Review Article

Polymeric Nanoparticles for Targeted Delivery in Cancer Treatment: An Overview

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Received: 05-08-2018; **Revised:** 28-08-2018; **Accepted:** 08-09-2018.

ABSTRACT

Advances in polymeric nanoparticles are rapidly progressing and offers new opportunities for targeted treatment of cancer. Nanoparticulate drug-delivery systems are capable of preferentially targeting large doses of chemotherapeutic agents or therapeutic genes into malignant cells while leaving the healthy cells free. Nanoparticles (NPs) used as drug delivery systems are submicron-sized particles or colloidal systems that are constructed from a large variety of materials such as polymers (polymeric NPs, micelles, dendrimers), lipids (liposomes) viruses (viral NPs) as well as organo-metallic compounds. Various polymers are currently being explored to modify the properties of polymeric matrix which enables greater encapsulation efficiency and high therapeutic load with controlled release ability for the treatment of cancer. The present review explores the various types of polymeric NPs and the different ways in which they can be utilized for the successful treatment of cancer. The targeting approaches namely passive targeting and ligand based targeting using NPs have been discussed in detail and the applications of the different types of NPs in the effective treatment of cancer has been exemplified.

Keywords: targeted delivery, nanoparticles, cancer treatment, polymeric nano-carriers.

INTRODUCTION

Cancer is the second most common cause of death in the world [1]. The most commonly diagnosed cancers throughout the globe are lung cancer (accounting for 12.7% of the total number of cases), breast cancer (10.9%) and colorectal cancer (9.7%) and the most common type of cancer leading to death are lung cancer (18.2% of the total), stomach cancer (9.7%) and liver cancer (9.2%) [2].

The implementation of nanomedicines in the field of drug delivery has led to exciting advances in the treatment of cancer. The barriers associated with the conventional cancer treatment includes non-specificity that causes off-target toxicity, thus destroying healthy cells, poor circulation times resulting in decreased efficacy and drug resistance leading to decreased intra-tumoural retention [3]. In this context, polymeric nanoparticles (NP) offer numerous advantages including high drug loading, controlled release profile, improved stability and prolonged circulation times with greater cellular uptake [4].

NPs have shown great promise as drug-delivery vectors that are capable of preferentially targeting large doses of chemotherapeutic agents or therapeutic genes into malignant cells while leaving the healthy cells free[5]. NPs that are used as drug delivery systems are submicro-sized particles (3-200nm) or colloidal systems of submicron size (<1µm) are constructed from a large variety of materials such as polymers (polymeric NPs, micelles, dendrimers), lipids (liposomes) viruses (viral NPs) as well as organo-metallic compounds [6]. Polymer systems offer immense flexibility in customization and optimization of nanocarriers to efficiently deliver new therapeutics and provide an integral step in aiding their progression to clinical practice [7]. Furthermore, advances in the development of polymeric NPs has also opened up new dimensions for the treatment of cancers as they can simultaneously target multiple sites on tumour cells and enables better specificity and selectivity, thus reducing the need for higher dose and harm to the patient [8].

The aim of the review is to explore the various types of polymeric NPs and the different ways in which they have been utilized for the successful treatment of cancer. The two primary ways for the targeted delivery of anticancer drugs by the NPs (passive targeting and ligand based targeting) has been highlighted and the application of the different types of podlymeric NPs in the effective treatment of cancer has been exemplified.

Pathways of Targeted Delivery of NPS

There are primarily two ways for the delivery of an anticancer drug to the target site by the NPs which are passive and ligand targeting (Figure 1). [1]

Passive targeting

Certain patho-physiological conditions, for example leaky vasculature, pH, temperature, and surface charge around the tumour are used for passive targeting of NPs [1].

Enhanced permeation and retention (EPR) effect

The unique pathophysiologic characteristics of tumour vessels are exploited by nano-particulate systems for passive targeting when the tumour volume exceeds 2mm^3, nutrition intake, waste excretion, and oxygen

delivery is impaired due to limited diffusion [9]. New blood vessels are generated by these rapidly growing cancerous cells. This phenomenon is called angiogenesis (or neovascularization). Some features of angiogenesis are irregular tortuosity, basement membrane abnormalities and the deficiency of pericytes lining up the endothelial cells. Due to such impairments, gaps in the leaky vessels are formed which range from 100 nm to 2µm depending upon the tumour type [10]. Poor lymphatic drainage also occurs in these tumours since there is high interstitial pressure at the core of the tumour rather than at the periphery. Thus, leaky vasculature and poor lymphatic flow together results in enhanced permeation and retention (EPR) effect. As the sizes of NPs are smaller than the fenestrated blood vessels, they preferentially localize in the cancerous cells and thus remain entrapped in the tumour because of their higher retention ability as compared to the normal tissues. [9, 10]

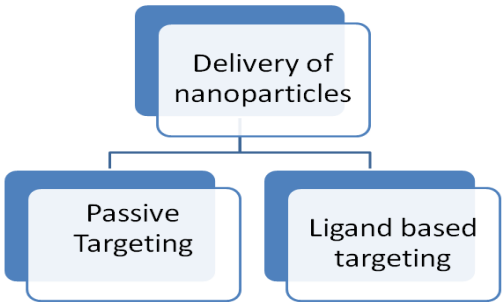

Figure 1: Approaches for Targeted Delivery of NPs[1].

Tumour microenvironment

The microenvironment around the tumour cells which is different from the normal cells can be utilized for passive targeting. The rate of metabolism is high in rapidly dividing cancer cells. An acidic environment is formed since these cells use glycolysis to maintain the supply of nutrients and oxygen [11]. pH-sensitive nanoparticulate systems are developed in a way so that they remain stable at a physiologic pH of 7.4 but then degrade for the release of the active drug target tissues have in lower pH values [12]. Hyperthermia is associated with many pathological conditions such as the human ovarian cancer. The design of a thermo-sensitive polymeric system consists of a polymer that exhibits a low critical solution temperature (LCST). Precipitation of the polymer occurs if the temperature in the tumour is above LCST with subsequent release of the drug. Using ultrasound or photothermal means, it is possible to induce localized hyperthermia in tumours [13].

Certain enzymes are also released by matrix metalloproteinases (MMPs) cancer cells. These are associated in the survival and movement mechanisms of the cancer cells [14].

Surface Charge

Surface charge is a critical factor for passive targeting of the tumour. Tumour cells have relatively high negative surface charge compared to normal cells. Therefore cationic nanoparticulate systems can be used that electrostatically bind to negatively charged phospholipid headgroups that are expressed on tumour endothelial cells [15]. The nature of polymeric surface charge (anionic, cationic or neutral governs cellular internalization and subcellular localization of the nanoparticulate systems and thus is responsible for the cytotoxicity potential of polymeric NPs. Cationic NPs have proven to be successful in delivering small interfering RNA (siRNA) to silence target gene in cancer cells and have also improved anticancer activity of Paclitaxel [16].

Ligand Based Targeting

In this type of targeting, ligands are conjugated at the periphery of the nanoparticulate system to bind with the proper receptors at the target tumour site. The targeting ligands can be classified as proteins (antibody and its fragments), peptides, nucleic acids (aptamers), or small molecules and others (vitamins and carbohydrates). These generally bind to the receptor that is over expressed by the tumour cells or vasculature[17]**.** These targeting ligands greatly influence cellular uptake of NPs which in turn is enhanced by the process of endocytosis. Due to the long circulation of NPs the NPs are able to efficiently deliver the therapeutic agent to the tumour site by EPR phenomenon [18].

