Chapter-2 Literature Review

Literature Review

2.1 Ovarian Cancer

Definition:

Cancer is defined as uncontrolled growth of body's own cells. Such cells become un-associated with the cell death pathway and continue to grow uncontrollably. The cell death of a normal one get initiated when the basic structural and functional element i.e. DNA gets damaged. However, for cancerous cells bearing this faulty DNA does not die and continue to grow producing multiple cells/ replica cells bearing the same faulty DNA at the site. Further, these cells gain the capacity to invade other parts of the body leading to their metastasis and spread. The DNA damage may be during the replication process, due to mutation or environmental challenge. The mutation may be acquired or inherited type. Whatever, the reason maybe it is to be noted that all the tumors produced due to uncontrolled division may not be cancerous. Based on the capacity to metastasize, tumors may be classified as benign or malignant. Further, for malignant tumors, are named according to their site of origin rather than their site of metastasizing.

For the tumors originating in the cells of the ovary are called as ovarian cancer (OC). Based on the type of cells the ovaries are made up of the tumors can either be of epithelial cell, germ cell or stromal cell tumors. Of the three types, epithelial cell tumors are the most common with incidence of 90% of cases (1). It can also be noted that the development process for ovarian carcinomas follows peculiar and unique dissemination process (2). Two types of OC: Type I and II based on the extent of growth which may be either slow or rapid respectively (Figure 2-1). A chart for various histologic subtypes of ovarian carcinoma is presented in the Figure 2-2. Advanced stage of disease with widely dissemination of tumor nodes throughout the peritoneal cavity are observed in about 70% of patients that are diagnosed. Presence of ascitic fluids may facilitate dissemination of cancer cell in peritoneal cavity (3),(4).

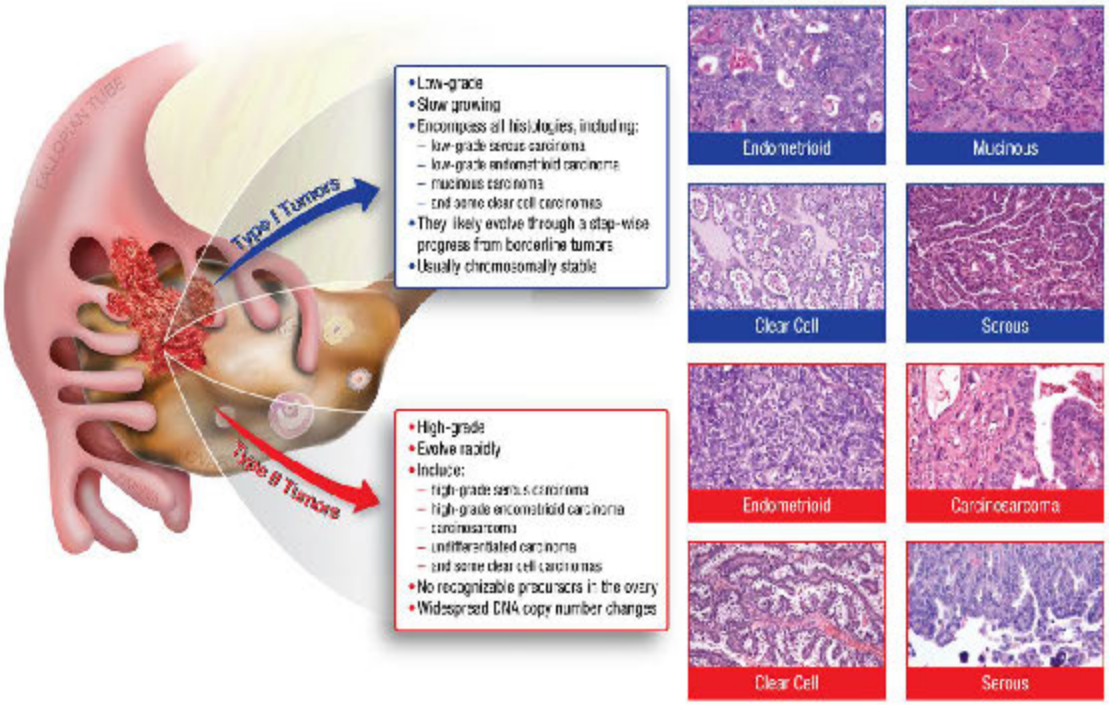


Figure 2-1: Subtypes of Ovarian cancer tumors

Ovarian cancer is often referred to as "a cancer that whispers", as the disease progression is not comprehended easily by symptoms and the symptoms are detected at a later stage after tumor has metastasized. Further, despite the progress made in chemotherapy, only 15% of cases are detected at early stage where the tumor is still in localized state and at this stage the 5-year survival rate is 92%. However, in majority of the cases the disease is diagnosed only after metastasis. Further, in most of the cases patients show relapses after treatment with the first line drug along with development of resistance to the chemotherapeutic and this associated with lack of effective second line drug to treat the relapses can be regarded as the main cause of poor survival rate in OC patients. The 5-year survival rate in OC patients with metastatic form is only 20-30% (5).

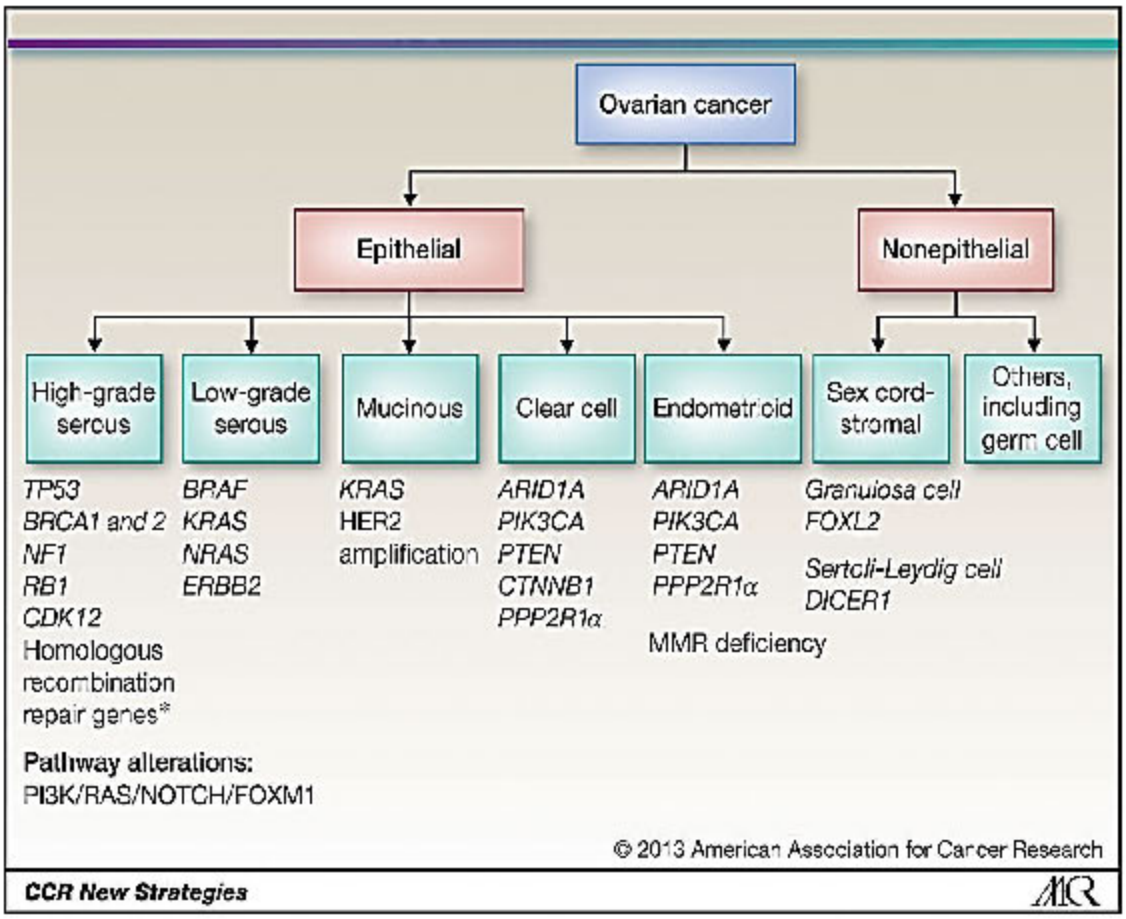


Figure 2-2: Histologic subtypes of epithelial ovarian carcinoma and associated mutations/molecular aberrations

Key statistics and facts about Ovarian Cancer:

United States: OC ranks fifth in cancer deaths among women, accounting for more deaths than any other cancer of the female reproductive system. A woman's risk of getting ovarian cancer during her lifetime is about 1 in 75 (6). The American Cancer Society estimates about 22,280 women will receive a new diagnosis of ovarian cancer and about 14,240 women will die from ovarian cancer in 2016. Overall, the 5-year relative survival rates for ovarian cancer patients are 46%. Generally, only 15% of cases are diagnosed at a local stage, for which 5-year survival is 92% (6), (7).

United Kingdom: Each year in the UK there are approximately 7,000 cases of OC and 4,300 cases of deaths from OC (8).

Australia: Estimated number of new cases of OC diagnosed in 2016 in Australia are 1,480 and estimated number of deaths from ovarian cancer in 2016 is 1,040 with 43% chance of surviving at least 5 years (9).

India: The OC survival rates have declined in India from 23% in 1995-99 to 14% in 2005-09 (10). In most of the population-based cancer registries in India, OC is the third leading cancer among women, trailing behind cervix and breast cancer and has the worst prognosis among all gynecological malignancies (11), (12).

Symptoms:

The initiation and progression of OC occurs with no specific symptoms until the disease gets metastasized. However, infrequently some common symptoms those observed are abdominal distention, bloating, pain and urinary urgency.

Diagnosis:

Detection of disease at an early stage is very difficult and no screening test are available for detection of OC in general population. Procedure available for diagnosis can be either imagining technique i.e. transvaginal ultrasound, CT scan, MRI or Positron Emission Tomography (PET). Only two markers that are approved by FDA for monitoring the disease progression are CA125 (Mucin 16) and HE4 (Human Epididymis protein 4). Other tumor markers that have been tested are SMRP, CA72-4, activin, inhibin, osteopontin, epidermal growth factor (EGFR), ERBB2 (Her2), CA15-3 and macrophage colony stimulating factor (M-CSF) (13). Herein, combination of one or more marker increases the sensitivity and specificity of detection. A list of tumor markers associated with OC is summarized in Table 2.1

Table 2.1: Tumor marker associated with OC.

