Medicinal chemistry and nanomedicine for reproductive cancer therapeutics

Debatosh Majumdar[1], Sunil Singhal[2]

[1]Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University School of Medicine, Emory University, Atlanta, GA 30322, USA, [2]Division of Thoracic Surgery, University of Pennsylvania School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

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1. ABSTRACT

Nanomedicine is an interdisciplinary research field where chemistry, physics, biology, engineering, nanotechnology, and medicine meet one other. Many novel nanoparticles have been designed, synthesized, and evaluated for selective targeting of cancer cells, delivering, and releasing the anticancer drugs in a controlled manner. In this review article we discuss the current status and future prospects of medicinal chemistry of nanomedicine with particular attention to nanoparticle systems that are in various stages of development for cancer therapy in reproductive medicine.

2. INTRODUCTORY REMARKS

Cancer is a deadly disease causing large number of death worldwide. The total number of cancer cases is increasing globally, with more than 10 million new cases each year (1). Rigorous and concerted effort has been made to diagnose, prevent, and cure cancer. Recent development of genetics, molecular biology, medicinal chemistry, material science, engineering, and nanotechnology has greatly accelerated the progress in molecular cancer imaging (2). With the discovery of new biomarkers and development of novel nanoparticles it has become much more convenient to pursue targeted cancer therapy (3-34).

Table 1. Examples of nanoparticle/nanocarrier-based therapeutics

Compounds	Commercial name	Nanoparticle/Nanocarrier	Symptoms/Diseases
Paclitaxel	Abraxane	Albumin-bound paclitaxel nanoparticles	Metastatic breast cancer
Doxorubicin	Doxil/Caelyx	PEG-ylated liposomes	Recurrent breast cancer, ovarian cancer, refractory Kaposi's sarcoma
Doxorubicin	Myocet	Non-pegylated liposomes	Combinatorial therapy of recurrent breast cancer, ovarian cancer, AIDS-related Kaposi's sarcoma
Daunorubicin citrate	DaunoXome	Liposomes	For AIDS-related Kaposi's sarcoma
Vincristine	OncoTCS	Liposomes	Relapsed aggressive non-Hodgkin's lymphoma (NHL)
Anti-CD20 antibody tositumomab conjugated to iodine-131	Bexxar	Radio-immunoconjugate	CD-20 antigen-expressing relapsed or refractory, low-grade, follicular, or transformed non-Hodgkin's lymphoma
Anti-CD20 antibody ibritumomab tiuxetan conjugated to yttrium-90 or, indium-111	Zevalin	Radio-immunoconjugate	Relapsed or refractory, low-grade, follicular, or transformed non-Hodgkin's lymphoma
An engineered protein combining interleukin-2 and diphtheria toxin	Ontak (Denileukin diffitox)	Immunotoxin (fusion protein)	Cutaneous T-cell lymphoma (CTCL)
PEG-granulocyte colony-stimulating factor (G-CSF) analog filgrastim	Neulasta /PEG-filgrastim	Conjugate of filgrastim protein and PEG	Stimulate the level of neutrophils. Prevention of chemotherapy-associated neutropenia.
PEG-L-asparaginase	Oncaspar	L-asparaginase enzyme-PEG conjugate	Acute lymphoblastic leukemia (ALL)
Styrene co-maleic acid polymer-neocarzinostatin (SMANCS)	Zinostatin/Stimalmer	Neocarzinostatin-polymer conjugate	Hepatocellular carcinoma

Many of the chemotherapeutics are poorly soluble in water and orally inactive, hence they require modification prior to intravenous administration. Excipients can cause undesired side effects while increasing the treatment cost. For example, paclitaxel is currently supplied with the excipient containing Cremophor EL and dehydrated ethyl alcohol at a 1:1 (v/v) ratio. Cremophor presents a number of serious concerns when administered intravenously, including various intrinsic toxic side effects, limiting the amount of paclitaxel that can be safely administered. The most common morbidity is acute hypersensitivity reaction characterized by dyspnea, rash, flushing, and generalized urticaria. Studies have shown that Cremophor, when administered intravenously, alters the pharmacokinetic profile of many drugs including paclitaxel. Moreover, systemic use of paclitaxel in large doses can cause hematologic and neurologic toxicity. Life-threating hypersensitivity reactions have been observed in about 3% of patients despite appropriate premedication like histamine H2 antagonists, dexamethasone, and diphenhydramine. In order to reduce the undesired side effect, increase the efficacy of chemotherapeutics, and reduce the cost of treatment, it is necessary to develop chemotherapeutic agents which are suitable for selective local delivery with limited or, no toxic side effects.

3. NANOTECHNOLOGY

There are several potential solutions that use nanotechnology to overcome these toxicity and efficacy issues. One approach is to formulate anti-cancer drugs as nanoparticles/nanocarriers. Alternatively, therapeutic agents can be covalently conjugated to the nano-size carrier molecules. Therapeutic agents-containing nanoparticles/nanocarriers can be conjugated to the targeting ligands (Table 1, Figure 1) (9). Ultimately, the long term goal is to develop nanomedicine that is highly target-specific, biocompatible, and has optimum pharmacokinetic and pharmacodynamic profiles. In order to synthesize target specific nanomedicine, various targeting ligands such as small molecules,

antibodies, peptides and aptamers will need to be employed. There are two approaches to deliver the anticancer drug to the tumor site: active targeting and passive targeting (Figure 2) (9). Generally tumors contain "leaky" blood vessels and poor lymphatic drainage. In case of passive targeting, the nanocarriers/nanoparticles containing the drugs can extravasate into the tumor tissue via the leaky vasculature due to enhanced permeability and retention (EPR) effect. Moreover, lymphatic drainage in the tumors can retain the nanoparticles/nanocarriers, and allow the nanoparticles to release the drugs near the tumor cells (9). In case of active targeting, the targeting ligand can selectively bind to the surface marker (specific receptor or antigen on the tumor cell surface), enter into the tumor cell and then release the drug.

Nanomedicine in cancer therapeutics is a vast and one of the most rapidly expanding fields of research, and no single review article could cover its depth entirely. To cope with this challenge, this review attempts to summarize several examples of various approaches of nanomedicine for cancer therapeutics in reproductive medicine.

4. CANCER THERAPEUTICS IN REPRODUCTIVE MEDICINE

The most commonly used anticancer drugs are paclitaxel, docetaxel, doxorubicin, and cisplatin (Table 2 and Figure 3).

5. NANOMEDICINES IN REPRODUCTIVE CANCER THERAPEUTICS

5.1. Paclitaxel

Paclitaxel (commonly known as Taxol) is a diterpenoid isolated from the western yew, Taxus brevifolia, has promising anticancer activity. Paclitaxel is now widely used to treat patients with lung, breast, ovarian cancer, head and neck cancer. In addition, many types of cardiovascular stents are impregnated with paclitaxel in order to prevent restenosis.
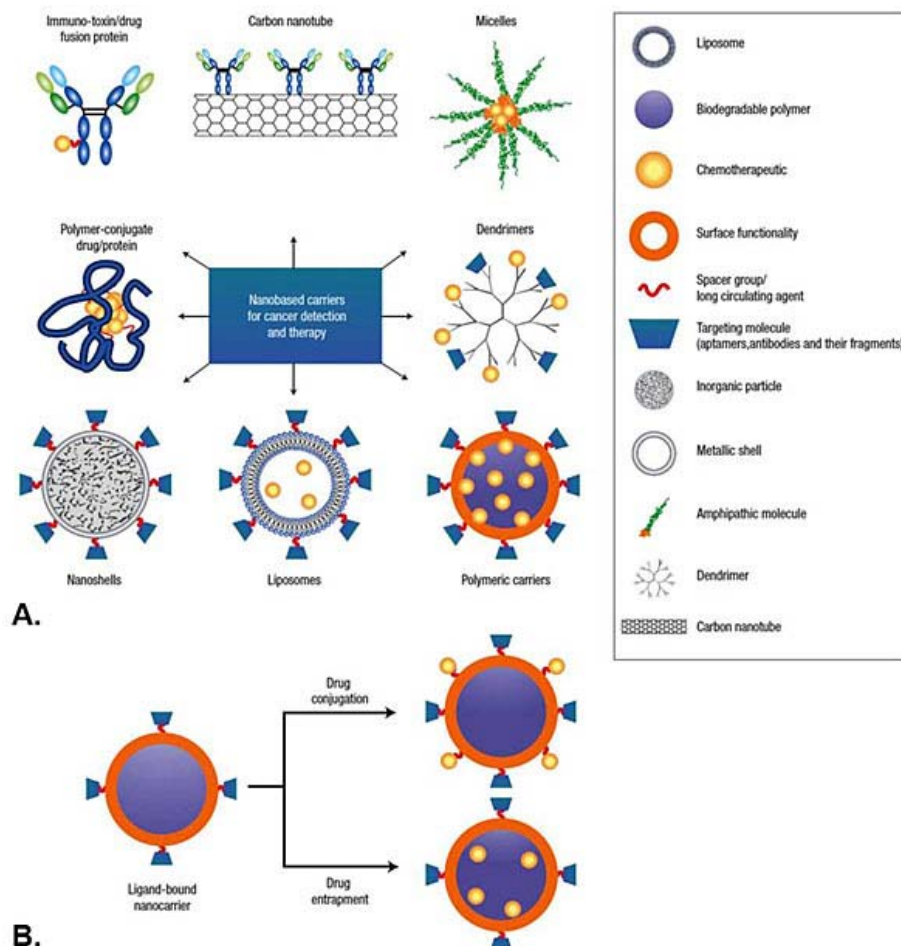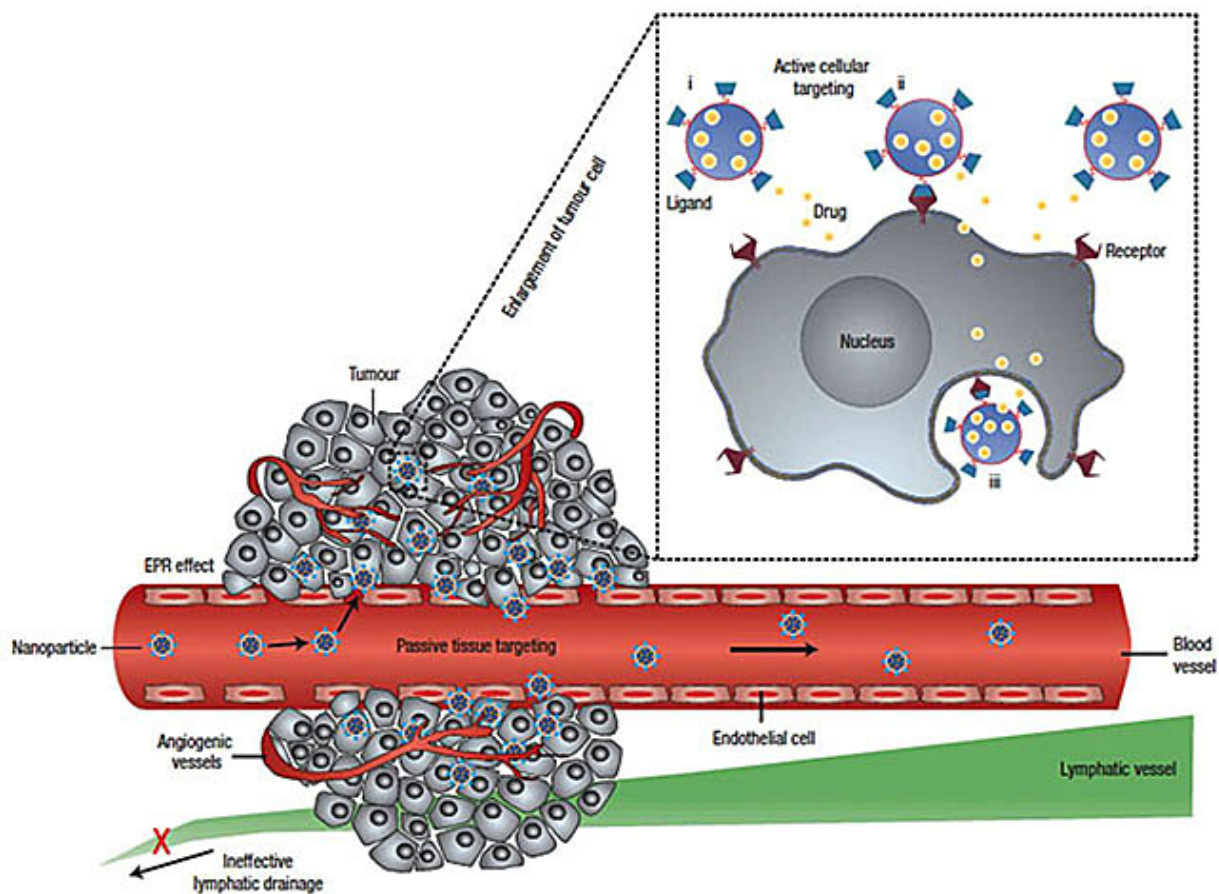
Figure 1. Schematic representations of examples of different nanocarriers for targeting cancer. a), a whole range of delivery agents are possible but the main components typically include a nanocarrier, a targeting moiety conjugated to the nanocarrier, and a cargo (such as the desired chemotherapeutic drugs). b), schematic diagram of the drug conjugation and drug entrapment processes. The chemotherapeutics could be bound to the nanocarrier, as in the use of polymer–drug conjugates, dendrimers and some particulate carriers, or they could be entrapped inside the nanocarrier. Reproduced with permission from (9).