Antibody based targeting

Antibodies fall into the category of targeting ligands owing to their availability and their attributes as specific and *in vivo* targeting ligands without depending on tumour enhanced permeability and retention (EPR) [19]. The two epitope binding sites present in a single molecule of antibody permits greater selectivity and binding affinity for the target of interest [20].

Various therapeutic antibodies have been developed and approved by the FDA such as Rituximab (Rituxan®) for non-Hodgkin's lymphoma treatment Rituximab (Rituxan®) [21], Trastuzumab (Herceptin®) for breast cancer treatment[22], Bevacizumab (Avastin®) designed to inhibit angiogenesis[23], and Cetuximab (Erbitux®) for advanced colorectal cancer treatment[24]. However, these antibodies are expensive to manufacture, and variations may occur from, batch to batch. Potential immunogenic responses may also be induced by antibodies and therefore to mitigate this response, antibodies have been engineered to produce humanized, chimeric, or fragmented forms of antibodies[20].

One of the most common pharmaceutical carriers for targeted drug delivery is immune-liposomes (antibody-directed liposomes). They have the unique ability to encapsulate both hydrophilic and hydrophobic therapeutic agents and their preparation is relatively simple. *In vivo* lung cancer targeted immuno-liposomes was developed by Wu *et al* [25].

However, there are certain challenges associated for the targeting of NPs such as antigen binding (monoclonal antibodies/mAb should have high target specificity and the NPs should not affect the desired specificity), conjugation (antibody-nanoparticle/Ab-NP linkage should be highly efficient and site specific) and circulation time (the mAb-NP conjugate linker must be stable when circulating in the body). Immunogenicity and purity are additional factors as well. The body is likely to recognize the antibodies as foreign particles and thus can nullify the action of the targeted NPs. Therefore, many conjugation techniques can be utilized such as exploiting lysine side-chain amines and cysteine sulfhydryl groups that produce heterogeneous mixtures of targeted NPs, each with different molar ratios of antibody and NPs (Ab:NP), with a safe pharmacokinetic profile[19].

Peptide based targeting

Due to smaller size, low immunogenicity and ease of manufacture at relatively low costs, peptides are considered to be attractive targeting molecules[26]. There are various methods for identifying peptide-based targeting ligands. Generally, peptides are obtained from the binding regions of the protein of interest. It is also possible to identify peptide based-targeting ligands by utilizing phage display techniques. In the phage display technique, a phage display screen is used for the bacteriophages to display a range of targeting peptide sequences in a phage display library (approximately 10^{11} sequences) and a binding assay is used to select the target peptides[27].

At present Cilengitide, which is a cyclic peptide that has a binding affinity for integrin is under phase II clinical trials for the treatment of non-small cell lung and pancreatic cancer [28]. Adnectins are a new class of therapeutic proteins based on the fibronectin and designed to bind with high affinity and specificity to relevant targets. An adnectin for human VEGF receptor 2, a 40 amino acid thermostable and protease-stable oligopeptide, is under phase I clinical trials for several oncology indications (Lipovsek, 2010). Even though peptides have certain disadvantages such as target affinity being low and easily susceptible to proteolytic cleavage, these conditions can be avoided by synthesis of the peptides with D-amino acids [20]. Peptides have been recently used to design multifunctional NPs for the purpose of targeted cancer imaging and therapy[20]. A breast tumour targeted nanodrug was designed to transfer siRNA to the human breast cancer which allowed the non-invasive monitoring of siRNA delivery concurrently[29].

Aptamer based targeting

Oligonucleic acids (RNA or DNA) which have unique three-dimensional structures and the potential to bind with many biochemical targets from small molecules to large proteins are known as aptamers[19]. Aptamers that are capable of binding to a target of interest are selected through Systematic Evolution of Ligands by Exponential enrichment (SELEX)[30]. The method involves rounds of target binding, partitioning binding from non-binding sequences, and multiplication of the enriched binding sequences the bioactive forms of target proteins on the surface of the cell[31]. Aptamers which have a high affinity and specificity for a target can be selected from libraries of 10^{15} random oligonucleotides.

Aptamers have greater advantages in terms of NPs targeting compared to antibodies. They can be synthesized with a specific functional moiety of carboxylate, amino, sulfhydryl or aldehyde at only one end of the nucleic acid that serves as an aptamer. This type of synthesis allows for conjugations which are site specific without the production of heterogeneous mixtures and with hardly any variations from batch to batch[19]. Aptamers exhibit non-immunogenic properties, are non-toxic and can be modified for stability in circulation[32]. The aptamers can be selected *in vitro* and *in vivo* and can be subjected to repeated and reversible denaturation. Since aptamers are not animal or immune-response dependent, they can be chosen against weak immunogenic targets and toxins[33]. They have a much smaller size (150kDa) in contrast to antibodies and they can produce compact structures which enable them to bind clefts, binding sites and enzymatic active sites[34]

More than 200 aptamers have been isolated till present[35]. Example of FDA approved aptamer is Pegaptanib ($VEGF_{165}$) in 2004 for the treatment of neovascular macular degeneration. Another aptamer which is a nucleolin targeted aptamer is under phase II clinical trial[36]. However, due to the degradation of the nuclease, rapid blood clearance may occur which can be lethal to the patient. This problem can be overcome by modification of pyrimidine at 2'-fluorine position or chemical modification with PEG can be done to increase bioavailability and pharmacokinetic properties[37].

2'-fluoro-pyridine-RNA aptamers which are produced against the extracellular domain of prostate-specific membrane antigen (PSMA) are considered to be the best aptamers for targeted delivery[38]. Self-assembled polymeric NPs of doxorubicin have been delivered by using these aptamers[39].

Small- molecule based targets

The small molecules with variable structures and properties are cheaper to produce and have proven to be a class of the more prevalent targeting ligands conjugated to NPs. The different types of small molecules used are folate, carbohydrates, riboflavin, EGFR, transferrin and lectins[20].

Folate

Folate, a water soluble vitamin B6, is one of the most widely studied molecules for drug delivery as they are inexpensive, non-toxic, non-immunogenic with high binding affinity, stability upon storage and during circulation and are easily conjugated to nanocarriers[40]. It

is an essential vitamin in humans for cell division and embryonic development [27]. The folate receptor has been considered as a potential tumour marker that binds vitamin folate and folate-drug conjugates with a high affinity and transfers these bound molecules into the cells through receptor-mediated endocytosis[41]. Since folate receptors are overexpressed in cancers, folate allows for the targeted delivery of imaging and therapeutic agents to the tumour due to its high binding affinity for the folate receptors. Certain theranostic agents have been produced by the combination of folate with drug delivery vehicles or inorganic NPs[20]. A new folate receptor-targeted nanoparticle formulation of Paclitaxel was formulated using heparin as a carrier (heparin-folate-Taxol (Paclitaxel), HFT) and was tested in animal models which showed more potent activity against the growth of tumour xenografts compared to binary heparin-Taxol or the free Paclitaxel drug [42].

Carbohydrates

Carbohydrates are another type of small molecule targeting ligands which can distinguish selectively the cell surface receptors such as lectin [43]. Hepatocytes have a high density of the asialoglycoprotein receptor (ASGP-R) (500,000 receptors per cell) and easily binds carbohydrates such as galactose, mannose, arabinose which can effectively act as liver-targeted drug delivery systems [43].

