NANO-SIZED DRUG DELIVERY PLATFORMS FOR TAXANE ANTINEOPLASTIC AGENTS

D. Momekova, G. Momekov, N. Lambov

Faculty of Pharmacy, Medical University of Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria

Abstract. The prototype taxane antineoplastic agent paclitaxel, was first isolated from the bark of the Western yew tree in 1971. It and its congener, the semisynthetic derivative docetaxel, display unique pharmacodynamics as mitotic spindle poisons, differing from the vinca alkaloids and colchicine derivatives in that they bind to a distinct site on β-tubulin and promote rather than inhibit microtubule self-assembly. These drugs are essential for the contemporary chemotherapy of breast cancer and a variety of other solid malignancies such as ovarian, lung, esophageal, bladder, and head and neck cancers. Both drugs however suffer from unfavorable pharmaceutical peculiarities, necessitating their dissolution in surfactant containing vehicles prior to infusion, which in turn lead to hypersensitivity reactions. These problems have fuelled much research interest towards the design and development of nano-sized, targeted drug delivery platforms based on nanoparticles, liposomes and so on. These advanced nanopharmaceutical carriers for targeted delivery of taxanes are briefly outlined in the presented review.

Key words: paclitaxel, docetaxel, taxanes, targeted drug delivery, liposomes, nanoparticles, polymeric micelles, dendrimers, multi-arm stars

Introduction

The prototype taxane antineoplastic agent paclitaxel is a diterpenoid alkaloid with complex structure, comprising an 8-membered taxane ring as its core scaffold [1]. The side chain tethered to C13 position of the taxane ring has been well appreciated as critical for its antineoplastic activity [2, 3]. Eventual alteration of the side chain has led to identification of the more potent analog, docetaxel, which generally shares the spectrum of clinical antineoplastic activity of paclitaxel, but has distinct toxicity profile [1]. While originally purified as the parent molecule from the bark of the Pacific yew (*Taxus brevifolia*), paclitaxel now can be feasibly obtained for commercial purposes by a straightforward semisynthetic route starting from 10-desacetylbaccatin, a precursor found in the leaves from the cosmopolite species *Taxus baccata* [1, 3].

Taxanes are mitotic spindle poisons with unique mode of antineoplastic activity. They bind explicitly to the β-tubulin subunit of microtubules and hamper the disassembly of the latter. This ultimately leads to mitotic arrest and induction of programmed cell death through apoptosis [1-3].

Albeit the clinical success of paclitaxel has attracted much attention this drug has very limited aqueous solubility and its conventional dosage form (Taxol®) must be administered in a vehicle of 50% ethanol and 50% Cremophore EL™ (polyethoxylated castor oil, a non-ionogenic surfactant), a formulation likely accountable for the high rate of hypersensitivity reactions, associated with paclitaxel use and necessitating mandatory premedication with corticosteroids and antihistamines [1-3].

The other taxane derivative in widespread clinical use docetaxel (Taxotere®), which is rather more soluble, is administered using an alternative vehicle based on polysorbate 80, the latter associated with quite lower incidence of hypersensitivity reactions [1]. In order to alleviate the toxicity of the formulation components and to improve the pharmacokinetics of the parent compounds a variety of new taxanes have been developed and subset to clinical evaluation [3].

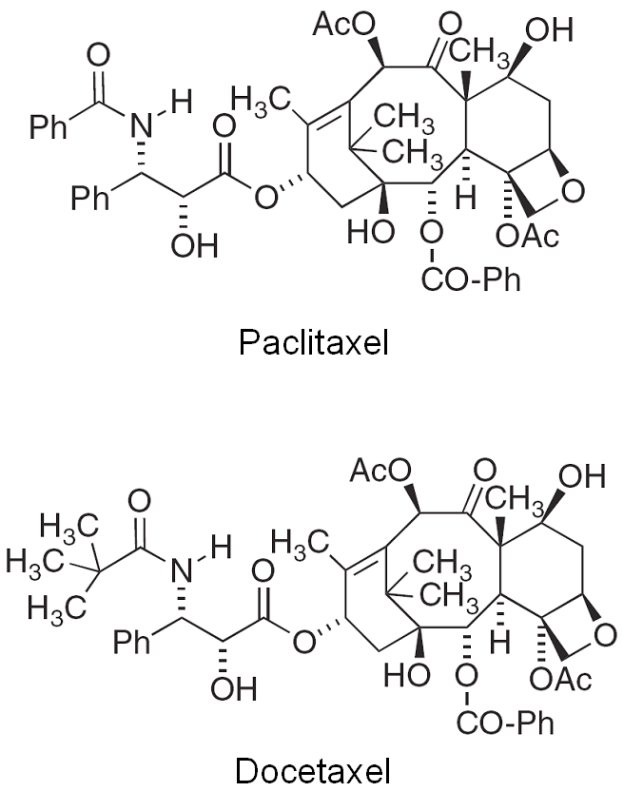

Fig. 1. Chemical structure of the taxane antineoplastic agents

Paclitaxel is administered as an intravenous infusion. It drug undergoes extensive hepatic biotransformation (principally *via* CYP2C8 with a contribution of CYP3A4), and less than 10% of a dose is excreted renally in unchanged form. The principle metabolite is 6-OH paclitaxel, which is pharmacologically inactive, but numerous additional hydroxylation products have been identified in plasma as well [1]. The clearance of the drug is nonlinear and has been found to decrease with escalating dose intensity or infusion rate, which presumably is related to its dissolution in the commercially available non-aqueous vehicle [1, 2].

The pharmacokinetic behavior of docetaxel appears to be analogous to those of paclitaxel. Its elimination half-life is roughly 12 hours. The drug is cleared hepatically principally *via* CYP3A4- and CYP3A5-mediated hydroxylation to pharmacologically inert biotransformation products. Contrary to paclitaxel, the pharmacokinetics or docetaxel appears to be linear up to doses of 115 mg/m^2 [1].

The taxane anticancer agents have become essential components of chemotherapeutic regimens for the management of solid tumors such as metastatic ovarian, breast, lung, and head and neck carcinomas [1-3]. Moreover, docetaxel has noteworthy activity in combinations with estramustine for the chemotherapy of hormone-refractory prostate cancer [1]. In current regimens, either drug is administered once weekly or once every 3 weeks, with comparable response rates and somewhat different patterns of toxicity. Docetaxel produces greater leukopenia and peripheral edema, while paclitaxel causes a higher incidence of hypersensitivity, muscle aching, and neuropathy (particularly when used in combination with a platinum analog) [1, 2, 4].

The hallmark toxicity of paclitaxel is myelosuppression. Neutropenia arises usually within 10 days after dosing and thereafter promptly reverses by days 15 to 21. If the drug is used in combination G-CSF, high dose regimens of up to 250 mg/m^2 over 24 hours are well tolerated, and peripheral neuropathy become dose limiting. Many individuals develop myalgias for several days after paclitaxel application. In high-dose regimens, or with protracted use, sensory neuropathy can be troublesome, particularly in patients with coinciding neuropathy or concurrent treatment with neurotoxic drugs, e.g. platinum compounds. Hypersensitivity reactions are frequent event in patients treated with short duration paclitaxel infusions (up to 6 hours), but are effectively avoided by antihistamine and corticosteroids premedication. The latter obligatory, however with 96-hour infusions [1].