Paclitaxel is an antimicrotubule agent which promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions.

Nanotechnology based approach has been explored to formulate the albumin-bound paclitaxel nanoparticles that is used to treat breast cancer (35). Albumin-bound paclitaxel nanoparticle is composed of an outer layer of albumin surrounding an inner core of hydrophobic paclitxel (35). The average diameter of nanoparticles is about 130 nm. Due to their small size, these nanoparticles can be safely administered intravenously. When in circulation, these albumin-bound paclitaxel nanoparticles bind gp60 receptors on vascular endothelial cell. The activated gp60 receptor interacts with caveolin 1 protein leading to formation of caveolae. Then this caveolae transports the albumin-loaded paclitaxel to the tumor interstitium where it remains trapped. Many tumors secrete a specialized protein into tumor interstitium and onto the surface of tumor cell. This secreted protein is commonly referred to as SPARC (Secreted Protein Acidic and Rich in Cysteine). SPARC acts as a receptor to specifically attract and bind the albumin-bound paclitaxel and facilitates the accumulation of high concentration of paclitaxel at the tumor site/tumor cell membrane. Once the albumin-bound paclitaxel approaches fatty lipid cell membrane, paclitaxel is released by a novel spring release mechanism activated by fatty acids. Upon release, the active free drug diffuses into the nucleus and initiates cell death. Due to the close structural similarity of paclitaxel and docetaxel, albumin-bound docetaxel nanoparticle can also be formulated for cancer therapy. One potential formulation to deliver paclitaxel would be conjugating folic acid and cyclic RGD to the albumin of the albumin-bound paclitaxel nanoparticle to give albumin-bound paclitaxel-folic acid and albumin-bound paclitaxel-cyclic RGD

Figure 2. Schematic representation of different mechanisms used by nanocarriers to deliver drugs to tumours. Polymeric nanoparticles are shown as representative nanocarriers carrying the drugs (shown in circles). Passive tissue targeting is achieved by extravasation of nanoparticles through increased permeability of the tumour vasculature and ineffective lymphatic drainage (commonly known as EPR effect). Active cellular targeting (inset) can be achieved by functionalizing the surface of nanoparticles with targeting ligands that promote cell-specific recognition and subsequent binding. The nanoparticles can (i) release their drug contents in close proximity to the target cells; (ii) attach to the membrane of the cell and act as an extracellular sustained-release drug depot; or (iii) internalize into the cell. Reproduced with permission from (9).

nanoparticles respectively. Albumin-bound paclitaxel-folic acid and albumin-bound paclitaxel-cyclic RGD nanoparticles can be explored to target the folate receptors and integrin receptors respectively in targeted cancer therapy.

Delie and co-workers prepared the paclitaxel loaded poly (DL-lactic acid) nanoparticles (NP-Tx) and conjugated the humanized monoclonal anti-HER2 antibodies to the nanoparticle surface to give the cytotoxic anti-HER2 immunonanoparticles (NP-Tx-HER) of size 237 ± 43 nm (Figure 4) (36). *In vitro* studies on HER2 overexpressing SKOV-3 ovarian cancer cells demonstrated that cytotoxic anti-HER2 immunonanoparticles (NP-Tx-HER) had much higher cytotoxicity in comparison to free paclitaxel (Tx) and paclitaxel loaded poly (DL-lactic acid) nanoparticles (NP-Tx). Results of cell viability studies showed that at a concentration of 1ng Tx/mL, viability of cells is much lower in case of NP-Tx-HER (77.32 ± 5.48 %) than that of NP-Tx (97.4 ± 12 %) and Tx (92.3 ± 9.3

%). Since, the therapeutic index of paclitaxel increased *in vitro* by this immunonanoparticle formulation, these immunonanoparticles may have the potential use.

CD44 is a cell-surface glycoprotein involved in cell differentiation, cell proliferation, cell migration, angiogenesis, and presentation of cytokines, chemokines and growth factors to the appropriate receptors, as well as signaling for cell survival (37). These properties are crucial to the physiological activities of normal cells, and they are also associated with the pathologic activities of cancer cells. Certain variations in CD44 have been reported as cell surface markers for some breast and prostate cancer stem cells. CD44 receptor can interact with various ligands such as hyaluronic acid, osteopontin, collagens, and matrix metalloproteinases (MMPs). Overexpression of Hyaluronan-mediated motility receptor (RHAMM or, CD168) is implicated in the metastases of certain cancers such as colorectal cancer. RHAMM interacts with CD44 to facilitate the migration of neighboring endothelial cells towards the tumor promoting angiogenesis.

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Table 2. Various cancer therapeutics used in reproductive medicine

| Cancer Type	Chemotherapy	Immunotherapy	Targeted Therapy	Hormonal Therapy
Breast Cancer	paclitaxel, doxorubicin, carboplatin, cyclophosphamide, methotrexate, gemcitabine, vinorelbine, docetaxel, anastrozole, exemestane, letrozole		bevacizumab (blocks vascular endothelial growth factor A), trastuzumab (interferes with HER2/neu receptor)	tamoxifen, toremifene, lapatinib
Cervical Cancer	cisplatin, paclitaxel, vinorelbine, topotecan, carboplatin, docetaxel, gemcitabine	human papillomavirus vaccine (Gardasil and Cervarix)		
Ovarian Cancer	paclitaxel, etoposide, gemcitabine, vinorelbine, cisplatin, ifosfamide, carboplatin, cyclophosphamide		bevacizumab (blocks vascular endothelial growth factor A)	tamoxifen
Prostate Cancer	docetaxel, satraplatin, vinblastine, vinorelbine, mitoxantrone, estramustine, cyclophosphamide			luprolide, flutamide goserelin, bicalutamide
Renal Cell Carcinoma		interferon α-2b, interleukin-2	temsirolimus, sorafenib, sunitinib, pazopanib, bevacizumab (blocks vascular endothelial growth factor A), everolimus	goserelin, flutamide
Testicular Cancer	carboplatin, bleomycin, etoposide, cisplatin, epirubicin, paclitaxel, gemcitabine			

In order to target cancers that overexpress CD44, Park and co-workers synthesized conjugates of paclitaxel and hyaluronic acid (HA) which self-assembled in aqueous solution to form nanoparticles of size 183 ± 17 nm (Figure 5) (38). *In vitro* antitumor activities of this nanoparticle have been studied using three different cell lines (MCF-7, HCT-116, and NIH-3T3). MCF-7 and HCT-116 cancer cells overexpress CD44, whereas NIH-3T3 cell is deficient in CD44. Cell viability study using CCK-8 showed that the paclitaxel-hyaluronic acid nanopaticle is more cytotoxic towards MCF-7 and HCT-116 cancer cells, and relatively less cytotoxic towards NIH-3T3 cell. This data suggests that the CD44 receptor mediated endocytosis of the nanoparticle and subsequent cellular internalization has caused the higher cytotoxic effect in MCF-7 and HCT-116 cancer cells. The apoptosis-inducing effect of paclitaxel-hyaluronic acid nanoparticle has been confirmed by confocal microscopy and flow cytometric analyses for HCT-116 cancer cells. Confocal images demonstrate that this approach causes nuclear changes such as DNA fragmentation, segregation and fragmentation of cell nucleus into dense and tiny granules. Flow cytometric analysis demonstrates that the paclitaxel-hyaluronic acid nanoparticle induces a significant increase in G2/M cell population (64.9%), in comparison to that of the hyaluronic acid (16.1%) and paclitaxel (31.2%). This type of nanoparticle can be used to efficiently deliver other anticancer drugs to the cancer cells that overexpress hyaluronic acid receptors.

Samyang pharmaceuticals have formulated the polymeric micelle-type nanoparticles of paclitaxel using polylactic acid polyethylene glycol copolymer (39). These nanoparticles commonly known as Genexol-PM have diameters of 20-50 nm. Genexol-PM is undergoing Phase II clinical studies in breast and lung cancers.