Riboflavin

An essential vitamin for cellular metabolism is riboflavin and thus, the carrier protein of riboflavin (RCP) is highly upregulated in metabolically active cells[44]. Therefore, an endogenous RCP ligand known as the flavin mononucleotide (FMN), was used for the metabolically active cancer or endothelial cells[45]. FMN- coated ultra-small super paramagnetic iron oxide nano particles (FLUSPIO) synthesized by Kiessler and co-workers to be used as MRI/optical dual probes for cancer detection which was stabilized by guanosine monophosphate[46]. An intense fluorescence emission was observed at 530 nm due to FMN. Through MRI, TEM and fluorescence microscopy of PC3 cells and HUVEC cells the cellular uptake of FLUSPIO was investigated in vitro. After 1-hour incubation with FLUSPIO and non-targeted USPIO, both PC3 and HUVEC cells depicted a higher R2 relaxation rate. This uptake was reduced by the competitive blocking of RCP with free FMN. After the incubation period, a green fluorescence in the cells was observed. This suggested that since there was endosomal localization of the NPs indicating that FMN could be considered as a building block for imaging and therapeutic agent for tumours[20].

Epidermal Growth Factors

The EGFRs are part of a group of tyrosine kinase receptors. These receptors are highly upregulated on tumour cell surfaces. EGFR binds to six known endogenous ligands, namely: EGF, transforming growth factor-α, amphiregulin, betacellulin, heparin-binding EGF, and epiregulin[47]. When EGFR is activated by one of these ligands intracellular signalling processes are stimulated which increases tumour growth and progression involving multiplication, angiogenesis, invasion, and metastasis. Overexpression of EGFR is evident in breast, lung, colorectal, and brain cancers [48].

Transferrin

Transferrin is a serum non-heme iron-binding glycoprotein which acts as a transporter which aids in the transport of iron through blood and into proliferating cells by its attachment to the transferring receptor. When the transferrin internalizes via receptor mediated endocytosis, iron is released in the acidic environment of the cell. The receptor is very important since it is responsible for iron homeostasis and regulation of cell growth. Therefore, due to the increased demand for iron as compared to the normal cells, transferrin receptors are overexpressed in metastatic and drug resistant cancer cells which make them an attractive target for cancer therapy and tumour-specific drug delivery[49]. Transferrin-conjugated paclitaxel loaded [poly(lactic-co-glycolic-acid) polymer] NPs inhibited cell growth to a greater extent compared to free paclitaxel[50]. Transferrin conjugated to liposomes promoted transfection efficacy of glycoprotein p53 leading to sensitization of transfected cancer cells [51].

Lectins

Lectins are proteins that can identify and bind specifically to the carbohydrate moieties of glycoproteins present on the extracellular side of plasma membrane. The glycoproteins of cancer cells are different from the ones expressed on the normal cells [1]. Therefore, lectins can be used as targeting molecules for site-specific delivery of drugs[41].

Lectin targeting based on lectin-carbohydrate interaction can be characterized into two types, known as direct lectin targeting and reverse lectin targeting[1,41]. Direct lectin targeting incorporates lectins into NPs as ligand to target cell surface carbohydrates and reverse lectin targeting that involves conjugating nano-system with carbohydrate moiety to target lectins. Lectin based targeting is primarily applied for targeting colon[52].

Polymer Based Nanocarriers for Targeted Cancer Therapy

Polymeric NPs offer numerous advantages for drug delivery by enabling high loading efficiencies and ability to target tumours to provide enhanced antitumour efficacy through triggered release inside tumours with minimum side effects [41]. Additionally, these NPs provide a shielding effect by protecting the drugs from rapid clearance by liver, kidney and reticuloendothelial system which enhances stability of the drug and target specificity. The polymers used for the development of these nanocarriers may be either natural or synthetic[1]. The polymeric NPs can be classified as solid polymeric NPs, polymeric micelle, polymer conjugate, dendrimer,

polymersome, polyplex and polymer-lipid hybrid system[1]. The different types of NPs characterized by their unique physicochemical structures and their applications in the treatment of cancer have been discussed in the following sections.

Polymeric NPs

Polymeric NPs are solid colloidal systems in which the therapeutic agent is either dissolved, entrapped, encapsulated or adsorbed onto the constituent polymer matrix[1]. The structures of polymeric NPs depend on the process of formation of NPs and vary from nanospheres to nanocapsules. Nanospheres are matrix systems in which the drug is dispersed throughout the particles whereas nanocapsules refer to vesicles in which the drug is entrapped within an aqueous or oily cavity which is further surrounded by a monolayer of polymeric membrane [53].

A wide range of polymers can be used to fabricate polymeric NPs which includes natural or synthetic substances composed of macromolecules such as poly (lactide-coglycolide), polyethylene glycol, poly (lactic acid), poly(ε-caprolactone), albumin, chitosan, and poly (alkyl cyanoacrylates)[54]. The composition of the NPs varies for the delivery of specific drugs to the surface of specific cells[55] .The first step for using a polymeric carrier is to ensure that the polymeric structure is biodegradable and can retain its properties *in vivo* for a prolonged period of time. Biodegradability is a critical factor so that polymeric carriers are degraded into smaller fragments that can be metabolized and excreted from the body. In comparison with other drug delivery systems, polymeric nano-carriers are non-toxic, non-immunogenic, relatively easier to synthesize, cheaper, water soluble and provide better biodegradability and biocompatibility[56].

A nanoparticle formulation of paclitaxel which contains serum albumin as a carrier, commercially known as Abraxane, has been used for the treatment of metastatic breast cancer[57]. Abraxane has not only been used for metastatic breast cancer but also has been assessed in other clinical trials for non-small-cell lung cancer (Phase II trial) and advance non-hematologic malignancies (Phase I and pharmacokinetics trials)[41].

NPs loaded with paclitaxel (PTX) and tamoxifen (TMX) containing biodegradable polyethylene oxide (PEO) conjugated with poly(ε-caprolactone) (PCL) proved to be efficient in overcoming multidrug resistance in the treatment of ovarian adenocarcinoma. Substantial enhancement in antitumour efficacy was observed and there was hardly any toxicity associated upon intravenous administration of nanoparticulate formulations of PTX-TMX in combination with PEO-PCL[58].

An aptamer-functionalized poly (lactide-coglycolide) conjugated with polyethylene glycol (PLGA-PEG) NPs was developed against prostate-specific membrane antigen (PSMA) overexpressing cancer cells by Cheng et al. The delivery of the NPs to the tumours was increased by 3.77 fold in 24 hours compared to NPs without the aptamer[59].

Polymeric Micelles

Polymeric micelles are formed as a result of self-assembling of amphiphilic di- or tri- block copolymers into spherical nanosized core/shell structure in aqueous media[41]. The hydrophobic core acts as a reservoir for hydrophobic drugs and allows for encapsulation of anticancer drugs whereas the hydrophilic shell is responsible for stabilizing the hydrophobic core which makes the polymer water-soluble and an attractive drug delivery system for intravenous route of administration. The hydrophilic layer also provides stealth property to the system which prolongs the circulation time of the micelle in the blood as it averts the uptake by the reticuloendothelial system [1,41]. The micelles that are derived from polymer have greater stability and lower critical micellar concentration value(in order of 10^{-6} M) when compared to surfactant-based micelles[60]. The loading of the drug within the micelle can be carried out in two ways -physical encapsulation and chemical covalent attachment [61].

