



Folate-Mediated Paclitaxel Nanodelivery Systems: A Comprehensive Review

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ABSTRACT

Paclitaxel (PTX) is used as a viable cancer medication in the chemotherapy of breast, ovarian, lung, bladder, neck, head, and esophageal tumors. The focus of this review is to survey various folate-targeting PTX-loaded nanopreparations in both research and clinical applications. There are diverse nanopreparations, including liposomes, micelles, polymeric nanopreparations, lipid nanopreparations, lipoprotein nanocarriers, and other inorganic nanopreparations for folate-associated PTX tumor targeting. Here, the folate targeting PTX-loaded nanopreparations, which have promising results in the constructive treatment of cancer by reducing toxic side-effects and/or improving effectiveness, was mainly reviewed.

Key words: Paclitaxel, anticancer, folate receptor, tumor targeting, nanopreparations

INTRODUCTION

Cancer is a huge group of diseases in which a part of the body can be affected. As *per* World Health Organization (WHO), globally cancer is the most common cause of death. In 2020, about 10 million deaths are caused by cancer. About 0.685 million deaths occur due to breast cancer, 0.83 million are due to liver cancer, 0.769 million are due to stomach cancer, while 1.80 million are due to lung cancer. Also, about a population of 2.26 million are being affected by breast cancer, 1.41 million from prostate cancer, 1.93 million from colorectal cancer, 1.20 million from non-melanoma skin cancer, and 1.09 million from stomach cancer.¹ Nowadays, cancer can be treated by surgery, photodynamic therapy, radiotherapy, photothermal therapy, and by using chemotherapeutic agents.² Chemotherapy includes the usage of various drugs for killing purpose of cancerous cells, but along with the affected cells, they kill healthy cells and, thus, cause toxicity. This toxicity is because to less targeting of the cancerous cells, and therefore, there is a need to develop the chemotherapeutics for effective targeting of cancerous cells, either by active targeting or by passive targeting. The active targeting is achieved by incorporating a molecule or ligand that can bind to overexpressed receptors on the targeted cancerous cells.³

Amongst the taxane group of drugs, paclitaxel (PTX) is the first to be used as a chemotherapeutic agent.⁴ PTX is a diterpenoid available as a white crystalline powder, isolated from the bark of *Taxus brevifolia* Nutt. (Taxaceae), known as the Northwest Pacific yew tree, with a melting point of ~210°C having formula $C_{47}H_{51}NO_{14}$ and it was first revealed by "Mrs. Manroe E. Wall and Mansukh C. Wani".⁵ The chemotherapeutic agent, PTX has a huge spectrum of activity over several cancers such as metastatic breast cancer, non-small-cell lung cancer (NSCLC), AIDS-related Kaposi's sarcoma, refractory ovarian cancer, head and neck malignancies,⁶ malignant lymphoma, and lymphoblastic leukemia.⁷ It exerts a cytotoxic effect by inhibiting late G2 or mitosis phases of the cell division through stabilization of microtubule.⁴

PTX is highly hydrophobic and poorly soluble in water (~0.4 µg/mL), thus, to enhance its solubility and make it bioavailable, the commercial formulation Taxol® was formulated. Taxol® is the parenteral solution containing 6 mg/mL PTX in a combination of polyoxyethylated castor oil (Cremophor EL) and dehydrated ethanol at a ratio of 1:1 v/v. Before *i.v.* administration, the above solution is diluted 5 to 20 fold with 0.9% sodium chloride injection or with other aqueous *i.v.* solutions.⁸ Cremophor EL causes severe side effects such as neurotoxicity, hypersensitivity

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reaction, nephrotoxicity, and cardiotoxicity⁹. Besides, it also affects endothelial and vascular muscles causing vasodilation, labored breathing, lethargy, and hypotension.⁸ To minimize the side effect of Taxol[®], it is given with pretreatment using corticosteroids (*e.g.* dexamethasone), diphenhydramine, and H₂-receptor antagonists (*e.g.* cimetidine and ranitidine).¹⁰ The major problem that arises for successful chemotherapy is due to the toxic effect of conventional surfactant used and the availability of the drug. Thus, the successful chemotherapy is mainly based upon the development of a novel delivery system.¹¹ For enhancing the solubility and tumor targeting of PTX, several investigations were done including liposomes, microspheres, nanoparticles (NP), polymeric micelles,¹⁰ CD complexes,⁶ nanospheres, emulsions, and polymeric conjugates.¹¹

Site-specific drug delivery to a target organ, tissue or cell is known as drug targeting. The therapeutic effect of the drug is to enhance by either delivering the drug to the target site or by reducing the drug delivery to the site other than the target site.¹² Besides, the binding of an active targeting moiety or cancer cell-specific ligand to the surface of a drug can boost the uptake of drug in tumor cells, hence, it also enhances the therapeutic efficacy and reduces the side effects.¹³ Among the variety of methods, ligand-mediated targeting of cancerous cells by targeting the receptors overexpressed on tumor cells was found to be most effective in complimenting therapeutic effectiveness and lowering the side effects.¹⁴ Ligand-mediated targeting is achieved by chemically modifying the drug with tumor-targeting signaling molecules such as transferring, sugar, peptides, folic acid (FA), and antibody.¹⁵ As a tumor targeting-ligand, FA has various advantages; FA has a high binding attraction towards folate receptors (FR) and these receptors are expressed in large numbers on various tumor cells of the brain, ovary, lungs, kidney, myelogenous cells, and breast. Along with organic compounds, FA has a high compatibility in aqueous solvents, while also low immunogenic. Due to its low molecular weight, it can be chemically modified easily and has low cost.¹⁶

FA and FR

FA is a naturally occurring vitamin B9 and is also the synthetic form of folate. In several metabolic pathways, FA is required for the one-carbon reaction. It helps making DNA and genetic material by biosynthesizing nucleotide bases, thus, FA is consumed in larger amounts by proliferating cells.¹⁷ FA shows a dual mechanism for tumor specificity, as because FR is mainly expressed on the outside of the apical membrane of epithelial cells, it is inaccessible to the chemicals, which are formed in the blood cells and becomes inaccessible to the drug in circulation hence it provides local targeting. Upon transformation of epithelial cell, the polarity of cell loss and thus FR, is accessible to the drug in circulation. These all make FA a popular ligand for targeting.¹⁸

FR is a glycosylphosphatidylinositol-anchored membrane glycoprotein and known as “high-affinity membrane folate-binding protein” having an apparent molecular weight of 38-40 kDa.¹⁹ FR exists in three isoforms, *e.g.* hFR α , hFR β , and hFR γ .