Jih Ru Hwu and co-workers have employed iron oxide nanoparticle (IONP) for delivery of paclitaxel (40). They conjugated paclitaxel to the IONP through a phosphodiester linkage. Cancer cells tend to dephosphorylate faster than normal cell which gives it a metabolic advantage. Chemotherapeutic agents containing phosphate functionality preferentially interact with cancer cells, where dephosphorylation often takes place much faster than that in the normal cell. In their study, they found that in ten days about 91% of the paclitaxel-IONP conjugate underwent phosphodiesterase-mediated hydrolysis to release free paclitaxel. Cytotoxicity assay with oral cancer cell (OEMC1) and normal cell (HUVEC) showed that the paclitaxel-IONP is 1x104 times more toxic than to cancer cell in comparison to the normal cell. Since paclitaxel is used for breast, cervical, ovarian and testicular cancers, this IONP-paclitaxel prodrug can have potential application in these cancers. Since docetaxel is an analog of paclitaxel, similar chemistry can be performed to synthesize the IONP-docetaxel prodrug. Moreover, this IONP-paclitaxel or, IONP-docetaxel in combination with appropriate targeting ligand can be used for targeted drug delivery as well as magnetic resonance imaging.

Iwao Ojima and co-workers have developed novel single-walled carbon nanotube based tumor-targeted delivery system, which consists of a functionalized single-walled carbon nanotube (SWNT) conjugated to paclitaxel via a cleavable linker, and a biotin conjugated to SWNT via the diaminobutane linker (Figure 6) (41). Cellular uptake of this conjugate was examined using a leukemia cell line, L1210FR, that overexpresses biotin receptors. Confocal fluorescence microscopy (CFM) images and the flow cytometry analysis have demonstrated that receptor-mediated endocytosis is by far the predominant mechanism accounting for internalization, while nanotube diffusion being the minor contributing pathway. Tumor-targeting specificity through receptor-mediated endocytosis and cytotoxicity assessment of biotin-SWNT-taxoid-fluorescein conjugate were performed using three different cell lines leukemia cell line (L1210FR), murine leukemia cell line (L1210), and human lung fibroblast cell line (WI38). L1210 and WI38 cell lines do not express biotin receptors.
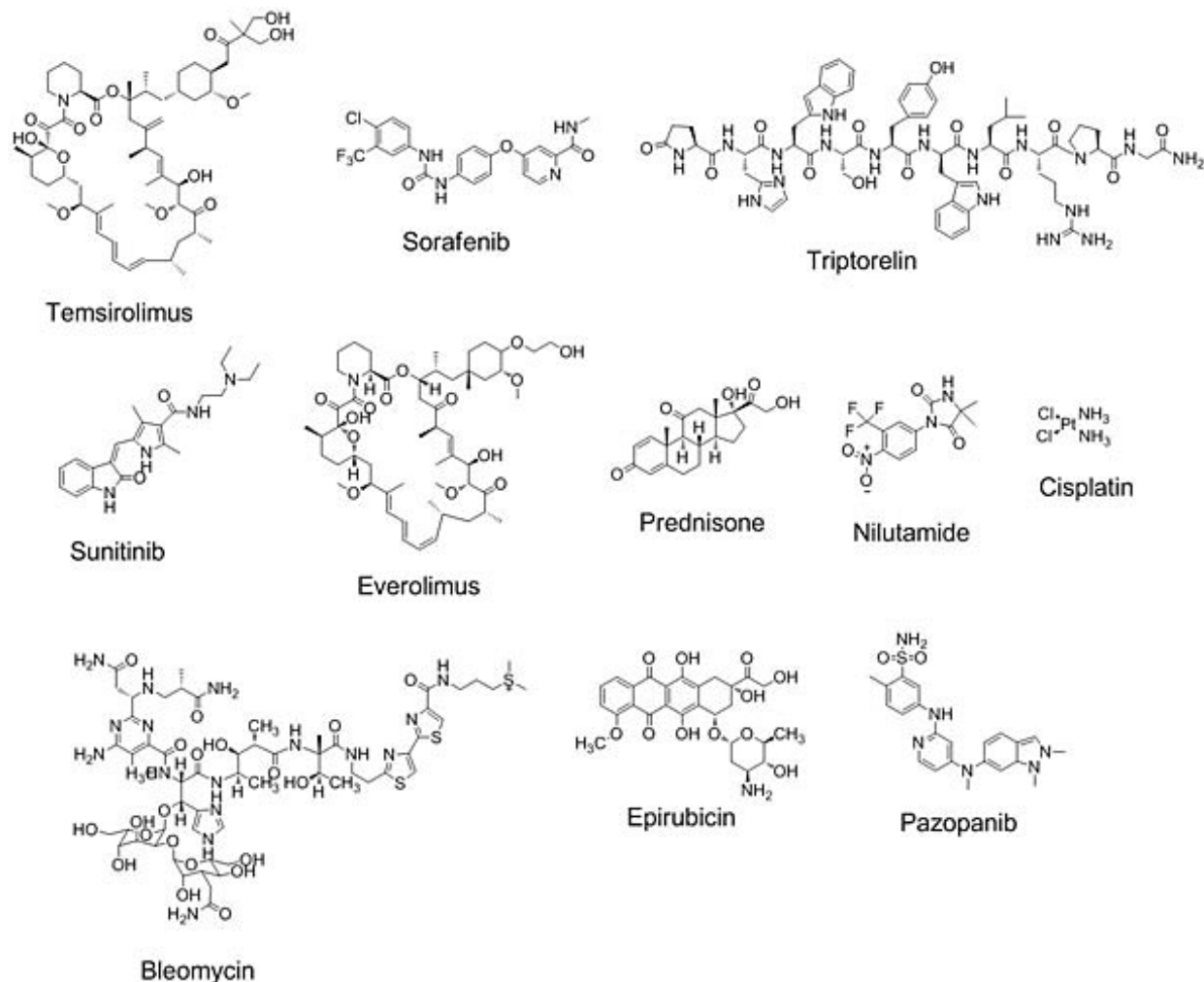
Figure 3. Anticancer drugs used in reproductive medicine.
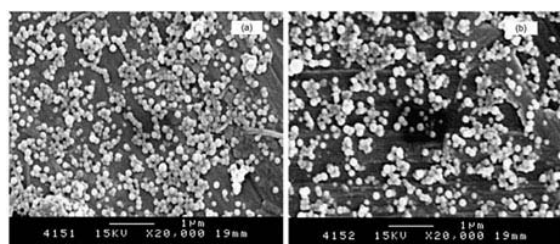
Figure 4. SEM images of (a) NPs-Taxol and (b) NPs-Taxol-HER. Reproduced with permission from (36).

Under identical conditions, the cellular uptake of Biotin-SWNT-taxoid-fluorescein conjugate in L1210FR was much higher than in L1210 and WI38 cells. As a result, L1210FR cells showed much stronger fluorescene intensity than L1210 and WI38 cells. Cytotoxicity of Biotin-SWNT-taxoid-fluorescein conjugate against three cell lines (L1210FR, L1210 and WI38 cells) was determined by MTT assay which demonstrated that Biotin-SWNT-taxoid-fluorescein conjugate was most cytotoxic against L1210FR cell in comparison to other cells (See Table 3 for IC50 values). Biotin receptors are believed to be overexpressed in certain cancers such as breast cancer, renal cell cancer, colon cancer, and leukemia. Hence such Biotin-SWNT-taxoid and Biotin-SWNT-taxotere can be used to target various cancers of reproductive system.

Shuming Nie and Debatosh Majumdar have synthesized a nanosize conjugate of paclitaxel, heparin and folic acid for targeted delivery of paclitaxel (Figure 7) (42).

5.2. Docetaxel
Docetaxel is a structural analog of paclitaxel. It's mode of action is similar to that of paclitaxel. Prostate-specific membrane antigen (PSMA) is overexpressed on the surface of prostate cancer cells. Recently Robert Langer and co-workers reported synthesis of docetaxel-encapsulated nanoparticles formulated with biocompatible biodegradable poly (D, L-lactic-co-glycolic acid)-block-poly (ethylene glycol) copolymer (Figure 8) (43). The surface of the nanoparticles was functionalized with the A10 2' fluoropyrimidine RNA aptamers that could recognize the extracellular domain of PSMA. In vitro
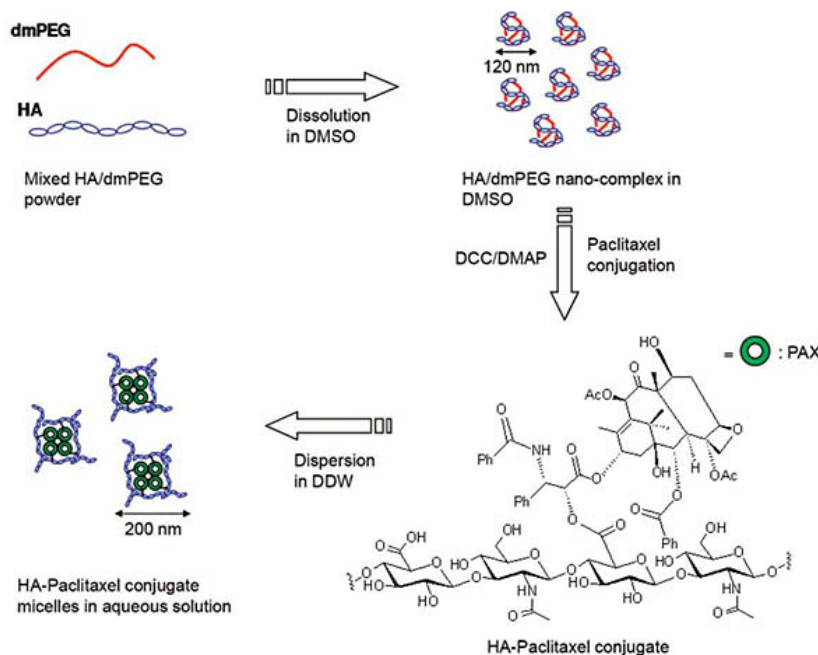
Figure 5. Synthetic scheme of HA-paclitaxel conjugation. Reproduced with permission from (38).
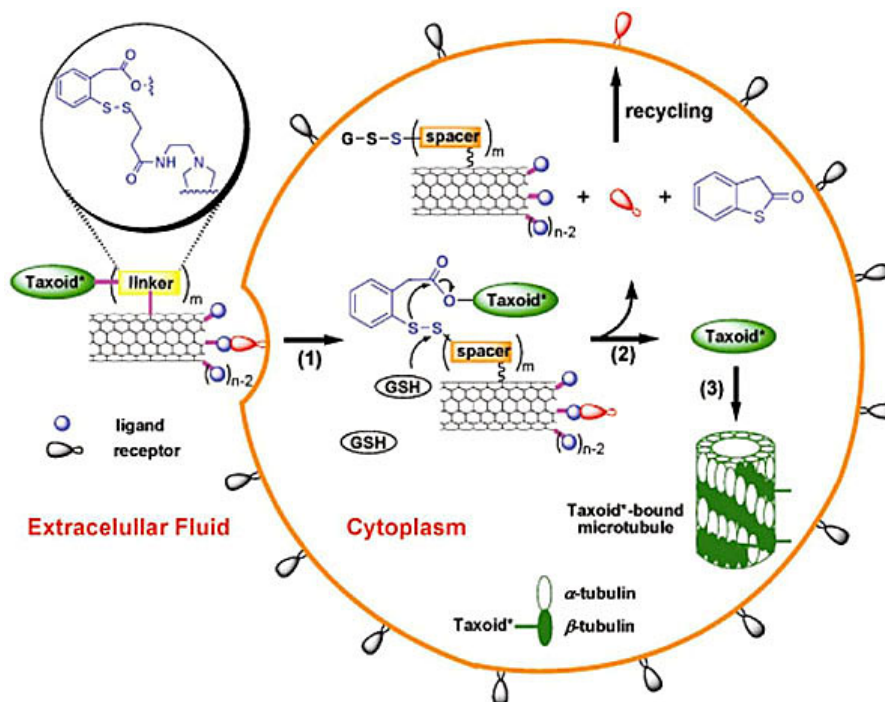
Figure 6. Schematic representation of three key steps involved in the tumor-targeted drug delivery of biotin-SWNT-taxoid conjugate 3: Step (1) internalization of the whole conjugate via receptor-mediated endocytosis; Step (2) drug release through cleavage of the disulfide linker moiety by intracellular thiol, e.g., GSH; Step (3) binding of the free taxoid molecules to tubulins/microtubules, forming stabilized microtubules that block cell mitosis and trigger apoptosis. Reproduced with permission from (41).