Docetaxel appears to cause more disabling, but short-lived, neutropenia as compared to paclitaxel. It is associated with milder peripheral neuropathy and asthenia, and due to the lack of Cremophore EL™ in the vehicle is less prone to induce hypersensitivity reactions. Fluid retention is a cumulative toxicity, arising with multiple cycles of chemotherapy. It progresses to peripheral edema, pleural and peritoneal fluid retention, and in extreme occasions to pulmonary edema. Oral dexamethasone, 8 mg/day, commenced 1 day before docetaxel infusion and continued over 3 days, has been well documented to alleviate fluid retention reactions. In rare occasions cases, docetaxel may cause a dramatic progressive interstitial pneumonitis, necessitating treatment cessation [1].

While taxanes are unambiguously essential components of the state-of-the-art antineoplastic drug armamentarium the aforementioned side effects and unfavorable pharmaceutical peculiarities are an important impediment hindering the realization of their complete therapeutic potential. These issues have

fuelled much research interest towards the design and development of nano-sized, targeted drug delivery platforms based on albumin-nanoparticles, liposomes, polymeric micelles, dendrimers, and so on. These advanced nanopharmaceutical carriers for targeted delivery of taxanes are briefly outlined in the presented review.

I. Albumin-bound-nanoparticulate paclitaxel (Abraxane™)

Tumors have adapted multiple mechanisms to address their increased demand for nutrients. One of these is the gp60 dependent pathway, by which nutrients are preferentially transported across the endothelial barrier when attached to albumin [5-9]. Tumor cells also secrete a specialized protein called SPARC (Secreted Protein Acidic and Rich in Cysteine) into the tumor's interstitium [10, 11]. The SPARC protein acts as a highly charged receptor to distinctively attract and bind albumin and albumin-bound nutrients to eventually capture and concentrate them within the tumor's interstitium [7, 11]. On the basis of these mechanisms, nanoparticle albumin-bound technology has been developed to attain selective tumor targeting of albumin-immobilized antineoplastic agents [12]. An exemplary nanoparticulate drug delivery system has been developed on the basis of albumin-bound nanoparticulate (d=130 nm) paclitaxel (Abraxane™), which has been commercialized for the treatment of metastatic breast cancer. By virtue of the excellent biocompatibility of albumin and the eliminiation of the solubilizing agents Abraxane™ is characterized by a superior safety profile, as compared to the conventional formulation of paclitaxel while preserving the clinical efficacy of the drug [3, 13-19]. Abraxane™ has been demonstrated to be superior to an equitoxic dose of conventional paclitaxel with a significantly lower incidence of toxicities in a large, international, randomized phase III trial [13].

II. Plain, coated and targeted liposomal carriers for taxanes

Liposomes are spherical vesicles comprising either a single phospholipid bilayer or alternating tightly packed aqueous compartment and lipid bilayers, which enclose a central aqueous reservoir. Liposomes are characterized by excellent biocompatibility, biodegradability, non-immunogenicity and generally low toxicological and safety pharmacological potential [20-23]. Due to the abundance of lipid and aqueous compartments liposomes are capable of accommodating both polar and non-polar compounds based on their solubility and partitioning characteristics [24, 25]. Lipophilic agents such as taxanes are typically encapsulated within the lipid bilayer of liposomal membranes. Over the last three decades the liposomes platforms have evolved from plain or conventional liposomes to long circulating "stealth" liposomes and ultimately to targeted and stimuli-responsive liposomes [26]. Moreover, the possibilities for complex surface modification, conjugation of permeation enhancing or homing moieties, incorporation of pH-responsive components etc. condition the possibilities for designing sophisticated drug delivery platforms with hybrid tumor- targeted and triggerable release properties [27].

Exemplary conventional liposomal formulations of paclitaxel agents that are currently in clinical development with the respective indications are summarized in **Table 1**.

Long circulating (Stealth™) liposome strategy is based on the process of steric stabilization, i.e. creating a repulsive polar coating around vesicles by grafting liposomal membranes with lipid-anchored hydrophilic polymers, most often poly-ethylene glycol [32]. As a proof of the principle pegylated liposomes are characterized by significantly increased plasma half-lives [33]. The feasibility of the stealth liposome concept for attaining optimized tumor-site bioavailability and its comprehensive experimental and clinical justification have fuelled immense and enduring efforts for elaboration of pegylated-liposomal platforms for taxanes [34-36].

The Stealth™ liposome could be further advanced to site-specific targeted liposomes, which are decorated with different types of homing moieties to in-

Table 1. Conventional liposomal formulations of anticancer drugs in clinical trials.

Product Name	Lipid composition	Indications
LEP-ETU	Plain liposomes; DOPC, CHOL, cardiolipin (90:5:5)	Ovarian, breast and lung cancers [28]
EndoTAG-1	Plain cationic liposomes DOTAP, DOPC and paclitaxel (50:47:3)	Anti-angiogenic properties, breast cancer (Phase II) [29-31]

crease the rate of liposomal drug accumulation in the ultimately targeted tissues/cells *via* interactions with cancer cell receptors/antigens [21, 37, 38] or tumor vasculature-specific epitopes [35, 39-42]

Targeted stealth liposomes most are most often based on pendant-type folate (FA) ligands conjugated to the distal ends of PEG-chains. This functionalization allows both significant tumor accumulation, due to the overexpression of folate receptors (FR) in tumor cells and increased cellular uptake *via* FR-mediated endocytosis. In an exemplary report Zhai *et al.* have presented a FA-targeted stealth liposomal formulation of docetaxel. The lipid composition comprised egg phosphatidylcholine, cholesterol, DSPE-PEG_{2000}, folate-PEG_{3350}-cholesteryl hemisuccinate (folate-PEG-CHEMS) at molar ratios of 80:15:4.5:0.5. The lipophilic Docetaxel was incorporated into the lipid bilayer at a drug-to-lipid ratio of 1:20 (wt/wt). FR-targeted liposomes of the same lipid composition loaded with the fluorescent probe calcein exhibited prominent uptake by FR(+)oral squamous cell carcinoma derived cell line KB. The cytotoxicity assessment in the same cell line showed that the targeted docetaxel-loaded liposomes exerted 4.4-fold greater cytotoxicity relative to non-targeted liposomes. A comparative pharmacokinetic study revealed that both liposomal formulations showed significantly prolonged half-lives (4.92 h for FR-targeted and 6.75 h for non-targeted) than the commercially available docetaxel formulation (Tween 80/ethanol solution) (1.09 h) [43].

Another targeted platform for docetaxel has been presented by Yuan et al. based on cholesterol-PEG-folate as surface modifying and targeting moiety. These docetaxel-loaded, folate-conjugated PEG-liposomes showed superior cytotoxicity against MCF-7 breast cancer derived cells as compared to an analogous non-targeted formulation. The pharmacokinetics of the docetaxel loaded folate-conjugated PEG-liposomes in rats showed a prominent, near 7-fold prolongation of $T_{1/2}$, relative to the free drug. Moreover, the targeted formulation significantly lowered the docetaxel exposure in the heart, brain and kidneys, which is a prerequisite for amelioration of the side effects of the taxane drug [44].

