2 Literature review

2.1 Nanotherapy for cancer treatment

Conventional chemotherapy is the most appealing approach for cancer treatment because of its noninvasive nature and, therefore, better patient compliance. However, most anticancer drugs cannot be delivered orally because of their poor oral bioavailability [27]. When these drugs are administered via the oral route, only a fraction of the administered dose is available to the systemic circulation. For example, the oral bioavailability of paclitaxel and docetaxel are 1% and <10%, respectively, [28, 29], whereas doxorubicin is <5% [30]. The poor bioavailability of these orally administered anticancer drugs is due to the extensive first-pass metabolic effect by the cytochrome P-450 liver microsomal enzyme system, as well as by their efflux by an overexpressed plasma membrane transporter P-glycoprotein efflux pump (P-gp), which are also responsible for the observed multi-drug resistance for anticancer drugs [31].

Nano-engineered drug delivery systems have shown their potential to increase the oral bioavailability, therapeutic efficacy, and safety profile of anticancer drugs. The maintenance of an optimal drug concentration in plasma and in the vicinity of tumors is the prime requirement of effective cancer therapy. The principal advantages of nanocarriers include their increased solubilization potential, superior encapsulation, altered absorption pathways, prevention of metabolic degradation within the gastrointestinal tract, chemical versatility of materials eligible for nanomedicine, flexibility in surface functionalization, drug and disease-specific tailor design capability, targeting potential and ability to incorporate a wide variety of drug substances [32]. The nanocarriers based approach is also worthwhile in case of oral delivery of various combination therapeutic regimens. The potential drug-drug interactions between various combinatorial therapeutic regimens of anticancer drugs could be fruitfully avoided by utilization of a carrier based approach where drugs are encapsulated within carrier matrices [32, 33]. In addition, it also prevents the

cytotoxic effects on the gastrointestinal tract, which is very critical for patients on chronic cancer therapy via the oral route.

2.2 Nanomedicine for targeting overexpressed hormonal receptors

Nanomedicine is the medical application of nanotechnology, which offers a wide variety of approaches for diagnosing, visualization, imaging, and delivering anticancer agents to the cancer site with improved efficacy and minimised toxicity, thereby improving anticancer activity [34]. Nanomedicine is an integration of medicine, pharmaceutics, engineering, material science, molecular biology and information technology. The application of nanotechnologies to medicine allows for a more thorough investigation of the physiological system and a better understanding of the numerous mechanisms associated [35]. Nanomedicine can solve many problems faced by conventional chemotherapeutic agents, such as solubility, instability, short circulation half life, drug resistance, and cytotoxicity. Nanomedicine can differentiate cancerous cells and normal cells, which helps to reduce the systemic toxicity and severe side effects of chemotherapy. Nanomaterials drug delivery systems can effectively aggregate in tumor sites due to the EPR effect, and tailoring the nanoparticle surface with an appropriate targeting ligand can increase selectivity and cellular absorption in target cells [36]. Some key features must be addressed while designing an efficient nanoparticle for drug delivery to the tumor location. The nanoparticle must be manufactured from biocompatible and easily derivatized compounds that are also well defined, soluble, have prolonged circulation, no agglomeration, and have a high uptake effectiveness by target cells [37].

Currently, research is being carried out in the area of novel nanomedicines, which focus on making chemotherapy curative long-lasting and also improving the therapeutic efficacy by using surface engineered nanocarrier with suitable targeting ligands that will selectively bind to the overexpressed receptors for delivering the chemotherapeutic agent [38]. In breast cancer, there are several receptors found to be overexpressed and can be

targeted which include hormonal receptors (ER, PR, AR), HER2, folate receptor, etc. Here we will focus mainly on nanomedicine designed to target overexpressed hormonal receptors. Among various types of nanocarriers that can be used for designing and developing nanomedicine liposomes, micelles, polymeric nanoparticles, solid lipid nanoparticles, and gold nanoparticles are widely employed in the treatment of hormone receptor overexpressed breast cancer [39].

2.2.1 Liposomes

Liposomes are the most commonly used carrier in nanomedicine for targeted and controlled drug delivery, and it was first introduced by Bangham in 1965 [40]. They are small vesicles composed of an aqueous inner core and membranous lipid bilayer that can be produced from phospholipids and cholesterol, which make them biodegradable and bio-compatible. The phospholipids organized in the form of bilayer structure increase the solubility and stability of anticancer drugs, and the cholesterol reduces the fluidity of nanoparticles and, intensifies the penetrability of hydrophobic molecules through the plasma membrane, and refines the stability of nanoparticles in blood [41]. Liposomes can encapsulate hydrophobic drugs in the lipid bilayer and hydrophilic drugs, genes, and siRNA into an aqueous core [42]. Liposomes serve as a promising delivery system that shows both passive and active targeting of cancer cells. Passive targeting utilizes the EPR effect, whereas active targeting is achieved by attaching specialized molecules, such as antibodies or ligands, that target specific receptors present in tumor tissues. Ligand coupled liposomes have longer circulation half-life, targeting properties and sustained release of drugs, thereby protecting normal healthy tissues from the side effects of anticancer agents.