cellular cytotoxicity assay (MTT assay) of docetaxel-encapsulated nanopartcle-aptamer bioconjugates (Dtxl-NP-Apt) and docetaxel-encapsulated nanopartcle (Dtxl-NP) was performed using LNCaP cells, which express the PSMA protein. Dtxl-NP-Apt bioconjugates are significantly more cytotoxic in comparison to the control

Table 3. IC$_{50}$ Values of Biotin-SWNT-Taxoid-Fluorescein Conjugate for Different Cell Lines

Cell line	L1210FR	L1210	WI38
IC$_{50}$ (µg/mL)	0.36±0.04	>50	>50

Table 4. IC$_{50}$ values of free DTX and DTX-derivatives (45)

IC$_{50}$ ± SD (DTX equivalent in nM)			
Cell Line	Free DTX	PEG-DTX	DTX loaded PEG-DTX nanoparticles
H460	1.1 ± 0.05	3 ± 0.2	1.1 ± 0.05
SKOV-3	26 ± 2.4	79 ± 35	47 ± 9
MCF-7	15 ± 4.7	37 ± 0.8	18 ± 3

Dtxl-NP lacking the A10 PSMA Apt (cell viability of 61% vs. 42% at 30 min and 48% vs. 30% at 2 h for Dtxl-NP vs. Dtxl-NP-Apt, respectively). *In vivo* efficacy and toxicity studies were performed using mouse LNCaP xenograft model of prostate cancer. Tumor was developed by s.c. injection of LNCaP cells in the flank BALB/c nude mice. The results of this study showed that a single intratumoral administration of Dtxl-NP-Apt bioconjugates is significantly more efficacious in tumor reduction in comparison to the nontargeted NPs and controls. It is believed that binding of Dtxl-NP-Apt bioconjugates to the PSMA proteins on prostate cancer cell surface, internalization into cell, and subsequent intracellular delivery of Dtxl has contributed to the cytotoxicity and enhanced efficacy in tumor. This study demonstrated the *in vitro* and *in vivo* efficacy of NP-Apt bioconjugates in targeted delivery of anticancer drugs.

Yaping Li and co-workers have synthesized docetaxel loaded targeted nanoassembly (TNA) formed by polyethylene glycol-distearoylphosphatidylethanolamine (PEG-DSPE) and surface-modified with EGF (44). In comparison to the nontargeted nanoassemblies (NNA), TNA demonstrated substantial improvement for cell-specific uptake and internalization, and displayed much higher cytotoxicity against breast cancer cell line MDA-MB-468. *In vivo* studies using BALB/c mice bearing breast tumor xenograft showed that TNAs inhibited the tumor growth much more effectively than that of the NNAs. In case of mice treated with TNA, tumor disappeared at a dose of 10 mg/Kg. In case of mice treated with NNA (10 mg/Kg), the tumor volume was much bigger than those of mice treated with TNAs (5 mg/Kg) (relative tumor volume in mice treated with 10 mg/Kg NNAs = 1.71 and 5 mg/Kg TNAs = 0.99), whereas in the docetaxel (10 mg/Kg) treated mice group tumor regression was much slower (relative tumor volume in mice treated with 10 mg/Kg taxotere = 4.20, 10 mg/Kg NNAs = 1.71, and in control mice = 9.21). This result demonstrates that docetaxel-encapsulated nanoparticles surface-modified with EGF ligand have potential application as a drug delivery system for clinical breast cancer chemotherapy.

Christine Allen and co-workers have synthesized nano-sized micelles made of PEG-Docetaxel conjugate and encapsulated docetaxel (Figure 9) (45). These nanoparticles have size in the range of 70-95 nm. *In vitro* cytotoxicity of docetaxel (DTX), PEG-DTX, DTX loaded PEG-DTX nanoparticles have been evaluated and the IC$_{50}$ values have been determined in human non-small cell lung cancer (H460 cell), human ovarian cancer (SKOV-3) and human breast cancer cell (MCF-7). It was found that the cytotoxicity of PEG-DTX is lower than that of free DTX as demonstrated by the two- to threefold increase in the IC$_{50}$ values in all three cell lines (Table 4). On the other hand, DTX loaded PEG-DTX nanoparticles and free DTX have quite similar IC$_{50}$ values, which is probably due to the rapid release of free DTX from the micelle-type nanoparticles. At physiological pH the *in vitro* half-life (t$_{1/2}$) for hydrolysis of DTX loaded PEG-DTX nanoparticles (t$_{1/2}$ ~96h) is seven-fold higher than that of PEG-DTX (t$_{1/2}$ ~13h). Future studies will include determination of *in vivo* therapeutic efficacy, development of new spacer to obtain more stable micelle-type nanoparticles with improved blood circulation half-life.

Christine Allen and co-workers also conjugated docetaxel (DTX) to the hydrophobic block of polyethylene glycol-b-poly (ε-caprolactone) copolymers (PEG-b-PCL) (46). Docetaxel has been encapsulated in PEG-b-PCL-DTX to obtain micelle-type nanoparticles of various morphologies. Drug release studies showed the initial rapid release of physically encapsulated docetaxel, whereas the release of core-conjugated docetaxel was much slower-released over the course of one week. Conjugation of appropriate ligand and further biological studies can make them suitable for use in targeted cancer therapy.

5.3. Doxorubicin

Doxorubicin is an anthracycline antibiotic with antineoplastic activity commonly used to treat cancers of the bladder, breast, ovaries, stomach, lung, thyroid, and soft tissue sarcoma as well as some leukemias such as Hodgkin's lymphoma.

Doxorubicin has been formulated as nanoparticles popularly known as Doxil and Myocet (47-48). Doxil is polyethylene glycol (PEG) derivatized distearoyl-phosphatidyl ethanolamine based liposome-encapsulated form of doxorubicin. The size of the nanoparticles is about 80-120 nm. PEG coating on the surface of these nanoparticles reduce the possibility of opsonization and RES-uptake, and increase the blood circulation time. Doxil nanopartilce is less cardiotoxic than free doxorubicin. Doxil exhibits enhanced efficiency in some cancers in comparison to free doxorubicin, as nanoparticles accumulate in the tumor interstitium of the target tumors through the enhanced permeability and retention effect (EPR), resulting in the delivery of increased drug payloads at the tumor site. Doxil has been approved for treatment of ovarian cancer, breast cancer and multiple myeloma.

Myocet is a non-pegylated liposome-encapsulated doxorubicin-citrate nanocarrier (48). Clinical studies of Myocet nanocarrier in women with breast cancer have shown significantly reduced cardiac toxicity in comparison to conventional doxorubicin, because unlike parental doxorubicin this nanocarrier localizes into tumor
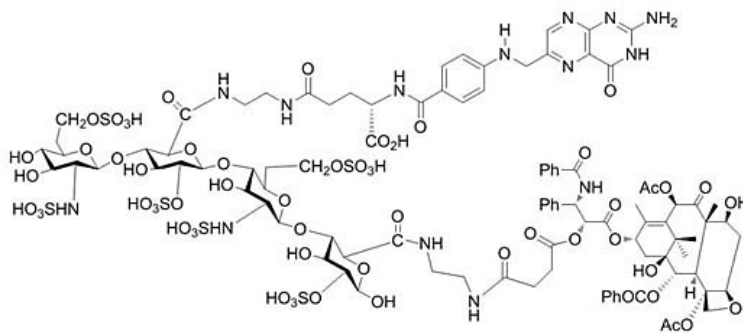
**Figure 7.** Conjugate of paclitaxel, heparin, and folic acid (42).
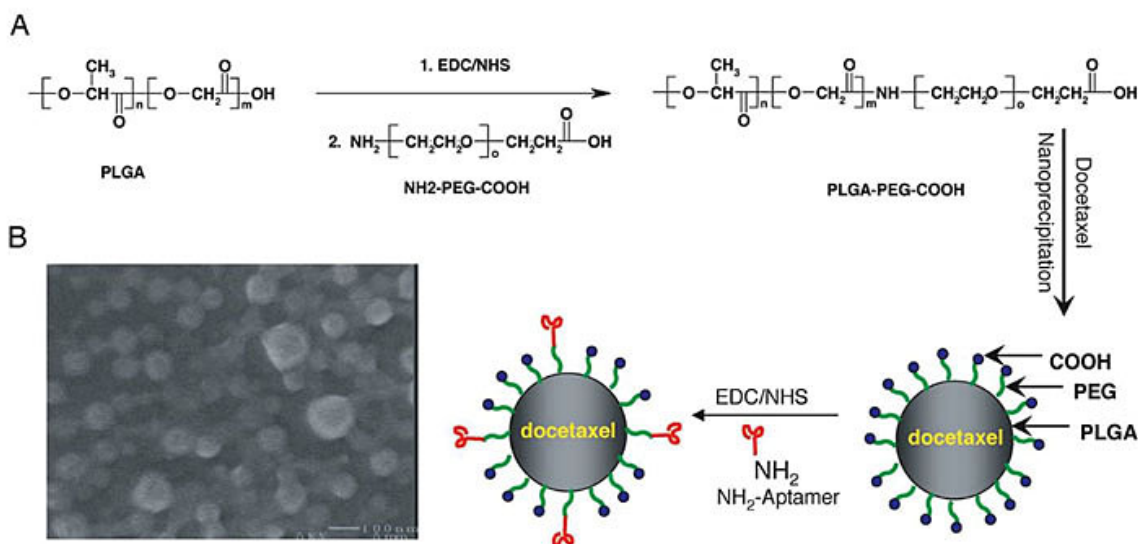
Figure 8. Development of Dtxl-encapsulated pegylated PLGA NP-Apt bioconjugates. (A) Schematic representation of the synthesis of PLGA-PEG-COOH copolymer and strategy of encapsulation of Dtxl. We developed Dtxl-encapsulated, pegylated NPs by the nanoprecipitation method. These particles have a negative surface charge attributable to the carboxylic acid on the terminal end of the PEG. The NPs were conjugated to amine-functionalized A10 PSMA Apt by carbodiimide coupling chemistry. (B) Representative scanning electron microscopy image of resulting Dtxl-encapsulated NPs is shown. Reproduced with permission from (43).