Marker associated with OC	**Example**
Mucin related glycoprotein	CA-125 (Mucin 16); OVX1; HE4; Mesothelin (MES)
Hepatic and acute phase protein	Haptaglobin α; Bikunin; C-reactive protein; Apolipoprotein A1, Transthyretin Inter-α-trypsin inhibitors
Cytokines and growth factors	Vascular endothelial growth factors (VEGF); Macrophage colony stimulating factor (M-CSF); Osteopontin (OPN)
Serum proteases	Human Kallikreins; Prostasin.

Over and above those stated, antibodies have also been screened and though in its infancy have emerged as reliable markers for OC diagnosis. Termed as Radio

immune conjugates, these have capability to detect premalignant lesions also. Some examples of antibodies or their antibody fragment used include: B72.3 mAb; HMFG2 mAb; H317; mAb 170; OC-125 F(ab'); MOv18 mAb; Anti CEA Ab; Igovomab F(ab')2mAb (Indimacis 125); CYT-103 mAb; C0C183B2; Nanobody (2Rs15d).

Treatment and Management:

For effective management of OC the following treatment alternatives are available: often, 2 or more different types of treatments are used. This includes Chemotherapy; Surgery; Hormone therapy; Targeted therapy and/or Radiation therapy.

Chemotherapy is the use of drugs to treat cancer. Most often, chemo is a systemic treatment – the drugs are given in a way that allows them to enter the bloodstream and reach all areas of the body. Systemic chemo can be useful for cancers that have metastasized (spread). Most of the time, systemic chemo uses drugs that are injected into a vein (IV) or given by mouth. For some cases of ovarian cancer, chemotherapy may also be injected through a catheter directly into the abdominal cavity. This is called intraperitoneal (IP) chemotherapy. Drugs given this way are also absorbed into the bloodstream, so IP chemotherapy is also a type of systemic chemo. This is discussed in more detail later in this section.

The standard approach is the combination of a platinum compound, such as cisplatin or carboplatin, and a taxane, such as paclitaxel (Taxol®) or docetaxel (Taxotere®). For IV chemotherapy, most doctors favor carboplatin over cisplatin because it has fewer side effects and is just as effective. The typical course of chemo for epithelial ovarian cancer involves 3 to 6 cycles. A cycle is a schedule of regular doses of a drug, followed by a rest period. Different drugs have varying cycles; your doctor will let you know what kind of schedule is planned for your chemotherapy.

Chemotherapy and Cytoreductive surgery are the currently employed therapeutic approach for OC. First line chemotherapeutic agents employed are either plant derivatives i.e. Taxol – Paclitaxel and Docetaxel; or platinum containing agents Carboplatin and Cisplatin (14). For Epithelial tumors, either single or combination of vinblastine, bleomycin and cisplatin may be employed. The preferred treatment

strategy for epithelial tumors is by surgery which may be used in combination with radiation therapy to obtain higher cure rates. Subsequently, the surgery has to be accompanied by chemotherapy to prevent relapse (15).

Surgery:

This is the main method that is used for treatment. Goals of the surgery are: staging and debulking. Staging of OC is done based on the spread of cancer to ovaries or fallopian tubes or omentum or farther parts like pelvis or abdomen for which biopsy of target organ is done. The other goal of the surgery is debulking that is removal of tumor as much as possible. Thus, surgery can be helpful in removing solid tumors such a OC.

Ovarian Cancer treatment: Research and clinical status:

Scientists around the globe continue to research on the risk factors, causes, prevention, early detection and treatment of OC. Some of the promising recent research strategies are described below;

Early detection: Detection of cancer associated antibodies and radiolabeled antibody conjugates are forming the basis for early diagnosis of ovarian cancer. Only one diagnostic RIC, 111In satumomab pendetide (OncoScint CR/OV®), targeting TAG-72 was developed however, the commercialization of the RIC was discontinued (16).

Chemotherapy: New chemotherapy (chemo) drugs and drug combinations are being tested. The drugs trabectedin (Yondelis®) and belotecan have shown promise in some studies. Although carboplatin is preferred over cisplatin in treating ovarian cancer if the drug is to be given IV, cisplatin is used in intraperitoneal (IP) chemotherapy. Studies are looking at giving carboplatin for IP chemo. Drugs known as PARP inhibitors are in clinical trials for treatment of patients with platinum-sensitive recurrent OC.

Immunotherapy: Antibodies when given alone act as an anti-angiogenic agent or utilize the protective role of immune system against OC. In addition, when given in combination with chemotherapy, antibodies have turned out to sensitize chemo-

resistant tumors. Bevacizumab is the only FDA approved antibody for OC therapy which has shown a marked safety and efficacy profile in clinical trials (16).

Hormone therapy: For treatment of recurrent or later-stage ovarian cancer, tamoxifen (Nolvadex, Soltamax), aromatase inhibitors, and enzalutamide (Xtandi), a blocker of the androgen receptor, are being used.

Gene therapy: A new area of research is discovering how damaged genes in ovarian cancer cells can be corrected or replaced. Researchers are studying the use of specially designed viruses that carry normal genes into the core of cancer cells and then replace the defective genes with the functional ones.

Tumor Targeted nanocarrier: The concept of site specific drug delivery for treatment of localized disease in the body to improve therapeutic index of the drug is considered as perennial challenge to the formulator in modern formulation design. Antibodies are playing cardinal role in design of highly potent class of targeted therapies, such as antibody-drug conjugates and clinically viable tumor targeted drug nanocarriers.

2.2 Liposomes as a drug delivery carrier

Since their discovery by Bangham et al. about 50 years ago (17), liposomes have drawn a lot of interest as pharmaceutical carriers for drugs and genes. Liposomes are composed of a bilayer structure of either natural or synthetic phospholipids. Phospholipids are a key structural component of the cell membrane. They are amphiphilic in nature, possessing both hydrophilic and hydrophobic regions. Amphiphilic phospholipids self-assemble into bilayers (18), (19) by arranging their hydrophilic groups outward to interact with aqueous environments and arranging their lipophilic groups toward the center of the bilayer. Liposomes can be unilamellar or multilamellar depending on the number of lipid bilayers formed (20).

Liposome surface properties can be easily manipulated for drug delivery. Surface charge can be modified by adjusting the lipid composition to add either neutral character or cationic charge using cationic lipids, which directly influences liposome interactions with the negatively charged cell membrane. Neutral liposomes have no significant cell membrane interaction, and the neutral charge results in

liposome aggregation (18). Aggregation is a key issue for drug delivery because particle aggregates are rapidly cleared by the phagocytosis, which greatly reduces drug delivery efficiency. Anionic liposomes are internalized through clathrin-mediated endocytosis (21)), while cationic liposomes deliver their contents by membrane fusion and/or by endocytosis.

Liposomes can be used to carry water- or lipid-soluble drugs and its surface can be modified according to the requirement Figure 2-3. Unilamellar liposomes have an aqueous core that is used to carry water-soluble drugs (22), while multilamellar liposomes have lipophilic layers between hydrophobic tail groups that are used to carry lipid-soluble drugs. One of the major drawbacks of classical liposomes was their rapid clearance from circulation, due to adsorption of plasma proteins (opsonins) to the naked phospholipid membrane, triggering recognition and uptake of liposomes by the receptors present in the mononuclear phagocytic system. A breakthrough in the field of liposomes came with the development of sterically stabilized (stealth) liposomes, which utilize a surface coating of hydrophilic carbohydrates or polymers, usually a lipid derivative of polyethylene glycol (PEG), to help evade mononuclear phagocytic system recognition. PEG attracts a water layer to the liposome surface, thus providing hydrophilic repulsion to opsonin adsorption. Stealth liposomes are capable of passive accumulations in various pathological sites, such as solid tumors and infarcted areas, via the so-called enhanced permeability and retention effect (23). This effect is based on the fact that the pathological vasculature, unlike vasculature of normal healthy tissues, is 'leaky', that is, penetrable for large molecules and even for small particles, which allows for their extravasation and accumulation in an interstitial tumor space. In addition, solid tumors have elevated interstitial pressure and impaired lymphatics that hinder the diffusion of colloidal particles such as liposomes from the tumor. Once inside the tumor interstitium, cytotoxic drugs are released from the liposomes in a sustained manner, killing the neighbouring cells.

Some of the marketed products of liposomes incorporating cancer drugs available are, Doxil, DaunoXome, LipoDox, and Myocet, and many others are in clinical trials (24). Amphiphilic drugs that are weak bases or weak acids can also be loaded into the liposome interior using remote loading methods like the ammonium sulphate method for doxorubicin or the pH gradient method for vincristine. Doxil and its second-generation drug, LipoDox, both contain doxorubicin encapsulated within a liposome with a PEG-modified surface. Doxil has been proven effective in treating

drug resistant tumors in clinical trials (25). Both Doxil and LipoDox have been used successfully to treat many cancers including Kaposi's sarcoma, ovarian cancer, and metastatic breast cancer (26). Myocet is an unpegylated liposomal doxorubicin approved for use in Canada and Europe to treat metastatic breast cancer (27). DaunoXome, which is a pegylated liposomal daunorubicin, has been approved to treat blood tumors (24).