Out of these isoforms, hFR α is expressed in a large amount in an expansive range of cancerous cells of the uterus, ovary, cervix, breast, kidney, testis, colon, brain, and pituitary gland, at the same time, hFR β leukemias, and activated macrophages.²⁰ There is almost no expression of FR in healthy cells, whereas it is highly expressed in undifferentiated metastatic cancer sites (Figure 1).²¹

Folate-mediated PTX NPs

A. PTX prodrug NPs

Because of limited lipophilicity and poor aqueous solubility formulation of lipid-based delivery system for PTX is difficult. To overcome this complication, Stevens et al.²² synthesized a prodrug of PTX that is “PTX-7-carbonyl-cholesterol (Tax-Chol)” with enhanced lipophilicity, also incorporated into lipid nanoparticles (LNs) formulation containing “folate-polyethyleneglycol-cholesterol (f-PEG-Chol)” as a ligand that targets FR. The drug-to-lipid ratio of FR-targeted LN formulation was 1:20. The resulting LNs had a smaller particle size (130 nm), higher entrapment efficiency (>90%), exhibited excellent colloidal stability, and *in vitro* therapeutic activity against tumor cells. The LNs displayed a better uptake and cytotoxicity in FR-targeted and FR-positive KB-cells and M109-cells than FR non-targeted cells. Also, the *in vivo* FR-targeted LNs notably augmented the antitumor activity along with animal survival compared to non-targeted LNs and Cremophor EL containing PTX formulation used for treating FR-positive M109 tumors bearing mice. The PTX pro-drug (Tax-Chol) incorporated LNs formulations show a greater potential for treating FR positive tumors cell than parent drug formulation.²²

A conjugation of PTX-poly-ethyl ethylene phosphate (PTX-PEEP) with FA (PTX-PEEP-FA) forms a novel prodrug that is soluble in water, has also been reported for targeted PTX delivery.²³ The steps involved in the synthesis of this prodrug are that, firstly, the amphiphilic pro-drug PTX-PEEP was formed by a ring opening polymerization reaction of 2-ethoxy-2-oxo-1,3,2-dioxaphospholane monomer, in which the catalysis of stannous octoate [Sn(Oct)₂] was started at PTX. In the next step, the

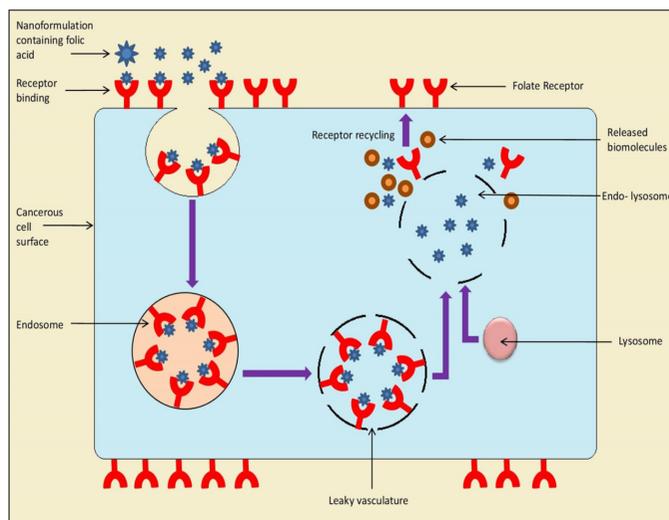


Figure 1. Receptor-mediated endocytosis of a drug conjugated to folates

process of esterification leads to form covalent conjugate of FA with PTX-PEEP. This conjugate is known as PTX-PEEP-FA amphiphilic polymeric pro-drug and is biodegradable. In an aqueous solution, the resulting PTX-PEEP-FA pro-drug was self-assembled and converted into micelles. The micelles had PTX in the hydrophobic core and PEEP in a hydrophilic coat, that as confirmed under transmission electron microscopy (TEM) and dynamic light scattering analysis (DLS). The micellar formulation was found to have smaller particle size (<130 nm), greater stability during systemic circulation, and exhibited better *in vitro* sustained-release behavior as compared to free doxycyclin (DOX) or PTX. The phosphoesterase-I degrade PEEP chain therefore the polyphosphoester-based pro-drug displayed lesser cytotoxicity of the parent drug up to the degradation of PEEP. The surface FA moiety enhances the selectivity, targeting, and efficiency of drug delivery, which were assessed *via* live-cell imaging system, by observing the cellular uptake of DOX-loaded PTX-PEEP-FA micelles for HeLa and KB cells, respectively. The endocytosis process, which is mediated by the FR, accelerates the cellular uptake of the drug formulation; hence, PTX-PEEP-FA micelles is the promising formulation for the targeted drug release intracellularly.

B. Copolymeric NPs

The copolymeric micelles are formed, when amphiphilic copolymer having both polar and non-polar segments is exposed to an aqueous environment it get self-assemble and forms a core and shell structure. As they contain both polar and non-polar portions, they may become effective targeting carriers for various water-soluble and water-insoluble amphiphilic drugs and genes to cancer cells.²⁴ The hydrophobicity and non-targeting nature of the drug-like PTX need to encapsulate in functionalize polymeric micelles for better therapeutic activity. The polymeric NPs can be prepared by covalent coupling²⁵ and physical encapsulation.²⁶ Physical encapsulation has the advantage like maximum drug loading efficiency of NPs, but it also has disadvantages such as easy leaking tendency at the time of delivery to the target site.²⁷ However, the NPs cannot achieve an adequately high concentration of drug in the cells of the tumor. For effective targeting and reducing side effects, introducing targeting moieties such as FA into NPs are required.

1. Poly-lactide (PLA) NPs

PLA is a matrix material used mostly for the formulation of polymeric NPs because of its biodegradability and safety. Wang et al.²⁸ effectively targeted the poorly water-soluble PTX to cancer cells by developing the folate associated hybrid polymeric NPs (FD-NPs). These FD-NPs were composed of monomethoxy-PEG-*b*-poly-lactide-PTX (MPEG-PLA-PTX) and D-R-tocopheryl PEG 1000 succinate folate (TPGS-FOL). As it remains an amphiphilic polymer the MPEG-PLA-PTX may self-assemble into NPs, even after PTX is in chemical conjugation with MPEG-PLA molecule. PTX could be delivered by physical encapsulation and chemical conjugation from FD-NPs. TPGS is a non-ionic water-soluble PEG-derivative of natural vitamin E and is one of the Food and Drug Administration (FDA) approved safe pharmaceutical adjuvants used in drug formulation. The

usage of TPGS in a formulation of NPs can enhance the drug loading as well as absorption of drug-like PTX.²⁸