Another docetaxel delivery system was developed using folate-poly(PEG-cyanoacrylate-co-cholesteryl cyanoacrylate) (FA-PEG-PCHL) as a novel hybrid steric stabilizing and targeting moiety. The docetaxel-loaded liposomes modified by FA-PEG-PCHL were prepared by an organic solvent injection method and lyophilized to obtain freeze-dried FA-PEG-PCHL-docetaxel liposomes (FA-PDCT-L). Compared with docetaxel solution and docetaxel-loaded non-targeted liposomes, FA-PDCT-L demonstrated the strongest cytotoxicity against MCF-7 and A-549 cancer cell lines, the greatest intracellular uptake especially in the nucleus, as well as the most powerful apoptogenic efficacy. An *in vivo* pharmacokinetic study showed that the AUC of FA-PDCT-L was increased 3.8 and 6.2 times relative to docetaxel-loaded non-targeted liposomes and free docetaxel, respectively. This was concomitant with lowest concentration of FA-PDCT-L in the MPS organs liver and spleen, and a significantly higher intratumoral levels indicative for excellent targeting capacity of the presented nanopharmaceutical [45].

An extensively explored approach for targeting solid tumors is based on liposome modification with RGD-peptide (arginine-glycine-aspartic acid) or similar ligands selectively binding the $\alpha_v\beta_3$-integrin receptors overexpressed in some tumor cells and in tumor vasculature, or with similar angiotropic homing moieties [35, 39, 42, 46, 47]. Zhao *et al*. evaluated a RGD-modified sterically stabilized liposomal formulation of paclitaxel (RGD-SSL-PTX) as a tumor targeted platform. *In vitro* bioassays using SKOV-3 ovarian cancer cell lines showed that RGD-SSL-PTX exhibited significant cytotoxicity (3.5 times lower IC_{50}) and *ca*. 6 fold higher intracellular accumulation *vs*. a non-targeted stealth formulation of the drug. RGD-SSL-PTX showed also superior tumor growth inhibition in BALB/c nude mice xenografted with SKOV-3 solid tumor, concomitant with no significant alterations of the body weight of the animals [39].

In another recent report RGD grafted docetaxel loaded liposomes (RGD-PEG-LP-DC) were prepared and subjected to thorough pharmacological and pharmacokinetic studies as drug delivery platform in breast cancer. The RGD-PEG-LP-DCs exhibited significant cytotoxicity against BT-20 and MDA-MB-231 cell lines. As evidenced by a flow-cytometric study the 48 h treatment with RGD-PEG-LP-DC induced almost complete G_2 phase arrest of the cycling population at low nanomolar concentration. Interestingly the cell death mechanism proved to be greatly dependent on exposure conditions – necrosis was the predominant at low concentration (1 nM docetaxel), whereas with increasing incubation time and concentration the relative contribution of apoptotic cell death was amplified. Biodistribution studies further demonstrated the targeting potential of RGD-PEG-LP-DC which displayed improvement in site specific drug distribution, $T_{1/2}$, and mean residence time (MRT) [35].

Protein transduction domains (PTD) are protein

domains with potent ability to mediate traversing cellular membranes. HIV-derived TAT-peptides are cell penetrating PTDs which have been widely exploited in the drug delivery field for their capacity to impart cell penetrating properties when attached to the steric stabilizing-coating of liposomes [48]. Thus in a recent study Zhao *et al*. have presented a sophisticated dually targeted system based on folic acid and TAT peptide conjugated on the octadecyl-quaternized, lysine-modified chitosan-cholesterol polymeric liposomes (FA-TATp-PLs) and studied its feasibility as drug delivery platform for paclitaxel. Confocal laser scanning microscopy in folate receptor (FR)-positive KB nasopharyngeal epidermal carcinoma cells and FR-deficient A549 lung cancer cells showed that FA-TATp-PLs had a significantly high efficient intracellular uptake in both KB and A549 cells, superior to either non-targeted liposomes or mono-TAT and FR-targeted ones. Moreover, paclitaxel-loaded FA-TATp-PLs exhibited superior cytotoxicity *in vitro* and tumor-inhibiting properties in vivo as compared to the commercially available preparation of the drug (Taxol®). These data revealed the synergistic targeting effects of combined folate / TAT peptide decoration [49].

III. Polymeric micelles as drug carriers for taxanes

Polymeric micelles comprise biodegradable spherical or nearly spherical nano-carriers with a usual size range of 10–200 nm. They are formed by the spontaneous self-assembly of block copolymers, consisting of two or more polymer domains with dissimilar polarity. As with surfactant based systems such copolymers spontaneously self-assemble yielding core-shell structures in an aqueous dispersion to minimize the free energy of the disperse system. The hydrophobic segments form the hydrophobic inner core, whereas, the polar domains (e.g. PEG) build up the outer hydrophilic corona-like shell, which by providing solvated hydrophilic repulsive barrier around the micelle imparts steric stabilization in analogy to the stealth liposomes. Drug loading could be *via* co-valent linking to the micelles [50, 51] or by intermolecular interactions with the carrier [52].

Based on their morphological, physicochemical and biopharmaceutical characteristics micelles are regarded as advantageous drug carriers and are subject to intensive research in the field of anticancer drug delivery [50, 53-56], as extensively reviewed by Deng *et al*. [52]. Their hydrophobic domains could be loaded with lipophilic drugs which are entrapped in the inner compartments of the particle with high loading efficiency. This is especially advantageous because the diphillic nature of micelles conditions their efficiency as solubilizing agents, eliminating the necessity of co-solvent or surfactants as formulation aids in case of poorly soluble anticancer drugs, such as the taxanes [57]. This is of special interest having in consideration the toxicological risks these excipients pose e.g. the well established propensity of Cremophor EL (non-ionic surfactant used as an excipient in the commercially available paclitaxel formulation Taxol®) to induce severe hypersensitivity reactions necessitating aggressive pretreatment regimens with corticosteroids and antihistamines [1]. The elimination of adjuvants when the drug is physically loaded in micellar formulations is expected to conversely minimize the associated risks for local irritation or systemic anaphylactic reactions. Noteworthy, the polar shell domain not only imparts a steric hindrance barrier, leading to increase plasma stability and circulation half-times, but also provides physical space and functional groups suitable for drug loading or specific micelle decoration with targeting ligands [58, 59].

Apart from physical loading drugs could be also covalently immobilized to the micelle forming macromolecular prodrugs [50, 51]. An intriguing new concept is based on constructing amphiphilic polymeric micelles whereby the hydrophobic portion of the polymeric micelles is replaced by highly lipophilic drugs themselves, forming a new micellar drug delivery system. By grafting hydrophobic drugs of paclitaxel onto the surface of hydrophilic hyperbranched poly(ether-ester) (HPEE), we constructed an amphiphilic copolymer (HPEE-paclitaxel). HPEE-paclitaxel could self-assemble into micellar nanoparticles in aqueous solution with tunable drug contents from 4.1 to 10.7%. Moreover, the hydrolysis of HPEE- paclitaxel in serum resulted in the cumulative release of paclitaxel. In vivo evaluation indicated that the dosage toleration of paclitaxel in mice had been improved greatly and HPEE-paclitaxel micellar nanoparticles could be used as an efficient prodrug with satisfactory therapeutic effect [60] .

