One of the proteins expressed abundantly and more or less selectively in ovaries is the follicle-stimulating hormone receptor (FSHR). FSHR is a G-protein coupled receptor with seven-transmembrane domains, present in granulosa cells of ovary is a unique transmembrane cell surface receptor. The expression of this receptor is maintained and even overexpressed on ovarian cancer cells. Further, FSHR, an internalizing receptor, is selectively expressed on the surface of the blood vessels of various carcinogenic tumors. FSH is observed to promote growth of OC and has been shown to induce vascular endothelial growth factor (VEGF) in granulosa cells promoting angiogenesis. Thus, FSHR may also contribute to the development of metastatic disease (38). The position of FSHR on the luminal endothelial surface suggests a potential role in tumor intravasation. Intravasation is a key component of the metastatic process in which malignant cells penetrate the endothelium and enter the circulation (39). FSHR which was previously believed to be expressed primarily in the ovary and testis, was recently found to be expressed in the tumor blood vessels of many solid tumor types, including prostate adenocarcinoma, urothelial carcinoma, and renal cell carcinoma (40).

Key characteristics of FSHR as an attractive therapeutic target in OC

- ✓ Limited expression of FSHR in normal tissues.
- ✓ Expression in the neovasculature of all genitourinary cancer specimens analysed to date.
- ✓ Potential functional role in angiogenesis and in the development of metastatic disease.
- ✓ Accessible to circulating ligands and internalization in endothelial cells upon binding.
- ✓ Binding sites for FSHR has been found in 50–70% of ovarian cancer tissues (41).

Hence, FSHR presents as an ideal candidate for targeted therapies to ovaries. Some domains including amino acid fragments 1–15, 33–53, 51–65, and 81–95 of  $\beta$  chain of FSH peptide have been reported to specifically bind FSHR for site specific drug delivery in ovarian cancer. The specific recognition and binding of these amino acid fragments to their receptors provide a means to use these hormone receptors as

new targeting sites, which allows therapeutic drugs to be concentrated at FSHR positive tumors while reducing nonspecific systemic distribution and toxic side effects (42), (43).

In the present study, we have selected the monoclonal antibody/mAb targeting FSHR. N-terminal of extracellular domain of receptor is involved in hormone as well as antibody binding to receptor. Binding of anti-FSHR antibody to FSHR was shown to block binding of FSH in reversible concentration dependent manner. Thus, binding of antibody and not the peptide may control binding of FSH and related growth promoting effects. Also, an attempt is being made to use Fab' fragment instead of whole antibody as a targeting ligand as it eliminates the immunogenic effect of Fc portion and the increased RES clearance through specific recognition by the phagocytic cells carrying Fc receptors. Smaller size of Fab' also improves tumor penetration. Fab' fragments also allow better way of conjugation to the liposomes containing DSPE-PEG-Mal through unique thiol groups in the hinge region (44).

#### **1.4 Aim of the proposed work**

Aim is to develop and characterize fab' fragment functionalized targeted immunoliposomal formulation of paclitaxel for ovarian cancer.

#### **1.5 Rationale**

The goal of chemotherapy in OC is to deliver drug efficiently and specifically to the ovarian tumorous cells. Chemotherapy using PTX, a first line drug, despite various improvements made in its delivery after its first marketed formulation using – a solubilized form for intravenous administration i.e. Taxol® (consisting of solubilizers and co-solvent in higher amount), and another recent one being nanoparticle formulation i.e. Abraxane® (protein nanoparticle formulation of PTX) still faces unmet challenges of non-specific distribution, short half-life and serious adverse effects. Thus, there is a need for suitable pharmaceutical alternative to currently marketed PTX formulations.

Currently, liposomal formulations have gained increased importance for delivery of variety of chemotherapeutic drugs with few already marketed and many under clinical trials. However, the carrier capacity of liposome for lipophilic drugs such as PTX, is limited due to the low loading efficiency in bilayer. Further, instability of the formulation due to crystallization of drug in bilayer is an issue. To overcome the above challenge of loading, simultaneous incorporation of drug in liposomal core by pre-encapsulation of PTX in the form of complex with cyclodextrin along with incorporation in bilayer presents promising strategy.