vasculature and avoids homing to the heart. Myocet is already a registered drug in Canada. In Europe, it is used in first line metastatic breast cancer in combination with cyclophosphamide. In USA, Myocet is being investigated in combination with Trastuzumab and Paclitaxel for treatment of HER2-positive metastatic breast cancer.

Hagen von Briesen and co-workers have developed target-oriented nanoparticles based on biodegradable human serum albumin (HAS) loaded with anticancer drug doxorubicin (Figure 10) (49). The surface of the nanoparticles was modified by covalent conjugation of trastuzumab to give Dox-NP-trastuzumab. Breast cancer cells (SK-Br-3) overexpressing HER2 were incubated with Dox-NP-trastuzumab and Dox-NP-IgG. It was found that there was specific targeting with Dox-NP-trastuzumab (73.80% positive cells for the 100% cross-linked

nanoparticles and 73.07% for 40% cross-linked nanoparticles), on the other hand Dox-NP-IgG nanoparticles had a marginal cellular binding (5.57% positive cells). SK-Br-3 cells were incubated with increased concentrations of DOX-NP-trastuzumab nanoparticles for 4h at 37ºC, and the cellular binding was found to increase in a concentration-dependent manner. This is due to high target specificity of Dox-NP-trastuzumab nanoparticles. Therapeutic effects of these nanoparticles were determined by WST-1 assay using SK-Br-3 cells. Dox-NP-trastuzumab and Dox-NP-IgG nanoparticles were incubated with SK-Br-3 cells in PBS for 4 h at 37ºC at a concentration of 15 mM with respect to doxorubicin concentration. Cell viability assay showed a viability of 20.1% for cells incubated with Dox-NP-trastuzumab whereas Dox-NP-IgG nanoparticles showed a viability of 56.5%. This demonstrates that Dox-NP-trastuzumab nanoparticles have
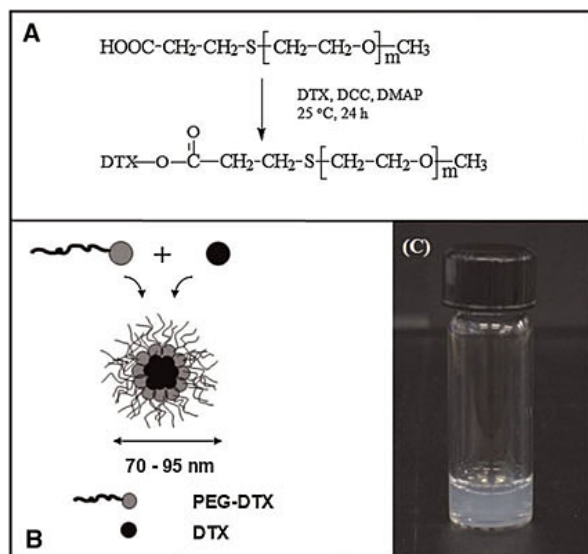
Figure 9. Formulation of docetaxel (DTX) loaded methoxy poly (ethylene glycol)-docetaxel (PEG–DTX) micelles: (A) synthetic scheme for PEG–DTX, (B) formulation of PEG–DTX micelles that physically entrap free DTX, (C) image of DTX loaded PEG–DTX micelle solution. Reproduced with permission from (45).
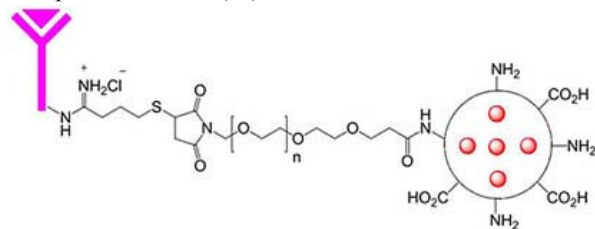
Figure 10. Covalent linkage of thiolated antibody to cross-linker-activated Doxorubicin-loaded nanoparticle. Reproduced with permission from (49).

higher cell-specific uptake as well as higher cytotoxic activity. This nanoparticle is a potential candidate for therapeutic application.

Tae Gwan Park and co-workers have formulated polymer encapsulated doxorubicin (DOX) nanoparticles (50). They separately synthesized conjugates DOX-PLGA-mPEG and PLGA-PEG-Folic Acid. These two conjugates were physically mixed with free DOX in an aqueous solution to form mixed micelles. The size of these micelle-type nanoparticles is about 100 nm. Folic acid (FA) is exposed on the micellar surface whereas DOX is physically and chemically trapped in the core of the micelles. Confocal image and flow cytometry analysis demonstrated that folate-conjugated mixed micelle-type nanoparticle displayed much greater extent of cellular uptake in comparison to the folate unconjugated micelles against the KB cells that overexpress the folate receptors. Enhanced cellular uptake occurred by the folate receptor mediated endocytosis. *In vivo* animal experiment using tumor xenograft nude mouse model displayed that the systemic administration of DOX nanoparticles significantly reduced

the tumor volume. These micelle-type nanoparticles have the potential clinical applications.

5.4. Cisplatin

Cisplatin forms adducts with DNA in several different ways, subsequently causing the cell to undergo apoptosis. Stephen J. Lippard and co-workers reported synthesis of cisplatin-encapsulated nanoparticles formulated with biocompatible biodegradable poly (D, L-lactic-co-glycolic acid)-block-poly (ethylene glycol) copolymer (Figure 11) (51). The surface of the nanoparticles was functionalized with the A10 2' fluoropyrimidine RNA aptamers that could recognize the extracellular domain of PSMA. *In vitro* cellular cytotoxicity assay of cisplatin-encapsulated nanopartcle-aptamer bioconjugates (Pt-NP-Apt) and cisplatin-encapsulated nanopartcle (Pt-NP) was performed using LNCaP cells, which express the PSMA protein. Under the same conditions, the IC_{50} values of Pt-NP-Apt, Pt-NP, and cisplatin are 0.03 mM, 0.13 mM, 2.4 mM respectively. Pt-NP-Apt bioconjugates are about 4 times more cytotoxic in comparison to the control Pt-NP lacking the A10 PSMA Apt, and 8 times more cytotoxic than that of free cisplatin. These results demonstrated the successful use of nanoparticle delivery system in aptamer-targeted delivery of a Pt (IV) prodrug to PSMA-expressing LNCaP cells.

5.5. Tamoxifen

Steroidal hormone 17β-estradiol (E2) binds to estrogen receptor (ER) which is essential to normal cell proliferation and cell differentiation in women. Hormone receptors such as estrogen receptor (ER) or, progesterone receptor are overexpressed in 75-80% of all breast cancers. The breast cancer treatment drug tamoxifen competes with 17β-estradiol for binding to ER, conformationally preventing adoption of associated transcription cofactors and subsequently initiating programmed cell death.

Robert J. Nicolosi and co-workers have formulated a water-soluble nanoemulsion of highly lipid-soluble drug tamoxifen (52). It has been demonstrated that nanoemulsions of tamoxifen have average particle size of 47 nm, inhibited cell proliferation 20-fold greater and increased cell apoptosis 4-fold greater relative to the suspension of tamoxifen in the breast cancer cell line HTB-20.

Paclitaxel and cisplatin are first choice for the treatment of ovarian cancer. Unfortunately about 70% of the patients show resistance to paclitaxel therapy. Acquisition of drug resistance to various chemotherapeutic drugs is believed to be due to phenotypic alterations in cancer cells owing to micro environmental selection pressure.