Besides, Xiong et al.²⁹ developed folate-conjugated interfacially crosslinked biodegradable micelles composed of poly(ethylene glycol)-*b*-poly(acryloyl carbonate)-*b*-poly(D, L-lactide) (PEG-PAC-PLA) and FA-PEG-PLA block copolymers for the delivery of PTX *via* receptors into KB-cells. In these crosslinked biodegradable micelles, the PEG-PAC-PLA was produced to crosslink micelles at an interface in presence of ultraviolet (UV) radiation, while FA-PEG-PLA was used to target cancer cells that over expresses FR. The crosslinked micelles were found to have better physicochemical properties and stability compared to non-crosslinked controls. Likewise, it displayed sustained-release properties at low micelles concentration. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was conducted to evaluate the toxicity of crosslinked micelles in KB-cells and it was confirmed that increasing the concentration of folate in crosslinked or non-crosslinked micelles enhances the toxicity of PTX. The FA conjugated crosslinked micelles were evaluated for their cellular uptake by flow cytometry on KB-cells. The folate-decorated fluorescein isothiocyanate labeled linked micelles were found to have significant greater cellular uptake compared to micelles, which did not have folate ligands, revealing that the FA-conjugated crosslinked micelles of PEG-PLA are a promising way to target cancer therapy.²⁹

Thu et al.³⁰ also prepared folate decorated PTX-loaded PLA-tocopheryl PEG 1000 succinate (TPGS) NPs (Fol-PTX-PLA-TPGS NPs) by a solvent evaporation or modified emulsification method. For the formulation of NPs, the ring-opening method was used to synthesize PLA-TPGS copolymer, and folate was attached covalently to TPGS (TPGS-Fol). The confirmation of NPs was done by DLS method, fourier-transform infrared (FTIR), and field emission scanning electron microscopy (SEM). In physical appearance, the NPs were seen to be spherical in shape having 50 nm size and showed a narrow size distribution. In the *in vitro* study on the HeLa cell line, the Fol-PTX-PLA-TPGS NPs demonstrated better targeting efficiency than free PTX and PTX-PLA-TPGS NPs. Also the *in vivo* analysis was performed on the colorectal tumor-bearing nude mice to investigate the inhibition activity of NPs on tumor growth, and it was found that Fol-PTX-PLA-TPGS NPs inhibit tumor growth most efficiently compared to only PTX and PTX-PLA-TPGS NPs. The results of both *in vitro* and *in vivo* analysis clearly indicated that the use of folate as a targeting agent effectively enhances delivery of PTX at the targeted site.³⁰

2. Poly(lactide-co-glycolic acid) (PLGA) NPs

Due to many advantages of it in manufacturing of nanodelivery systems, PLGA is known as the best biodegradable copolymer. In the body, it produces non-toxic products of lactic and glycolic acids by hydrolysis, and at the end it produces carbon dioxide and water. As the body effectively deals with these degradants, PLGA is less prone to cause the systemic toxicity. In this view, He et al.³¹ and a co-worker have attempted to synthesize the amphiphilic copolymer, FA conjugated poly(ethylene glycol)-

poly(lactic-co-glycolic acid) (FA-PEG-PLGA) NPs for treating FR overexpressing tumor cells. FA-PEG-PLGA NPs are expressed as excellent carriers for combinational therapy of PTX and cisplatin (cis-diaminodichloro platinum, CDDP). As the NPs formed by conjugation of FA show active targeting and more uptake of NPs at target site, this improves the efficacy of PTX and CDDP and reduces the side effects associated with it.³¹

3. Polyacrylamide NPs

The monomer of polyacrylamide is used in the preparation of thermal sensitive, pH-sensitive, and water-swallowable preparation. In response, Seow et al.¹⁰ fabricated a new amphiphilic copolymer, cholesterol-grafted poly(*N*-isopropylacrylamide-co-*N*, *N*-dimethylacrylamide-co-undecenoic acid) [P(NIPA-DMA-UA)-*g*-cholesterol]. The folate decorated P(NIPA-DMA-UA)-*g*-cholesterol conjugate was formed by conjugation of folate to the polar segment of the prepared copolymer. The ¹H-NMR technique was used to confirm the synthesis of the polymer and it also shows a lower critical micelle concentration (CMC) of ~ 20 mg/mL. In the presence of cholesterol, the polymer self-assembled and formed micelles *via* membrane dialysis techniques. A highly hydrophobic drug such as PTX was encapsulated into the hydrophobic cores of these micelles, thus, the solubility of PTX in water significantly increased. The lower consolute temperature and particle size of the micelles containing the drug depended on external pH values. Similarly, the micelles released PTX more rapidly at a pH 5.0 *i.e.*, in an acidic environment than normal extracellular pH 7.4, thus it was confirmably shows pH-responsive thermal sensitivity.

The *in vitro* cytotoxicity assay was performed to determine therapeutic potential against FR overexpressing KB cells, which provided evidence that the PTX-loaded functionalized micelles more effectively killed KB cells due to the FR-assisted endocytosis process. In the prepared copolymer, no significant cytotoxicity was assessed with the polymeric carriers without drug and the targeted micellar formulation exhibited potential targeting efficiency and intracellular delivery.¹⁰

4. Pluronic NPs

Because of its amphiphilic nature and triblock structure pluronic is most widely used in the preparation of polymeric NPs. The triblock structure [poly(ethylene oxide) (PEO)-poly(propylene oxide) (PPO)-PEO] is made up of hydrophilic PEO blocks and hydrophobic PPO blocks. Zhang et al.³² (2011) developed mixed micelles with FA functionalized pluronic P123/F127 and PTX encapsulated in it (FPF-PTX). All these were tested *in vivo* and *in vitro* for selective targeting using pluronic P123/F127 mixed micelles loaded with PTX (PF-PTX) and taxol as control. The size of particles of the prepared FPF-PTX micelles was decreased up to 20 nm and found to a spherical shape with a higher entrapment efficiency. In an *in vitro* study of cellular uptake, it was investigated that the FPF-PTX micelles show cellular uptake in time-dependent way and was more due to the endocytosis mediated by FR compared to PF-PTX. The effect of FPF-PTX on cell apoptosis, cytotoxicity, and cell-division cycle arrest was studied in KB and KBv cells and it confirmed that the

prepared FPF-PTX was founded more efficient than Taxol® and PF-PTX. In the pharmacokinetic study, it was also shown that the bioavailability of FPF-PTX NPs in rats was 3 fold greater than that of Taxol®.

The *in vivo* study revealed that the antitumor efficiency of FPF-PTX group was more effective in KBv multi-drug resistant (MDR) tumor-bearing BALB/c mice than those of the Taxol® and PF-PTX treated groups. The additive effect of MDR inverting ability of pluronic block copolymers and active targeting by FA and FR, the therapeutic efficacy of FPF-PTX was enhanced.³²

C. Other nanoparticles

1. Albumin/albumin moiety NPs

The folate-conjugated chemotherapeutic showed poor remedial adequacy caused by limited blood circulation or suboptimal pharmacokinetics. The use of albumin to enhance the pharmacokinetics of the drug is an optimistic approach; furthermore, it improves the circulation time of the drug through blood and accumulation of drug in tumors. Albumin is a protein present abundantly in plasma and is the important carrier for delivery of drugs derived from endogenous and exogenous substances.³³ Conjugating drugs to albumin³⁴ and albumin-binding moieties³⁵ are the most prosperous approaches as they enhance the efficiency of delivery of antitumor drugs and lower the side effects. Longer PTX blood circulation time and improved pharmacokinetic properties of PTX were reported by decorating the folate to PTX-loaded biodegradable bovine serum albumin (PTX-BSA-NPs)³⁴ and Evans blue (EB) an albumin-binding moiety conjugated to FA-PTX.³⁵ The small molecule EB exhibits a greater binding affinity toward blood circulating albumin. The formation of bifunctional prodrug by binding of albumin and albumin binding moieties to folate for both active and passive targeted PTX delivery results in high antitumor activity and lower toxicity.