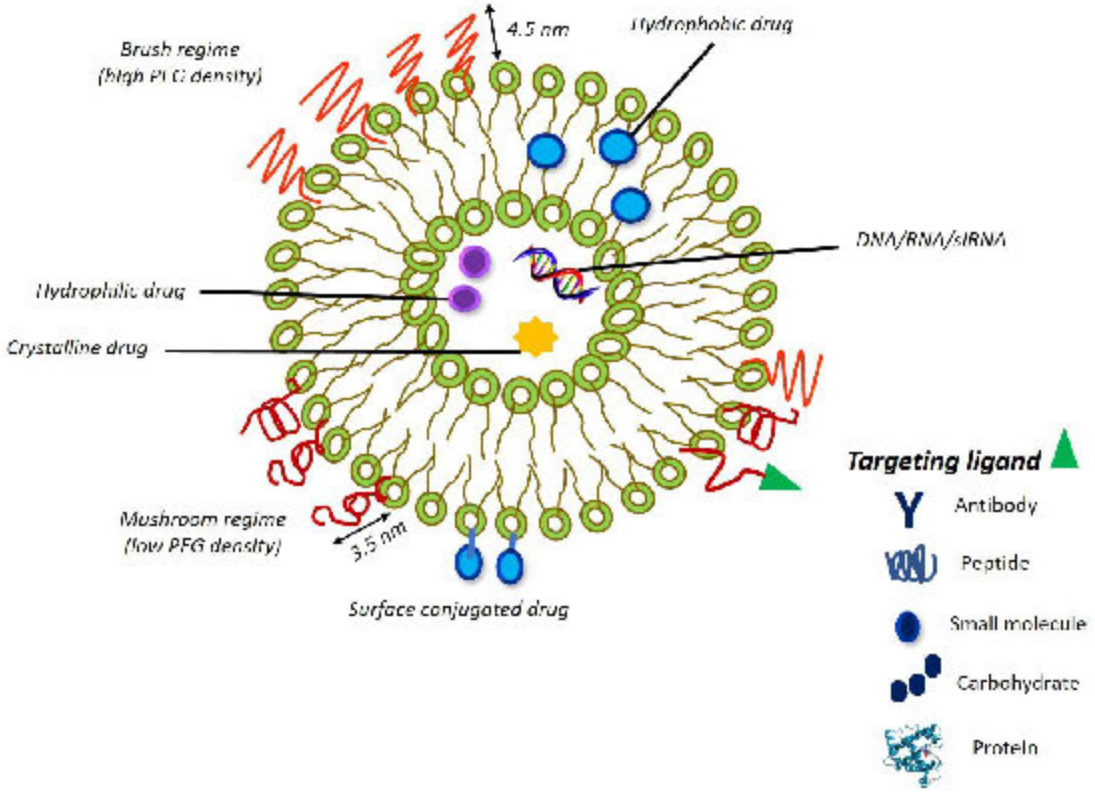


Figure 2-3: Liposomes as drug delivery carriers

2.3 Cyclodextrins as a drug delivery carrier

Cyclodextrins are chemically and physically stable macromolecules produced by enzymatic degradation of starch. They are water-soluble, biocompatible in nature with hydrophilic outer surface and lipophilic cavity (28). They have the shape of truncated cone or torus rather than perfect cylinder because of the chair conformation of glucopyranose unit (29). The CDs of biomedical and pharmaceutical interest are cyclic oligosaccharides made up of six to eight dextrose units (α-, β-, and γ-CDs, respectively) joined through one to four bonds. The most common natural

cyclodextrins are α, β, and γ consisting of 6, 7, and 8 glucopyranose units (30). The derivatives of β-cyclodextrin are listed in Table 2.2.

Cyclodextrin molecules are relatively large with a number of hydrogen donors and acceptors and, thus, in general they do not permeate lipophilic membranes. Cyclodextrins are widely used as "molecular cages" in the pharmaceutical, agrochemical, food and cosmetic industries (31). In the pharmaceutical industry they are used as complexing agents to increase the aqueous solubility of poorly soluble drugs and to increase their bioavailability and stability (32). Cyclodextrin consists of (α-1,4)-linked α-D-glucopyranose unit with a lipophilic central cavity and the structures of various CDs are as shown in Figure 2-4. Due to the chair formation of the glucopyranose units, cyclodextrin molecules are shaped like cones with secondary hydroxyl groups extending from the wider edge and the primary groups from the narrow edge. This gives cyclodextrin molecules a hydrophilic outer surface, whereas the lipophilicity of their central cavity is comparable to an aqueous ethanolic solution. The naturally occurring cyclodextrins have limited aqueous solubility due to the strong intermolecular hydrogen bonding in the crystal state. Substitution of the H-bond forming -OH group has improved their solubility (33).

Inclusion complexes are formed when the "guest" molecule usually a drug is partially or fully included inside the "host's cavity" (34). Owing to the hydrophobic cavity, cyclodextrins as host offer the guest a suitable environment for interaction. The outer sphere of cyclodextrins is compatible with water, which allows hydrogen bonding cohesive interactions (35), (36). Due to this feature, CDs form inclusion complexes with a wide variety of hydrophobic compounds and change the physicochemical and biological properties of guest molecules (37). These changes may enhance the therapeutic potential of drugs by diminishing their decomposition before they enter tissues and by altering how they enter tissue. The ability of a CD to form an inclusion complex is a function of steric as well as thermodynamic factors. The driving force for complexation involves the removal of water molecule from hydrophobic cavity and formation of Vander Waal forces, hydrophobic, and hydrogen bond interactions (38). The approach used for complexation is phase solubility study as described by Higuchi and Connors, which examines the effect of cyclodextrin (solubilizer/ligand) on the drug being solubilized (substrate) and several examples of cyclodextrin enhanced solubility for various drugs are listed in Table 2.3

Table 2.2: Pharmaceutical derivatives of β-cyclodextrins

Cyclodextrin	R=H or
B-Cyclodextrin	-H
2-Hydroxypropyl-β-cyclodextrin	$-CH_2CHOHCH_3$
Sulfobutylether-β-cyclodextrin sodium salt	$-(CH_2)_4SO_3^-Na^+$
2,6 dimethylated-β-cyclodextrin	$-CH_3$
Branched β-cyclodextrin	Glucosyl or maltosyl group

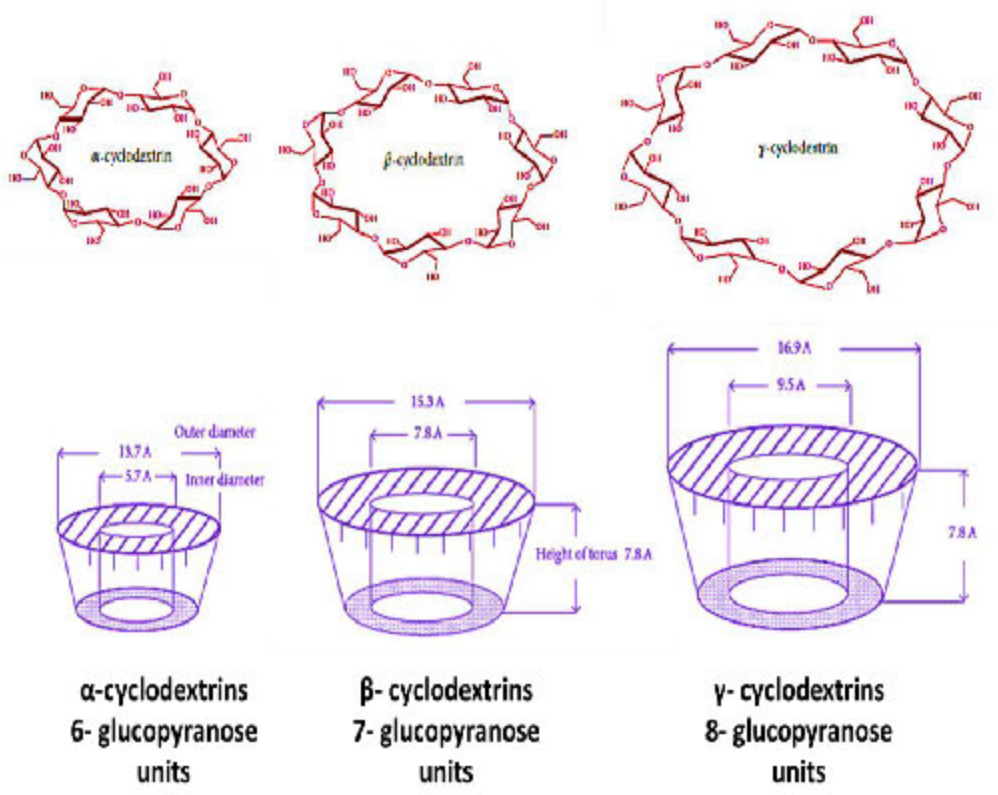


Figure 2-4 Types of Cyclodextrins

Table 2.3: Examples of Cyclodextrin-enhanced solubility and dissolution

Improvements	Drug
β-cyclodextrin	Nimesulide, sulfomethiazole, lorazepam, Ketoprofen, Griseofulvin, ibuprofen
α-cyclodextrin	Praziquantel
γ-cyclodextrin	Omeprazole, digoxin
HP-β-cyclodextrin	Albendazole, Ketoprofen, Itraconazole, Carbamazepine, Phenytoin, Rutin
DM-β-cyclodextrin	Naproxen, Campotothesin
SBE--β-cyclodextrin	Danazol, Fluasterone, Spironolactone
RM-β-cyclodextrin	ETH-615, Tacrolimus
Randomly acetylated amorphous -β-cyclodextrin	Naproxen

2.3.1 Cyclodextrin Complexation with Anticancer Drugs

Chemotherapy for cancer, particularly for recurrent and metastasis disease, has limited therapeutic effect. Limited aqueous solubility (hydrophobicity), degradation in gastrointestinal fluids, insufficient *in vitro* stability (shelf life), low bioavailability, short *in vivo* stability (half-life), affinity for intestinal and liver cytochrome P450 (CYP3A4) and Pglycoprotein (P-gp) in the intestinal barrier, poor intestinal permeabilities, and strong dose dependent side effects of promising anticancer drug candidates have long been obstacles in treatment of cancer (39). Lack of selectivity and short blood circulation time which cause various toxic side effects are also issues of major concern (40). The narrow therapeutic index of some anticancer drugs and the fact that these cytotoxic drugs damage not only cancer cells but also normal and healthy tissue is a major challenge. Multidrug resistance, due to increased efflux pumps such as P-glycoprotein (Pgp) in the cell membrane, which transport most of anticancer drugs out of the cell, is also major problem (41). Thus, there is a need to develop such a delivery system, which combines safety, efficacy, and convenience. Cyclodextrins are competent enough to overcome certain forms of above associated drawbacks of anticancer drugs. The lack of efficient treatment has created the need to develop and implement novel technology based on combination strategy of cyclodextrin complexation and nanotechnology with a view to make the therapy more useful and acceptable. The formation of inclusion complex with nontoxic agents leads to improvement in physicochemical properties of drug. Most of the anticancer drugs have been complexed with cyclodextrin and their derivatives to improve/enhance the solubility and stability, increase the bioavailability and dissolution, reduce the toxicity, and modify the physicochemical characteristics (42), (43), (44), (45). Complexation of doxorubicin with γ-CD and HP-γ-CD led to an increase in permeability across blood brain barrier, due to the disruption of the membrane (46). Similarly, the β-CD-PEG folic acid conjugate increased the solubility of chlorambucil. Complexation of 9- nitro camptothecin with HP-β-CD led to significant enhancement in antitumor activity with low toxicity (47).