Mansoor Amiji and co-workers investigated the function of intracellular ceramide modulation in reversing the tumor multidrug resistance (53). Higher cytotoxic effect was observed in case PEO-PCL encapsulated paclitaxel nanoparticles showed much higher cytotoxicity than that of free paclitaxel during *in vitro* studies SKOV3 ovarian
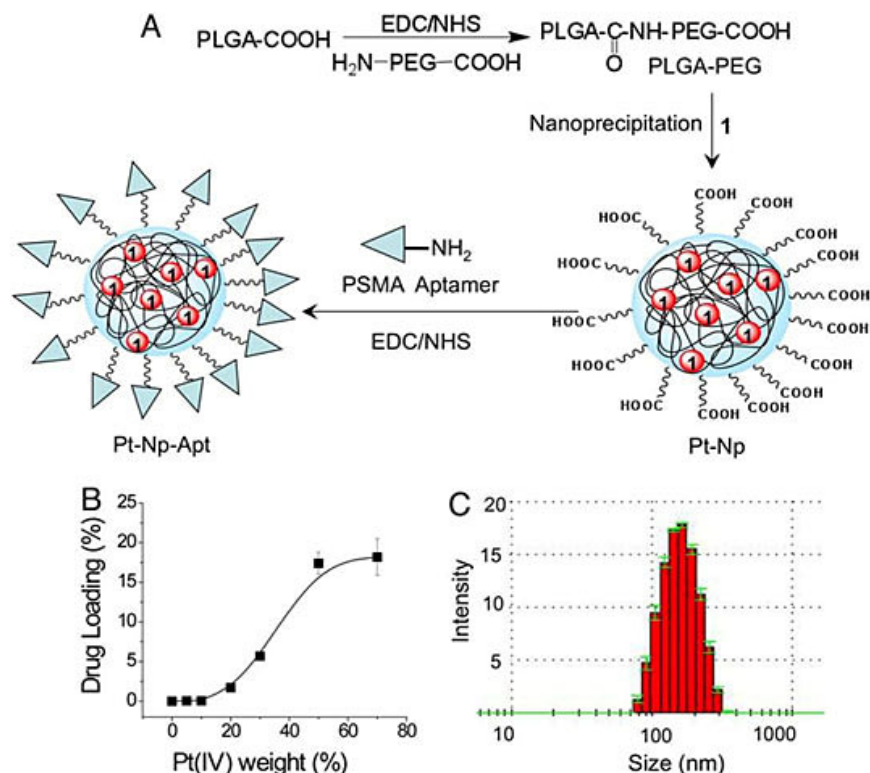
Figure 11. Construction and properties of aptamer-functionalized Pt (IV) nanoparticles. (A) Synthesis of Pt (IV)-encapsulated PLGA-b-PEG-COOH nanoparticles by nanoprecipitation and conjugation of PSMA aptamer to NP. (B) Loading of 1 in the PLGA-b-PEG-COOH nanoparticles. (C) Size of the Pt (IV)-encapsulated nanoparticles. Reproduced with permission from (51).
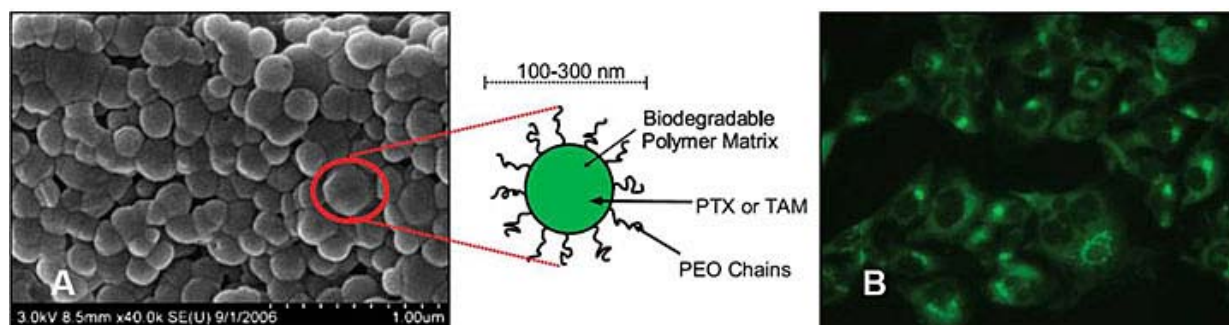

Figure 12. Polymer-based engineered nanoparticle formulations. (A), scanning electron micrograph and schematic illustration of the PEO-PCL nanoparticle system with encapsulated paclitaxel (PTX) and tamoxifen (TAM) for single and combination therapy in SKOV3 ovarian adenocarcinoma model. (B), fluorescence microscopy analysis of intracellular delivery of Oregon Green-labeled paclitaxel in PEO-PCL nanoparticles to SKOV3 cells. Reproduced with permission from (53).

cancer cell lines (Figure 12). Moreover co-administration of PEO-PCL-based paclitaxel and tamoxifen nanoparticles resulted in sensitization of drug resistant SKOV3TR cells to paclitaxel. Combination of PEO-PCL-based nanoparticles substantially reduced the tumor growth and increased the cellular apoptosis in sensitive SKOV3 and drug resistance SKOV3TR xenograft tumor models. Results of this study demonstrated that paclitaxel and tamoxifen nanoparticles can have the potential to increase the cytototoxicity of these drugs and overcome the drug resistance in ovarian cancer patients.

Mansoor Amiji and co-workers have also developed and characterized tamoxifen-loaded nanoparticle formulation using poly (ε-) caprolactone (Figure 13) (54). Significant amount of nanoparticles were uptaken by the ER (+) breast cancer cell line, MCF-7, resulting an increased local concentration of tamoxifen.

Mostafa El-Sayed, Adegboyega Oyelere, and co-workers have synthesized conjugates of gold nanoparticle and thiol-pegylated tamoxifen to selectively target and deliver plasmonic gold nanoparticle to estrogen receptor
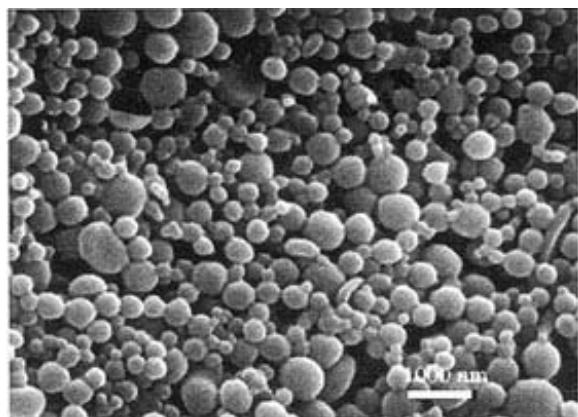
Figure 13. Results of particle size analysis by scanning electron micrograph of PCL nanoparticles. The nanoparticles were prepared by solvent displacement method. Reproduced with permission from (54).
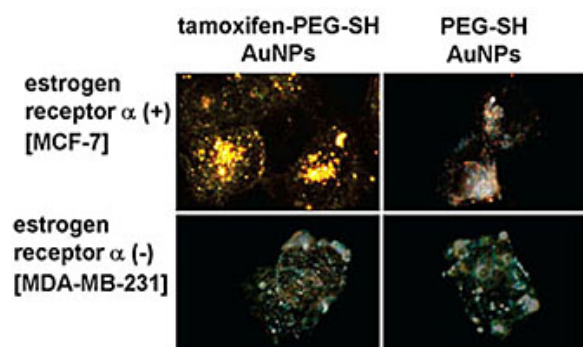
Figure 14. Dark-field scattering microscopy showing ligand- and receptor-dependent intracellular targeting of breast cancer cells by gold nanoparticle conjugates. Representative dark-field scattering images of ERα (+) (MCF-7, top) and ERα (-) (MDA-MB-231, bottom) human adenocarcinoma cells incubated for 24 h with 1 μM TAM-PEG-SHAuNP (left) and PEG-SH-AuNP (right) conjugates (ca. 1.2x104 TAM-PEG-SH per AuNP). Reproduced with permission from (55).
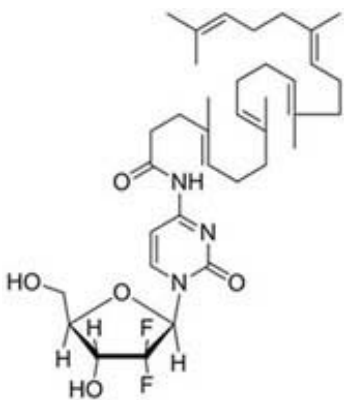
Figure 15. Structure of 4- (N)-trisnorsqualenoylgemcitabine (SQdFdC). Reproduced with permission from (56).
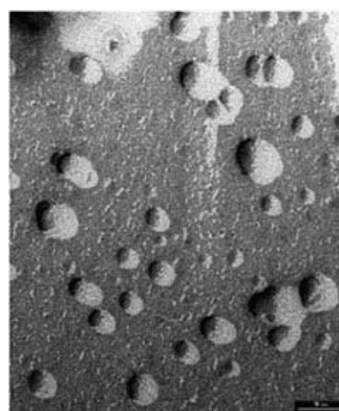
Figure 16. Transmission electron micrograph of 4- (N)-trisnorsqualenoylgemcitabine (SQdFdC) nanoassemblies after freeze-fracture. Reproduced with permission from (56).

positive breast cancer cells (55). ERα is overexpressed in breast cancer cell line, MCF-7, whereas it is absent in another breast cancer cell line, MDA-MB-231. In order to investigate the intracellular nanoparticle uptake, breast cancer cells were incubated for 24h with 1mM TAM-PEG-SH-Au NPs and PEG-SH-Au NPs, and dark-field scattering microscopy was performed. MCF-7 cells demonstrated high degree of intracellular and perinuclear localization of TAM-PEG-SH-Au NPs, whereas MDA-MB-231 cells showed no such labeling (Figure 14). Since, PEG-SH-Au NP does not contain any tamoxifen, so it does not show any cellular labeling or uptake for MCF-7 and MDA-MB-231 cells. A comparative study of the time-dependent IC_{50} values obtained for the free drug, TAM-PEG-SH, TAM-PEG-SH-Au NPs shows that TAM-PEG-SH-Au NP has 1.3-2.7-fold enhanced drug potency *in vitro*. Time-dependent dose-response data, receptor-selective and estrogen-competitive cytotoxicity and uptake of nanoparticle conjugates suggest that increased activity of TAM-PEG-SH-Au NPs is due to plasma membrane-localized ER-α-mediated endocytotic transport of TAM-PEG-SH-Au NP. Targeted intracellular delivery and increased potency of tamoxifen-gold nanoparticle conjugate provide immense scope to further increase the therapeutic potential by employing multimodal endocrine treatment strategies and adjunctive laser photothermal therapy.

5.6. Gemcitabin

Gemcitabine has shown antineoplastic activity against a wide range of solid tumors, including breast, cervical, ovarian, bladder, and testicular cancers. However, gemcitabine is metabolized intracellularly and extracellularly by deoxycytidine deaminase into chemotherapeutically inactive uracil derivative, resulting in a short plasma half-life (~1.5 h) and drug resistance thus limiting therapeutic efficacy of gemcitabine.