Additional advantage of polymeric micelles is their ability for concurrent co-delivery of two or more therapeutic agents, e.g. combination of anticancer drugs, or of cytotoxic agent plus MDR-modulator *etc*. For instance efficient micellar co-delivery has been described for paclitaxel in synergistic combination with therapeutic genes [61], VEGF-gene silencing siRNA [62], TRAIL (TNF-related apoptosis inducing ligand) [63], cyclosporin (as a MDR-reversal agent) [55]; a more sophisticated system for simultaneous

co-delivery of paclitaxel, 7-allylamino-17-demethoxygeldanamycin (17-AAG), and rapamycin has been reported as well [64].

Besides the stabilization of micelles different elaborate strategies for improved targeting and effective cytosolic delivery (e.g. surface decoration and stimuli-responsiveness) have been employed [52, 58, 59, 65]. These are based on essentially the same principles already described for liposomal carriers and hence will be subject to only topical description in the light of recent findings.

Folate has been widely employed as a targeting motif for effective tumor accumulation and intracellular delivery of micelle-loaded paclitaxel [57, 66-74]. Other recently exploited targeting motifs for efficient targeting and cellular uptake of micelles loaded with cytotoxic agents include transferrin [75], somatostatin and analogues [76-78], RGD-motifs and other tumor vasculature seeking homing ligands [79-82], cancer-cell specific phage proteins [83], galactose for efficient targeting to hepatoma cells [84], aptamers targeting the prostate specific membrane antigene (PSMA) [85-87], among others.

A vastly explored strategy for controlled destabilization and release of anticancer drugs from micelles takes advantage of the lower pH inside tumors and in endosomal compartments [52, 65, 88]. In order to impart pH-responsiveness to micelle-based nanopharmaceuticals, the drug is usually chemically immobilized to the polymer core or alternatively to the shell domains of the micelle via an acid cleavable bonding such as hydrazone linkage [51]. These polymer-drug conjugates are referred to as a polymeric prodrug and allow the cytotoxic agent to remain pharmacologically and toxicologically inert until cleavage from the polymer carrier [51]. An exemplary recent exemplary pH-responsive system was developed on the basis of 2-(omega-methoxy)PEGyl-1,3-dioxan-5-ylamine onto poly(N-(acryloyloxy)succinimide-co-butyl methacrylate) graft co-polymer (mPEG-g-p(NAS-co-BMA)). Pseudo in situ cross-linking of the mPEG-g-p(NAS-co-BMA) was carried out by an acid-labile diamine cross-linker bearing two symmetrical cyclic orthoesters. The hydrolysis rate of the prepared CL micelles proved to be much more rapid at mildly acidic than physiological conditions. PTX was successfully loaded into the CL micelles and a controlled and pH-dependent release behavior was observed. The non-loaded micelles themselves proved to be non-toxic and biocompatible [89]. Another pH-responsive system is based on polymers exhibiting pH-dependent shift in swelling-shrinking behavior leading to slow release of PTX, without prompt disintegration of the carrier [90].

Thermoresponsive micelle-based drug delivery systems have been presented in recent reports as efficient triggerable carriers for paclitaxel [91, 92]. Reversibly SS-crosslinked micelles whose destabilization and drug release could be triggered by pharmacological intervention, i.e. concurrent administration of the GSH precursor/ mimic N-acetylcysteine have been developed and characterized as feasible drug carrier for an on-demand delivery of paclitaxel [93].

IV. Dendrimers and multiarm-star-like polymers as carriers for taxanes

Dendrimers comprise a class of globular, highly branched, synthetic macromolecules with tunable size and architecture [94-96]. They comprise multiple layers with large number of chemically active surface groups, also known as generations, which emanate out of an initiator core, denoted as generation zero (G0). Typically the size of dendrimer particles ranges 1–15 nm and these are characterized by significant homogeneity in terms of size distribution and morphology [95]. Dendrimers have many attractive properties which make them advantageous drug carriers as compared to both linear and hyperbranched polymer-based systems [95, 97, 98]. The unique highly regular branching architecture and the multiple arms of dendrimers provide a large multivalent backbone whereby anticancer drugs, targeting moieties or solubilizing groups can be feasibly attached through covalent conjugation or electrostatic
adsorption [95, 99].

Kesharwani evaluated the comparative efficacy and feasibility of folate-, dextran- and galactose as targeting ligands for polypropylenimine (PPI) dendrimers loaded with paclitaxel. Based on MTT and flow cytometric *in vitro* bioassays in HeLa and SiHa cells the authors found that the targeting potential among the tested homing moieties decreases in the following order: folate, dextran and galactose, whereby the folate loaded paclitaxel was invariably far more active as compared to the free drug [100].

Luo *et al.* have developed a well-defined and biocompatible amphiphilic telodendrimer system (PEG-b-dendritic oligo-cholic acid) which self-assembles into multifunctional micelles in aqueous solution for efficient delivery of hydrophobic drugs e.g. paclitaxel. A series of telodendrimers with variable length of PEG chain and number of cholic acid in the dendritic blocks were synthesized. The sizes of the micelles, with and without paclitaxel loading,

could be tuned from 11.5 to 21 nm and from 15 to 141 nm, respectively. In vivo studies in xenograft models demonstrated preferential tumor uptake of the smaller paclitaxel-loaded micelles (17-60 nm) whereas the larger dendrimer-based micelles (150 nm) were distributed to the liver and lungs. The in vivo toxicity profile and antitumor efficacy of the paclitaxel-loaded micelles proved to be superior to those of the marketed paclitaxel formulation Taxol® [101].

The same group further advanced the aforementioned system to reversible disulfide cross-linked micelles (DCMs) that can be triggered to release drug at the tumor site or inside cancer cells. The telodendrimer based DCMs were globular with a uniform size of 28 nm, and were characterized by superior paclitaxel loading capacity of up to 35.5% (w/w, drug/micelle). Cross-linking of the micelles within the core reduced their apparent critical micelle concentration and greatly enhanced their colloidal and biopharmaceutical stability in non-reductive physiological conditions. The release of PTX from the DCMs was significantly slower than that from non-cross-linked micelles (NCMs), but proved to be gradually facilitated by increasing the concentration of reduced glutathione (GSH) to concentrations relevant to its actual intracellular levels. The empty DCMs demonstrated superior pharmacokinetic and safety profiles in nude mice, when compared to NCMs. DCMs preferentially accumulated at the tumor site in nude mice bearing SKOV-3 ovarian cancer xenograft. Moreover, the disulfide cross-linked micellar formulation of paclitaxel (paclitaxel-DCMs) was more efficacious than both free drug and the non-cross-linked formulation of paclitaxel at equivalent doses of the free drug in the xenograft model. The antineoplastic effect of paclitaxel-DCMs could be further enhanced by triggering the release of paclitaxel on-demand by the administration of the clinically used reducing agent N-acetylcysteine, after paclitaxel-DCMs have attained the tumor site [93].

A series of pegylated polyglycerol dendritic polymers proved to significantly increase the solubility of paclitaxel in water (up to 10,000 fold) and thereafter the drug release rate from the dendrimers was a function of the dendrimer generation [102].