BSA magnetic nanocomposites have also been reported for PTX delivery and tumor diagnosis. A simple modification process was used to develop BSA magnetic nanocomposites of PTX with carboxymethyl cellulose (CMC) (PTX-BSA-CMC-FA) and chitosan (CS) (PTX-BSA-CS-FA). The BSA-CMC-FA and BSA-CS-FA conjugates were prepared by an esterification reaction of FA to CMC and CS, respectively. The nickel ferrite (NiFe₂O₄) nanocores (NFs) PTX-NFs-BSA-CMC-FA and PTX-NFs-BSA-CS-FA were prepared *via* thermolysis of nickel acetylacetonate and PTX loading by the diffusion process. Irrespective of FA-modified surface the fabricated multifunctional nanoconjugates demonstrated better dispersibility, excellent transversal R2 relaxation rate, along with FR targeted and magnetically guided functions. Tumor diagnosis and tumor inhibition rate of PTX-NFs-BSA-CMC-FA, and PTX-NFs-BSA-CS-FA nanoconjugates were effectively enhanced by the application of an external magnetic field.³⁶

Besides, Chen et al.³⁷ generated lipoprotein-mimicking nanocomplex for dual targeting therapy through the electrostatic attraction of FA modified BSA (FB) and LNs loaded with PTX (PTX-LNP). The thin-film hydration method was used to prepare

PTX-LNP and the FB complex was prepared by conjugation of FA with BSA. SPARC-albumin interaction leads to increased gp60-mediated transendothelial transport along with accumulation of the drug in tumor cells thus BSA provides specific targeting to tumor cell, hence, it is employed as a protein in lipoprotein mimicking nanocomplex FB-PTX-LNP. Further, the conjugated FA to BSA accomplished the active dual targeting delivery. *In vitro* cytotoxicity assay was performed against MCF-7 and HepG2 cells and the study revealed that FB-PTX-LNP and BSA-PTX-LNP exhibited considerably a more cytotoxic effect compared to PTX-LNP. Flow cytometry analysis was performed to determine cellular uptake of the drug by MCF-7 cells and it indicated that FB-coumarin-6-LNP get quickly uptaken compared to BSA-coumarin-6-LNP and coumarin-6-LNP. In the *in vivo* analysis on mice bearing MDA-MB-231 tumor, it appeared that FB-PTX-LNP indicated better ability to target tumor cells with promising anti-tumor activity. In preparation of dual-targeted PTX-loaded protein lipid nanocomplex FB-PTX-LNP, BSA, and FA both play a considerably important role.³⁷

2. Heparin NPs

Heparin is a versatile natural polymer that commonly attaches to angiogenic growth factors and was used to initiate the self-assembly of nanomicelles from designated amphiphilic molecules of peptide.³⁸ Heparin also improved response to drug and survival period in patients taking chemotherapy for cancer,³⁹ and inhibited the tumor growth related to binding of growth factors.⁴⁰ In response, Wang et al.⁴¹ developed a novel drug delivery system that enhanced efficiency and decreased the adverse effects of PTX by fabricating heparin-FA-PTX (HFT), a ternary conjugate loaded with extra PTX (T). *In vitro* cytotoxicity study was done on FR-positive human head and neck tumor cell line KB-3-1, and the study indicates that the HFT-T NPs exhibit higher cytotoxicity as compared to free PTX. In a xenograft model of subcutaneous KB-3-1, HFT-T NPs were found to selectively target the FR overexpressing tumor tissue and extraordinarily increase the antitumor efficiency of PTX. The same results were exhibited in average tumor-volume evaluation, HFT-T treated and free PTX-treated mice group average tumor volume was $92.9 \pm 78.2 \text{ mm}^3$ and $1670.30 \pm 286.10 \text{ mm}^3$, respectively. The PTX tumor recurrence was not observed in HFT-T-conjugated NP treatment, which indicated that the tumor was inhibited more effectively by HFT-T NPs from developing resistance to the drug. No significant acute systemic toxicity was found in the xenograft model. All these results lead us to believe that using ternary-structured NPs (HFT-T), PTX can be delivered to FR-overexpressed tumor cells is a favorable strategy to boost the efficacy of chemotherapy and lower the adverse effects.⁴¹

The inhibitory effect of heparin on tumor growth is investigated so the use of a heparin-based self-assembled, folate-conjugated heparin-poly(β -benzyl-laspartate) (HP) amphiphilic copolymer containing nanoparticulate system for PTX delivery. The folate-PEG-conjugated HP (FHP) NPs have a greater ability to serve as potential carriers for PTX targeting in cancer therapy compared to the PTX-loaded HP and PTX-loaded folate-HP. NPs

which was formed get recognized easily and effectively by the FR because the PEG spacer between the heparin backbone and the folate ligand of the FPHP-PTX NPs increases the targeting moiety length.⁴²

3. Chitosan NPs

In recent years, numerous micelles of CS PTX have been studied, such as amphiphilic carboxymethyl CS-quercetin PTX micelles,⁴³ *N*-octyl-*N*-(2-carboxylbenzoyl) CS PTX micelles,⁴⁴ α -tocopherol succinate-modified CS PTX micelles,⁴⁵ and *N*-succinyl-palmitoyl-CS PTX micelles.⁴⁶ But higher toxicity on normal cells the use of these PTX micelles was greatly constrained. To increase cancer cell targeting and minimize the side effects of PTX, Wang et al.⁴³ introduced a modified biodegradable micellar delivery of PTX via deoxycholic acid-*o*-carboxymethylated CS-FA conjugate (DOMC-FA). *o*-Carboxymethylated chitosan (OCMC) is a type of carboxymethylated derivative of CS and deoxycholic acid (DOCA) is amphiphilic natural bile acids. However, DOCA and OCMC interaction induces the self-associated self-assemble micelles for hydrophobic drugs. The DOMC-FA micelles loaded with PTX were effectively prepared and referred as a novel system for drug targeting. The covalently-bonded FA is employed as a ligand for cell membrane targeting and for improving DOMC-FA-PTX NP endocytosis through the FR. The commercially available injections of PTX (Taxol®), plain micelles, and folate conjugated micelles were tested for their cytotoxicity and ability to target tumor cells and were confirmed by studies on cellular uptake, morphological changes, apoptosis, and MTT assay in MCF-7 cells with overexpression FR. The positive results of this formulation confirmed that the DOMC-FA micelles loaded with PTX are beneficial for targeting and reducing the side effects of PTX.⁴³