Liposomes have been widely investigated for targeting antineoplastic agents in breast cancer therapy. Doxorubicin (DOX) loaded pegylated liposomes (Doxil) was the first FDA-approved nanomedicine for cancer treatment in 1995; nowadays, it is used for treating Kaposi Sarcoma, multiple myeloma, ovarian cancer, and metastatic breast cancer

[43]. After the success of Doxil, several research studies are undergoing for developing anticancer agents loaded targeted liposomal nanomedicine. Paliwal et al. reported the successful utilization of Estrogen Receptor (ER) for the delivery of anticancer drug DOX encapsulated within pegylated liposomes for the treatment of breast cancer. Estrone (ES) was anchored as a ligand on the pegylated or stealth liposomes (ES-SL-DOX) to target estrogen receptor overexpressed breast cancer. The *in vivo* biodistribution study on female albino rats showed that estrone appended liposomes (ES-SL-DOX) accumulates significantly higher concentrations in ER positive breast cancer cells and were found to be 13.9% higher than free DOX and non-targeted DOX loaded liposomes. Also, the accumulation of ES-SL-DOX in the heart tissue was significantly lowered compared to free DOX [44]. Tang et al. developed epirubicin (EPI) and paclitaxel (PTX) encapsulated estrogen functionalized pegylated liposomes to enhance the antitumor effect of drugs against breast cancer cells and reduce the adverse off target effects. Liposomes with an average size of 120 nm and PDI more than 0.02 were prepared by adding soy phospholipids (SPC) cholesterol and DSPE.mPEG2000 and were functionalized with ES. These targeted liposomes have excellent drug loading capacity, cellular uptake, and release profile. ER-targeted SSL-EPI/PTX injected into tumor bearing mice showed remarkable tumor growth inhibiting effects on breast cancer with out any visible systemic toxicity. The targeting capacity of ES and internalization of high con of EPI and PTX enhances therapeutic responses against cancer cells [45]. Jain et al. used well known ER antagonist Tamoxifen (TMX) for incorporation within the liposomal surface as a targeting ligand in liposome loaded with DOX (TMX-DOX-liposomes). TMX-DOX-liposomes exhibit significant inhibition of MCF-7 cell based tumor growth compared to free DOX and non-targeted DOX loaded liposomes [46]. Wang et al. developed nanomedicine based combination therapy using a penta peptide QLPVM conjugated liposomal tamoxifen (TMX) and

doxorubicin (DOX). Quantitative and qualitative approaches demonstrated the enhanced endocytosis and cytotoxicity of QLPVM conjugated sterically stabilized liposomes (QLPVM-SSL) invitro and *in vivo*. QLPVM significantly enhances drug release from the liposomes during their interaction with tumor cells. Estrogen receptor modulator TMX and chemotherapy agent DOX encapsulated in QLPVM-modified stealth liposomes achieved nanomedicine based combination therapy against ER positive breast cancer [47]. Mitoxantrone (MTO) is an anthraquinone drug that has been approved for the treatment of numerous cancers; much clinical data currently available shows its application in the treatment of advanced breast cancer. Due to a range of toxicities such as bone marrow suppression, GI toxicity, and cardiotoxicity, its utilization is limited; therefore, it is necessary to develop a safe, effective, and stable drug delivery system to promote clinical applications [48]. Xu et al. had showed that Mitoxantrone (MTO) loaded ER targeting sterically stabilized liposomes (ES-SSL-MTO, ES act as ligand for binding to ER). exhibits a very high antiproliferative effect against ER positive breast cancer with IC_{50} value of 0.7ng/ml. ES-SSL could effectively enter into ER expressing tumor cells and be accumulated to prolong circulation in vivo, thereby reducing the toxic effects and enhancing the bioavailability compared to free MTO [49]. Agardan etal developed new liposomal formulation containing TMX for oral treatment of breast cancer. This was developed using absorption enhancers dimethyl-β-cyclodextrin (DM-β-CD) and sodium taurochlorate (NaTc). The aim of the study was to increase the oral treatment efficiency of tamoxifen by using a liposomal formulation. Antitumoral activity and MMP-2 inhibition efficiency were investigated in breast cancer cell lines MCF-7 and MDA-MB-231. Treatment of tumor bearing rat with TMX-DM-β-CD liposomes formulation resulted in 92% tumor area and represented 50% treatment efficiency [50]. Several research were

taking place with liposomal nanocarriers to develop effective and safe nanomedicine to improve the chemotherapy of breast cancer.

2.2.2 Polymeric Micelles

Polymeric Micelles are self-assembled or colloidal nanocarriers with a size usually in the range of 5-100 nm [51]. They are composed of amphiphilic graft/block copolymers that form self-assembled nanostructured core-shell architecture in an aqueous medium at or above a particular concentration called critical micelle concentration(CMC), the amphiphilic polymer exist in two distinct regions mostly hydrophilic head and hydrophobic tail, these self assembled architecture is called polymeric micelle in which hydrophobic core is stabilized by hydrophilic head [52]. Polymer selection of micelles is based on the properties of both hydrophobic and hydrophilic block copolymers [53]. The hydrophilic shell of the micelle provides steric stability as well as avoids rapid uptake of the nanocarrier by the reticuloendothelial system (RES), resulting in the prolonged circulation of drugs in the body. Poly(ethylene glycol)(PEG) is the most commonly used hydrophilic polymer; it is water soluble, highly hydrated, efficient steric protector, bio-compatible, and has low toxicity [54]. Hydrophobic block copolymers should have good compatibility with the incorporated drug and must possess high drug loading capacity; the most widely used polymers for hydrophobic core formation are polyesters, polyethers, and poly aminoacids. Frequently used core-forming molecules are poly(propylene oxide) (PPO), poly(D,L-lactic acid) (PDLLA), poly(ε-caprolactone) (PCL), poly(L-aspartate) and poloxamers [55]. Polymeric micelles have been widely recognized as promising nanocarriers in targeting chemotherapeutic agents for cancer therapy, accordingly a wide range of therapeutic molecules, including hydrophobic drugs, charged compounds, metal complexes etc, can be efficiently incorporated into the micelle core, and their release can be controlled in a sustained or environment sensitive manner. Being small in size, micelles can penetrate easily through leaky tumor vasculature and retain them for longer, thereby delivering drugs

selectively at the target site. Installation of functional moieties such as targetable ligands on the block copolymers along with drug molecules will enhance the therapeutic efficiency of polymeric micelles [56]. The advantages of using polymeric micelles in designing nanocarriers for delivery of chemotherapeutic agents to tumor tissues include high drug encapsulation efficiency, high drug loading capacity, high cellular uptake, improved stability of drugs, easy elimination of nanocarrier from biological environment after bio-degradation, protect normal body cells from drug toxicity, useful for combination therapy and improve the pharmacokinetic properties of encapsulated drug molecules [57]. Various types of nanoformulation are presented in **figure 2.1**.