The core–shell type star polymers bearing hyperbranched cores and multi-arm shell of linear polymers bearing active end functionalities are an attractive class of drug delivery systems [103, 104]. These new macromolecules display an "unimolecular micelle" behavior in aqueous medium, whereby the covalently linked interior and shell domains remain stable independently of concentration, abundance of interactive solutes and temperature [67, 105-109]. The structural stability and multifunctionality of the stars conditions the significant scope for their elaboration as drug delivery systems, since they provide opportunities for either chemical/ electrostatic immobilization, or physical encapsulation of anticancer drugs [67, 109].

An elaborate hybrid therapeutic/diagnostic system for folate targeted co-delivery of paclitaxel and a Gd-based NMR-contrast agent has been presented by Li et al. The system was based on a fractionated fourth-generation hyperbranched polyester (Boltorn H40™) core, with a hydrophobic poly-varepsilon-caprolactone (PCL) inner layer, and a polar co-polymer corona based on oligo(ethylene glycol) monomethyl ether methacrylate (OEGMA) and 3-azidopropyl methacrylate (AzPMA). The prepared H40-PCL-b-P(OEGMA-co-AzPMA stars were terminally decorated by click reaction with alkynyl-functionalized cancer cell-targeting moieties, alkynyl-folate, and T1-type MRI contrast agent alkynyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakisacetic acid-Gd, affording H40-PCL-b-P(OEGMA-Gd-FA). In aqueous solution, the amphiphilic multiarm star block copolymer proved to behave as well defined, structurally stable unimolecular micelles. The H40-PCL-b-P(OEGMA-Gd-FA) stars were loaded with PTX (6.67 w/w% loading content) and exhibited sustained release of up to 80% of the loaded payload over ca. 120 h. Moreover, in vivo MR imaging experiments in rats revealed good accumulation of unimolecular micelles within rat liver and kidney, prominent positive contrast enhancement, and relatively long duration of blood circulation [67].

Conclusion and prospectus

Breast carcinoma represents the most common type of neoplastic disease diagnosed in women. Noteworthy, in the metastatic setting this malignancy is still incurable. The clinically applied taxanes represent an essential class of antineoplastic agents, which have proven to be central for the treatment of advanced and early-stage breast cancer. Nevertheless the clinical success of taxanes has been limited by their unfavorable pharmaceutical properties and most importantly by their prominent hydrophobicicity. To surmount this poor water solubility, lipid- and polymer based solvents have been used as a vehicle, and new systemic formulations have been developed, mostly for paclitaxel, which are Cremophor™-free and increase the circulation time of the drug with a concomitant avoidance of the surfactant-associated

safety issues. The development and commercialization of new nanosized formulations with beneficial safety profiles would allow clinicians to fully take advantage of the therapeutic potential of taxanes.

References

1. Chabner BA, Amrein PC, Druker BJ, Michaelson D, Mitsiades CS, Goss PE, et al. Chemotherapy of neoplastic disease. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman & Gilman's The pharmacological basis of therapeutics. 11 ed. New York: McGraw Hill; 2006. p. 1315 - 403.
2. Anonymous. Antineoplastics. In: Swettman SC, editor. Martindale: The Complete Drug Reference, 36th Ed. London: Pharmaceutical Press; 2009. p. 635-790.
3. Avendaño C, Menéndez JC. Medicinal Chemistry of Anticancer Drugs. Amsterdam: Elsevier; 2008.
4. Grever MR, Grieshaber CK. Toxicology by organ system. In: Bast Jr RC, Kufe DW, Pollock RE, Weichselbaum RR, Holland JF, Frei III E, editors. Cancer Medicine 5th Edition. Hamilton: B.C. Decker Inc.; 2000. p. 602-11.
5. John TA, Vogel SM, Minshall RD, Ridge K, Tiruppathi C, Malik AB. Evidence for the role of alveolar epithelial gp60 in active transalveolar albumin transport in the rat lung. J Physiol. 2001;533(Pt 2):547-59.
6. Tiruppathi C, Song W, Bergenfeldt M, Sass P, Malik AB. Gp60 activation mediates albumin transcytosis in endothelial cells by tyrosine kinase-dependent pathway. J Biol Chem. 1997;272(41):25968-75.
7. Schnitzer JE, Oh P. Antibodies to SPARC inhibit albumin binding to SPARC, gp60, and microvascular endothelium. Am J Physiol. 1992;263(6 Pt 2):H1872-9.
8. Schnitzer JE. gp60 is an albumin-binding glycoprotein expressed by continuous endothelium involved in albumin transcytosis. Am J Physiol. 1992;262(1 Pt 2):H246-54.
9. Siflinger-Birnboim A, Schnitzer J, Lum H, Blumenstock FA, Shen CP, Del Vecchio PJ, et al. Lectin binding to gp60 decreases specific albumin binding and transport in pulmonary artery endothelial monolayers. J Cell Physiol. 1991;149(3):575-84.
10. Hsiao YH, Lien HC, Hwa HL, Kuo WH, Chang KJ, Hsieh FJ. SPARC (osteonectin) in breast tumors of different histologic types and its role in the outcome of invasive ductal carcinoma. Breast J. 2010;16(3):305-8.
11. Tai IT, Dai M, Owen DA, Chen LB. Genome-wide expression analysis of therapy-resistant tumors reveals SPARC as a novel target for cancer therapy. J Clin Invest. 2005;115(6):1492-502.
12. Lluch A, Alvarez I, Munoz M, Segui MA, Tusquets I, Garcia-Estevez L. Treatment innovations for metastatic breast cancer: nanoparticle albumin-bound (NAB) technology targeted to tumors. Crit Rev Oncol Hematol. 2014;89(1):62-72.
13. Miele E, Spinelli GP, Tomao F, Tomao S. Albumin-bound formulation of paclitaxel (Abraxane ABI-007) in the treatment of breast cancer. Int J Nanomedicine. 2009;4:99-105.
14. Roy V, LaPlant BR, Gross GG, Bane CL, Palmieri FM. Phase II trial of weekly nab (nanoparticle albumin-bound)-paclitaxel (nab-paclitaxel) (Abraxane) in combination with gemcitabine in patients with metastatic breast cancer (N0531). Ann Oncol. 2009;20(3):449-53.
15. Gardner ER, Dahut W, Figg WD. Quantitative determination of total and unbound paclitaxel in human plasma following Abraxane treatment. J Chromatogr B Analyt Technol Biomed Life Sci. 2008;862(1-2):213-8.
16. Lobo C, Lopes G, Silva O, Gluck S. Paclitaxel albumin-bound particles (abraxane) in combination with bevacizumab with or without gemcitabine: early experience at the University of Miami/Braman Family Breast Cancer Institute. Biomed Pharmacother. 2007;61(9):531-3.
17. Altundag K, Dede DS, Purnak T. Albumin-bound paclitaxel (ABI-007; Abraxane) in the management of basal-like breast carcinoma. J Clin Pathol. 2007;60(8):958.
18. Stinchcombe TE, Socinski MA, Walko CM, O'Neil BH, Collichio FA, Ivanova A, et al. Phase I and pharmacokinetic trial of carboplatin

and albumin-bound paclitaxel, ABI-007 (Abraxane) on three treatment schedules in patients with solid tumors. Cancer Chemother Pharmacol. 2007;60(5):759-66.