Recent studies on few of the micellar based anticancer drugs have shown their potential as drug carriers in oncotherapy[60]. Genexol-PM (PEG-poly(D,L-lactide)-paclitaxel) is the first polymeric micelle formulation of paclitaxel. It is cremophor-free (i.e. free from poly-ethoxylated castor oil which is used in other formulations of paclitaxel). This particular polymeric micelle formulation has undergone Phase I and pharmacokinetic studies in patients with advanced refractory [62]. Multifunctional polymeric micelles coupled with targeting ligands and imaging and therapeutic agents are being developed as well[63].

Curcumin was encapsulated using polymeric micelles of cross-linked and random copolymers of N-isopropylacrylamide with N-vinyl-2-pyrrolidone and PEG monoacrylate. Anticancer efficacy was observed *in vitro* when compared with free curcumin against human pancreatic cancer cells lines by these newly formulated micelles [64].

Cisplatin incorporated within PEG-polyglutamic acid block copolymer micelles showed 20-fold higher blood circulation and accumulation in solid tumours compared to free cisplatin in lung carcinoma cells. It provided sufficient stability that led to prolonged circulation and showed sustained drug release pattern after accumulation at the delivery site through enhanced permeability retention effect. Furthermore, complete destruction of the tumour occurred and no significant loss in body weight was observed after treatment with polymeric micelles compared to free cisplatin which causes approximately 20 percent loss of body weight and tumour survival at an equivalent dose[65].

Polymeric micelles consisting of PEG-phosphatidyl ethanolamine conjugated to antitumour monoclonal

antibody that had a nucleosome-restricted specificity for different cancer cells were developed for target-specific delivery of Paclitaxel (PTX). These immune micelles were able to recognize and efficiently bind to various cancer cells such as the murine lung carcinoma cells, lymphomacells and human breast adenocarcinoma cell lines *in vitro*. The tumour specific immune-micelles loaded with PTX demonstrated an increase in accumulation of PTX in the tumour and enhanced the inhibition of the growth rate of the tumour upon intravenous administration into mice with lung carcinoma by a factor of 2.5 times compared to free PTX or TAXOL in non-targeted micelles[66].

Folic acid was coupled with doxycycline loaded PEG-PLGA micelles by covalently attachment of the ligand through its γ -carboxyl group to form polymeric micelles. Enhanced cellular uptake as well as cytotoxicity against human nasopharyngeal epidermal carcinoma cell lines was observed in comparison to non-targeted micelles. In addition, there was a two times reduction in the rate of tumour growth showing significant improvement in antitumour activity in contrast to non-targeted micelles[67].

In order to overcome multidrug resistance (MDR) in human breast cancer, pluronic copolymers were studied as micellar carriers for delivery of Paclitaxel. Pluronic micellar paclitaxel resulted in a remarkable decrease in IC_{50} of Paclitaxel in multidrug resistance cell lines when compared with free Paclitaxel, thus proving its potential to cause cytotoxic effects in multidrug resistance cells compared to nonresistant cell lines. Enhanced uptake by the folate receptors led to greater internalization of the polymeric micelles and therefore pluronics based micelles to overcome MDR and folate-mediated uptake can be applied for the treatment of MDR solid tumours[68].

Dendrimers

A dendrimer is a synthetic polymeric macromolecule of nanometer dimensions. They are composed of repeatedly branched monomers that extend from the central core resembling a tree-like structure[1,41].

They can serve as attractive carriers for anticancer therapeutics because of their distinct properties such as mono-disperse size, modifiable surface functionality that facilitates the encapsulation/ conjugation of therapeutic agent in the internal cavity or on the surface multivalency as well water solubility[69].

Poly (glycerol succinic acid) dendrimers were investigated as potential carriers for Campotothecin. Camptothecin encapsulated dendrimer exhibited anticancer activity which was assessed by using the human breast adenocarcinoma (MCF-7), colorectal adenocarcinoma (HT-29), non-small-cell lung carcinoma (NCI-H460), and glioblastoma (SF-268). When compared to camptothecin dissolved in dimethyl sulfoxide, the dendrimer carrier camptothecin demonstrated an increased cytotoxicity as well as a decrease in IC_{50} in two-to-seven-fold range. Cellular uptake was also increased by 16-fold compared to the free drug in MCF-7 cells[70]. Melamine based dendrimers reduced the organ toxicity of anticancer agents such as methotrexate and 6-mercapturine which causes hepatotoxicity. Alanine transaminase levelALT) was reduced to 27 percent in case of methotrexate encapsulated dendrimers, while for 6-mercaptopurine was reduced to 36 percent as compared to the non-encapsulated drugs [71]

Poly (ε- caprolactone) and polyethylene glycol (PEG) were explored as a carrier for the anticancer drug etoposide and showed comparable toxicity to free etoposide when tested on porcine kidney cells. The dendrimer most widely used as a scaffold is poly-amido amine dendrimer. A polyamidoamine (PAMAM) dendrimer was conjugated with cisplatin formed by sodium carboxylate synthesizing dendrimer palatinate which released platinum slowly in vitro. The dendrimer Pt was 8-fold less toxic than the free drug and showed higher activity against melanoma bearing mice when compared to the free drug upon intraperitoneal administration. Free Cisplatin was inactive after IV administration to treat melanoma whereas the dendrimer-Pt showed anticancer activity[72].

Polymersomes

Polymer vesicles composed of synthetic amphiphilic block copolymers that are self-assembled and contain separate hydrophilic and hydrophobic blocks are known as polymersomes. They are similar to liposomes in terms of architecture and structured design but have greater stability, storage capability and enhanced circulation time compared liposomes even though structurally they are similar. Doxycycline (DOX) can be encapsulated within the aqueous center of the polymer vesicles. The efficiency of DOX-loaded PEO-block-PCL in terms of its therapeutic activity was studied in xeno-transplanted tumour-bearing mice and their potential to retard tumour growth was assessed. When compared to commercially available DOXIL, which is a clinically administered liposomal formulation of DOX, DOX-loaded polymersomes were able to inhibit the tumour growth in a similar manner[73].

For breast cancer therapy polymersomes based on phosphazene were researched on as the delivery systems of hydrophilic DOX hydrochloride or hydrophobic DOX base which allowed higher encapsulation of DOX or DOX-HCl. In comparison to free DOX-HCL, the DOX-HCL loaded polymersomes increased life safety upon in vivo administration of these polymersomes in mice with xenograft tumours[74]. Efficient passive delivery to human breast tumour-bearing mice can be obtained by co-encapsulation of PTX (in hydrophobic bilayer) and DOX (in hydrophilic core) by utilizing polymersomes. The dual drug loaded polymersome increases the synergistic anticancer effect, increases the suppression of the tumour and also shows a higher maximum tolerated dose as compared to the free drug [75].