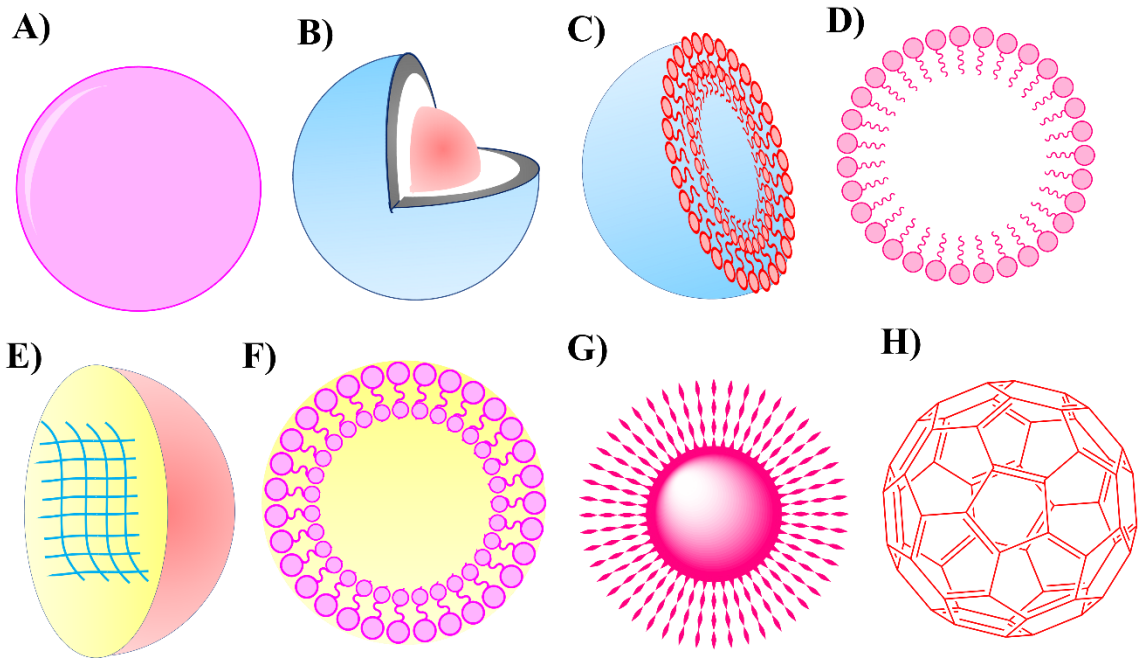


Figure 2.1 Schematic representation of various nanomedicines: (A) Polymeric nanoparticles (B) Core-shell polymeric nanoparticles (C) Liposomes (D) Micelles (E) Nanofibers (F) Nanovesicles (G) Stimuli-response supramolecular nanomedicine (H) Host-guest nanocomplex

With recent progress in polymer science and nano-biotechnology, polymeric micelles as drug carriers have gained an increasing interest in cancer chemotherapy. Due to the versatile and tailor made design of the polymeric micelles, several researches are taking place related to developing anticancer nanomedicine using micelles as nanocarriers.

Numerous micellar nanomedicine formulations are developed for targeting breast cancer and research are ongoing for developing new targeted nanomedicine using polymeric micelles as drug carriers. Peto et al. developed polymeric micelles to protect the drug from aggregation and destruction by phagocytosis; the synthesized PTX micelles were utilized in treating metastatic breast cancer and demonstrated improved therapeutic outcome [58].

Zheng et al., synthesized amphiphilic methoxy-poly(ethylene glycol) grafted polyphosphazene with glycine ethyl ester side groups (PPP-g-PEG/GlyEt) and utilized them for the preparation of DOX loaded polymeric micelles system. DOX loaded polymeric micelles have demonstrated better loading sustained release with significantly higher uptake in human breast cancer MCF-7 cell lines [59]. Melittin, a principal component in bee venom, is a cytotoxic peptide to breast cancer cells consisting of 26 amino acids. The anti-tumor activity of melittin, owing to its potent membrane disrupting activity, has been demonstrated in several studies. However, direct administration of melittin is not possible due to its hemolytic effect on normal cells [60]. Melittin delivery with nanocarrier is crucial in order to overcome the side effects and enzymatic degradation. Raveendran et al., developed an estrone appended polyion complex (PIC) micelle to deliver melittin. Estrone-conjugated poly(ethylene glycol) methyl ether methacrylate-b-poly tert-butyl methacrylate (PEGMEMA-PtBuMA) could complex with melittin to form polyion complex (PIC) micelles of particle size around 60 nm due to the electrostatic interaction of the deprotected polymer and melittin in aqueous media. The estrone conjugated PIC micelles show improved cytotoxicity in 2D and 3D cellular models of MCF 7 cells, and also flowcytometry demonstrated an enhanced cellular uptake; these results demonstrated the potential of estrone appended PIC micelles for targeting hormone receptor positive breast cancer [61]. Teniposide is a semisynthetic derivative of podophyllotoxin with a broad spectrum of *in vivo* antitumor activity. The mechanism of this drug is related to the

inhibition of topoisomerase II activity, thus damaging DNA in the replication process and inducing cellular apoptosis [62]. But, poor solubility and adverse effects of commercial formulation (VM-26) restrict its clinical application. Chu et al., worked on teniposide-loaded polymeric micelles to improve their therapeutic activity; they prepared micelles based on monomethoxy-poly(ethylene glycol)-poly(e-cap-rolactone-co-D,L- lactide) (MPEG-PCLA) copolymers through a thin-film hydration method to improve the hydrophilic and reduce the systemic toxicity. The prepared MPEG-PCLA micelles showed slow and sustained release of teniposide, improved terminal half life, exhibits enhanced cellular uptake, and exhibited stronger antitumor effect than VM-26 on studies performed invitro using MCF 7 cells [68]. Guo et al. prepared methoxy poly(ethylene glycol)-poly(D,L Lactide) (mPEG-PDLA) copolymer based micelles with docetaxel and resveratrol at 1:1 fixed ratio for drug resistant breast cancer therapy. The loading of both drugs in micelles resulted in a prolonged release profile and improved cytotoxicity *in vitro* [63].