19. Green MR, Manikhas GM, Orlov S, Afanasyev B, Makhson AM, Bhar P, et al. Abraxane, a novel Cremophor-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer. Ann Oncol. 2006;17(8):1263-8.
20. Al-Jamal WT, Kostarelos K. Liposomes: from a clinically established drug delivery system to a nanoparticle platform for theranostic nanomedicine. Acc Chem Res. 2011;44(10):1094-104.
21. Andresen TL, Jensen SS, Jorgensen K. Advanced strategies in liposomal cancer therapy: problems and prospects of active and tumor specific drug release. Prog Lipid Res. 2005;44(1):68-97.
22. Elizondo E, Moreno E, Cabrera I, Cordoba A, Sala S, Veciana J, et al. Liposomes and other vesicular systems: structural characteristics, methods of preparation, and use in nanomedicine. Prog Mol Biol Transl Sci. 2011;104:1-52.
23. Jolck RI, Feldborg LN, Andersen S, Moghimi SM, Andresen TL. Engineering liposomes and nanoparticles for biological targeting. Adv Biochem Eng Biotechnol. 2011;125:251-80.
24. Lasic DD, Vallner JJ, Working PK. Sterically stabilized liposomes in cancer therapy and gene delivery. Curr Opin Mol Ther. 1999;1(2):177-85.
25. New RRC. Introduction. In: New RRC, editor. Liposomes: a practical approach. Oxford: Oxford University Press; 1994. p. 1-32.
26. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov. 2005;4(2):145-60.
27. Bibi S, Lattmann E, Mohammed AR, Perrie Y. Trigger release liposome systems: local and remote controlled delivery? J Microencapsul. 2012;29(3):262-76.
28. Chang H-I, Yeh M-K. Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy. Int J Nanomedicine. 2012;7:49–60.
29. Bode C, Trojan L, Weiss C, Kraenzlin B, Michaelis U, Teifel M, et al. Paclitaxel encapsulated in cationic liposomes: a new option for neovascular targeting for the treatment of prostate cancer. Oncol Rep. 2009;22(2):321-6.
30. Strieth S, Eichhorn ME, Werner A, Sauer B, Teifel M, Michaelis U, et al. Paclitaxel encapsulated in cationic liposomes increases tumor microvessel leakiness and improves therapeutic efficacy in combination with Cisplatin. Clin Cancer Res. 2008;14(14):4603-11.
31. Strieth S, Nussbaum CF, Eichhorn ME, Fuhrmann M, Teifel M, Michaelis U, et al. Tumor-selective vessel occlusions by platelets after vascular targeting chemotherapy using paclitaxel encapsulated in cationic liposomes. Int J Cancer. 2008;122(2):452-60.
32. Moghimi SM, Szebeni J. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. Prog Lipid Res. 2003;42(6):463-78.
33. Zeisig R, Shimada K, Hirota S, Arndt D. Effect of sterical stabilization on macrophage uptake in vitro and on thickness of the fixed aqueous layer of liposomes made from alkylphosphocholines. Biochim Biophys Acta. 1996;1285(2):237-45.
34. Pohlen U, Buhr HJ, Berger G. Improvement of biodistribution with PEGylated liposomes containing docetaxel with degradable starch microspheres for hepatic arterial infusion in the treatment of liver metastases: a study in CC-531 liver tumor-bearing WAG RIJ rats. Anticancer Res. 2011;31(1):153-9.
35. Naik S, Patel D, Chuttani K, Mishra AK, Misra A. In vitro mechanistic study of cell death and in vivo performance evaluation of RGD grafted PEGylated docetaxel liposomes in breast cancer. Nanomedicine. 2012;8(6):951-62.
36. Niu R, Zhao P, Wang H, Yu M, Cao S, Zhang F, et al. Preparation, characterization, and antitumor activity of paclitaxel-loaded folic acid modified and TAT peptide conjugated PEGylated polymeric liposomes. J Drug Target. 2011;19(5):373-81.
37. Storm G, Wilms HP, Crommelin DJ. Liposomes and biotherapeutics. Biotherapy. 1991;3(1):25-42.
38. Storm G, Crommelin DJA. Liposomes: quo vadis? PSST. 1998;1(1):19-31.
39. Zhao H, Wang JC, Sun QS, Luo CL, Zhang Q. RGD-based strategies for improving antitumor activity of paclitaxel-loaded liposomes in nude

mice xenografted with human ovarian cancer. J Drug Target. 2009;17(1):10-8.

40. Jiang J, Yang SJ, Wang JC, Yang LJ, Xu ZZ, Yang T, et al. Sequential treatment of drug-resistant tumors with RGD-modified liposomes containing siRNA or doxorubicin. Eur J Pharm Biopharm. 2010;76(2):170-8.
41. Zhang YF, Wang JC, Bian DY, Zhang X, Zhang Q. Targeted delivery of RGD-modified liposomes encapsulating both combretastatin A-4 and doxorubicin for tumor therapy: in vitro and in vivo studies. Eur J Pharm Biopharm. 2010;74(3):467-73.
42. Garg A, Kokkoli E. pH-Sensitive PEGylated liposomes functionalized with a fibronectin-mimetic peptide show enhanced intracellular delivery to colon cancer cell. Curr Pharm Biotechnol. 2011;12(8):1135-43.
43. Zhai G, Wu J, Xiang G, Mao W, Yu B, Li H, et al. Preparation, characterization and pharmacokinetics of folate receptor-targeted liposomes for docetaxel delivery. J Nanosci Nanotechnol. 2009;9(3):2155-61.
44. Yuan Z, Chen D, Zhang S, Zheng Z. Preparation, characterization and evaluation of docetaxel-loaded, folate-conjugated PEG-liposomes. Yakugaku Zasshi. 2010;130(10):1353-9.
45. Li X, Tian X, Zhang J, Zhao X, Chen X, Jiang Y, et al. In vitro and in vivo evaluation of folate receptor-targeting amphiphilic copolymer-modified liposomes loaded with docetaxel. Int J Nanomedicine. 2011;6:1167-84.
46. Herringson TP, Altin JG. Increasing the antitumor efficacy of doxorubicin-loaded liposomes with peptides anchored via a chelator lipid. J Drug Target. 2011;19(8):681-9.
47. Pohlen U, Buhr HJ, Berger G, Ritz JP, Holmer C. Hepatic arterial infusion (HAI) with PEGylated liposomes containing 5-FU improves tumor control of liver metastases in a rat model. Invest New Drugs. 2012;30(3):927-35.
48. Metselaar JM, Mastrobattista E, Storm G. Liposomes for intravenous drug targeting: design and applications Mini Rev Med Chem. 2002;2(4):319-29.
49. Zhao P, Wang H, Yu M, Cao S, Zhang F, Chang J, et al. Paclitaxel-loaded, folic-acid-targeted and TAT-peptide-conjugated polymeric liposomes: in vitro and in vivo evaluation. Pharm Res. 2010;27(9):1914-26.
50. Kratz F, Muller IA, Ryppa C, Warnecke A. Prodrug strategies in anticancer chemotherapy. ChemMedChem. 2008;3(1):20-53.
51. Bildstein L, Dubernet C, Couvreur P. Prodrug-based intracellular delivery of anticancer agents. Adv Drug Deliv Rev. 2011;63(1-2):3-23.
52. Deng C, Jiang Y, Cheng R, Meng F, Zhong Z. Biodegradable polymeric micelles for targeted and controlled anticancer drug delivery: Promises, progress and prospects. Nano Today. 2012;In press.
53. Gaucher G, Marchessault RH, Leroux JC. Polyester-based micelles and nanoparticles for the parenteral delivery of taxanes. J Control Release. 2010;143(1):2-12.
54. Sawant RR, Torchilin VP. Multifunctionality of lipid-core micelles for drug delivery and tumour targeting. Mol Membr Biol. 2010;27(7):232-46.
55. Sarisozen C, Vural I, Levchenko T, Hincal AA, Torchilin VP. PEG-PE-based micelles co-loaded with paclitaxel and cyclosporine A or loaded with paclitaxel and targeted by anticancer antibody overcome drug resistance in cancer cells. Drug Deliv. 2012;19(4):169-76.
56. Sawant RR, Torchilin VP. Polymeric micelles: polyethylene glycol-phosphatidylethanolamine (PEG-PE)-based micelles as an example. Methods Mol Biol. 2010;624:131-49.
57. Xu W, Cui Y, Ling P, Li LB. Preparation and evaluation of folate-modified cationic Pluronic micelles for poorly soluble anticancer drug. Drug Deliv. 2012;19(4):208-19.
58. Brewer E, Coleman J, Lowman A. Emerging technologies of polymeric nanoparticles in cancer drug delivery. J Nanomaterials. 2011:1-10.
59. Riggio C, Pagni E, Raffa V, Cuschieri A. Nano-oncology: clinical application for cancer therapy and future perspectives. J Nanomaterials. 2011;2011:1-10.
60. Li G, Liu J, Pang Y, Wang R, Mao L, Yan D, et al. Polymeric micelles with water-insoluble drug as hydrophobic moiety for drug delivery. Biomacromolecules. 2011;12(6):2016-26.
61. Nam K, Nam HY, Kim PH, Kim SW. Paclitaxel-conjugated PEG and arginine-grafted bioreducible poly (disulfide amine) micelles for co-delivery of drug and gene. Biomaterials. 2012;33(32):8122-30.