The selectivity of the formulation to OC cells was conferred by targeting a receptor that is specifically over-expressed at tumour site i.e. FSHR. In present research, an attempt was made to use Fab' fragment of anti-FSHR antibody as a targeting ligand and was grafted on the surface of liposomes. The functionalized formulation having capability to specifically target tumor cells was expected to deliver PTX specifically and efficiently to tumor cells thus leading to higher efficacy than non-targeted formulations.

### **1.6 Hypothesis**

It was hypothesized that the selectivity conferred by functionalizing the prepared liposomal formulation using receptor specific antibody fragment may lead to better anti-tumor efficacy and increase the response rates along with simultaneous improvement in toxicity profile for the chemotherapeutic agent PTX.

### **1.7 Research Plan and objectives**

The major objective of the study is to formulate nano sized immunoliposomal formulation double loaded with PTX and PTX-DM $\beta$ CD inclusion complex to improve efficacy and to decrease toxicity of chemotherapeutics by developing targeted drug delivery system for ovarian cancer.

The objectives of the proposed work are:

1. To formulate and evaluate stable complexes of PTX with DM $\beta$ CD for improving the aqueous solubility of PTX.
2. To develop and characterize PEGylated liposomes loaded with PTX in bilayer and optimize for maximum drug entrapment, minimum particle size, and enhanced steric stability.
3. To develop and characterize PEGylated double loaded liposomes incorporating PTX in bilayer and aqueous core and optimize for maximum drug entrapment, minimum particle size and stability.
4. To develop and characterize immunoliposomes by conjugating Fab' fragments of anti-FSHR monoclonal antibody, as the recognition moiety, over the PEGylated liposomes using functionalized PEG derivative (DSPE-PEG<sub>2000</sub>-Maleimide) via thioether linkage.
5. To evaluate developed formulation for cell uptake abilities, targeting efficiency and toxicity in ovarian cancer cell lines.
6. To evaluate the developed formulation in vivo in animals.

In present work, an attempt has been made to pre-encapsulated an anti-cancer drug, PTX, in cyclodextrin before loading into the liposomes. The PTX and PTX-DM $\beta$ CD (paclitaxel-Heptakis (2,6-di-O-methyl)-beta-cyclodextrin) inclusion complex have been loaded into phospholipid bilayer and aqueous core of PEGylated liposomes respectively. The strategy was furthered by developing PEGylated immunoliposomes decorated with an antibody fragment to the distal end of PEG chain, for site specific delivery of PTX in OC. In present research, we explored Fab' fragment of anti-FSHR antibody as a targeting ligand to graft on the surface of liposomes. Thus, Immunoliposomes double loaded with PTX and PTX-DM $\beta$ CD inclusion complex is hypothesized to actively target OC cells that may reduce side effects of current chemotherapy and may improve intra-tumor drug accumulation for efficient therapy of OC.