Numerous research are taking place for developing suitable nanomedicine with polymeric micelles as drug carriers for the targeted delivery of chemotherapeutic agents in breast cancer therapy as well as other types of cancers and also, many nanoformulations are now undergoing clinical trials; we expect the approval of those micellar anticancer agents and generations of innovative micellar nanomedicine with smart functionalities in the near future.

2.2.3 Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are lipid based submicron sized colloidal carriers used for controlled and targeted delivery of therapeutic agents. They are made up of a lipid matrix which remains solid at physiological temperature**.** The most widely used materials to form lipid cores are mono, di and triglycerides of fatty acids, fatty alcohols, and waxes. These lipids exhibit good biocompatibility and there melting point is above body

temperature; the formation of a solid core is essential, and it determines the relevant properties of SLNs, such as controlled drug release and particle stability. The main features of SLNs are high biocompatibility, biodegradability, excellent physical stability, protection of incorporated therapeutic agent from degradation, good tolerability, controlled release, site specific targeting, and feasibility of large scale production. SLNs can be used for the formulation of both hydrophilic and lipophilic drugs; cell specific ligands can be easily incorporated into the surface of nanoparticles inorder to improve the cellular uptake and targeting efficiency [64]. SLNs are now considered an attractive nanocarrier for formulating targeted chemotherapeutic agents for cancer treatment as they can overcome several physiological barriers that hinder the drug delivery to tumor cells; SLNs are capable of overcoming the multidrug resistance characteristic of cancer cells. This will help to increase the concentration of anticancer drugs in the tumor site, thereby enhancing the antitumor activity reduces the toxicity and side effects caused to normal cells and tissues due to the drug therapy; hence, SLNs are able to overcome the limitations of other nanocarriers such as liposomes, polymeric NPs etc.[65]. Schematic drawing of solid lipid NPs for imaging and therapy of tumor in **figure 2.2**.

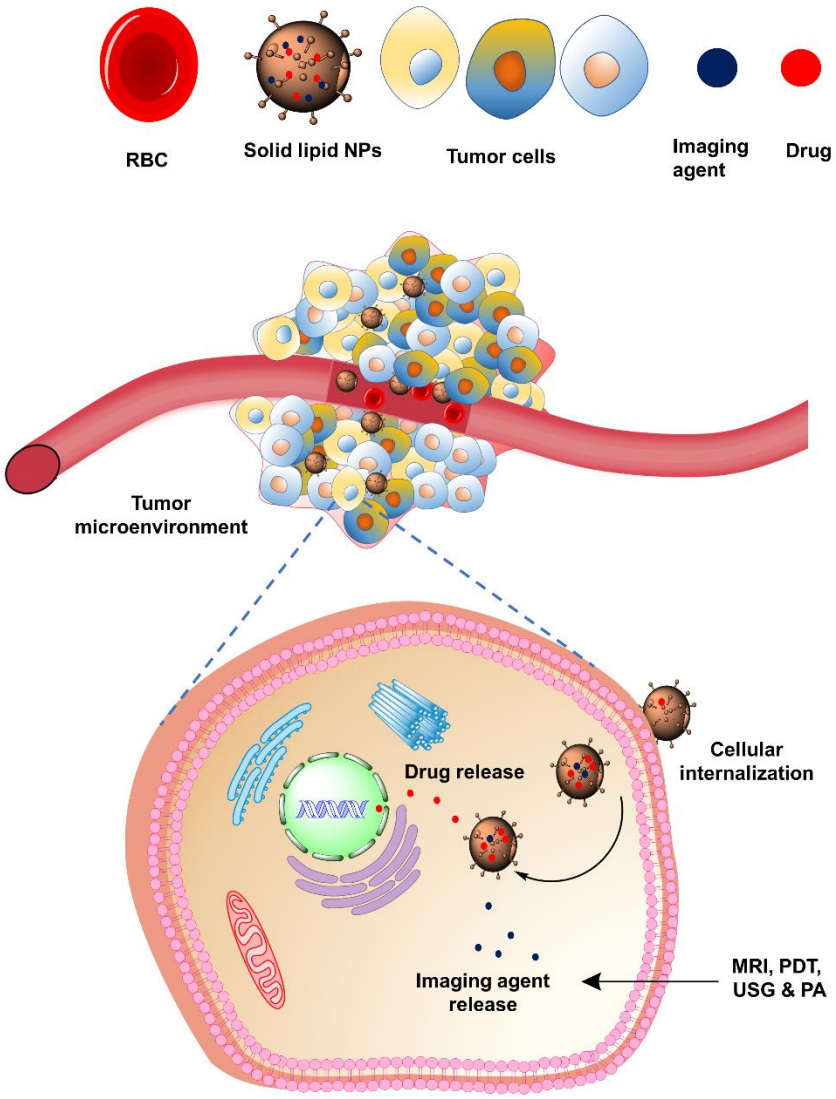


Figure 2.2 Schematic drawing of polymeric solid lipid NPs for imaging and therapy of tumor.