62. Zhu C, Jung S, Luo S, Meng F, Zhu X, Park TG, et al. Co-delivery of siRNA and paclitaxel into cancer cells by biodegradable cationic micelles based on PDMAEMA-PCL-PDMAEMA triblock copolymers. Biomaterials. 2010;31(8):2408-16.

63. Lee AL, Wang Y, Pervaiz S, Fan W, Yang YY. Synergistic anticancer effects achieved by co-delivery of TRAIL and paclitaxel using cationic polymeric micelles. Macromol Biosci. 2011;11(2):296-307.

64. Shin HC, Cho H, Lai TC, Kozak KR, Kolesar JM, Kwon GS. Pharmacokinetic study of 3-in-1 poly(ethylene glycol)-block-poly(D, L-lactic acid) micelles carrying paclitaxel, 17-allylamino-17-demethoxygeldanamycin, and rapamycin. J Control Release. 2012.

65. Wu Y, Chen W, Meng F, Wang Z, Cheng R, Deng C, et al. Core-crosslinked pH-sensitive degradable micelles: A promising approach to resolve the extracellular stability versus intracellular drug release dilemma. J Control Release. 2012.

66. Jiao Z, Wang X, Chen Z. Folate-conjugated methoxy poly (ethylene glycol)/poly (L-Alanine) amphiphilic block copolymeric micelles for targeted delivery of paclitaxel. Drug Deliv. 2011;18(7):478-84.

67. Li X, Qian Y, Liu T, Hu X, Zhang G, You Y, et al. Amphiphilic multiarm star block copolymer-based multifunctional unimolecular micelles for cancer targeted drug delivery and MR imaging. Biomaterials. 2011;32(27):6595-605.

68. Liu Y, Sun J, Cao W, Yang J, Lian H, Li X, et al. Dual targeting folate-conjugated hyaluronic acid polymeric micelles for paclitaxel delivery. Int J Pharm. 2011;421(1):160-9.

69. Rezaei SJ, Nabid MR, Niknejad H, Entezami AA. Folate-decorated thermoresponsive micelles based on star-shaped amphiphilic block copolymers for efficient intracellular release of anticancer drugs. Int J Pharm. 2012;437(1-2):70-9.

70. Wan Y, Zheng Y, Song X, Hu X, Liu S, Tong T, et al. Anti-tumor activity of biodegradable polymer-paclitaxel conjugate micelles on lewis lung cancer mice models. J Biomater Sci Polym Ed. 2010.

71. Wang F, Chen Y, Zhang D, Zhang Q, Zheng D, Hao L, et al. Folate-mediated targeted and intracellular delivery of paclitaxel using a novel deoxycholic acid-O-carboxymethylated chitosan-folic acid micelles. Int J Nanomedicine. 2012;7:325-37.

72. Wang F, Zhang D, Zhang Q, Chen Y, Zheng D, Hao L, et al. Synergistic effect of folate-mediated targeting and verapamil-mediated P-gp inhibition with paclitaxel -polymer micelles to overcome multi-drug resistance. Biomaterials. 2011;32(35):9444-56.

73. Wu W, Zheng Y, Wang R, Huang W, Liu L, Hu X, et al. Antitumor activity of folate-targeted, paclitaxel loaded polymeric micelles on a human esophageal EC9706 cancer cell line. Int J Nanomedicine. 2012;7:3487-502.

74. 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(11):2894-906.

75. Yue J, Liu S, Wang R, Hu X, Xie Z, Huang Y, et al. Transferrin-conjugated micelles: Enhanced accumulation and antitumor affect for transferrin-receptor-overexpressing cancer models. Mol Pharm. 2012.

76. Huo M, Zou A, Yao C, Zhang Y, Zhou J, Wang J, et al. Somatostatin receptor-mediated tumor-targeting drug delivery using octreotide-PEG-deoxycholic acid conjugate-modified N-deoxycholic acid-O, N-hydroxyethylation chitosan micelles. Biomaterials. 2012;33(27):6393-407.

77. Niu J, Su Z, Xiao Y, Huang A, Li H, Bao X, et al. Octreotide-modified and pH-triggering polymeric micelles loaded with doxorubicin for tumor targeting delivery. Eur J Pharm Sci. 2012;45(1-2):216-26.

78. Zhang Y, Zhang H, Wang X, Wang J, Zhang X, Zhang Q. The eradication of breast cancer and cancer stem cells using octreotide modified paclitaxel active targeting micelles and salinomycin passive targeting micelles. Biomaterials. 2011;33(2):679-91.

79. Javali NM, Raj A, Saraf P, Li X, Jasti B. Fatty acid-RGD peptide amphiphile micelles as potential paclitaxel delivery carriers to alpha(v)beta (3) integrin overexpressing tumors. Pharm Res. 2012;In press.

80. Shahin M, Ahmed S, Kaur K, Lavasanifar A. Decoration of polymeric micelles with cancer-specific peptide ligands for active targeting

of paclitaxel. Biomaterials. 2011;32(22):5123-33.