To increase the efficiency and decrease toxicity of PTX, Cheng et al.⁴⁷ also developed an amphiphilic injection system for PTX (FACC-PTX micelles) using a biocompatible and biodegradable FA-cholesterol-chitosan (FACC) polymer conjugates. The aminoacylation reaction of the primary amino group of CS leads to synthesize FACC polymeric conjugate and the dialysis method was used to prepare FACC-PTX micelles. FACC polymer had a critical concentration 64.13 low $\mu\text{g}/\text{mL}$ and could self-built in an aqueous environment. In the *in vitro* release study the micelles of FACC-PTX showed that the drug release at the tumor site, where the environment is weak acidic was higher and at the normal environment of cells was low. Hence, all these results indicated that the formulation was less toxic. By *in vitro* cytotoxicity study against HeLa (FR-positive) and A549 (FR-negative cells), the results of cytotoxicity and targeting efficiency of FACC-PTX micelles were found to be significantly optimistic compared with Taxol®.⁴⁷

The octadecyl quaternized lysine-modified chitosan (OQLCS) is a derivative of CS, soluble in water and organic solvent, has an amino group for functional group attachment and is easily reconstituted in liposomes. Based on OQLCS and cholesterol, Zhao et al.³⁵ synthesized PTX or calcein-loaded folic acid-modified TAT peptide-conjugated polymeric liposomes (PTX

loaded FA-TATp-PLs). 11% Feed ratio of PTX to FA-TATp-PLs conjugate achieved a drug loading of 9.55%, and encapsulation efficiency of 86.83%. The particle size of PTX-FA-TATp-PLs and the cellular uptake of PLs were directly proportional to each other. *In vitro* study revealed that the PTX-loaded FA-TATp-PLs showed 80% drug released in two weeks and indicated that FA-TATp-PLs displayed more ability to endocytosis in both KB cells with overexpression of FR and in FR deficient A549 cells compared to PLs. *In vitro* cytotoxicity study was conducted on KB cells (FR-positive) and the PTX-loaded FA-TATp-PLs showed higher cytotoxicity than PTX containing FA-PLs, Taxol®, and PLs. Similarly, as compared to Taxol®, the FA-TATp-PLs loaded with PTX exert promising antitumor activity under *in vivo* study conducted on the mice bearing nasopharyngeal tumor. Due to its high efficacy for delivery of TAT peptide and FA target specificity, in the future, this formulation will become a promising therapy for tumor targeting.³⁵

4. Graphene oxide (GO) NPs

GO is a graphite derivative with exceptional biocompatibility, having electronic flexibility, large specific surface area, mobility, better thermal conductivity, and mechanical strength that allows it to facilitate chemical modification and functionalization.⁴⁸ Recently, it has been largely used in drug and gene delivery, photothermal cancer therapy, and as biosensors.⁴⁹

Vinothini et al.⁵⁰ prepared GO through modified Hummers method. A novel GO-methyl acrylate-folic acid- PTX (GO-MA-FA-PTX) nanocarrier was fabricated *via* conjugation of the targeting ligand, FA, and methyl acrylate (MA) with the GO surface *via* ether and amide linkage. PTX was attached through hydrophobic interaction and π - π stacking on the GO-MA-FA carrier surface. The FT-IR and XRD analyses confirmed the conjugation and structural modification (GO-MA-FA-PTX) by indicating a chemical change in the GO structure. The SEM, TEM, and atomic force microscopy images confirm the surface modification of GO. On thermogravimetric analysis (TGA), it was confirmed that the GO and PTX-loaded GO-MA-FA-PTX nanocarrier demonstrated better stability. In the *in vitro* study, PTX release was higher in the acidic microenvironment as compare with the physiological compartment. MTT assay was done on MDA-MB-231 human cell line of breast cancer to determine the cytotoxicity of the prepared nanocarriers and it was confirmed that the GO-MA-FA-PTX had 39% cytotoxicity. Likewise, an *in vivo* study was performed on rats suffering with DMBA-induced breast cancer and on treatment with GO-MA-FA-PTX, it was seen that the nanocarriers increased the depleted level of mitochondrial citric acid enzymes to normal. This result reveals that the GO-MA-FA-PTX nanocarrier was a significantly more potent and specific targeted delivery system for an anticancer drug.⁵⁰

5. Hydroxyapatite (HAp) NPs

HAp is a biocompatible, biodegradable material and it has been used as a delivery system for several drugs and therapeutic agents. Venkatasubbu et al.⁵¹ reported the synthesis of FA decorated PEG functionalized HAp NPs (Hap-PEG-FA) for the successful delivery of the anticancer drug PTX. UV spectroscopy

and TGA confirmed the conjugation and structural modification of HAp-PEG-FA NPs by comparing the changes in the chemical structure of a pure component. The TEM images confirmed the absence or presence of residual components in NPs. The FT spectroscopic analysis indicated the functionalization of NPs with a polymer and its chemical adsorption. In *in vitro* study, PTX was rapidly released in the initial stage and then followed a slow, steady, and controlled release. All these results revealed that use of HAp in the formulation of drug delivery system makes it a promising one.⁵¹

6. Hyaluronic acid (HA) NPs

HA is a non-toxic, natural, and biodegradable polysaccharide. In biotechnological and biomedical fields, it has wide applications and this is because of its powerful affinity towards the markers, which make the cell surface specific, such as glycoprotein CD44 and receptors for motility mediated by HA, which are overexpressed mostly on various types of malignant solid tumor surface.⁵² Recently, HA is also used as dual receptor targeting strategy for effective drug delivery. The most important benefit of dual targeting nanocarrier systems is to overcome MDR. Dual targeting therapy can be developed using FA targeting to folate and HA targeting to CD44 receptors. As both of these receptors are overexpressed on malignant tumor cells, it is possible to develop a dual-targeting drug delivery system. A similar dual targeting system was developed by a Chinese scientist Yanhua Liu et al.⁵³, using FA, HA, and C18 conjugates. MTT assay was performed on MCF-7 and A-549 cell lines with three samples *i.e.* taxol solution, FA-C18, and HA-FA-C18 micelles, and it was indicated that the cytotoxicity of taxol solution was much lower compared to conjugated micelles. Also, the pharmacokinetic study stated that conjugated micelles exhibit much longer circulation compared to taxol solution. This study suggests that these conjugates are biodegradable, biocompatible, and dual-targeting nanostructure carriers for delivery of hydrophobic anticancer drugs intercellularly.¹¹ In 2014, similar conjugates were evaluated for comparison of single targeting and dual-targeting micelles for eliminating multidrug resistance using MCF-7 and MCF-7/Adr cells. The efflux of the drug mediated by the P-gp transporter is a crucial reason for the resistance of PTX. The result of the study showed that targeting micelles significantly increased the drug uptake in drug resistance cells as compared to taxol solution. Furthermore, *in vitro* cytotoxicity study and intracellular uptake studies demonstrated that CD44 and FAR dual-mediated endocytosis played a vital role in overcoming MDR. These studies indicated that targeting therapy is a promising therapy for overcoming MDR for PTX drug delivery.⁵³