2.3.2 "Drug in Cyclodexrtin in Liposome" as a drug delivery carrier

Liposomes can encapsulate hydrophilic as well as hydrophobic drug in its aqueous core and lipid bilayer respectively. Additionally, their composition can be adapted to achieve predetermined release and circulation in biological environment. In comparison with ICs, liposomes can encapsulate and retain drug molecule with better stability profile, has long circulation time when PEGylated and are able to deliver drug pay load to the specific sites. But, it is observed that the amount of drug loaded in the bilayer is limited and is determined by the drug to lipid molar ratio which is generally low. Further, lipophilic drugs incorporated in higher amount in bilayer may destabilize the membrane thus impacting formation of stable bilayer thus releasing the content prematurely (48).

CD and liposomes have been extensively explored as carrier containers to deliver drugs efficiently. However, they differ in their structural properties, drug encapsulation and retention efficiencies and in vivo behavior. "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 (48). The concept, would allow entrapment of water soluble cyclodextrin ICs of water insoluble drugs such as PTX in aqueous core of liposomes. The extent of loading efficiency depends on the entrapment of drug into CD cavity, the concentration of ICs in solution during liposomal formulation and the method of preparation of liposomes. This strategy has been explored by some of the researches to encapsulate water insoluble drugs in aqueous compartment of liposomes and seems to be promising in improving drug to lipid molar ratio and loading efficiency as compared to conventional incorporation of drugs in lipid phase (49), (50). Herein, a suitable alternative is to encapsulate drug as CD complex in the aqueous core. Incorporation of lipophilic drug in form of water soluble complex only in core though improves the stability of the system but present limitation in the amount being loaded as the volume of aqueous compartment is very low compared to the volume of bilayer. Such strategy has been widely used to accommodate a variety of lipophilic drug and has shown improved physicochemical and pharmaceutical properties compared to the conventional liposomes.

Considering the probability of encapsulating drug in both bilayer and core of liposome for maximizing the drug load, double loading of liposomes was proposed.

Herein, a certain amount of drug is incorporated in the bilayer during the film formation stage and the additional amount of drug is then incorporated in the core as aqueous soluble supramolecular species associated with cyclodextrin. Thus, using this strategy potential increase in drug to lipid molar ratio has been achieved and further fast onset and a prolonged effect can be attained.

2.4 Targeted therapeutics for cancer

The goal of targeted cancer therapy is (1) to deliver a high dose of an anticancer drug directly to the site of a tumor, (2) to enhance drug uptake by malignant cells, and (3) to minimize drug uptake by non-malignant cells. The general approach for designing targeted cancer therapies is to design the drug delivery system to exploit the features that are unique to tumor cells and tumor tissues. Targeted delivery research has focused on unique features of the tumor microenvironment, such as leaky vasculature, overexpressed cell surface receptors, and intra-tumoral pH differences, as well as features of the cell uptake process, such as endosomal pH. Advances in cancer research in combination with advances in biomaterials and nanotechnology have enabled the development of targeted anticancer drug delivery and a more tailored approach to treating individual cancer types. A multidisciplinary approach that includes cancer biology, biomaterials, and nanotechnology has the potential to improve treatment outcomes while minimizing harmful side effects. The design of an effective targeted therapy will require optimization of therapeutic particles, cancer cell targeting, and drug release mechanisms. Targeting moieties, ligands that bind to receptors overexpressed on malignant cells, can be conjugated to particles to increase cellular uptake, and as a result, enhance treatment efficacy.

2.4.1 Passive targeting

Passive targeting, first described in 1986, takes advantage of the greater vascular permeability and poor lymphatic drainage of tumors that result in the accumulation of micro- and nano-particles in tumor tissue (51). The particles accumulate through passive diffusion, a phenomenon known as the enhanced permeability and retention (EPR) effect (52). Enhanced permeability of the EPR effect is the result of the leaky vasculature in tumor tissue. Vessels in tumors are

irregularly shaped, leaky, and dilated due to rapid growth and abnormal blood flow (53)). Endothelial junctions, gaps in the endothelium that mediate passage of macromolecules from the blood to tissue, vary between non-malignant and malignant tissue. In normal vasculature, endothelial junctions between cells are narrow, ranging from 5 to 10 nm in width (20). However, in tumor tissue, these junctions range from 100 to 780 nm depending on the tumor type (54). These large gaps allow extravasation of particles out of circulation and into the tumor tissue.

2.4.3 Design criteria for targeting anticancer drugs

When cancer drugs enter the body, they face many physiological barriers that can prevent them from reaching the target site and achieving these design goals (59). These physiological barriers dictate the design parameters for targeted cancer treatments. A thorough understanding of these barriers is necessary for the development of effective targeting. Table 2.4 represents various strategies to overcome barriers faced in achieving higher efficacy of nano-formulation.

Table 2.4: Barrier faced and strategies to enhance efficacy of nano-formulations

Barrier	Design feature	Strategy
Opsonization and MPS	Hydrophobicity	Increase hydrophilicity
	Size	Decrease particle size (<200 nm)
Extravasation	Surface charge	Use cationic particles to increase extravasation and uptake
	Size	Tailor particle size to endothelial junction size of tumor tissue (<200 nm, with enhanced tissue penetration and retention at ~50–100 nm)
Drug uptake and release	Ligand conjugation	Conjugate ligands to particles that target overexpressed receptors on malignant cells. Use multiple ligands and oblique particles to increase binding avidity
	Stimuli-responsive element	Use pH, temperature, or enzyme-responsive polymers or linkers to trigger release
Multidrug resistance	Particle encapsulation	Encapsulate therapeutics using particles such as micelles, liposomes, polymeric particles, dendrimers, or carbon nanotubes
Particle elimination	Biodegradable	Use biodegradable materials to avoid accumulation in the liver and spleen
	Size	Particles <20 nm are excreted, while larger, nondegradable particles accumulate in the liver and spleen

Targeting moieties:

Targeting moieties are ligands that bind to receptors that are overexpressed on cancer cells (Figure 2-5). Conjugating targeting moieties to the surface of particles promotes uptake and intracellular retention of particles by malignant cells, both of which enhance therapeutic efficacy (60).

Active targeting of malignant cells using ligands promotes receptor-mediated endocytosis (Figure 2-6). After a ligand binds to its corresponding cell surface receptor, the receptor–ligand–particle complex is endocytosed. Active targeting promotes direct cell kill and enhances cytotoxicity of anticancer drugs against malignant cells, while passive targeting promotes accumulation of particles in tumor tissue (61). Targeting moieties discussed here include folate, transferrin, monoclonal antibodies (mAbs), peptides, EGF, and aptamers. Ideally, unique cell surface antigens

would be expressed exclusively on, and homogeneously among, cancer cells (62). However, receptors overexpressed on cancer cells are also expressed on non-malignant. The choice of targeting ligand can be crucial to the success of targeting applications. Variables that must be considered include the degree of receptor expression; whether the ligand is internalized or not; choice of antibody, antibody fragments or non-antibody ligands; and binding affinity of the ligand. The antigen or receptor should also not be shed or downregulated (63). The various active and passive targeting systems under clinical trial for ovarian cancer are presented in Table 2.5

Types of targeting agents

Targeting agents can be broadly classified as proteins (mainly antibodies and their fragments), nucleic acids (aptamers), or other receptor ligands (peptides, vitamins, and carbohydrates). Targeting cancer with a mAb was described by Milstein in 1981 (64). Over the past two decades, the feasibility of antibody-based tissue targeting has been clinically demonstrated (reviewed in (65) with 17 different mAbs approved by the US Food and Drug Administration (FDA) (66). Today, over 200 delivery systems based on antibodies or their fragments are in preclinical and clinical trials (67). Recent developments in the field of antibody engineering have resulted in the production of antibodies that are of animal and human origins such chimeric mAbs, humanized mAbs (those with a greater human contribution), and antibody fragments. Antibodies may be used in their native state or as fragments for targeting. However, use of whole mAbs is disadvantageous because the presence of two binding sites (within a single antibody) gives rise to a higher binding avidity. Furthermore, when immune cells bind to the Fc portion of the antibody, a signalling cascade is initiated to kill the cancer cells. However, the Fc domain of an intact mAb can also bind to the Fc receptors on normal cells, as occurs with macrophages. This may lead to increased immunogenicity — the ability to evoke an immune response — and liver and spleen uptake of the nanocarrier. An additional advantage of whole/intact antibodies is their ability to maintain stability during long-term storage. Although antibody fragments including antigen-binding fragments (Fab), dimers of antigen-binding fragments (F(ab')2), single-chain fragment variables (scFv) and other engineered fragments are less stable than whole antibodies, they are considered safer

when injected systemically owing to reduced non-specific binding (63), (67). To rapidly select antibodies or their fragments that bind to and internalize within cancer cells, phage display libraries that involve a high throughput approach may be used (68), (69). This method generates a multitude of potentially useful antibodies that bind to the same target cells but to different epitopes (a part of a macromolecule that is recognized by antibodies; one receptor may have several epitopes that will be recognized by multiple antibodies). For example, through a selective process, scFv antibodies have been identified for superior binding and internalization properties for prostate cancer cells (70).