Patric Couvier and co-workers have developed a new strategy where they conjugated gemcitabine with 1, 1', 2-trinorsqualenic acid to give 4- (N)-trinorsqualenoylgemcitabine (SQdFDC) (Figure 15) (56). SQdFDC nanoassemblies (SQdFDC NA) were prepared by

nanoprecipitation method employing acetone solution of SQdFDC and dextrose solution (5%) (Figure 16) (57). Anticancer activity of SQdFDC NA and free gemcitabine have been evaluated on MCF-7 and KB-3 cancer cell line using MTT assay. For MCF-7 breast cancer cell line, the IC_{50} values are 29±5.8 mM (gemcitabine) and 4.8±3.9 mM (SQdFDC NA). For KB-3 cell line, the IC_{50} values are 50.8±9.2 mM (gemcitabine) and 8.8±4.1 mM (SQdFDC NA). Results of this study demonstrated that SQdFDC NA are about 6- to 8-fold more cytotoxic than free gemcitbine. SQdFDC NA has certain advantages over the free gemcitabine, i) it displays 6- to 8-fold higher anticancer activity *in vitro* and its metabolism in plasma has slowed down, ii) formation of nanoassemblies allows much safer intravenous administration. Anticancer activity of SQdFDC NA and gemcitabine was evaluated after intravenous injection of 5 mg/Kg and 15 mg/Kg gemcitabine equivalent in P388 leukemia mouse model. SQdFDC NA displayed better control over leukemia progression, and led to long term survival for the treated mice in comparison to free gemcitabine treatment. In order to understand the apoptosis and higher efficacy of SQdFDC NA *in vivo*, a P388 leukemia ascites mouse model was developed and extent of apoptosis was determined 3 days after injection of SQdFDC NA and free gemcitabine (5 mg/Kg gemcitabine equivalent). This study showed that SQdFDC NA-treatment resulted in 19.6-fold greater induction of apoptosis in ascitic cells than that of the gemcitabine treatment. It is believed that the prolonged intracellular liberation of gemcitabine and its active phosphorylated form are reason for enhanced efficacy of SQdFDC NA. Squalenoyl nucleoside gemcitabine displayed higher anticancer activity than that of the free nucleoside analog both *in vitro* and *in vivo* after oral or intravenous administration. Moreover squalene is suitable for oral delivery, and so its bioavailability is expected to be higher compared to the free molecules. It has been found that gemcitabine nanoassemblies displayed more efficient cytotoxicity compared to gemcitabine against breast cancer (MCF7, NCI/ADR-RES, MDA-MB-468, MDA-MB-231/ATCC, HS578T, MDA-MB-435, T-47D, MDA-MB-468), prostate cancer (PC-3), ovarian cancer (IGROV1, OVCAR-3, OVCAR-4, SK-OV-3), and renal cancer cell (786-0, ACHN, CAKI-1, RXF-393, TK10, UO-31) (57).

5.7. Etoposide

Etoposide is used in treatment of testicular cancer. Effort has been to formulate etoposide containing nanoparticles. R.S.R. Murthy and co-workers have formulated etoposide containing nanoparticles and have studied tumor uptake of these nanoparticles in Daltons lymphoma bearing mice (58).

Lamprecht and co-workers have synthesized lipid nanoparticlecs containing etoposide and investigated their role in tumor growth inhibition and concomitant P-glycoprotein inhibition (59). Therapeutic potential of etoposide nanoparticles can be explored for treatment of ovarian and testicular cancer.

5.8. Anastrazole

Anastrazole is an aromatase inhibitor which interrupts the synthesis of estrogen. It is used to treat estrogen receptor- positive and hormone-sensitive breast cancer in postmenopausal women.

M. J. Habib and co-workers have developed poly (d,l-lactic-co-glycolic acid) (PLGA)-based anastrozole microparticles in the size range of 75 to 420 mm. It was found that anastrazole microparticles have higher encapsulation efficiency, and their exterior surface is amenable to ligand conjugation (60). PLGA-encapsulated anastrozole microparticles have achieved a prolonged release for as long as 35 days.

Recently Hu Yang and co-workers have developed dendrimer-based stealth nanoparticles composed of PAMAM dendrimers core and outer PEG layer (61). PAMAM dendrimer provides the hydrophobic core which can entrap the hydrophobic anastrozole, and the outer PEG layer provides an improved circulating half-life by avoiding RES uptake. The diameter of these dendrimers ranges from 1.5 nm to 14.5 nm. PAMAM dendrimer-based stealth nanoparticles have shown upto 7.4 wt% anastrazole encapsulation and a favorable release profile for over 5 days. Such stealth nanoparticles with appropiately surface-functionalalized PEG can be conjugated to various targeting ligands for application in targeted breast cancer therapy.

5.9. Triptorelin

Luteinizing hormone releasing hormone (LHRH) receptor is overexpressed in breast, ovarian, endometrial, and prostate cancer cells. Triptorelin is a decapeptide analog of luteinizing hormone releasing hormone (LHRH), currently used in the treatment of hormone responsive cancers such as prostate cancer and breast cancer.

E. Fattal and co-workers have designed and synthesized triptorelin-loaded PLGA nanospheres for transdermal ionotophoretic administration (62). Encapsulation efficiency reached as high as 83% under optimum reaction conditions. The average diameter of the nanospheres was about 750 nm. These nanospheres displayed favorable release profiles over 15 days with the absence of any burst phase. Moreover, the free carboxylic acid functionalities on the surface of the nanospheres make them amenable to conjugation with various targeting liganveds.

Kyung A. Kang and co-workers have conjugated iron oxide nanoparticles with triptorelin (63). The iron oxide-triptorelin conjugate has retained its binding affinity and biological activity for the LHRH receptor. Breast cancer cell lines (MCF-7 and MDA-MB231) upon treatment with conjugated nanoparticles resulted in 95-98% cell death and loss of cell viability within 24 hours; on the contrary no change in cell proliferation or cell apoptosis was observed in cells treated with equal amounts of either triptorelin or iron oxide nanoparticles.

6. LIMITATIONS

From the above discussion, it is clear that nanomedicine has many advantages for clinical applications. Nevertheless, newly developed nanoparticles still have various limitations and there are many barriers to

overcome. Like any new technology, the safety of nanotechnology is continuously being tested. Implications of nanoparticles for health, safety, and environment have raised concerns. Some nanoparticles are too toxic (quantum dot contains toxic cadmium, carbon nanotube caused tissue damage in animal studies) for *in vivo* applications; nanoparticles without appropriate polymer surface coating are taken up non-specifically by mononuclear phagocytic cells and other immune cells; small nanoparticles (less than 5nm) undergo quick renal clearance; nanoparticles with different surface charges behave differently under the physiological condition (64-65). Various other *in vivo* conditions affect the biodistribution and targeting of nanoparticles, pharmacokinetic and pharmacodynamic properties which have not been completely understood. In spite of the increasing effort in developing new nanotherapeutics, pharmacokinetic and toxicological studies are limited due to lack of proper techniques. Moreover, the information on the release and accumulation of potentially toxic by-products is also not always complete. The advancement of in silico methods, mathematical models, and nano-QSAR could be of immense help in predicting the potential hazard of nano products (65).

7. CONCLUSIONS

One of the most challenging aspects of pharmacology in delivering cancer therapeutics is formulating agents for minimal side effects with better delivery. Due to highly heterogeneous and continuously evolving nature of tumor microenvironment, the optimal design of nanoparticle is a daunting task, especially if we consider the differences from one tumor to another, from primary tumor to its metastasis, dynamics of the same tumor before treatment and after treatment. Nanomedicine has emerged to meet this challenge. In this review, we described the immense potential of nanomedicine for applications in clinical settings. Nanoparticle formulation has enormous power of altering the pharmacokinetics and pharmacodynamics of traditional chemotherapeutics. A joint effort from medicinal chemists, molecular biologists, biomedical engineers, pharmaceutical scientists, and medical doctors will continue to address these issues. Nanotechnology will incorporate appropriate characteristics into the nanoparticles/nanocarriers to develop novel nanomedicine with improved therapeutic outcomes at an affordable cost.

8. ACKNOWLEDGEMENT

9. REFERENCES

1. http://www.who.int/cancer/en/, http://uicc.org/, http://www.iarc.fr, http://nano.cancer.gov

2. D. Majumdar, X. H. Peng, D. M. Shin. The medicinal chemistry of theragnostics, multimodality imaging and applications of nanotechnology in cancer. *Curr Top Med Chem* 10, 12, 1211-26 (2010)

3. D. A. LaVan, D. M. Lynn, R. Langer. Moving smaller in drug discovery and delivery. *Nat Rev Drug Discov* 1, 1, 77-84 (2002)

4. D. A. LaVan, T. McGuire, R. Langer. Small-scale systems for *in vivo* drug delivery. *Nat Biotechnol* 21, 10, 1184-91 (2003)

5. M. A. Moses, H. Brem, R. Langer. Advancing the field of drug delivery: taking aim at cancer. *Cancer Cell* 4, 5, 337-41 (2003)

6. E. S. Kawasaki, A. Player. Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer. *Nanomedicine* 1, 2, 101-9 (2005)

7. O. C. Farokhzad, R. Langer. Nanomedicine: developing smarter therapeutic and diagnostic modalities. *Adv Drug Deliv Rev* 58, 14, 1456-9 (2006)

8. E. M. Pridgen, R. Langer R, O. C. Farokhzad. Biodegradable, polymeric nanoparticle delivery systems for cancer therapy. *Nanomedicine (Lond)* 2, 5, 669-80 (2007)

9. D. Peer, J. M. Karp, S. Hong, O. C. Farokhzad, R. Margalit, R. Langer. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol* 2, 12, 751-60 (2007)

10. S. Vijayaraghavalu, D. Raghavan, V. Labhasetwar. Nanoparticles for delivery of chemotherapeutic agents to tumors. *Curr Opin Investig Drugs* 8, 6, 477-84 (2007)

11. C. Wei, W. Wei, M. Morris, E. Kondo, M. Gorbounov, D. A. Tomalia. Nanomedicine and drug delivery. *Med Clin North Am* 91, 5, 863-70 (2007)

12. A. Pope-Harman, M. M. Cheng, F. Robertson, J. Sakamoto, M. Ferrari. Biomedical nanotechnology for cancer. *Med Clin North Am* 91, 5, 899-927 (2007)

13. E. M. Pridgen, R. Langer, O. C. Farokhzad. Biodegradable, polymeric nanoparticle delivery systems for cancer therapy. *Nanomedicine (Lond)* 2, 5, 669-80 (2007)

14. S. Nie, Y. Xing, G. J. Kim, J. W. Simons. Nanotechnology applications in cancer. *Annu Rev Biomed Eng* 9, 257-88 (2007)

15. S. Parveen, S. K. Sahoo. Polymeric nanoparticles for cancer therapy. *J Drug Target* 16, 2, 108-23 (2008)

16. H. N. Banerjee, M. Verma. Application of nanotechnology in cancer. *Technol Cancer Res Treat* 7, 2, 149-54 (2008)

17. N. Sanvicens, M. P. Marco. Multifunctional nanoparticles--properties and prospects for their use in human medicine. *Trends Biotechnol* 26, 8, 425-33 (2008)

18. E. Angeli, R. Buzio, G. Firpo, R. Magrassi, V. Mussi, L. Repetto, U. Valbusa. Nanotechnology applications in medicine. *Tumori* 94, 2, 206-15 (2008)

19. W. E. Bawarski, E. Chidlowsky, D. J. Bharali, S. A. Mousa. Emerging nanopharmaceuticals. *Nanomedicine* 4, 4, 273-82 (2008)

20. T. Lammers, W. E. Hennink, G. Storm. Tumour-targeted nanomedicines: principles and practice. *Br J Cancer* 99, 3, 392-7 (2008)