7. Cyclodextrin NPs

Amphiphilic CDs and their derivatives are one of the promising tools for the delivery of nanoscale drug molecules. It is widely used in designing various novel functionalize materials for biomedical applications due to its biocompatibility, unique inclusion capability, and powerful functionalization capacity.⁵⁴ In aqueous solutions, the amphiphilic CDs get self-modified

and, hence, it has a great ability to interact with biological membranes.⁵⁵

Erdoğar et al.¹⁵ performed esterification and altered the CD derivative at the primary and secondary phases by substitution of C₆ alkyl chains and developed FCD-1 and FCD-2 folate conjugated CDs. Each derivative (FCD-1 and FCD-2) carries one folate residue on the substituted face at the termination of the C₆ linker chain, which was joined to the mother amphiphilic CD to give effective targeting efficiency to FR overexpressed on cancer cells. The optimized PTX loaded, actively targeted NP formulation was obtained through a specific modifications using 3² factorial designs. In water, the prepared FCD-1 and FCD-2 derivatives can self-organize in NPs having size (smaller than 100 nm) with narrow size distribution and in this carrier, up to 60% PTX should be encapsulated by the nanoprecipitation method. The PTX-loaded FCD-1 and FCD-2 NPs were more stable than the other nanoparticulate systems and delayed the drug release even more. No cytotoxicity of the blank NPs was found against L929 cells. PTX-loaded NPs exhibited a more anticancer efficacy because of the good interaction with the FR-positive T-47D and ZR-75-1 human breast cancer cells. Therefore, these novel folate conjugated CD NPs are considered a promising formulations for effective and safe delivery of PTX with a folate dependent mechanism.¹⁵

8. Gene therapy

In the efforts of the development of folate conjugated chemotherapeutics, development has been made in the field of folate-targeted gene therapy, in which both viral and non-viral vectors have been examined.¹² Gene therapy is introduced as an effective method for treating ovarian cancer and contains small interfering RNA (siRNA).⁵⁶ Relapse and resistance are commonly seen obstacles in ovarian cancer treatment, which are attempted to be overcome by various siRNA combination therapies, currently being studied.⁵⁷ Jones et al.⁵⁸ incorporates targeted delivery of siRNA and PTX to FR overexpress ovarian cancer cells through the tri-block copolymer micelleplexes consisting of PEI- graft-polycaprolactone-block-poly(ethylene

glycol) (PEI-g-PCL-*b*-PEG-Fol) that overcome toll-like receptor 4 (TLR4)-driven chemotherapy resistance. The optimized targeted delivery of siRNA micelleplexes was explored by altering different molecular weights of PEG as well as different grafting degrees of the (g-PCL-*b*-PEG-Fol) chains to PEI. Western blotting and flow cytometry analysis demonstrated the effective delivery of siRNA *via* PEI-g-PCL-*b*-PEG-Fol conjugates, which is responsible for efficient protein destruction of TLR4. TLR4-mediated chemotherapy resistance is overcome by destruction of TLR4 within SKOV 3 cells, which makes them sensitive toward PTX treatment and increases apoptosis.⁵⁸

CONCLUSION

PTX is the anticancer drug found to be most effective against a variety of cancers such as NSCLC, refractory ovarian cancer, metastatic breast cancer, head and neck malignancies, AIDS-related Kaposi's sarcoma, malignant lymphoma, and lymphoblastic leukemia. Apart from the effectivity that was found to be toxic due to Cremophor EL and ethanol used in the formulation, as solvent hence to overcome the formulation-related problem researchers innovate some nanodelivery systems for delivering PTX, such as lipid-based formulations, polymeric NPs, inorganic NPs, polymer conjugates, carbon nanotubes, CD NPs, and nanocrystals. To overcome PTX-related problems such as its low solubility, pharmacokinetic profile, and targeting, researchers found some targeting moiety and targeting sites like FA and FRs which are overexpressed in cancerous cells. This review contains overview about a various folate targeted PTX containing nanodelivery systems such as PTX pro-drug NPs includes Tax-Chol prodrug and water-soluble polymeric prodrug, copolymeric NPs includes PLA NPs, PLGA NPs, polyacrylamide NPs, pluronic NPs, and there are some other NPs such as albumin/albumin moieties NPs, heparin NPs, CS NPs, GO NPs, HAp NPs, HA NPs, CD NPs, and gene therapy along with it, the review also focuses on ongoing research on targeting therapy for PTX (Table 1).

Table 1. Folate-mediated paclitaxel nanodelivery system

S. no	Formulation category	Formulation (ligand)	Reference
A. PTX Pro-drug NPs			
1.	Tax-Chol prodrug	f-PEG-Chol	22
2.	Water-soluble polymeric prodrug	PTX-PEEP-FA	23
B. Copolymeric NPs			
1.	Poly(lactide) PLA NPs	MPEG-PLA-PTX & TPGS-Fol	28
		PEG-PAC-PLA & FA-PEG-PLA	29
		Fol-PTX-PLA-TPGS	30
2.	Poly(lactide-co-glycolic acid) (PLGA) NPs	FA-PEG-PLGA	31
3.	Polyacrylamide NPs	f-P(NIPA-DMA-UA-g-Cholesterol)	10
4.	Pluronic NPs	FPF-PTX	32
C. Other nanoparticles			
1.	Albumin/albumin moieties NPs	PTX-BSA NPs	36
		EB-FA-PTX	
		PTX-NFs-BSA-CS-FA	
		PTX-NFs-BSA-CMC-FA	
2.	Heparin NPs	HFT-T	41
		PTX-FPHP-PTX NPs	42
3.	Chitosan NPs	DOMC-FA/PTX	43
		FACC-PTX micelles	47
		PTX loaded FA-TATp-PLs	35
4.	Graphene oxide (GO) NPs	GO-MA/FA-PTX	50
5.	Hydroxyapatite NPs	HAp-PEG-FA	51
6.	Hyaluronic acid NPs	FA-HA-PTX	11
7.	Cyclodextrin NPs	PTX loaded FCD-1 & FCD-2 NPs	15
8.	Gene therapy	siRNA & PTX via PEI-g-PCL-b-PEG-Fol	58

PTX: Paclitaxel, NPs: Nanoparticles

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REFERENCES

- World Health Organization (2021). Factsheet on Cancer. <https://www.who.int/en/news-room/fact-sheets/detail/cancer> [accessed 3 March 2021].
- Zhou L, Dong K, Chen Z, Ren J, Qu X. Near-infrared absorbing mesoporous carbon nanoparticle as an intelligent drug carrier for dual-triggered synergistic cancer therapy. *Carbon*. 2015;82:479-488.
- Cancer Facts & Figures 2007, (American Cancer Society, Atlanta, 2007).
- Marupudi NI, Han JE, Li KW, Renard VM, Tyler BM, Brem H. Paclitaxel: a review of adverse toxicities and novel delivery strategies. *Expert Opin Drug Saf*. 2007;6:609-621.
- Louage B, De Wever O, Hennink WE, De Geest BG. Developments and future clinical outlook of taxane nanomedicines. *J Control Release*. 2017;253:137-152.
- Kim SC, Kim DW, Shim YH, Bang JS, Oh HS, Wan Kim S, Seo MH. *In vivo* evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy. *J Control Release*. 2001;72:191-202.