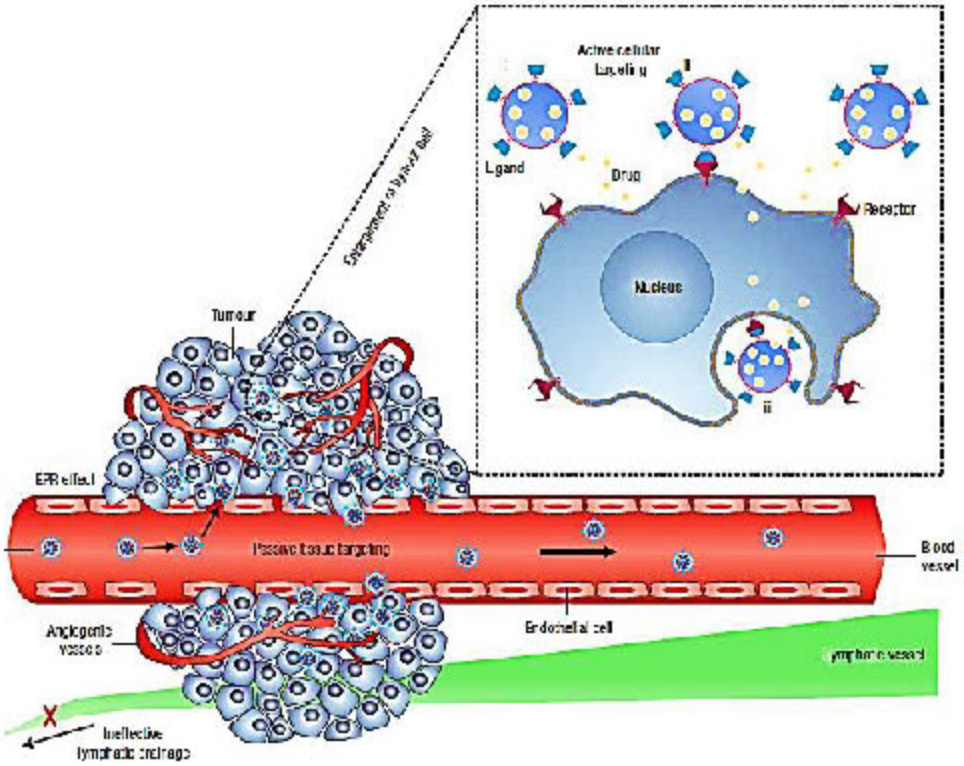


Figure 2-5: Schematic representation of targeting approach and mechanisms by which nanocarriers (circles) can deliver drugs to tumours.

The nanocarriers may get internalized or attach to receptor at cell surface to act as depot to release therapeutic slowly or may release content in proximity of target cells (71).

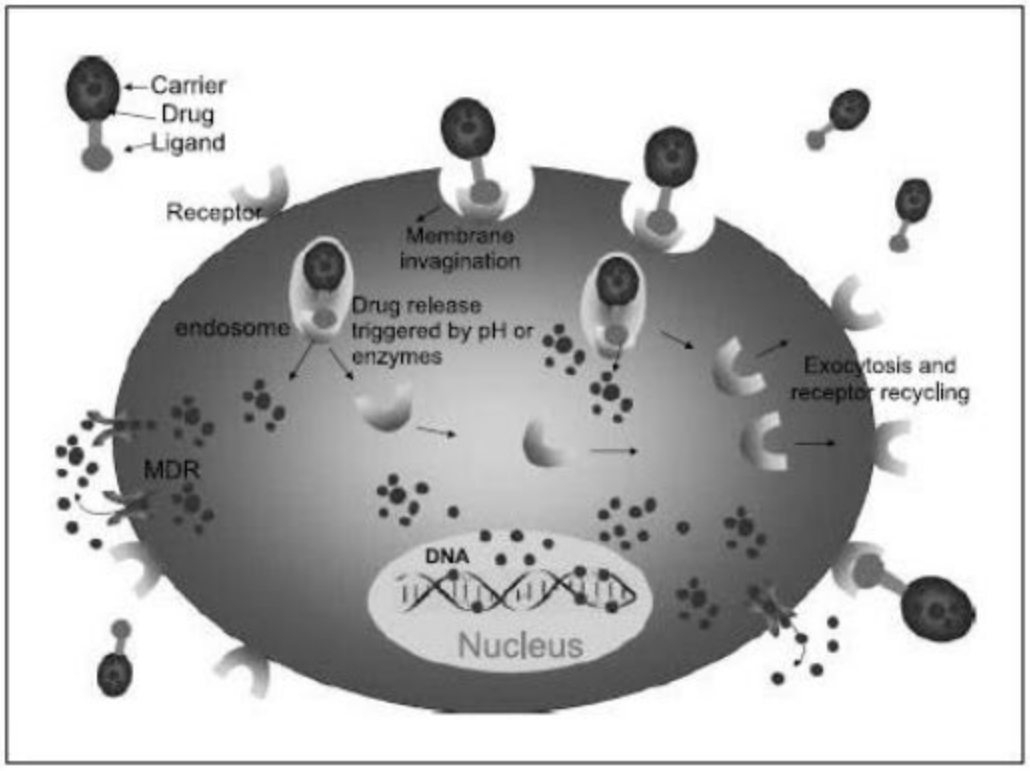


Figure 2-6: Receptor mediated endocytosis of nanocarrier.

Steps: particle internalization by endocytosis via receptor interaction → acidic pH of endosome → release of drug in cytoplasm (P-gp efflux bypass leading to high intracellular concentration).

Table 2.5: Nanocarrier based active and passive targeting systems for OC under clinical trials.

Targeting	Name	Company	DDS	Drug	Status	Clinical Trial
Active Targeting	-	Calando Pharmaceuticals	Cyclodextrin	siRNA anti-RRM2	Phase I	CALAA-01, NCT00689065
Passive Targeting	Doxil®/ Caelyx®	Janssen	Liposome	Doxorubicin	Marketed, breast and ovarian cancer multiple myeloma and Kaposi syndrome	-
	Nanotax®	CritiTech Inc.	Nanocrystals	Paclitaxel	Phase I	NCT00666991
	-	National Cancer Institute	Lipid Nanoparticle	siRNA anti-PLK1	Phase I	NCT01437007
	-	National Cancer Institute	Albumin stabilized nanoparticle	Lapatinib ditosylate + paclitaxel	Phase I	NCT00313599
	-	Cerulean Pharma Inc	Cyclodextrin	Campthotecin	Phase I & II	CRLX101, NCT00333502
	Genexol-PM®	Samyang	Micelles	Paclitaxel (+carboplatin)	Phase II	NCT00886717, NCT01276548, NCT00877253

	Myocet®	Cephalon Europe	Liposomes	Doxorubicin	Marketed, breast cancer; Phase II: Ovarian Cancer	NCT01100372
	Xylotax®	Cell Therapeutics	Conjugate	Paclitaxel	Phase II & III	NCT00045682, NCT00017017, NCT00108745, NCT00060359, NCT0069901
	Paclical®	Oasmia Pharmaceuticals AB	Micelles xr17	Paclitaxel	Phase III	NCT00989131
	Abraxane®	Abraxis Bioscience SAS	Conjugate	Paclitaxel	Marketed, breast cancer, Phase I, II & III: Ovarian Cancer	74 active studies
	Volasertib	Boehringer Ingelheim	Liposomes	Paclitaxel	Phase II	NCT01121406
	-	Boehringer Ingelheim	Pegylated Liposomes	Doxorubixin + carboplatin + BIBF1120	Phase I	NCT01314105

2.4.2 Active targeting

Active targeting uses ligands to specifically target receptors that are overexpressed on malignant cells. Ligands are molecules, such as folate, transferrin, epidermal growth factor (EGF), and aptamers, which bind to receptors on the surface of a cell. Ligands are conjugated to anticancer drugs or particle-encapsulated drugs to target malignant cells or tumor endothelium. Conjugation is the physical or chemical attachment of a ligand directly to an anticancer drug or attachment to a particle encapsulating an anticancer drug. Ligand candidates for cancer treatment target receptors that are overexpressed on malignant cells. The folate receptor (FR) and the epidermal growth factor receptor (EGFR) are two examples of receptors that are overexpressed on many types of malignant cells. Therefore, conjugation of these ligands to drugs or particles will result in receptor-mediated active targeting and higher drug or particle concentration in malignant cells than in non-malignant cells.

Active targeting promotes internalization of ligand-conjugated drug carriers into a cell via receptor-mediated endocytosis. The lack of tumor selectivity of anticancer drugs and the development of multidrug resistance (*mdr*) have given impetus to the development of target-specific agents and new classes of cytotoxic compounds that may be able to overcome *mdr (55).* The drug may be released either at the surface of the cell or upon internalization. The ligand-conjugated particle and receptor are first internalized via invagination, and then an endosome is formed. The anticancer drug must escape the endosome before it fuses with the lysosome to avoid being damaged or destroyed by lysosomal enzymes. After release of the drug and receptor from the endosome, some receptors are recycled back to the surface of the cell where they will be available for another cycle of endocytosis. The active-targeting approach addresses many of the goals for improving cancer therapies. Ligands conjugated to cancer drugs often help protect the cancer drug from degradation and enhance the physical and chemical stability of the drug (56). Ligand binding also increases the drug dose delivered to malignant cells, which permits systemic administration of smaller doses. Particle internalization that occurs by active targeting has been shown to enhance therapeutic effects (57), an important advantage over passive targeting. However, active targeting alone will not achieve optimal results. If an anticancer drug is delivered systemically, the ligand-conjugated drug or

drug–particle complex must first reach the cancer tumor before the advantages of active targeting can be realized. Passive-targeting mechanisms using the EPR effect are still necessary for extravasation and drug or particle accumulation in tumor tissue. Therefore, it is necessary to use a combination of active and passive targeting in designing drug carriers to improve targeted delivery of cancer therapeutics (58).