Bio-resorbable polymersomes were developed for efficient and targeted delivery of cisplatin to human colon

cancer cells that overexpress α(5)β(1) integrin [76]. PEO-block-poly(ɣ-methyl-ε-caprolactone) polymersomes were attached with α(5)β(1) integrin specific targeting peptide named PR_b which enabled target binding and enhanced uptake into the cancer cells. As a comparison to the conventional RGD-targeting peptides which bind to different types of integrins. Higher cytotoxity was observed in colon cancer cells. Self-assembled polymersomes from PEO-b-poly(butadiene) di-block copolymers functionalized with PR_b employed for delivery of therapeutic protein named tumour necrosis factor-α(TNF-α) to prostate cancer cells. PR_b polymersomes attached to the $\alpha_5\beta_1$integrins which were expressed on prostate cancer cells led to efficient internalization of polymersomes and enhanced cytotoxic potential compared with non-targeted polymersomes[77].

Polyplexes

Polymeric systems which formed as a consequence of condensation and/or complexation of gene or siRNA through electrostatic interactions between the cationic groups of the polymer and the negatively charged nucleic acids are termed as polyplexes. The nucleic acids are protected from enzymatic degradation by the polyplexes and prevent the release at off-target sites. Transfection with the negatively charged cell surface may be enhanced by the polyplexes which contain an excess of positive charge. Site-specific delivery of therapeutic nucleic acids to the tumour sites is a promising strategy for the treatment of cancer[78].

Poly-l-lysine based vector was investigated for cancer-specific gene therapy by modifying the polymer (Zhao, Tanaka, & Kim, 2014). In order to impart endosome escape property, the polymer was modified with histidine group and with cationic peptide moiety to help in the formation of polyplex with pDNA and act as a substrate for protein kinase (α(PK(α) which is specifically activated in cancer cells. After application of the polyplexes into cancer cells, protein kinase responsive gene expression was observed which extended for 24 hours [79].

Polyplexes using phosphorylcholine-modified polyethyleneimine (PEI) has been utilized for efficient delivery of DNA in cancer therapy. These polyplexes enabled selective uptake by the liver cancer cells compared to PEGylated polyplexes and showed gene expression in liver cancer cells six folds greater than normal cells [80].

The ability of galactose-modified trimethyl chitosan-cysteine-based polymeric vectors to deliver siRNA were explored. The result showed that the polyplexes were efficient in persistent gene knockdown when in human liver cancer and human lung cancer cells. In addition, greater retardation of tumour growth, angiogenesis inhibition and apoptosis induction was observed with the use of these polyplexes [79]. For the delivery of prostate cancer cell-specific VEGF siRNA, a polymeric system was developed in which PEI was joined with the prostate cancer cell-targeting peptide by a PEG linker, to form stable polyplexes by condensing siRNA. Higher gene silencing ability was demonstrated by these newly formed polyplexes because of targeting peptide-mediated specific internalization in human prostate carcinoma cells[81].

Polymer hybrid systems

Polymer-lipid hybrid system

When polymeric NPs and liposomes are enjoined together, the resulting system is known as polymer-lipid hybrid system. The polymer-lipid hybrid system is composed of a biodegradable hydrophobic polymeric core that encapsulates water- soluble anticancer drugs for sustained-release action, which is surrounded by a hydrophilic shell responsible for providing stealth property that prevents them from recognition by the immune system and prolongs the systemic circulation. A third component known as the lipid monolayer is also present as part of the system that separates the hydrophobic core and hydrophilic shell to prevent diffusion of encapsulated agent and reduce the rate of water penetration into the NPs. This hybrid system offers the advantages of both the polymeric NPs and liposomes which are greater amount of drug encapsulation and loading efficiency, desirable sustained release drug profile, better stability and enables surface functionalization for targeting cancer cells [82].

An example of a polymer-lipid hybrid system is complexation of cationic doxorubicin with anionic soybean-oil-based polymer dispersed with lipid (stearic acid) in water. This particular hybrid system exhibited effective delivery of the anticancer drug doxorubicin and enhanced cytotoxicity by eight-fold against P-gp-overexpressing human breast cancer cell line. Furthermore, the incorporation of doxorubicin within the polymer-lipid hybrid nano-particulate showed enhanced uptake of the drug and retention in P-gp-overexpressing cells than free drug [83].

Polymer-surfactant hybrid system

Surfactants are agents that are responsible for reducing interfacial tension between two or more components in a system, thus promoting miscibility, colloidal stability, dispersion between water and oil, NPs and polymers, NPs and analytes (Heinz, et al., 2017). A polymer-surfactant nano-particulate hybrid system was developed by Chavanpatil et al. that comprised of sodium alginate as the polymer and dioctyl sodium sulpho-succinate as the anionic surfactant. The hybrid system resulted in enhanced cytotoxicity as a result of increased cellular uptake and drug accumulation in drug-resistant cells compared to doxorubicin solution[84].

Polymer cyclodextrin hybrid system

Cyclodextrins are glucopyranose units which are linked with α bonds[85]. The natural cyclodextrins are named as α, β, γ, based on the number of glucopyranose units, for

example α cyclodextrins contain 6 units of glucopyranose andβ and γ cyclodextrins contain 7 and 8 units respectively.

Cyclodextrins have a truncated cone shaped appearance and are polar exterior owing to the presence of primary and secondary hydroxyls groups as shown in Figure 4. The interior of the cyclodextrins is apolar which enables them to incorporate hydrophobic molecules within the cavity and form an inclusion complex in the molar ratio 1:1, 1:2 or 2:1 drug: cyclodextrin. Various physicochemical properties, such as the taste, solubility, stability, odor, side effects and physical state of the guest molecule can be altered using this cyclodextrin inclusion complex. Therefore, cyclodextrins has been widely used as excipients since it improves solubility of poorly water soluble drug, enhances the physical or chemical stability of active ingredient and avoids the side effects of drugs in parenteral, oral, nasal, buccal, topical and mucosal formulations [86].

It seems a promising approach to formulate NPs directly from cyclodextrins owing to the idea that cyclodextrins with amphiphilic property self-assemble at interfaces. Since natural or hydrophilic cyclodextrins are being used in NPs as coating material or in conjugation with polymers for the controlled release of drugs, amphiphilic cyclodextrins could also be used individually as a nanoparticle. Even though amphiphilic cyclodextrins can be acquired by the chemical grafting of long aliphatic chains to the primary and /or secondary faces with ester, ether, thio, fluoro or amido bonds, they can still form inclusion complexes with lipophilic drugs. Cyclodextrins can also form nanoparticle by techniques such as nanoprecipitation, double emulsion and detergent dialysis techniques[87].

A transferrin-modified, cyclodextrin polymer-based system for delivery of siRNA was formulated by Bellocqet. al. The hybrid system is composed of a cyclodextrin polycation condensed with nucleic acid along with the presence of polyethylene glycol at the surface for increasing the stability in biological fluids as well as transferrin used for targeting of cancer cells. The conjugate transferrin-PEG-adamantane self assembles with the NPs by admnatane(host) and cyclodextrin (guest) inclusion complex formation, resulting in a a four-fold enhancement of leukemia cells compared to non-targeted NPs[88].

Polymer conjugates

Polymer conjugates refer to water-soluble polymers either conjugated to anticancer drugs or proteins. They are considered as new chemical entities as they have a pharmacokinetic profile which differs from that of the parent drug[1]. Protein used carrier systems provide selective drug targeting and solves enzyme-induced degradation problem, thus increasing stability[56]. Proteins also offer numerous advantages such as decreased immunogenicity, greater plasma half-life and prolonged systemic circulation [1].

Polymer-drug conjugation promotes tumour targeting using the phenomenon of EPR effect and enables endocytic capture at cellular level, culminating in lysosomotropic drug delivery [1]. A targeting moiety or inclusion of a targeting ligand or antibody in polymer drug conjugate allows for specific targeting of tumours [56].