### 1.8 Expected Outcome

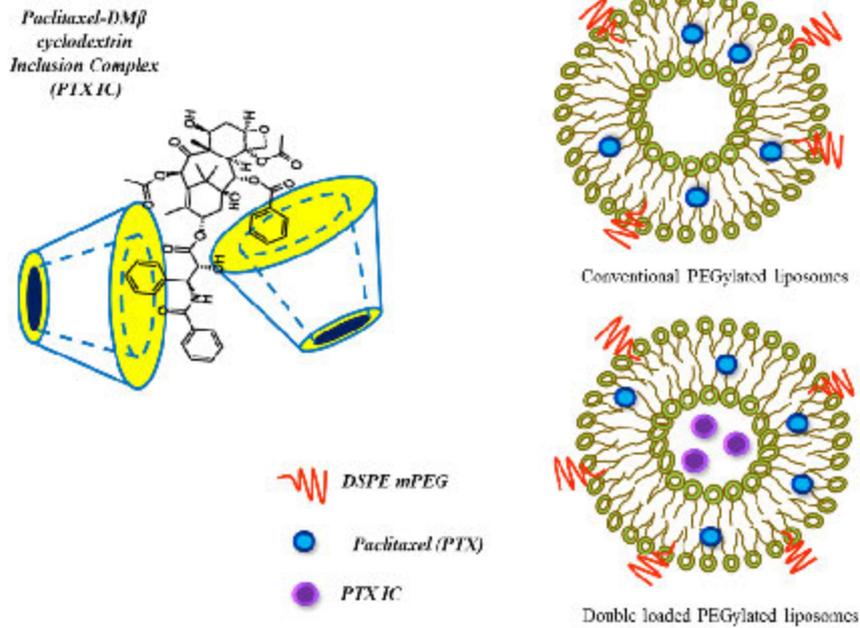
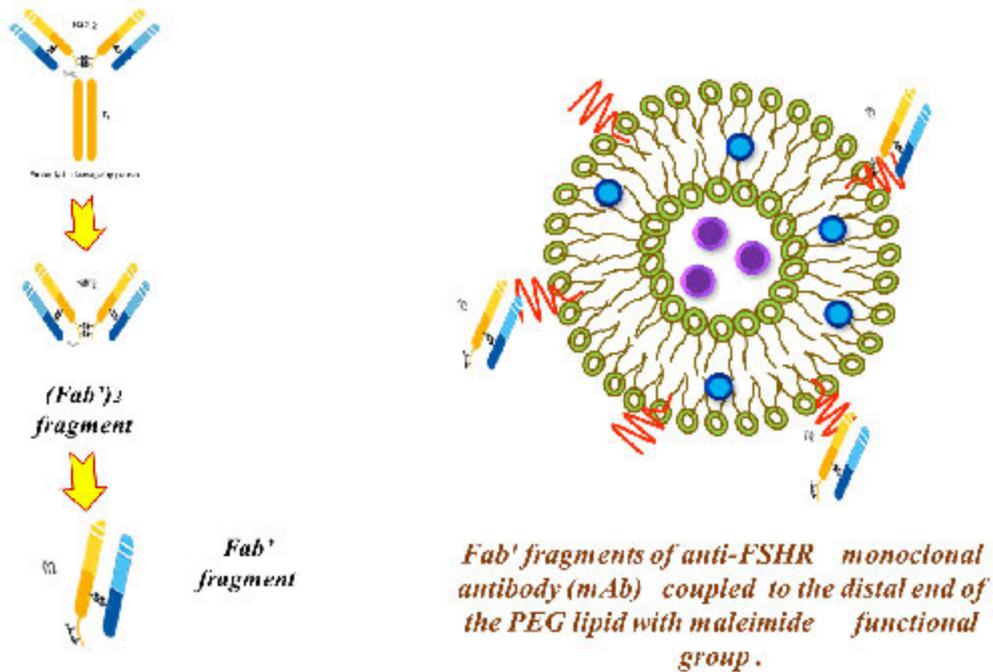
Presently, conventional dosage forms of PTX that are available in the market have low bioavailability, non-specific bio-distribution and high toxic effects. The dose can be reduced by improving the bioavailability and specific distribution,

thereby reducing cost of overall treatment. Favourable results from the present study would be a platform for clinical study and commercialization of nano-formulations like immunoliposomes containing PTX for effective treatment of ovarian cancer. Chemotherapeutic drug such as PTX formulations currently available in the market is not site specific and have side effects like agranulocytosis, thrombocytopenia, aplastic anaemia, bone marrow suppression etc. Development of a system capable of selectively delivering the drug to the intended target cells may be able to reduce these complications. Nanocarrier formulation like specific ligand (fragment of antibody) conjugated immunoliposomes of these drugs will help to reduce the associated side effect of the drug. PEGylation of liposomes will avoid macrophagic endocytosis and will provide long circulating time through i.v administration. The attached fragment of antibody will specifically target the diseased area. Here, the aim is to maximize anti-tumour efficacy of chemotherapeutic drug without compromising quality of life by the burden of toxicity. Development of such drug delivery system would establish as Platform Technology for the receptor targeting liposomes. The outcome of this study will provide base for researchers working on targeting therapy for ovarian cancer. It will be a way forward for developing formulations for the treatment of ovarian cancer. Thus, an effective formulation approach will be available for many coming drugs for the treatment most deadly diseases.

### 1.9 Plan of Work

- Literature survey.
- Analytical method development.
- Preparation and characterization of Inclusion Complex.
- Preparation and characterization of conventional PEGylated liposomes.
- Preparation and characterization of PEGylated double loaded liposomes.
- Preparation and characterization of Immunoliposomes.
- Determination of FSHR expression on OC cell lines.
- *In vitro* characterizations of PEGylated double loaded liposomes and Immunoliposomes.
- *In vivo* characterization of PTX.
- Summary and Conclusion.

## 1.10 Graphical overview

1. Improving the loading efficiency for PTX: Double loading in liposomes2. Active Targeting (Site Specific targeting) : Fab' armed liposome

### 1.11 References

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