2.5 Follicle Stimulating Hormone Receptor

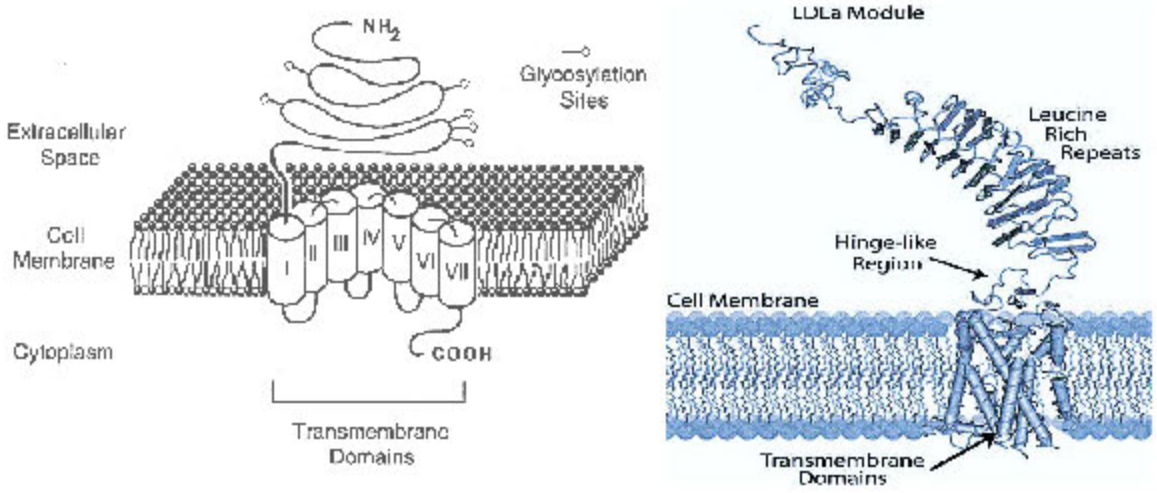


Figure 2-7 FSHR

Follicle Stimulating Hormone Receptor, a transmembrane cell surface receptor is a 30 kDa heterodimeric glycosylated transmembrane protein that belongs to the G protein-coupled receptor family. Structurally, these glycoproteins share a common alpha subunit, but have unique beta subunits that confer receptor specificity (72). Upon binding follicle stimulating hormone (FSH), FSHR transduces signal primarily via the adenylyl cyclase-cAMP-protein kinase A pathway. FSH binds to the FSH receptor, which belongs to the G-protein coupled superfamily characterized by their 7 hydrophobic transmembrane domains comprising intracellular and extracellular helices. The FSH receptor is coupled to the Gs subtype, which activates cyclic AMP (cAMP) when the receptor is activated by FSH (73). FSH, a central hormone of mammalian reproduction, is produced by the gonadotroph cells in the anterior pituitary gland. The target organs for FSH are the ovary and testis. FSHR is expressed by ovarian granulosa cells and is

critical for follicular maturation during the menstrual cycle and in the production of estradiol through aromatization of androgens (74). FSHR is also expressed by ovarian epithelial cells and the endometrium (75). In males, testicular Sertoli cells express FSHR, which is essential for Sertoli cell proliferation in immature testis and maintenance of qualitatively and quantitatively normal spermatogenesis in adults (76). FSHR is also expressed in human osteoclasts and monocytes (77). In the vasculature of non-malignant tissues, FSHR is only expressed in placental endothelial cells (78) and, to a lesser extent, in the endothelium of ovaries (79) and testis (80).

Recently, it was demonstrated that FSHR is selectively expressed on the surface of the blood vessels of a wide range of tumors. Results suggest that FSHR will be able to act as a general target for anti-cancer drugs as well as for agents which destroy or block blood vessels in tumours. Because FSHR is notably absent in most healthy tissues, its use could help minimize the damage that anti-cancer drugs do to surrounding tissue or organs. Expression of FSHR was confirmed by the microvasculature of metastatic tumors. This fact strongly increases FSHR potential relevance as a clinical target for cancer imaging and for therapy, especially for tumors that are highly resistant to currently available antiangiogenic treatments (81).

Remarkably, FSHR was identified in the neovasculature of every tumor evaluated. FSHR expression was identified almost exclusively at the tumor periphery, in a region that extended, 1 cm inside or outside of the tumor. Importantly, FSHR expression was largely limited to neoplastic tissue. Though 20% of samples from patients with benign prostatic hyperplasia (BPH) demonstrated FSHR expression in the endothelium, FSHR expression was not observed in the blood vessels of nonmalignant tissues, including conditions such as rheumatoid arthritis, chronic pancreatitis, Crohn's disease, and normal wound healing. Uninvolved normal tissues adjacent to tumor served as a negative control and, beyond several millimeters from the tumor, were always negative for FSHR. A mouse xenograft model was used to show that FSHR can be targeted by circulating ligands. Lymph node carcinoma of the prostate (LNCaP) tumors were grown on nude mice, and the mice were subsequently sacrificed and perfused with anti-FSHR antibody that had been coupled to colloidal gold. Upon evaluation with electron microscopy, the conjugated gold particles could be seen adhering to the neovasculature. These particles were internalized into

endothelial cells through coated vesicles, endosomes, and multivesicular bodies. These findings confirm that FSHR is expressed on the luminal endothelial surface and can bind to, and internalize, targeted ligands, characteristics critical to the development of drug- or radio-immunoconjugates and imaging agents targeting FSHR (82).

The presence of FSHR on tumor blood vessels raises the possibility that FSHR contributes to neoangiogenesis. FSH has been shown to induce vascular endothelial growth factor (VEGF) in granulosa cells and conceivably, a similar role of FSH-FSHR signaling could exist in the tumor endothelium (83). FSHR may also contribute to the development of metastatic disease. 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. The molecular mechanisms of this process are poorly understood. The expression of FSHR at the periphery of tumors, where the tumor interacts with stroma, further suggests that FSHR may be of relevance to the metastatic process. The epithelial-to-mesenchymal transition (EMT) is believed to be critical for the formation of metastases (84), and stromal elements interacting with tumor cells at the tumor periphery are thought to contribute to EMT (85).

The ability of gonadotropins to act on and regulate normal ovarian surface epithelial (OSE) cells and ovarian cancer cells was investigated. Observations support the hypothesis that gonadotropins may influence some ovarian cancers. In summary, the current study demonstrates the novel observation that both the FSHR and LHR are expressed by bovine OSE and selected ovarian cancers. Interestingly, the actions of FSH and LH to promote OSE growth may in part be mediated indirectly through an elevation in the expression of autocrine growth factors (KGF, HGF, and KL). Ovarian cancer is more common in conditions with elevated gonadotropins such as post-menopausal women. Therefore, gonadotropin actions on the OSE are postulated to be a potential factor in the onset and progression of some ovarian cancers (86).

Aurelian Radu at Mount Sinai School of Medicine in New York and colleagues looked for FSHR expression in 1336 human tumour samples, including

prostate, breast, lung and liver cancers. The group applied colour-labelled antibodies for the FSH receptor to the samples. They found that in every sample, the antibodies bound to blood vessels around the periphery of the tumour. Additionally, it was postulated that by attaching a cancer drug to an FSH receptor antibody, exclusive targeting to tumor cells would be possible (87). The group also found that the various stages of ovarian and types of tumor do not differ in terms of vascular expression of FSHR and are found in metastatized tumor from ovaries to brain (88).

Few examples for targeting FSHR approach are provided below:

A targeted nanoparticle delivery system for siRNA was developed using follicle-stimulating hormone (FSH) β 33–53 peptide as a targeting moiety that specifically recognized FSH receptor (FSHR) expressed on ovarian cancer cells to mediate silencing of growth regulated oncogene-α expression. This study indicated that a FSHR-mediated delivery system could mediate the highly selective delivery of siRNA into ovarian cancer cells and that silencing gro-α expression could be a potential choice for ovarian cancer treatment (89).

A poly(amidoamine) (PAMAM) dendrimers to selectively target the follicle stimulating hormone receptor (FSHR), which is overexpressed by tumorigenic ovarian cancer cells but not by immature primordial follicles and other non-tumorigenic cells were developed. Fluorescein-labeled generation 5 (G5) PAMAM dendrimers were conjugated with the binding peptide domain of FSH (FSH33) that has a high affinity to FSHR. The targeted dendrimers exhibited high receptor selectivity to FSHR-expressing OVCAR-3 cells, resulting in significant uptake and downregulation of an anti-apoptotic protein survivin, while showing minimal interactions with SKOV-3 cells that do not express FSHR. The selectivity of the FSH33-targeted dendrimers was further validated in 3D organ cultures of normal mouse ovaries. Immunostaining of the conjugates revealed their selective binding and uptake by ovarian surface epithelium (OSE) cells that express FSHR, while sparing the immature primordial follicles. In addition, an in vivo study monitoring tissue accumulation following a single intraperitoneal (i.p.) injection of the conjugates showed significantly higher accumulation of FSH33-targeted dendrimers in the ovary and oviduct compared to the non-targeted conjugates. These proof-of-concept findings highlight the potential of these FSH33-targeted dendrimers to serve as a

delivery platform for anti-ovarian cancer drugs, while reducing their systemic side effects by preventing nonspecific uptake by the primordial follicles (90). By using a peptide derived from FSH (amino acids 33–53 of the FSH B chain, named as FSH33), a conjugated nanoparticle, FSH33-NP, to target FSHR in ovarian cancer were developed. FSH33-NP-PTX displayed stronger antiproliferation and antitumor effects compared with free PTX or naked PTX-loaded nanoparticles (NP-PTX) both in vitro and in vivo. The delivery system showed very high selectivity and efficacy for FSHR-expressing tumor tissues. Therefore, the choice of conjugated antibody or peptide as target-specific ligand is also important for specific therapeutic purposes.

One study concluded that most of the internalized FSHFSHR complex is recycled back to the cell surface, that this recycling pathway is highly dependent on amino acid residues present near the C terminus of the FSHR, and that it is an important determinant of the extent of down-regulation of the FSHR (91).

Using a polypeptide of follicle-stimulating hormone (named as FSHP), a conjugated nanoparticle, FSHP-NP was developed to target FSHR in lymphatic metastasis of ovarian cancer. FSHP-NP was tested for recognition specificity and uptake efficiency on FSHR-expressing cells. A paclitaxel (PTX)-loaded FSHP-NP (FSHP-NP-PTX) was further developed and its anti-tumor effect was determined in vivo and in vitro. FSHP-NP-PTX displayed significantly stronger anti-proliferative and anti-tumor effects in a dose- and time-dependent manner when compared with free PTX or naked PTX- loaded nanoparticles (NP-PTX) in vitro. In vivo examinations showed that the size and weight of the lymph nodes were reduced in the FSHP-NP-PTX group. FSHR as a novel therapeutic target in ovarian cancer and delivery of PTX via conjugated nanoparticle (FSHP-NP) represents a new therapeutic approach in ovarian cancer (92).