7. Rivkin I, Cohen K, Koffler J, Melikhov D, Peer D, Margalit R. Paclitaxel-clusters coated with hyaluronan as selective tumor-targeted nanovectors. *Biomaterials*. 2010;31:7106-7114.
8. Weiss RB, Donehower RC, Wiernik PH, Ohnuma T, Gralla RJ, Trump DL, Baker JR Jr, Van Echo DA, Von Hoff DD, Leyland-Jones B. Hypersensitivity reactions from taxol. *J Clin Oncol*. 1990;8:1263-1268.
9. Liebmann J, Cook JA, Mitchell JB, Cremophor EL, solvent for paclitaxel, and toxicity. *Lancet*. 1993;342:1428.
10. Seow WY, Xue JM, Yang YY. Targeted and intracellular delivery of paclitaxel using multi-functional polymeric micelles. *Biomaterials*. 2007;28:1730-1740.
11. Liu Y, Sun J, Cao W, Yang J, Lian H, Li X, Sun Y, Wang Y, Wang S, He Z. Dual targeting folate-conjugated hyaluronic acid polymeric micelles for paclitaxel delivery. *Int J Pharm*. 2011;421:160-169.
12. Sudimack J, Lee RJ. Targeted drug delivery *via* the folate receptor. *Adv Drug Deliv Rev*. 2000;41:147-162.
13. de Wolf FA, Brett GM. Ligand-binding proteins: their potential for application in systems for controlled delivery and uptake of ligands. *Pharmacol Rev*. 2000;52:207-236.
14. Park EK, Kim SY, Lee SB, Lee YM. Folate-conjugated methoxy poly(ethylene glycol)/poly(epsilon-caprolactone) amphiphilic block copolymeric micelles for tumor-targeted drug delivery. *J Control Release*. 2005;109:158-168. Erratum in: *J Control Release*. 2006;112:145-146.
15. Erdoğar N, Esendağlı G, Nielsen TT, Şen M, Öner L, Bilensoy E. Design and optimization of novel paclitaxel-loaded folate-conjugated amphiphilic cyclodextrin nanoparticles. *Int J Pharm*. 2016;509:375-390.
16. Abdulrahman GO Jr, Rahman GA. Epidemiology of breast cancer in Europe and Africa. *J Cancer Epidemiol*. 2012;2012:915610.
17. Turk MJ, Breur GJ, Widmer WR, Paulos CM, Xu LC, Grote LA, Low PS. Folate-targeted imaging of activated macrophages in rats with adjuvant-induced arthritis. *Arthritis Rheum*. 2002;46:1947-1955.
18. Reddy JA, Low PS. Folate-mediated targeting of therapeutic and imaging agents to cancers. *Crit Rev Ther Drug Carrier Syst*. 1998;15:587-627.
19. Antony AC. Folate receptors. *Annu Rev Nutr*. 1996;16:501-521.
20. Wibowo AS, Singh M, Reeder KM, Carter JJ, Kovach AR, Meng W, Ratnam M, Zhang F, Dann CE 3rd. Structures of human folate receptors reveal biological trafficking states and diversity in folate and antifolate recognition. *Proc Natl Acad Sci USA*. 2013;110:15180-15188.
21. Lu Y, Low PS. Folate-mediated delivery of macromolecular anticancer therapeutic agents. *Adv Drug Deliv Rev*. 2002;54:675-693.
22. Stevens PJ, Sekido M, Lee RJ. A folate receptor-targeted lipid nanoparticle formulation for a lipophilic paclitaxel prodrug. *Pharm Res*. 2004;21:2153-2157.
23. Zhang G, Zhang M, He J, Ni P. Synthesis and characterization of a new multifunctional polymeric prodrug paclitaxel-polyphosphoester-folic acid for targeted drug delivery. *Polym Chem*. 2013;4515-4525.
24. Soppimath KS, Tan DW, Yang YY. pH-triggered thermally responsive polymer core-shell nanoparticles for drug delivery. *Adv Mater*. 2005;17:318-323.
25. Tong R, Yala L, Fan TM, Cheng J. The formulation of aptamer-coated paclitaxel-poly lactide nanoconjugates and their targeting to cancer cells. *Biomaterials*. 2010;31:3043-3053.
26. Hu Y, Xie J, Tong YW, Wang CH. Effect of PEG conformation and particle size on the cellular uptake efficiency of nanoparticles with the HepG2 cells. *J Control Release*. 2007;118:7-17.
27. Xie Z, Guan H, Chen X, Lu C, Chen L, Hu X, Shi Q, Jing X. A novel polymer-paclitaxel conjugate based on amphiphilic triblock copolymer. *J Control Release*. 2007;117:210-216.
28. Wang J, Liu W, Tu Q, Wang J, Song N, Zhang Y, Nie N, Wang J. Folate-decorated hybrid polymeric nanoparticles for chemically and physically combined paclitaxel loading and targeted delivery. *Biomacromolecules*. 2011;12:228-234.
29. Xiong J, Meng F, Wang C, Cheng R, Liu Z, Zhong Z. Folate-conjugated crosslinked biodegradable micelles for receptor-mediated delivery of paclitaxel. *J Mater Chem*. 2011;5786-5794.
30. Thu HP, Nam NH, Quang BT, Son HA, Toan NL, Quang DT. *In vitro* and *in vivo* targeting effect of folate decorated paclitaxel loaded PLA-TPGS nanoparticles. *Saudi Pharm J*. 2015;23:683-688.
31. He Z, Huang J, Xu Y, Zhang X, Teng Y, Huang C, Wu Y, Zhang X, Zhang H, Sun W. Co-delivery of cisplatin and paclitaxel by folic acid conjugated amphiphilic PEG-PLGA copolymer nanoparticles for the treatment of non-small lung cancer. *Oncotarget*. 2015;6:42150-42168.
32. Zhang W, Shi Y, Chen Y, Ye J, Sha X, Fang X. Multifunctional Pluronic P123/F127 mixed polymeric micelles loaded with paclitaxel for the treatment of multidrug resistant tumors. *Biomaterials*. 2011;32:2894-2906.
33. Rimac H, Debeljak Ž, Bojić M, Miller L. Displacement of drugs from human serum albumin: from molecular interactions to clinical significance. *Curr Med Chem*. 2017;24:1930-1947.
34. Shan L, Zhuo X, Zhang F, Dai Y, Zhu G, Yung BC, Fan W, Zhai K, Jacobson O, Kiesewetter DO, Ma Y, Gao G, Chen X. A paclitaxel prodrug with bifunctional folate and albumin binding moieties for both passive and active targeted cancer therapy. *Theranostics*. 2018;8:2018-2030.
35. Zhao D, Zhao X, Zu Y, Li J, Zhang Y, Jiang R, Zhang Z. Preparation, characterization, and *in vitro* targeted delivery of folate-decorated paclitaxel-loaded bovine serum albumin nanoparticles. *Int J Nanomed*. 2010;5:669-677.
36. Bano S, Afzal M, Waraich MM, Alamgir K, Nazir S. Paclitaxel-loaded magnetic nanocomposites with folate modified chitosan/carboxymethyl surface; a vehicle for imaging and targeted drug delivery. *Int J Pharm*. 2016;513:554-563.
37. Chen C, Hu H, Qiao M, Zhao X, Wang Y, Chen K, Chen D. Anti-tumor activity of paclitaxel through dual-targeting lipoprotein-mimicking nanocarrier. *J Drug Target*. 2015;23:311-322.
38. Rajangam K, Behanna HA, Hui MJ, Han X, Hulvat JF, Lomasney JW, Stupp SI. Heparin binding nanostructures to promote growth of blood vessels. *Nano Lett*. 2006;6:2086-2090.
39. Meister B, Kropshofer G, Klein-Franke A, Strasak AM, Hager J, Streif W. Comparison of low-molecular-weight heparin and antithrombin *versus* antithrombin alone for the prevention of symptomatic venous thromboembolism in children with acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2008;50:298-303.
40. Collen A, Smorenburg SM, Peters E, Lupu F, Koolwijk P, Van Noorden C, van Hinsbergh VW. Unfractionated and low molecular weight heparin affect fibrin structure and angiogenesis *in vitro*. *Cancer Res*. 2000;60:6196-6200.
41. Wang X, Li J, Wang Y, Cho KJ, Kim G, Gjyzezi A, Koenig L, Giannakakou P, Shin HJ, Tighiouart M, Nie S, Chen ZG, Shin DM. HFT-T, a targeting