Internalization usually takes place through receptor-mediated endocytosis. For example, an endosome is formed after the invagination plasma membrane engulfs the complex of the folate-targeted conjugate bound with the folate receptor on the cell surface. These endosomes are then transferred to target different organelles. The drug is released from the conjugate when the interior pH value of the endosome becomes acidic and the lysozymes are activated. Given that the drug has the suitable physic-chemical properties to cross through the endosomal membrane, it enters the cytoplasm. The released drugs are then taken up by their preferred target organelle. During this time, the folate receptor which was released from the conjugate starts a second round of transporting by binding to new folate-targeted conjugates by returning to the cell membrane[6].

The polymers used in the fabrication of polymer-drug conjugates include linear polymers such as N-(2-hydroxypropyl) methacrylamide copolymers, polyglutamic acid, PEG and polysaccharides (dextran) with drugs (DOX, PTX, camptothecin and platinate). The clinically approved polymeric NPs are listed in Table 1.

Table 1: Clinically approved polymeric nanomedicine for oncologic treatment.

Polymeric Platforms	Product Description	Therapeutic Agent	Commercial Name	Indication	Administration	Reference
Polymer-protein conjugate	SMANCS	Neocarzinostatin	Zinostatin® (Stimalmer)	Hepatocellular carcinoma	Intra-arterial	89
	PEG-l asparaginase	Asparaginase	Oncaspar ®	Acute lymphoblastic leukemia	Intravenous, intramuscular	90
	PEG-GCSF	GCSF	Neulasta®/PEGFllgrastim	Prevention of neutropenia associated with cancer chemotherapy	Subcutaneous	91
Polymeric micelle	Methoxy-PEG-poly (d,l-lactide)-paclitaxel micelle	Paclitaxel	Genexol®-PM	Metastatic breast cancer	Intravenous	92

CONCLUSION

The greater scope of chemically modifying the polymeric system has increased its potential to be utilized for targeted drug delivery of many therapeutic agents especially for the treatment of cancer. The therapeutic agent is either conjugated to the surface of the nanoparticle or can be encapsulated within the polymeric core. The targeted delivery of NPs can overcome the problems associated with the conventional cancer therapy such as lack of water solubility, nonspecific bio-distribution and lack of selectivity resulting in non-specific toxicity towards normal cells and lower dose of drugs delivered to cancer cells leading to poor bioavailability and low therapeutic indices. The NPs can be designed for optimal size and surface characteristics to prolong their circulation time in the bloodstream to improve the biodistribution of cancer drugs. Various polymers are currently being explored to modify the properties of polymeric matrix which enables greater encapsulation efficiency and high therapeutic load with controlled release ability for the treatment of cancer. Passive and active targeting strategies are used to deliver drugs to the target sites. NPs can utilize the enhanced permeability and retention effect and the tumour microenvironment to selectively target the anticancer drug to the cancer cells. In addition to this passive targeting mechanism, active targeting strategies using ligands and antibodies can increase the specificity of these therapeutic NPs. NPs can be used to decrease or overcome drug resistance which hinders the efficiency of molecularly targeted and conventional chemotherapeutic agents by escaping recognition from P- glycoprotein, one of the main mediators of multidrug resistance. Furthermore, progression in nanoscale imaging will allow for the development of multifunctional “smart” NPs that can facilitate personalized cancer therapy as they are capable of detecting malignant cells (active targeting moiety), visualizing of their location in the body (real-time *in vivo* imaging), destroying the cancer cells specifically and harming the normal cells leading to minimum side effects (active targeting and controlled drug release) and monitoring the outcome of treatments in real time. Although, a number of polymeric NPs have been clinically approved for oncologic treatment, yet these NPs for functionalization with targeting ligand must be evaluated and the safety of these nanocarriers must be assessed before proceeding to clinical study.

REFERENCES

1. Prabhu, R. H., Patravale, V. B. & Joshi, M. D., Polymeric nanoparticles for targeted treatment in oncology: current insights, International journal of nanomedicine, 10, 2015, 1001.
2. Ferlay, J. *et al.*, Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008, International journal of cancer, 127, 2010, 2893-2917.
3. Ho, D., Wang, C.-H. K. & Chow, E. K.-H., Nanodiamonds: The intersection of nanotechnology, drug development, and personalized medicine, Science advances, 1, 2015, e1500439.
4. Pridgen, E. M., Alexis, F. & Farokhzad, O. C., Polymeric nanoparticle technologies for oral drug delivery, Clinical Gastroenterology and Hepatology, 12, 2014, 1605-1610.
5. Shin, C. *et al.*, Transition behavior of block copolymer thin films on preferential surfaces, Macromolecules, 41, 2008, 9140-9145.
6. Leamon, C. P. & Reddy, J. A., Folate-targeted chemotherapy, Advanced drug delivery reviews, 56, 2004, 1127-1141.
7. Shroff, K. & Vidyasagar, A., Polymer nanoparticles: newer strategies towards targeted cancer therapy, J Phys Chem Biophys, 3, 2013, 2161-0398.1000125.
8. van Vlerken, L. E. & Amiji, M. M., Multi-functional polymeric nanoparticles for tumour-targeted drug delivery, Expert opinion on drug delivery, 3, 2006, 205-216.
9. Byrne, J. D., Betancourt, T. & Brannon-Peppas, L., Active targeting schemes for nanoparticle systems in cancer therapeutics, Advanced drug delivery reviews, 60, 2008, 1615-1626.
10. Maeda, Y., Tabata, H. & Kawai, T., Two-dimensional assembly of gold nanoparticles with a DNA network template, Applied Physics Letters, 79, 2001, 1181-1183.
11. Pelicano, H., Martin, D., Xu, R. & Huang, P., Glycolysis inhibition for anticancer treatment. Oncogene25: 4633–4646, View Article PubMed/NCBI Google Scholar, 2006.
12. Yatvin, M., Kreutz, W., Horwitz, B. & Shinitzky, M., pH-sensitive liposomes: possible clinical implications, Science, 210, 1980, 1253-1255.
13. Brewer, E., Coleman, J. & Lowman, A., Emerging technologies of polymeric nanoparticles in cancer drug delivery, Journal of Nanomaterials, 2011, 2011, 1.
14. Deryugina, E. I. & Quigley, J. P., Matrix metalloproteinases and tumor metastasis, Cancer and Metastasis Reviews, 25, 2006, 9-34.
15. Krasnici, S. *et al.*, Effect of the surface charge of liposomes on their uptake by angiogenic tumor vessels, International journal of cancer, 105, 2003, 561-567.
16. Boyer, C. *et al.*, Effective delivery of siRNA into cancer cells and tumors using well-defined biodegradable cationic star polymers, Molecular pharmaceutics, 10, 2013, 2435-2444.
17. Danhier, F., Feron, O. & Préat, V., To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery, Journal of Controlled Release, 148, 2010, 135-146.
18. Drummond, D. C., Hong, K., Park, J. W., Benz, C. C. & Kirpgtin, D. B., Liposome targeting to tumors using vitamin and growth factor receptors, Vitamins & Hormones, 60, 2000, 285-332.
19. D Friedman, A., E Claypool, S. & Liu, R., The smart targeting of nanoparticles, Current pharmaceutical design, 19, 2013, 6315-6329.
20. Yu, M. K., Park, J. & Jon, S., Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy, Theranostics, 2, 2012, 3.
21. James, J. & Dubs, G., FDA approves new kind of lymphoma treatment. Food and Drug Administration, AIDS treatment news, 1997, 2.
22. Albanell, J. & Baselga, J., Trastuzumab, a humanized anti-HER2 monoclonal antibody, for the treatment of breast cancer, Drugs Today (Barc), 35, 1999, 931-946.
23. Ferrara, N., Hillan, K. J. & Novotny, W., Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy, Biochemical and biophysical research communications, 333, 2005, 328-335.
24. Van Cutsem, E. *et al.*, Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer, New England Journal of Medicine, 360, 2009, 1408-1417.