21. B. Mishra, B. B. Patel, S. Tiwari. Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery. *Nanomedicine* 6, 1, 9-24 (2010)

22. M. Ferrari. Frontiers in cancer nanomedicine: directing mass transport through biological barriers. *Trends Biotechnol* 28, 4, 181-8 (2010)

23. F. Alexis, E. M. Pridgen, R. Langer, O. C. Farokhzad. Nanoparticle technologies for cancer therapy. *Handb Exp Pharmacol* 197, 55-86 (2010)

24. M. Wang, M. Thanou. Targeting nanoparticles to cancer. *Pharmacol Res* 62, 2, 90-9 (2010)

25. U. Kedar, P. Phutane, S. Shidhaye, V. Kadam. Advances in polymeric micelles for drug delivery and tumor targeting. *Nanomedicine* 6, 6, 714-29 (2010)

26. R. K. Jain, T. Stylianopoulos. Delivering nanomedicine to solid tumors. *Nat Rev Clin Oncol* 7, 11,653-64 (2010)

27. I. Ali, Rahis-Uddin, K. Salim, M. A. Rather, W. A. Wani, A. Haque. Advances in nano drugs for cancer chemotherapy. *Curr Cancer Drug Targets* 11, 2, 135-46 (2011)

28. F. Danhier, O. Feron, V. Préat. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *J Control Release* 148, 2, 135-46 (2010)

29. R. Seigneuric, L. Markey, D. S. Nuyten, C. Dubernet, C. T. Evelo, E. Finot, C. Garrido. From nanotechnology to nanomedicine: applications to cancer research. *Curr Mol Med* 10, 7, 640-52 (2010)

30. R. E. Serda, B. Godin, E. Blanco, C. Chiappini, M. Ferrari. Multi-stage delivery nano-particle systems for therapeutic applications. *Biochim Biophys Acta* 1810, 3, 317-29 (2011)

31. R. Arvizo, R. Bhattacharya, P. Mukherjee. Gold nanoparticles: opportunities and challenges in nanomedicine. *Expert Opin Drug Deliv* 7, 6, 753-63 (2010)

32. G. M. Das, C. Mohanty, S. K. Sahoo. Ligand-based targeted therapy for cancer tissue. *Expert Opin Drug Deliv* 6, 3, 285-304, (2009)

33. A. K. Gupta, R. R. Naregalkar, V. D. Vaidya, M. Gupta. Recent advances on surface engineering of magnetic iron oxide nanoparticles and their biomedical applications. *Nanomedicine (Lond)* 2, 1, 23-39 (2007)

34. R. R. Sawant, V. P. Torchilin. Multifunctionality of lipid-core micelles for drug delivery and tumour targeting. *Mol Membr Biol* 27, 7, 232-46 (2010)

35. http://www.abraxane.com/

36. A. Cirstoiu-Hapca, F. Buchegger, L. Bossy, M. Kosinski, R. Gurny, F. Delie. Nanomedicines for active targeting: physico-chemical characterization of paclitaxel-loaded anti-HER2 immunonanoparticles and *in vitro* functional studies on target cells. *Eur J Pharm Sci* 38, 3, 230-7 (2009)

37. T. C. Laurent. Biochemistry of hyaluronan. *Acta Otolaryngol Suppl* 442, 7-24 (1987)

38. H. Lee, K. Lee, T. G. Park. Hyaluronic acid-paclitaxel conjugate micelles: synthesis, characterization, and antitumor activity. *Bioconjug Chem* 19, 6, 1319-25 (2008)

39. S. C. Kim, D. W. Kim, Y. H. Shim, J. S. Bang, H. S. Oh, S. Wan Kim, M. H. Seo. *In vivo* evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy. *J Control Release* 72 (1-3), 191-202 (2001)

40. J. R. Hwu, Y. S. Lin, T. Josephrajan, M. H. Hsu, F. Y. Cheng, C. S. Yeh, W. C. Su, D. B. Shieh. Targeted Paclitaxel by conjugation to iron oxide and gold nanoparticles. *J Am Chem Soc* 131, 1, 66-8 (2009)

41. J. Chen, S. Chen, X. Zhao, L. V. Kuznetsova, S. S. Wong, I. Ojima. *J Am Chem Soc* 130, 49, 16778-85 (2008)

42. D. Majumdar, S. Nie. Nanotechnology in Cancer Therapy: Design and Synthesis of Nanoscale System for Targeted Delivery of Anti-cancer Drug. *National Cancer Institute Nanotechnology Alliance Investigators Meeting, Chapel Hill, NC.* 258 (October 16-18, 2007)

43. O. C. Farokhzad, J. Cheng, B. A. Teply, I. Sherifi, S. Jon, P. W. Kantoff, J. P. Richie, R. Langer. Functionalized single-walled carbon nanotubes as rationally designed vehicles for tumor-targeted drug delivery. *Proc Natl Acad Sci USA* 103, 16, 6315-20 (2006)

44. Y. Gao, L. Chen, W. Gu, Y. Xi, L. Lin, Y. Li. Targeted nanoassembly loaded with docetaxel improves intracellular drug delivery and efficacy in murine breast cancer model. Mol Pharm 5, 6, 1044-54 (2008)

45. J. Liu, P. Zahedi, F. Zeng, C. Allen. Nano-sized assemblies of a PEG-docetaxel conjugate as a formulation strategy for docetaxel. J Pharm Sci 97, 8, 3274-90 (2008)

46. A. S. Mikhail, C. Allen. Poly (ethylene glycol)-b-poly (epsilon-caprolactone) micelles containing chemically conjugated and physically entrapped docetaxel: synthesis, characterization, and the influence of the drug on micelle morphology. Biomacromolecules 11, 5, 1273-80 (2010).

47. http://www.doxil.com/

48. http://www.sopherion.com/

49. M. G. Anhorn, S. Wagner, J. Kreuter, K. Langer, H. von Briesen. Specific targeting of HER2 overexpressing breast cancer cells with doxorubicin-loaded trastuzumab-modified human serum albumin nanoparticles. *Bioconjug Chem* 19, 12, 2321-31 (2008)

50. H. S. Yoo, T. G. Park. Folate receptor targeted biodegradable polymeric doxorubicin micelles. *J Control Release* 96, 2, 273-83 (2004)

51. S. Dhar, F. X. Gu, R. Langer, O. C. Farokhzad, S. J. Lippard. Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt (IV) prodrug-PLGA-PEG nanoparticles. *Proc Natl Acad Sci USA* 105, 45, 17356-61 (2008)

52. J. B. Tagne, S. Kakumanu, D. Ortiz, T. Shea, R. J. Nicolosi. A nanoemulsion formulation of tamoxifen increases its efficacy in a breast cancer cell line. *Mol Pharm* 5, 2, 280-6 (2008)

53. H. Devalapally, Z. Duan, M. V. Seiden, M. M. Amiji. Modulation of drug resistance in ovarian adenocarcinoma by enhancing intracellular ceramide using tamoxifen-loaded biodegradable polymeric nanoparticles. *Clin Cancer Res* 14, 10, 3193-203 (2008)

54. J. S. Chawla, M. M. Amiji. Biodegradable poly (epsilon-caprolactone) nanoparticles for tumor-targeted delivery of tamoxifen. *Int J Pharm* 249 (1-2), 127-39 (2002)

55. E. C. Dreaden, S. C. Mwakwari, Q. H. Sodji, A. K. Oyelere, M. A. El-Sayed. Tamoxifen-poly (ethylene glycol)-thiol gold nanoparticle conjugates: enhanced potency and selective delivery for breast cancer treatment. *Bioconjug Chem* 20, 12, 2247-53 (2009)

56. P. Couvreur, B. Stella, L. H. Reddy, H. Hillaireau, C. Dubernet, D. Desmaele, S. Lepetre-Mouelhi, F. Rocco, N. Dereuddre-Bosquet, P. Clayette, V. Rosilio, V. Marsaud, J. M. Renoir, L. Cattel. Squalenoyl nanomedicines as potential therapeutics. *Nano Lett* 6, 11, 2544-8 (2006)

57. L. H. Reddy, J. M. Renoir, V. Marsaud, S. Lepetre-Mouelhi, D. Desmaele, P. Couvreur. Anticancer efficacy of squalenoyl gemcitabine nanomedicine on 60 human tumor cell panel and on experimental tumor. *Mol Pharm* 6, 5, 1526-35 (2009)

58. L. Harivardhan Reddy, R. K. Sharma, K. Chuttani, A. K. Mishra, R. S. Murthy. Influence of administration route on tumor uptake and biodistribution of etoposide loaded solid lipid nanoparticles in Dalton's lymphoma tumor bearing mice. *J Control Release* 105, 3, 185-98 (2005)

59. A. Lamprecht, J. P. Benoit. Etoposide nanocarriers suppress glioma cell growth by intracellular drug delivery and simultaneous P-glycoprotein inhibition. *J Control Release* 112, 2, 208-13 (2006)

60. A. S. Zidan, O. A. Sammour, M. A. Hammad, N. A. Megrab, M. J. Habib, M. A. Khan. Process analytical technology: non-destructive assessment of anastrozole entrapment within PLGA microparticles by near infrared spectroscopy and chemical imaging. *J Microencapsul* 25, 3, 145-53 (2008)

61. K. Sarkar, H. Yang. Encapsulation and extended release of anti-cancer anastrozole by stealth nanoparticles. *Drug Deliv* 15, 5, 343-6 (2008)

62. S. Nicoli, P. Santi, P. Couvreur, G. Couarraze, P. Colombo, E. Fattal. Design of triptorelin loaded nanospheres for transdermal iontophoretic administration. *Int J Pharm* 214 (1-2), 31-5 (2001)

63. S. S. Kakar, H. Jin, B. Hong, J. W. Eaton, K. A. Kang. LHRH receptor targeted therapy for breast cancer. *Adv Exp Med Biol* 614, 285-96 (2008)

64. B. Kim, G. Han, B. Toley, C. Kim, V. M. Rotello, N. S. Forbes. Tuning payload delivery in tumour cylindroids using gold nanoparticles. *Nat Nanotechnol* 5, 6, 465-72 (2010)

65. A. G. Cattaneo, R. Gornati, E. Sabbioni, M. Chiriva-Internati, E. Cobos, R. Marjorie, M. R. Jenkinsf, G. Bernardini. Nanotechnology and human health: risks and benefits. *J Appl Toxicol* 30, 8, 730-44 (2010)

Send correspondence to: Debatosh Majumdar, Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University School of Medicine, Emory University, Atlanta, GA 30322, USA, Tel: 404-778-5691, Fax: 404-778-5520, E-mail: majumdar.debatosh@gmail.com

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