2.6 Antibody and Engineered Fragments

Antibody, a glycoprotein, is made up of two light and two heavy chains linked by disulphide bonds. Antibodies have the constant region and the antigen-binding site (complementarity-determining regions/CDR or variable region). The rationale for using antibodies as a therapeutic agent in cancer treatment is their potential to induce tumor cell apoptosis. For clinical efficacy, use of murine

antibodies initially led to profound immunogenicity in human which was replaced by designing of humanized forms of antibody that was made possible through molecular and biotechnological advances thus leading to reduction in immunogenicity of mAbs (Figure 2-8). Besides this, efficacy of both immunescintigraphy and immunotherapy depends on the amount of antibody taken up in tumor. This again depends on pharmacokinetics, penetrability and retention of mAbs in the tumor cells. OC is solid tumor which varies from small deposits to large tumors of more than 10 cm in size (93). Tumors are characterized by heterogeneous and tortuous vasculature (especially in endometrioid OC) and high interstitial fluid pressure with high viscosity of tumor blood supply (94, 95). mAbs have to diffuse through this pressure gradient which ultimately depends on the molecular size of mAbs. Thus, the large size of mAbs because of the presence of Fc region is a nuisance in cases where tumor penetration is difficult due to heterogeneity.

Whole antibodies have a multimodular nature with each domain having a specific function i.e. Fab region for antigen binding and Fc domain for effector functions. Interaction of the neonatal Fc receptor (FcRn) and Fc domain of antibody helps to extend its biological half-life **(96)**. However, recently the concept of engineered antibody fragments has been developed by through selection of appropriate molecular domains of mAbs in attempts to control *in vivo* pharmacokinetics, valency, affinity, avidity and tissue penetration (97). Additionally, sometimes Fc mediated actions are not desired; for e.g. to confer short half-life to contrast imaging applications, to reduce excessive cytokine release and toxicity (98). Thus, such genetically engineered-customized monovalent (Fab, scFv, single variable VH and VL domains) or divalent ($F(ab)_2$, diabodies, minibodies, etc.) fragments are heralding onset of next wave of antibody-based reagents as diagnostics and therapeutics in OC (99), (100).

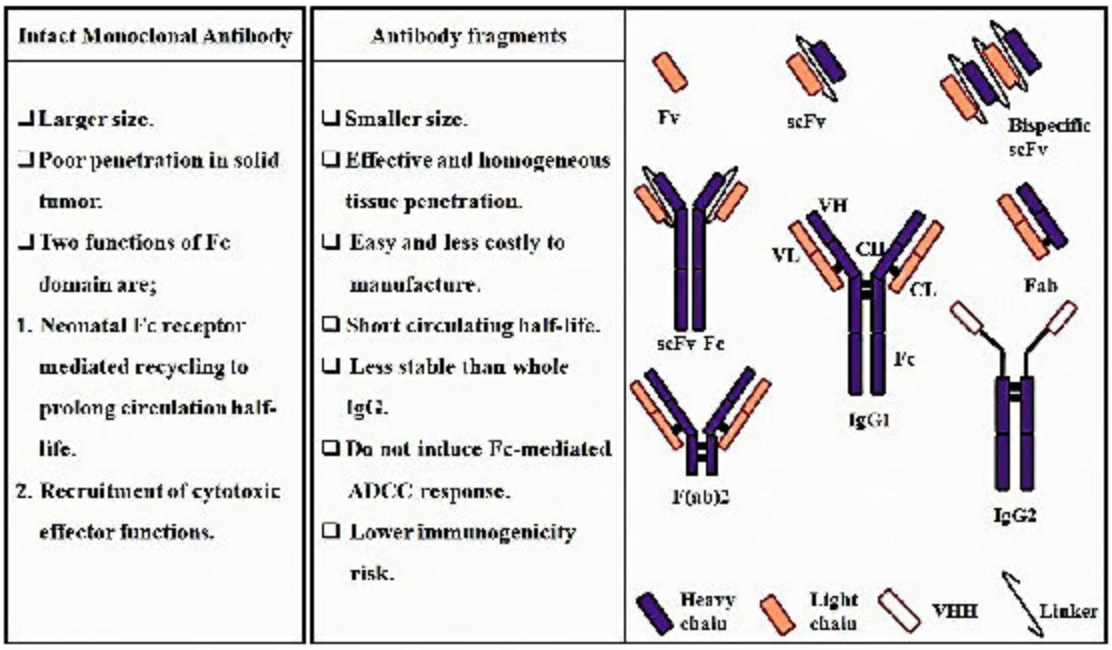


Figure 2-8: Progress in the generation of monoclonal antibodies from murine to human and various antibody fragments.

(Fv-Variable fragment, scFv-Single chain variable fragment, VH-Variable heavy region, VL-Variable light region, CH-Constant heavy region, CL- Constant light region, VHH-single domain antibody, ADCC-Antibody dependent cell-mediated cytotoxicity)

These fragments have retained natural antigen binding capability and have shown improved as well as homogeneous tumor penetration with faster blood clearance. In general, antibodies and its fragments >60 kDa which are above the renal threshold exhibit prolonged circulation, whereas smaller antibody fragments show a shorter serum half-life. In context to that the fusion of polyethylene glycol to the scFv (single chain variable fragment) (101) has improved antibody circulation time, stability and tumor targeting without increasing toxicity (102). Optimization of mAb glycosylation also offers chance to control half-life and effector function to enhance safety and efficacy (103). To improve serum half -life and avidity of scFvs, multimerization of antibody fragments was also introduced (100). Divalent scFvs were developed (diabodies) and multivalent scFvs were also generated by connecting two or more scFv molecules using a peptide linker (104),(105). Apart from monospecific antibodies directed against a single antigen, it is possible to unite the specificities of two antibodies into a single molecule called a bispecific antibody (BsAb) in which one arm is specific for the surface antigen on tumor cell, while the

other arm identifies and triggers the signalling receptor on the effector cells resulting in the killing of the targeted tumor cells. Most of bispecific antibodies are with one arm specific to CD3 to attract cytotoxic T-cells and the other arm is directed toward a tumor antigen such as Endothelial growth factor receptor (EGFR) , HER2/neu (Human Epidermal growth factor Receptor 2/neu), CA125 in OC (106, 107).

2.6.1 Immunoliposomes

The newer strategies in this field are in the direction of developing immunonanocarriers which further augment the therapeutic outcomes of the drug. An attractive strategy to enhance the therapeutic effect of an anticancer drug is to specifically deliver these agents to the tumor cells thereby keeping them away from non-malignant cells sensitive to toxic effects of drugs. This would allow for more effective treatments achieved with doses those are better tolerated. Among the colloidal drug delivery systems explores for site specific drug delivery, liposomes have gained considerable attention (108), (109), (110). Active targeting of liposomes to tumor cells is generally attempted by conjugating targeting ligands to the liposomal surface which allow a specific interaction with the tumor cells (111). Several types of targeting ligands have been explored for this purpose such as antibody, antibody fragments, vitamins, peptides, glycoproteins and oligonucleotides (112). The first report on antibody-targeted liposomes came from Torchilin et al. around four decades ago (113). The process of targeted drug delivery with immunoliposomes (Figure 2-9) can be roughly divided in two phases such as the transport phase and the effector phase. Transport phase deals with the transport of immunoliposomes from administration site mostly I.V. administration to the targeted tumor cells. While the effector phase covers the specific binding of immunoliposomes to target tumor cells and the subsequent delivery of loaded drug. Accessibility of tumor cells is a crucial challenge in development of immunoliposomes. Other examples for antibody targeted nanocarriers, investigated at preclinical phase are described in Table 2.6.

Table 2.6: Antibody/Antibody fragment conjugated nanocarriers evaluated in preclinical studies for treatment of ovarian cancer

Nanocarrier	Drug	Antibody	Evaluation	Remarks	Ref.
Liposomes	Doxorubicin	OA-TL3 Fab' fragment	*In vitro* (OVCAR-3 cell line) Preclinical (OVCAR-3 xenograft model in mice)	• i.p administration led to >70% binding to OVCAR-3 tumor at 24 hr. • Improved residence in tumor compared to non-targeted liposomes. • Efficacy similar to non-targeted liposomes due to leakage before target site binding.	(114, 115), (116)
Liposomes (biotinylated)	Doxorubicin	Cetuximab (Neutravidin conjugated)	*In vitro* (SKOV-3 cell line) Preclinical (SKOV-3 xenograft model in mice)	• Pre-targeting strategy through i.p administration of Neutravidin conjugated Cetuximab followed by biotinylated liposomes. • 22-38 times higher binding of targeted liposomes compared to non-targeted ones in SKOV-3 cells. • Less difference in cytotoxicity of targeted and non-targeted liposomes due to inefficient release of drug. • i.p administration superior over i.v administration for efficient targeting. • Negligible immunogenicity of pre-targeted carriers as compared to immunoliposomes directly.	(117)

Liposomes	Doxorubicin	Anti-nucleosome antibody	*In vitro* (SKOV-3) Preclinical (SKOV-3 sensitive and resistant cancer model)	• TAT peptide and pH sensitive triggering polymer provided improved cellular uptake in SKOV-3 cell-lines at cancer cell pH. • At least 2 folds smaller tumor volume was observed as compared to Lipodox alone on i.v. administration in both, drug resistant as well as in drug sensitive SKOV-3 tumors mice model.	(118)
Liposomes	Benzoporphyrin derivative	Cetuximab	*In vitro* (OVCAR-5 cell line)	• Photosensitizer and antibody were conjugated to preformed liposomes by physical adsorption • Photo-immuno-conjugate-associated-liposomes provided improved cellular uptake and cytotoxicity	(119)
Pegylated lipososmes	^{125}I labeled anthracycline dyt.	Anti-HER2 antibody	*In vitro* (SKOV3), *in vivo*, (SKOV3 i.p injected BALB mice	• Tumor specific delivery was achieved by targeted liposomes but not by non-targeted ones in biodistribution study.	(120)

(i.v- intravenous, i.p- intraperitoneal, OC- Ovarian cancer)