25. Lu, R.-M., Chang, Y.-L., Chen, M.-S. & Wu, H.-C., Single chain anti-c-Met antibody conjugated nanoparticles for in vivo tumor-targeted imaging and drug delivery, Biomaterials, 32, 2011, 3265-3274.

26. Wang, A. Z. *et al.*, Superparamagnetic iron oxide nanoparticle–aptamer bioconjugates for combined prostate cancer imaging and therapy, ChemMedChem, 3, 2008, 1311-1315.

27. McCarthy, J. R., Bhaumik, J., Karver, M. R., Sibel Erdem, S. & Weissleder, R., Targeted nanoagents for the detection of cancers, Molecular oncology, 4, 2010, 511-528.

28. Beekman, K. W. *et al.*, Phase II evaluations of cilengitide in asymptomatic patients with androgen-independent prostate cancer: scientific rationale and study design, Clinical genitourinary cancer, 4, 2006, 299-302.

29. Yigit, M. V. & Medarova, Z., In vivo and ex vivo applications of gold nanoparticles for biomedical SERS imagingi, American journal of nuclear medicine and molecular imaging, 2, 2012, 232.

30. Tuerk, C. & Gold, L., Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase, Science, 249, 1990, 505-510.

31. Daniels, D. A., Chen, H., Hicke, B. J., Swiderek, K. M. & Gold, L., A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment, Proceedings of the National Academy of Sciences, 100, 2003, 15416-15421.

32. de Campos, W. R. L., Coopusamy, D., Morris, L., Mayosi, B. M. & Khati, M., Cytotoxicological analysis of a gp120 binding aptamer with cross-clade human immunodeficiency virus type 1 entry inhibition properties: comparison to conventional antiretrovirals, Antimicrobial agents and chemotherapy, 53, 2009, 3056-3064.

33. Jayasena, S. D., Aptamers: an emerging class of molecules that rival antibodies in diagnostics, Clinical chemistry, 45, 1999, 1628-1650.

34. Khati, M., The future of aptamers in medicine, Journal of clinical pathology, 2010, jcp. 2008.062786.

35. Lee, J. F., Stovall, G. M. & Ellington, A. D., Aptamer therapeutics advance, Current opinion in chemical biology, 10, 2006, 282-289.

36. Bates, P. J., Kahlon, J. B., Thomas, S. D., Trent, J. O. & Miller, D. M., Antiproliferative activity of G-rich oligonucleotides correlates with protein binding, Journal of Biological Chemistry, 274, 1999, 26369-26377.

37. Bouchard, P., Hutabarat, R. & Thompson, K., Discovery and development of therapeutic aptamers, Annual review of pharmacology and toxicology, 50, 2010, 237-257.

38. Lupold, S. E., Hicke, B. J., Lin, Y. & Coffey, D. S., Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen, Cancer research, 62, 2002, 4029-4033.

39. Bagalkot, V., Farokhzad, O. C., Langer, R. & Jon, S., An aptamer–doxorubicin physical conjugate as a novel targeted drug-delivery platform, Angewandte chemie international edition, 45, 2006, 8149-8152.

40. Low, P. S. & Kularatne, S. A., Folate-targeted therapeutic and imaging agents for cancer, Current opinion in chemical biology, 13, 2009, 256-262.

41. Cho, K., Wang, X., Nie, S. & Shin, D. M., Therapeutic nanoparticles for drug delivery in cancer, Clinical cancer research, 14, 2008, 1310-1316.

42. Yang, T. *et al.*, Enhanced solubility and stability of PEGylated liposomal paclitaxel: in vitro and in vivo evaluation, International journal of Pharmaceutics, 338, 2007, 317-326.

43. Zhang, H., Ma, Y. & Sun, X. L., Recent developments in carbohydrate-decorated targeted drug/gene delivery, Medicinal research reviews, 30, 2010, 270-289.

44. Heinonen, I. M. & Turck, D., Dietary Reference Values for riboflavin, EFSA Journal, 2017.

45. Karande, A. A., Sridhar, L., Gopinath, K. & Adiga, P. R., Riboflavin carrier protein: a serum and tissue marker for breast carcinoma, International journal of cancer, 95, 2001, 277-281.

46. Jayapaul, J. *et al.*, FMN-coated fluorescent iron oxide nanoparticles for RCP-mediated targeting and labeling of metabolically active cancer and endothelial cells, Biomaterials, 32, 2011, 5863-5871.

47. Laskin, J. J. & Sandler, A. B., Epidermal growth factor receptor: a promising target in solid tumours, Cancer treatment reviews, 30, 2004, 1-17.

48. Acharya, S., Dilnawaz, F. & Sahoo, S. K., Targeted epidermal growth factor receptor nanoparticle bioconjugates for breast cancer therapy, Biomaterials, 30, 2009, 5737-5750.

49. Saba, N. F. *et al.*, Examining expression of folate receptor in squamous cell carcinoma of the head and neck as a target for a novel nanotherapeutic drug, Head & neck, 31, 2009, 475-481.

50. Sahoo, S. K. & Labhasetwar, V., Enhanced antiproliferative activity of transferrin-conjugated paclitaxel-loaded nanoparticles is mediated via sustained intracellular drug retention, Molecular pharmaceutics, 2, 2005, 373-383.

51. Xu, L., Pirollo, K. F., Tang, W.-H., Rait, A. & Chang, E. H., Transferrin-liposome-mediated systemic p53 gene therapy in combination with radiation results in regression of human head and neck cancer xenografts, Human gene therapy, 10, 1999, 2941-2952.

52. Minko, T., Drug targeting to the colon with lectins and neoglycoconjugates, Advanced drug delivery reviews, 56, 2004, 491-509.

53. Parveen, S., Misra, R. & Sahoo, S. K., Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging, Nanomedicine: Nanotechnology, Biology and Medicine, 8, 2012, 147-166.

54. Gao, W., Chen, Y., Zhang, Y., Zhang, Q. & Zhang, L., Nanoparticle-based local antimicrobial drug delivery, Advanced drug delivery reviews, 2017.

55. Ge, M.-Z. *et al.*, In situ plasmonic Ag nanoparticle anchored TiO 2 nanotube arrays as visible-light-driven photocatalysts for enhanced water splitting, Nanoscale, 8, 2016, 5226-5234.

56. Chen, C.-H., Chan, T.-M., Wu, Y.-J. & Chen, J.-J., Application of nanoparticles in urothelial cancer of the urinary bladder, Journal of medical and biological engineering, 35, 2015, 419-427.

57. Gradishar, W. J. *et al.*, Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil–based paclitaxel in women with breast cancer, Journal of clinical oncology, 23, 2005, 7794-7803.