Several studies and research are taking place for developing SLN formulations for targeting breast cancer. Jain et al. developed SLNs of beta-carotene (BS-SLNs) to enhance breast cancer treatment. *In vitro* MTT assay showed a significant inhibitory effect on tumor cells compared to free Beta-carotene. The formulation showed increased bioavailability, sustained release and prolonged circulation time in the body on oral administration [66]. Xu et al. developed PTX loaded SLNs in order to increase the cellular uptake of drugs in breast cancer cells. The anticancer effect of the formulation was determined by using MCF-7 breast cancer cell lines and its MDR variant MCF-7/ADR, and the cell penetration

mechanism was estimated using Rhodamine dye in both cell lines. The SLN-PTX formulation showed significantly higher anticancer potential against MCF-7/ADR than PTX administered with DMSO and cremophor EL-based vehicles. The SLN-PTX formulation also exhibits an improved tumor cell penetration effect, which is attributed to different endocytosis pathways by SLNs in MDR cancer cells [67]. Jadon et al., developed Docetaxel (DTX) loaded hybrid nanoparticles using lipid and polymer excipients (LPHNPs-DTX). Invitro studies of the formulation confirmed better cytotoxicity and high cellular penetration of DTX with lower IC50 in breast cancer cell lines. *In vivo* studies confirmed improvement in pharmacokinetics and target specificity with low biodistribution of DTX in normal cells and tissues as compared to free DTX [68].

2.2.4 Gold Nanoparticles

Gold Nanoparticles (GNPs) are nontoxic and inert, containing gold core with particle size below 150nm. GNPs are very suitable and efficient inorganic nanoparticles for gene therapy, drug delivery, and imaging applications. GNPs recently emerged as an attractive nanocarrier to deliver anticancer drugs because these nanoparticle conjugates can exhibit long circulating half life, increased targeting rapid transport kinetics, enhanced tumor cell uptake, and biocompatibility. GNP coating can act as a biomarker for cancer diagnosis, as a probe for transmission electron microscopy, and as radiosensitization and photothermal therapy [69]. The GNPs are resistant to oxidation under physiological conditions, which permits interactions in the biological environment, and the shape of GNPs enhances the cell membrane penetrability; when the gold nanoparticles are functionalized, they exhibit increased binding affinity, targeting selectivity with multiple targeting groups, leads to an increased tumor cell uptake of the therapeutic agent [70]. Many types of research studied inorganic nanocarriers for therapeutics and imaging, especially on GNPs, as it is found to decrease tumor size, effective accumulation of therapeutic agents at the target site, and prominent diagnosis of tumors. Sun et al. designed GNPs loaded with

DOX, which can effectively deliver the drug to breast cancer cells and significantly enhance anticancer efficiency. The study involved the rational design of DOX tethered gold nanoparticles with PEG and an acid labile hydrazine bond, which exhibits more significant potential for the delivery of DOX to breast cancer cells and results in the reduction of cancer initiation activity via P-gp efflux evasion mechanism. This nanocarrier shows approximately 32 times and 18 times lower tumor volume compared with the control group and free DOX treated group [71]. Dreaden et al., developed Tamoxifen (TAM)-PEG-thiol-GNPs to selectively target and deliver plasmonic GNPs to ER positive breast cancer cells. The TAM loaded Pegylated GNPs demonstrated 2.7 fold enhanced drug potency *in vitro* compared to convectional formulations [72].

2.2.5 Polymeric nanoparticles

Polymeric nanoparticles are solid colloidal systems having a particle size less than 200nm in which chemotherapeutic agents are dissolved, encapsulated, entrapped or adsorbed onto the composition of a polymeric matrix. Depending on drug entrapment, nanoparticles are divided into nanocapsules and nanosphere. Polymers used for making nanoparticles can be natural and synthetic. The most widely used natural polymers include cellulose, chitosan, alginate, and gelatin. Synthetic polymers commonly employed for the preparation of nanoparticles are Poly(D,L lactide co glycolic acid)(PLGA), Poly(D,L lactic acid) (PLA), Polyethyleneglycol(PEG), Poly-ε-caprolactone (PCL), N(2-hydroxypropyl) Methacrylamide (HPMC) etc. These polymers have a high rate of solubility, permeability and are biocompatible with slow degradation rate, good drug stability, and release. Polymeric nanoparticles are used as carriers for delivering therapeutic agents in many diseases such as cancer, CNS disorders, immune system diseases, etc., to enhance therapeutic agents' absorption, distribution, and bioavailability [73]. They have a high active substance loading capacity, thereby increasing the intracellular distribution of active substances and protecting the active substance entrapped in the polymer matrix from

degradation. Polymeric nanoparticles have a better ability to penetrate biological membranes, barriers, and tissues, the small size allows them to pass through narrow capillary vessels and enter the tumor cells and their ability to escape from phagocytosis enhances circulation time and also provides controlled release of drugs. Bioconjugation of nanoparticles with targeting moieties such as ligands, peptides, antibodies etc, can enhance the site-specific drug delivery, thereby enhancing the therapeutic outcome by increasing the cellular uptake, uniform distribution, biostability, and reduced toxic effects as normal cells and tissues are not affected by the therapeutic agents [74].