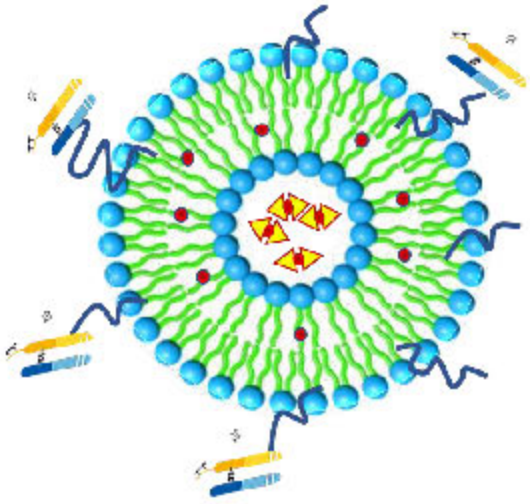


Figure 2-9: Structure of Immunoliposomes

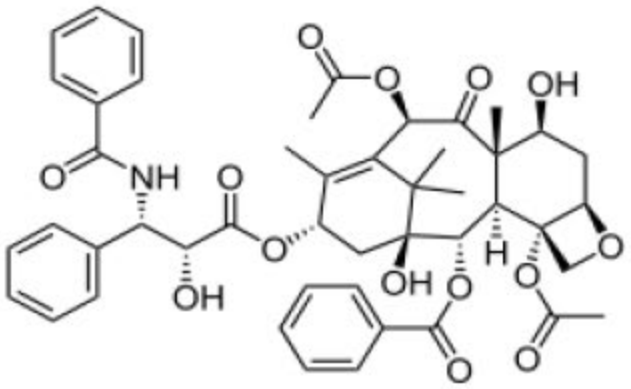
2.7 Drug Profile

Paclitaxel is a mitotic inhibitor used in cancer chemotherapy. It was discovered in a US 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. Later it was discovered that endophytic fungi in the bark synthesize paclitaxel. When it was developed commercially by Bristol-Myers Squibb (BMS), the generic name was changed to paclitaxel and the BMS compound is sold under the trademark Taxol. In this formulation, paclitaxel is dissolved in Kolliphor EL and ethanol, as a delivery agent. A newer formulation, in which paclitaxel is bound to albumin, is sold under the trademark Abraxane. [Wikipedia]

Chemical Name: 5β,20-Epoxy-1,2-α,4,7 β,10 β,13 α -hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine;

Benzenepropanoic acid, b-(benzoylamino)-a-hydroxy-, 6,12b- bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a, 12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-9-yl es- ter, [2aR-[2aa,4b,4ab,6b,9a(aR*,bS*),11a,12a,12aa,12ba]]-. (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12, 12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13, 13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]-benz[1,2-b] oxet-5-one 6,12b-diacetate, 12-benzoate, 9-ester with (2R,3S)- N-benzoyl-3-phenylisoserine.

Structure:



Formula: $C_{47}H_{51}NO_{14}$

Category: Antineoplastic agent

Available marketed formulation: Taxol®, Abraxane®

Molecular Weight: 853.91

Melting Point: 213.5-223°C

Appearance and Colour: White to off-white crystalline powder

logP and pKa value: LogP = 3; pKa = 10.36 (strongest acidic) & -1 (strongest basic)

Solubility:

Paclitaxel is soluble in DMSO (50 mg/ml); soluble in methanol (50 mg/ml); soluble in ethanol and acetonitrile; soluble in a mixture of 50% Cremophor EL and 50% anhydrous ethanol and has poor solubility in water.

Mode of Action:

Paclitaxel interferes with the normal function of microtubule growth by binding to β subunit of tubulin and thus stabilize microtubule structure against depolymerization which destroys the cell's ability to use its cytoskeleton in a flexible manner (Figure 2-10). The resultant microtubule- paclitaxel complex does not have the ability to disassemble and thus cell cycle arrest occurs.

PTX acts by blocking cells in the late G2-mitotic phase of the cell cycle by stabilizing the microtubule cytoskeleton. Thus, it promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. The stabilization leads to inhibition of the regular dynamic reorganization of the microtubule network that is needed for vital interphase and mitotic cellular functions (121). In addition, PTX also activates the intrinsic mitochondrial apoptotic pathway as like other anti-microtubule agents.

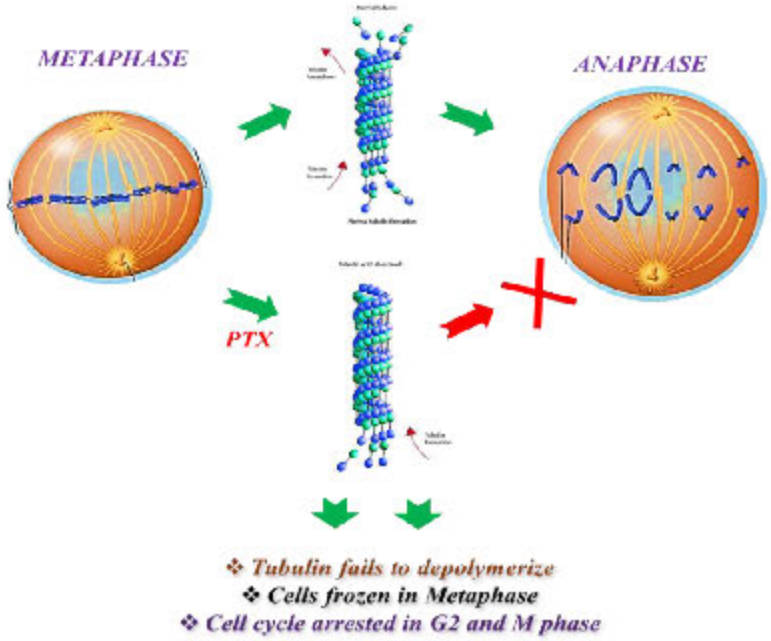


Figure 2-10: Mechanism of action of Paclitaxel

Physicochemical stability:

Paclitaxel converts to more stable isomer 7-*epi*-taxol upon heating in dry state as well as upon contact with organic solvents. Of the various solvent, the conversion was observed to be minimum in chloroform. In methanol or acidified methanol degradation occurs to 30% when drug solution is stored for two week at room temperature which is due to presence of traces of alkali in methanol. Addition of 0.1% acetic acid neutralizes this excess alkali in methanol whereby the stability increased to 7 days at room temperature and 3 months at 4°C (122). Further, the kinetics of degradation is dependent upon several parameters over and above that of temperature (123). It degrades rapidly in weakly alkaline solutions, suitable pH range where paclitaxel exhibits higher stability is 3-5.

Clinical pharmacology:

In vitro

Paclitaxel exhibits cytotoxic activity against a wide variety of both human and rodent tumor cell lines at IC_{50} concentration in the nM range. Paclitaxel disrupts microtubule cytoskeleton, and inhibits a variety of cell functions including chemotaxis, migration, cell spreading, polarization, generation of hydrogen peroxide and killing of phagocytosed microorganisms. In addition to its ability to induce microtubule polymerization, exposure of murine macrophages to paclitaxel results in the release of tumor necrosis factor-α (TNF-α) accompanied by down regulation of the receptor.

In Vivo

Paclitaxel has shown antitumor activity against many tumor models including leukemias and solid tumors and human solid xenografts.

Pharmacokinetics:

Bioavailability: 6.5% (oral)

Protein binding: 89 to 98%

Metabolism: Hepatic (CYP2C8 and CYP3A4)

Half-life: 5.8 hours

Excretion: Fecal and urinary

Volume of distribution: at steady state – 227 to 688 L/m^2 after 24-hour infusion.

Therapeutic dose: 135 and 175 mg/m^2

Clearance: Paclitaxel undergoes extensive non-renal clearance. After an infusion of 225-250 mg/m2 dose, 71% was excreted in feces and 14 % was recovered from urine.

Toxicology: Signs of toxicity in rats were lethargy, rough coat, thinness, hunched posture, neck abscesses, soft stool, decreased body weight, squinted eyes, alopecia. Signs of toxicity in dogs were decreased body weight.

Indications and Usage:

First line therapy for advanced ovarian carcinoma. It is given in combination with cisplatin for non-small cell lung cancer treatment as first line therapy in cases where

radiation therapy or curative surgery cannot be undertaken. It is also indicated as an adjuvant treatment administered post doxorubicin

chemotherapy in node positive breast carcinoma. It is indicated after failure of combination chemotherapeutic / relapse of adjuvant chemotherapy in breast cancer. It is also indicated for the second-line treatment of AIDS-related Kaposi's sarcoma.

Dosage and Administration:

Available as injectable solution: 6mg/mL

Premedication is required prior to administration of Taxol® to prevent hypersensitivity reactions: with dexamethasone 20 mg PO, diphenhydramine (or its equivalent) 50 mg IV, and cimetidine (300 mg) or ranitidine (50 mg) IV.

Table 2.7: Dosage regimen in treatment of various stages of Ovarian, Breast and Non-small cell lung cancer

Stage	Dosage
	For Ovarian Carcinoma (one regimen every 3 weeks)
Previously untreated	175 mg/m2 (3 hrs) followed by 75 mg/m2 cisplatin (every 3 weeks) or 135 mg/m2 (24 hrs) followed by 75 mg/m2 (cisplatin) (every 3 weeks)
Previously treated	135 mg/m2 / 175 mg/m2 (3 hours) (every 3 weeks)
After de bulking surgery	Intravenous paclitaxel 135 mg/m2 (24 hrs) IP cisplatin 100 mg/m2 (every 3 weeks) * 6 cycles.
	For Breast Carcinoma
Node positive breast cancer	175 mg/m2 IV over 3 hrs (every 3 weeks for 4 courses) after doxorubicin
Failure of chemotherapy or relapse	175mg/m2 (3 hrs every 3 weeks)
	For Non-small cell lung cancer
Recommended regimen	135 mg/m2 over 24 hrs + cisplatin 75 mg/m2.
	For AIDS- related Kaposi's sarcoma
Recommended regimen	135 mg/m2 by IV 3 hrs/2 weeks

Associated drawbacks-side effects:

Neutropenia (78-100%); Alopecia (55-96%); Anemia (47-96%); Arthralgia/myalgia (93%); Diarrhea (90%); Leukopenia (90%); Nausea/vomiting (9-88%); Opportunistic infections (76%); Peripheral neuropathy (42-79%); Thrombocytopenia (4-68%);Mucositis (5-45%); Hypersensitivity (2-45%); Renal impairment (34%); Hypotension (17%); Bradycardia (3%); Grand mal seizures; Cardiac conduction abnormalities; Pyrexia; Dehydration; Pancytopenia; Congestive heart failure; Left ventricular dysfunction; Stevens-Johnson syndrome, toxic epidermal necrolysis, and extravasation.

2.8 References

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