81. Yin J, Li Z, Yang T, Wang J, Zhang X, Zhang Q. Cyclic RGDyK conjugation facilitates intracellular drug delivery of polymeric micelles to integrin-overexpressing tumor cells and neovasculature. J Drug Target. 2011;19(1):25-36.
82. Zhao BJ, Ke XY, Huang Y, Chen XM, Zhao X, Zhao BX, et al. The antiangiogenic efficacy of NGR-modified PEG-DSPE micelles containing paclitaxel (NGR-M-PTX) for the treatment of glioma in rats. J Drug Target. 2010;19(5):382-90.
83. Wang T, Petrenko VA, Torchilin VP. Paclitaxel-loaded polymeric micelles modified with MCF-7 cell-specific phage protein: enhanced binding to target cancer cells and increased cytotoxicity. Mol Pharm. 2010;7(4):1007-14.
84. Yang R, Meng F, Ma S, Huang F, Liu H, Zhong Z. Galactose-decorated cross-linked biodegradable poly(ethylene glycol)-b-poly(epsilon-caprolactone) block copolymer micelles for enhanced hepatoma-targeting delivery of paclitaxel. Biomacromolecules. 2011;12(8):3047-55.
85. Dhar S, Gu FX, Langer R, Farokhzad OC, Lippard SJ. Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA-PEG nanoparticles. Proc Natl Acad Sci U S A. 2008;105(45):17356-61.
86. Dhar S, Kolishetti N, Lippard SJ, Farokhzad OC. Targeted delivery of a cisplatin prodrug for safer and more effective prostate cancer therapy in vivo. Proc Natl Acad Sci U S A. 2011;108(5):1850-5.
87. Min K, Jo H, Song K, Cho M, Chun YS, Jon S, et al. Dual-aptamer-based delivery vehicle of doxorubicin to both PSMA (+) and PSMA (-) prostate cancers. Biomaterials. 2011;32(8):2124-32.
88. Salmaso S, Bersani S, Pirazzini M, Caliceti P. pH-sensitive PEG-based micelles for tumor targeting. J Drug Target. 2010;19(4):303-13.
89. Yuan Z, Huang J, Liu J, Cheng S, Zhuo R, Li F. PEG-detachable and acid-labile cross-linked micelles based on orthoester linked graft copolymer for paclitaxel release. Nanotechnology. 2011;22(33):335601.
90. Lin W, Kim D. pH-Sensitive micelles with cross-linked cores formed from polyaspartamide derivatives for drug delivery. Langmuir. 2011;27(19):12090-7.
91. Gong C, Wang C, Wang Y, Wu Q, Zhang D, Luo F, et al. Efficient inhibition of colorectal peritoneal carcinomatosis by drug loaded micelles in thermosensitive hydrogel composites. Nanoscale. 2012;4(10):3095-104.
92. Nie S, Hsiao WL, Pan W, Yang Z. Thermoreversible Pluronic F127-based hydrogel containing liposomes for the controlled delivery of paclitaxel: in vitro drug release, cell cytotoxicity, and uptake studies. Int J Nanomedicine. 2011;6:151-66.
93. Li Y, Xiao K, Luo J, Xiao W, Lee JS, Gonik AM, et al. Well-defined, reversible disulfide cross-linked micelles for on-demand paclitaxel delivery. Biomaterials. 2011;32(27):6633-45.
94. Bharali DJ, Khalil M, Gurbuz M, Simone TM, Mousa SA. Nanoparticles and cancer therapy: a concise review with emphasis on dendrimers. Int J Nanomedicine. 2009;4:1-7.
95. Gillies ER, Frechet JM. Dendrimers and dendritic polymers in drug delivery. Drug Discov Today. 2005;10(1):35-43.
96. Sampathkumar SG, Yarema KJ. Targeting cancer cells with dendrimers. Chem Biol. 2005;12(1):5-6.
97. Guo R, Shi X. Dendrimers in cancer therapeutics and diagnosis. Curr Drug Metab. 2012;In press.
98. Gupta U, Dwivedi SK, Bid HK, Konwar R, Jain NK. Ligand anchored dendrimers based nanoconstructs for effective targeting to cancer cells. Int J Pharm. 2010;393(1-2):185-96.
99. Gul-e-Saba, Abdah A, Abdullah MA. Hyaluronan-mediated CD44 receptor cancer cells progression and the application of controlled drug-delivery system Int J Curr Chem. 2010;1(4):195-214.
100. Kesharwani P, Tekade RK, Gajbhiye V, Jain K, Jain NK. Cancer targeting potential of some ligand-anchored poly(propylene imine) dendrimers: a comparison. Nanomedicine. 2011;7(3):295-304.
101. Luo J, Xiao K, Li Y, Lee JS, Shi L, Tan YH, et al. Well-defined, size-tunable, multifunctional micelles for efficient paclitaxel delivery for cancer treatment. Bioconjug Chem. 2010;21(7):1216-24.
102. Ooya T, Lee J, Park K. Effects of ethylene gly-

col-based graft, star-shaped, and dendritic polymers on solubilization and controlled release of paclitaxel. J Control Release. 2003;93(2):121-7.

103. Kolhe P, Khandare J, Pillai O, Leih-Lai M, Kannan S, Kannan RM. Hyperbranched polymer–drug conjugates with high drug payload for enhanced cellular delivery. Pharm Res. 2004;21:2185–95.

104. Perumal O, Khandare J, Kolhe P, Kannan S, Lieh-Lai M, Kannan RM. Effects of branching architecture and linker on the activity of hyperbranched polymer–drug conjugates. Bioconjugate Chem. 2009;20:842–6.

105. Prabaharan M, Grailer JJ, Pilla S, Steeber DA, Gong S. Amphiphilic multi-arm-block copolymer conjugated with doxorubicin via pH-sensitive hydrazone bond for tumor-targeted drug delivery. Biomaterials. 2009;30(29):5757-66.

106. Prabaharan M, Grailer JJ, Pilla S, Steeber DA, Gong S. Folate-conjugated amphiphilic hyperbranched block copolymers based on Boltorn H40, poly(L-lactide) and poly(ethylene glycol) for tumor-targeted drug delivery. Biomaterials. 2009;30(16):3009-19.

107. Tian H, Chen X, Lin H, Deng C, Zhang P, Wei Y, et al. Micellization and reversible pH-sensitive phase transfer of the hyperbranched multiarm PEI-PBLG Copolymer. Chemistry. 2006;12(16):4305-12.

108. Wang J, del Rosario LS, Demirdirek B, Bae A, Uhrich KE. Comparison of PEG chain length and density on amphiphilic macromolecular nanocarriers: self-assembled and unimolecular micelles. Acta Biomater. 2009;5(3):883-92.

109. Zou J, Zhao Y, Shi W. Encapsulation mechanism of molecular nanocarriers based on unimolecular micelle forming dendritic core-shell structural polymers. J Phys Chem B. 2006;110(6):2638-42.

✉ **Corresponding author:**
Georgi Momekov,
Department of Pharmacology, Pharmacotherapy and Toxicology
Faculty of Pharmacy, Medical University of Sofia,
2 Dunav Str.,
1000 Sofia, Bulgaria
Tel.: +3592 9236 509;
Fax: +3592 9879 874;
e-mail: gmomekov@gmail.com; gmomekov@cetrh.org