In the treatment of breast cancer, numerous nanodrug delivery systems have been developed using polymeric materials to ensure targeted and controlled release (**figure 2.3**). Various studies have shown that anticancer chemotherapeutic agents such as doxorubicin, docetaxel, paclitaxel tamoxifen etc are encapsulated with different polymeric nanoparticles are effective in targeting breast cancer cells compared to conventional drug therapy, which shows nanodrug delivery systems have great potential in breast cancer treatment [75]. Mamnoon et al, formulated hypoxia responsive polymeric nanoparticles (HRPs), also called polymerosomes, functionalized with 17β estradiol (E2) for targeted delivery of DOX in ER positive breast cancer cells. Estradiol (E2) conjugated polymerosome-DOX-complex was prepared using the solvent exchange method. The targeted polymerosomes (E2-DOX-HRPs) exhibit high loading efficiency for DOX (59%), and cell viability studies on ER-positive MCF-7 cells showed higher cytotoxicity compared to non-targeted polymerosome as well as free drug (DOX). The merits of this targeted formulation is its ability to selectively target the ER positive breast cancer, break in hypoxic niches of microtumors, release the encapsulated drug, and reduce cancer cell viability. The novel estradiol-conjugated hypoxia-responsive polymersomes described here have the potential for targeted drug delivery in estrogen-receptor-positive breast cancer therapy [77]. Kulhari et

al., developed a Bombsein peptide (BBP) conjugated DTX loaded PLGA nanoparticle for the treatment of breast cancer. The BBP conjugated polymeric nanoparticle showed the sustained release of DTX over a period of 120 h. During cellular cytotoxicity assay in GPR-positive breast cancer cells (MDA-MB-231), BBP conjugated DTX nanoparticles were found to be 12 times more toxic than pure DTX. The IC_{50} value for pure DTX, DTX-NPs, and BBP conjugated DTX-NPs was >375, 142.23, and 35.53 ng/ml, respectively. The study showed that Bombesin conjugated nanocarrier system could be a promising carrier for active targeting of anticancer drugs in GRP receptors overexpressing cancer cells. Massadeh et al., successfully developed PEGylated polymer lipid hybrid nanoparticles (PLNPs) for the delivery of Anastrozole (ANS) to enhance its biopharmaceutical properties and overall efficacy. Biodegradable polymer Poly ε Caprolactone is used for formulating nanoparticles. ANS-loaded PLNPs were optimized to low particle size (>200nm), low polydispersity index, and high stability. It shows an apototic effect on ER positive breast cancer cell lines as compared with the free form of the drug [76]. Nicholas et al. developed polymeric nanocapsules of calcitriol for breast cancer, the formulation showed sustained release of calcitriol with significant accumulation of calcitriol in tumor cell [80]. Ravikumara et al., worked on polymeric nanoparticles and formulated poly (D,L lactic acid) nanoparticles to encapsulate TAM, which enhanced the antitumor efficacy of TAM, studies carried out on MCF-7 breast cancer cell lines [77]. This research and *in vitro* studies show that polymeric nanoparticle based targeted chemotherapy has great potential in breast cancer treatment.

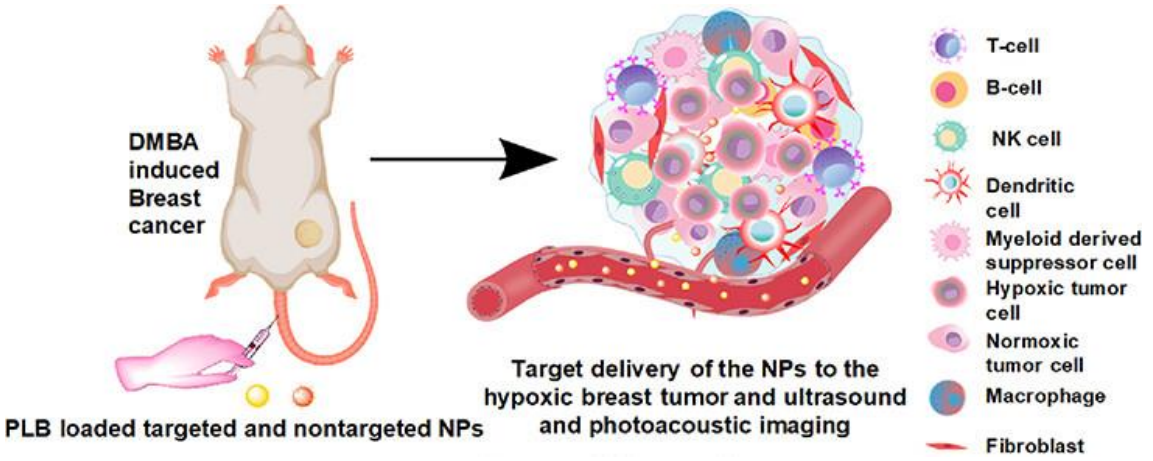


Figure 2.3. Schematic representation of the targeted polymeric NPs for PLB delivery to breast cancer cells.

2.3 Theranostic nanomedicine for imaging and therapy of hormonal receptor positive breast cancer.

Nanomedicine formulations are generally designed to improve biodistribution and target site accumulation of systemically administered therapeutic agents. To facilitate biodistribution and pharmacokinetic analysis and hence to improve the targeting of therapeutic agents to the target site, especially tumor cells, it would be highly useful if the organ accumulation and circulation time of nanomedicine could be able to visualize non-invasively in real time. To achieve this, several different types of nanomedicine have been formulated using drugs and imaging agents [78]. Nanomedicine with therapeutic agents and targeting agents for targeting the drug to specific tissue or organ and imaging agents for diagnosis and to determine the progress and effectiveness of therapy, loaded with in a single nanocarrier, such type of multifunctional nanomedicine is termed as theranostic nanomedicine. Theranostic nanomedicine has a significant ability to make personalized chemotherapy a clinical reality for cancer patients. Theranostic nanomedicine enables monitoring of pharmacokinetics, biodistribution, and tumor cell accumulation of the incorporated drug molecule, ensures therapeutic response, and also provides information about biological characteristics of tumor tissues; this will help the physicians to make decisions about dosage, frequency, drug choice, and treatment strategies by tracking

therapeutic responses with the help of imaging or diagnosing agent loaded in the nanomedicine. Non-invasive molecular imaging techniques can monitor theranostic nanomedicine. Diagnostic agents that are commonly used in theranostic nanocarriers include gadolinium, fluorescent dyes, quantum dots, super-paramagnetic iron oxides, radionuclides, heavy elements, such as iodine for optical imaging, magnetic resonance imaging (MRI), nuclear imaging, and computed tomography [79]. Various types of nanocarriers based on organic and inorganic materials have been developed to prepare theranostic nanomedicine, which is capable of loading diagnostic agents, targeting ligands and therapeutic agents, which include carbon nanotubes, gold nanoparticles, silver nanoparticles, liposomes, polymeric nanoparticles like micelles, dendrimers, polymersomes, hydrogels etc. [80].