- nanoparticle, enhances specific delivery of paclitaxel to folate receptor-positive tumors. *ACS Nano*. 2009;3:3165-3174.
42. Li L, Kim JK, Huh KM, Lee YK, Kim SY. Targeted delivery of paclitaxel using folate-conjugated heparin-poly (β -benzyl-L-aspartate) self-assembled nanoparticles. *Carbohydr Polym*. 2012;3:2120-2128.
 43. Wang X, Chen Y, Dahmani FZ, Yin L, Zhou J, Yao J. Amphiphilic carboxymethyl chitosan-querceetin conjugate with P-gp inhibitory properties for oral delivery of paclitaxel. *Biomaterials*. 2014;35:7654-7665.
 44. Li H, Liu J, Ding S, Zhang C, Shen W, You Q. Synthesis of novel pH-sensitive chitosan graft copolymers and micellar solubilization of paclitaxel. *Int J Biol Macromol*. 2009;44:249-256.
 45. Liang N, Sun S, Li X, Piao H, Piao H, Cui F, Fang L. α -Tocopherol succinate-modified chitosan as a micellar delivery system for paclitaxel: preparation, characterization and *in vitro/in vivo* evaluations. *Int J Pharm*. 2012;423:480-488.
 46. Yuan ZQ, Li JZ, Liu Y, Chen WL, Yang SD, Zhang CG, Zhu WJ, Zhou XF, Liu C, Zhang XN. Systemic delivery of micelles loading with paclitaxel using *N*-succinyl-palmitoyl-chitosan decorated with cRGDyK peptide to inhibit non-small-cell lung cancer. *Int J Pharm*. 2015;492:141-151.
 47. Cheng LC, Jiang Y, Xie Y, Qiu LL, Yang Q, Lu HY. Novel amphiphilic folic acid-cholesterol-chitosan micelles for paclitaxel delivery. *Oncotarget*. 2017;8:3315-3326.
 48. Babaei S, Girard-Lauriault PL. Tuning the surface properties of oxygen rich and nitrogen-rich plasma polymers: functional groups and surface charge. *Plasma Chem Plasma Process*. 2016;36:651-666.
 49. Ren W, Yan Y, Zeng L, Shi Z, Gong A, Schaaf P, Wang D, Zhao J, Zou B, Yu H, Chen G, Brown EM, Wu A. A near infrared light triggered hydrogenated black TiO₂ for cancer photothermal therapy. *Adv Health Mater*. 2015;4:1526-1536.
 50. Vinothini K, Rajendran NK, Ramu A, Elumalai N, Rajan M. Folate receptor targeted delivery of paclitaxel to breast cancer cells *via* folic acid conjugated graphene oxide grafted methyl acrylate nanocarrier. *Biomed Pharmacother*. 2019;110:906-917.
 51. Venkatasubbu GD, Ramasamy S, Avadhani GS, Ramakrishnan V, Kumar J. Surface modification and paclitaxel drug delivery of folic acid modified polyethylene glycol functionalized hydroxyapatite nanoparticles. *Powder Technol*. 2013;235:437-442.
 52. Yadav AK, Mishra P, Agrawal GP. An insight on hyaluronic acid in drug targeting and drug delivery. *J Drug Target*. 2008;16:91-107.
 53. Liu Y, Sun J, Lian H, Cao W, Wang Y, He Z. Folate and CD44 receptors dual-targeting hydrophobized hyaluronic acid paclitaxel-loaded polymeric micelles for overcoming multidrug resistance and improving tumor distribution. *J Pharm Sci*. 2014;103:1538-1547.
 54. Zhang J, Ma PX. Cyclodextrin-based supramolecular systems for drug delivery: recent progress and future perspective. *Adv Drug Deliv Rev*. 2013;65:1215-1233.
 55. Yin JJ, Zhou ZW, Zhou SF. Cyclodextrin-based targeting strategies for tumor treatment. *Drug Deliv Transl Res*. 2013;3:364-374.
 56. Thomas CE, Ehrhardt A, Kay MA. Progress and problems with the use of viral vectors for gene therapy. *Nat Rev Genet*. 2003;4:346-358.
 57. Li L, Gu W, Chen J, Chen W, Xu ZP. Co-delivery of siRNAs and anti-cancer drugs using layered double hydroxide nanoparticles. *Biomaterials*. 2014;35:3331-3339.
 58. Jones SK, Lizzio V, Merkel OM. Folate receptor targeted delivery of siRNA and paclitaxel to ovarian cancer cells *via* folate conjugated triblock copolymer to overcome TLR4 driven chemotherapy resistance. *Biomacromolecules*. 2016;17:76-87.