Recent developments in nanoscience allow us to design and develop multifunctional nanomedicine by functionalizing nanocarriers with targeting, diagnostic, and therapeutic agents, especially for cancer therapy, which are now heading towards more precise and targeted co-delivery of both diagnostic and therapeutic agents. These smart multifunctional nanoparticles encapsulating antitumor drugs and surface coated with specific ligand and imaging agents eventually bind with receptors overexpressed on the cancer site and destroy the cells, in addition molecular imaging helps us to simultaneously diagnose and treat cancer; the theranostic approach improves current cancer diagnosis and treatment [81]. Several researches are also going on for developing theranostic nanomedicine for breast cancer diagnosis and therapy. Cai et al, designed and fabricated a novel amphiphilic triblock N-2(hydroxypropyl methyl) acrylamide (HPMA) copolymer gadolinium-paclitaxel cyanine 5.5 (pHPMA-Gd-PTX-Cy5.5) conjugate and further studied its theranostic nanomedicine for breast cancer treatment. *In vivo,* MR imaging, florescence imaging, and Gd(III) histological distribution demonstrated that the residence time of

multifunctional conjugate-based nanomedicine had been significantly prolonged, and accumulation at the tumor site has been increased. The conjugate-based nanomedicine shows successful inhibition of tumor proliferation and induced apoptosis of 4T murine breast cancer cells in the xenograft model, and no obvious side effects were seen. The studies showed that enzyme-sensitive pHPMA-Gd-PTX-Cy5.5 conjugate opens a door for simultaneous therapeutic and MR imaging of breast cancer [82]. Abbasi et al, experimented with Mn Oxide and DTX co-loaded fluorescent polymer based nanoparticles for dual model imaging and therapy of breast cancer. The authors showed that this type of polymer based nanoparticles are good candidates for breast cancer theranostics [83]. Schematic of theranostics NPs for breast cancer targeted imaging and therapy has been presented in **figure 2.4**.

In this regard, a variety of nanocarriers are gaining increasing attention for their ability to co-encapsulate multiple therapeutic agents and synchronize their delivery to the diseased cells. A variety of novel technologies such as liposomes [84, 85], polymeric nanoparticles [86-89], polymer drug conjugates [90, 91], mesoporous silica nanoparticles [92], and dendrimers [93] have been investigated for the co-encapsulation of various therapeutic relevant combinations such as cytotoxic agents, chemosensitizer, small interference RNA (si-RNA) and antiangiogenic agents.

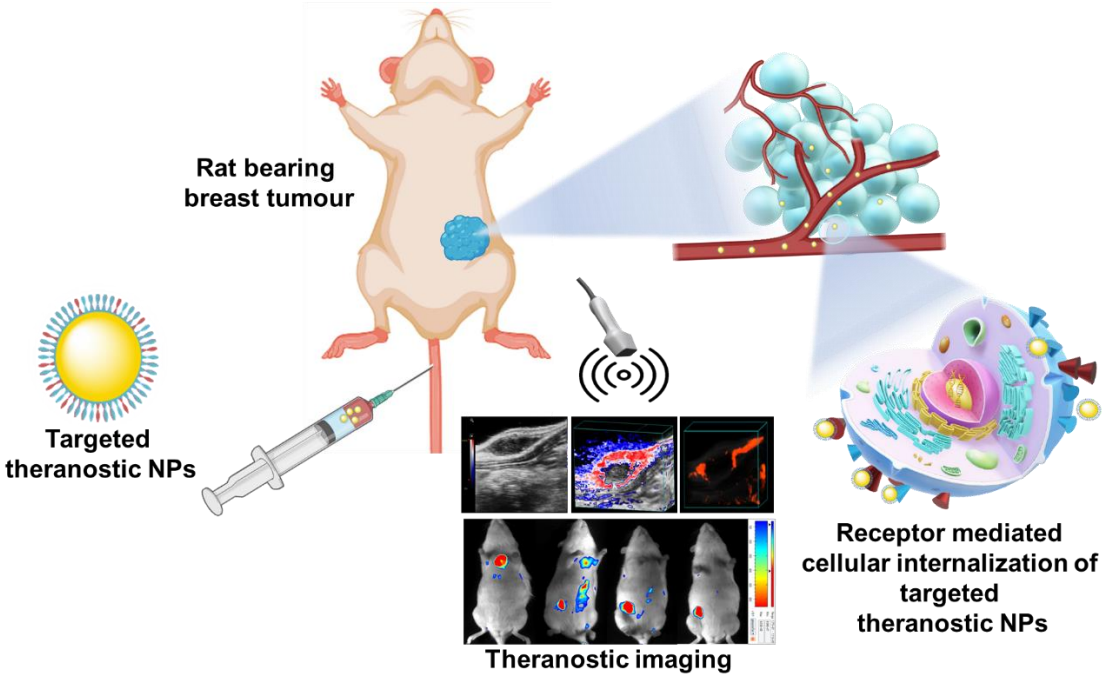


Figure 2.4. Targeted theranostic NPs for targeted breast cancer imaging and therapy

Subsequent to oral administration of polymeric nanocarriers, the M-cells (specialized cells staying over mucosa-associated lymphoid tissue) in Peyer's patches uptake the particles and transport them from the gut lumen to intra-epithelial lymphoid cells and afterward through the lymphatic system into the blood stream [94-96]. Owing to the aforementioned absorption mechanism, polymeric nanocarriers avoid the enzymatic degradation of the encapsulated drug(s) in enterocytes, first pass metabolism in the liver, therefore decreasing the dose and, ultimately the drug-related toxicity. Like all other nanoparticulate systems, chitosan-NPs also follow this special absorption pathway, enhancing encapsulated drugs' bioavailability. The said nanoparticulate system has been widely studied and evaluated for the oral delivery of chemotherapeutic agents. The principal advantage of this system includes its robust structural characteristics, imparting very high stability in the gastrointestinal tract. Furthermore, the hydrophobicity and hydrophilicity within the polymeric system can be manipulated to accommodate a wide variety of drug molecules [97]. Chitosan-NPs tend to show a very high degree of sustained release of encapsulated

drug(s), which could be of particular significance for oral delivery in terms of ensuring that no drug is released from the formulation till it reaches into systemic circulation, thereby bypassing various physiological barriers to oral delivery of difficult-to-deliver drugs [98].

2.4 Palbociclib for breast cancer therapy

For over a quarter of a century, Palbociclib (PLB) has been prescribed to patients with advanced stage of breast cancer and as a long-term prophylactic therapy to reduce the risk of recurrence in localized breast cancer. More specifically, it is a chronically administered preferable drug for the treatment of HR positive and HER2 negativetive breast cancer in both pre- and post-menopausal women [99]. Palbociclib was the first CDK4/6 inhibitor to be approved as a cancer therapy. Palbociclib, ribociclib, and abemaciclib have been approved in recent years for the treatment of endocrine-resistant MBC in combination with ET, considering their efficacy in prolonging progression-free survival (PFS), increasing clinical benefit rate (CBR) and response rate (RR) in different clinical context and treatment lines [100].

It is provided in both capsule and tablet forms, with a recommended oral dosage of 75-125 mg once daily for 21 consecutive days, followed by 7 days of treatment to comprise a complete cycle of 28 days.

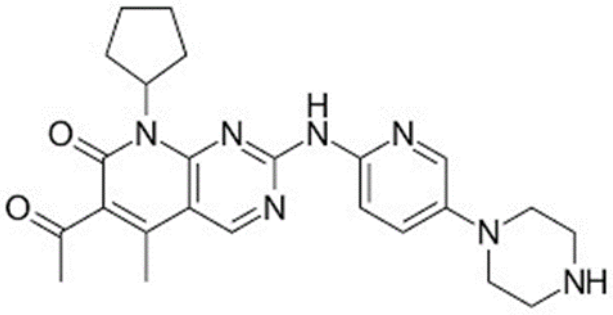
2.4.1 Mechanism of action for Palbociclib in breast cancer therapy

PLB is a selective inhibitor of CDK4 and CDK6, serine-threonine kinases that regulate the cell cycle progression. In fact, when the expression of D-type cyclins activates PLB, they initiate the phosphorylation of retinoblastoma tumor suppressor protein (pRb) with subsequent release of transcription factors from the E2F family **(Table 2.1)**. These factors coordinate a gene expression program that is required for determining cell cycle progression, DNA replication, and mitosis. CDK 4/6 inhibitors hamper the phosphorylation of CDK 4/6, leading to hypophosphorylation of pRb and hindering the activation of the transcription factors necessary for S-phase entry. Palbociclib also determines an arrest of

the progression of the cell cycle at the G1 phase, preventing DNA synthesis required for cellular replication.

The mechanisms of resistance to these molecules can derive from p16 hyperexpression (mediating intrinsic resistance), activation of alternative proliferative pathways such as mTOR and PI3K (acquired resistance), or deregulation of cyclin expression [100]

Table 2.1 Drug profile of Palbociclib (PLB)

Physicochemical properties	
Parameters	**Specifications**
Chemical Structure/IUPAC name	 6-Acetyl-8-cyclopentyl-5-methyl-2-[[5-(1-piperazinyl)-2-pyridinyl]amino]-pyrido[2,3-d]pyrimidin-7(8H)-one
Molecular formula	C24H29N7O2
Molecular weight	447.5328 g·mol−1
pKa (Strongest Acidic)	7.4 (secondary piperazine nitrogen) and 3.9 (pyridine nitrogen)
Log P	0.99
Melting point	263-266 ºC
Boiling point	711.5ºC
Solubility	pH-dependent solubility. The solubility of palbociclib in aqueous media decreases over the range of pH 4.3 to pH 9.0 from greater than 0.7 mg/mL to less than 0.002 mg/mL. At or below pH 4, palbociclib behaves like a high-solubility compound. Above pH 4, the solubility of the drug substance reduces significantly.
Pharmacokinetics properties	
Parameters	**Specifications**
Absorption	C_{max} and Tmax of PLB tablet is 6-12 hr. The mean absolute bioavailability of Palbociclib after an oral 125 mg dose is 46%.
Distribution	The binding of Palbociclib to human plasma proteins *in vitro* was ~85%, with no concentration dependence over the concentration range of 500 ng/mL to 5000 ng/mL. The geometric mean apparent volume of distribution (Vz/F) was 2583 (26%) L.
Metabolism	Extensively, hepatic metabolism after oral administration to major primary metabolic pathways for Palbociclib involved oxidation and sulfonation, with acylation and glucuronidation contributing as minor

	pathways. CYP3A and SULT2A1 are mainly involved in the metabolism of Palbociclib.
Excretion	The geometric mean apparent oral clearance (CL/F) of palbociclib was 63.08 L/h, and the mean plasma elimination half-life was 28.8 hours in patients with advanced breast cancer.
Adverse effects/toxicity	
Produce neutropenia, leukopenia, fatigue, decreased appetite, diarrhea, dry skin, hair loss or thinning of the hair, nausea, and fetal harm. (https://go.drugbank.com/drugs/DB09073)	