58. Devalapally, H., Duan, Z., Seiden, M. V. & Amiji, M. M., Modulation of drug resistance in ovarian adenocarcinoma by enhancing intracellular ceramide using tamoxifen-loaded biodegradable polymeric nanoparticles, Clinical cancer research, 14, 2008, 3193-3203.

59. Cheng, K. K. *et al.*, Curcumin-conjugated magnetic nanoparticles for detecting amyloid plaques in Alzheimer's disease mice using magnetic resonance imaging (MRI), Biomaterials, 44, 2015, 155-172.

60. Oerlemans, C. *et al.*, Polymeric micelles in anticancer therapy: targeting, imaging and triggered release, Pharmaceutical research, 27, 2010, 2569-2589.

61. Nakanishi, T. *et al.*, Development of the polymer micelle carrier system for doxorubicin, Journal of Controlled Release, 74, 2001, 295-302.

62. Kim, T.-Y. *et al.*, Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies, Clinical cancer research, 10, 2004, 3708-3716.

63. Nasongkla, N. *et al.*, Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems, Nano letters, 6, 2006, 2427-2430.

64. Bisht, S. *et al.*, Polymeric nanoparticle-encapsulated curcumin (" nanocurcumin"): a novel strategy for human cancer therapy, Journal of nanobiotechnology, 5, 2007, 3.

65. Nishiyama, N. *et al.*, Novel cisplatin-incorporated polymeric micelles can eradicate solid tumors in mice, Cancer research, 63, 2003, 8977-8983.

66. Torchilin, V. P., Lukyanov, A. N., Gao, Z. & Papahadjopoulos-Sternberg, B., Immunomicelles: targeted pharmaceutical carriers for poorly soluble drugs, Proceedings of the National Academy of Sciences, 100, 2003, 6039-6044.

67. Yoo, H. S. & Park, T. G., Folate-receptor-targeted delivery of doxorubicin nano-aggregates stabilized by doxorubicin–PEG–folate conjugate, Journal of Controlled Release, 100, 2004, 247-256.

68. Wang, Y., Yu, L., Han, L., Sha, X. & Fang, X., Difunctional Pluronic copolymer micelles for paclitaxel delivery: synergistic effect of folate-mediated targeting and Pluronic-mediated overcoming multidrug resistance in tumor cell lines, International journal of Pharmaceutics, 337, 2007, 63-73.

69. Tomalia, D. A., Reyna, L. & Svenson, S. (Portland Press Limited, 2007).

70. Morgan, M. T. *et al.*, Dendrimer-encapsulated camptothecins: increased solubility, cellular uptake, and cellular retention affords enhanced anticancer activity in vitro, Cancer research, 66, 2006, 11913-11921.

71. Chen, H.-T., Neerman, M. F., Parrish, A. R. & Simanek, E. E., Cytotoxicity, hemolysis, and acute in vivo toxicity of dendrimers based on melamine, candidate vehicles for drug delivery, Journal of the American Chemical Society, 126, 2004, 10044-10048.

72. Malik, N., Evagorou, E. G. & Duncan, R., Dendrimer-platinate: a novel approach to cancer chemotherapy, Anti-cancer drugs, 10, 1999, 767-776.

73. Levine, D. H. *et al.*, Polymersomes: a new multi-functional tool for cancer diagnosis and therapy, Methods, 46, 2008, 25-32.

74. Song, Y. *et al.*, Identification of genomic alterations in oesophageal squamous cell cancer, Nature, 509, 2014, 91-95.

75. Ahmed, F. *et al.*, Shrinkage of a rapidly growing tumor by drug-loaded polymersomes: pH-triggered release through copolymer degradation, Molecular pharmaceutics, 3, 2006, 340-350.

76. Ortega-Velazquez, R. *et al.*, Arg-Gly-Asp-Ser (RGDS) peptide stimulates transforming growth factor β1 transcription and secretion through integrin activation, The FASEB journal, 17, 2003, 1529-1531.

77. Demirgöz, D. *et al.*, PR_b-targeted delivery of tumor necrosis factor-α by polymersomes for the treatment of prostate cancer, Soft Matter, 5, 2009, 2011-2019.

78. Christie, R. J., Nishiyama, N. & Kataoka, K., Minireview: Delivering the code: polyplex carriers for deoxyribonucleic acid and ribonucleic acid interference therapies, Endocrinology, 151, 2010, 466-473.

79. Li, J., Wang, Y., Zhu, Y. & Oupický, D., Recent advances in delivery of drug–nucleic acid combinations for cancer treatment, Journal of Controlled Release, 172, 2013, 589-600.

80. Ghaffarzadehgan, K. *et al.*, Expression of cell adhesion molecule CD44 in gastric adenocarcinoma and its prognostic importance, World journal of gastroenterology: WJG, 14, 2008, 6376.

81. Mardis, E. R. *et al.*, Recurring mutations found by sequencing an acute myeloid leukemia genome, New England Journal of Medicine, 361, 2009, 1058-1066.

82. Zhang, L. *et al.*, Nanoparticles in medicine: therapeutic applications and developments, Clinical pharmacology & therapeutics, 83, 2008, 761-769.

83. Wong, H. L. *et al.*, A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer-lipid hybrid nanoparticle system, Journal of Pharmacology and Experimental Therapeutics, 317, 2006, 1372-1381.

84. Chavanpatil, M. D. *et al.*, Surfactant–polymer nanoparticles overcome P-glycoprotein-mediated drug efflux, Molecular pharmaceutics, 4, 2007, 730-738.

85. Memisoglu-Bilensoy, E., Bochot, A., Trichard, L., Duchene, D. & Hıncal, A., Amphiphilic cyclodextrins and microencapsulation, Microencapsulation-2nd and Revised Edition, 2005, 269-295.

86. Duchêne, D., Ponchel, G. & Wouessidjewe, D., Cyclodextrins in targeting: application to nanoparticles, Advanced drug delivery reviews, 36, 1999, 29-40.

87. Lemos-Senna, E., Wouessidjewe, D., Lesieur, S. & Duchene, D., Preparation of amphiphilic cyclodextrin nanospheres using the emulsification solvent evaporation method. Influence of the surfactant on preparation and hydrophobic drug loading, International journal of Pharmaceutics, 170, 1998, 119-128.

88. Bellocq, N. C., Pun, S. H., Jensen, G. S. & Davis, M. E., Transferrin-containing, cyclodextrin polymer-based particles for tumor-targeted gene delivery, Bioconjugate chemistry, 14, 2003, 1122-1132.

89. Vicent, M. J. & Duncan, R., Polymer conjugates: nanosized medicines for treating cancer, Trends in biotechnology, 24, 2006, 39-47.

90. Müller, H.-J. *et al.*, PEG-asparaginase (Oncaspar) 2500 U/m2 BSA in reinduction and relapse treatment in the ALL/NHL-BFM protocols, Cancer chemotherapy and pharmacology, 49, 2002, 149-154.

91. Dinndorf, P. A., Gootenberg, J., Cohen, M. H., Keegan, P. & Pazdur, R., FDA drug approval summary: pegaspargase (Oncaspar®) for the first-line treatment of children with acute lymphoblastic leukemia (ALL), The oncologist, 12, 2007, 991-998.

92. Lim, W. *et al.*, Phase I pharmacokinetic study of a weekly liposomal paclitaxel formulation (Genexol®-PM) in patients with solid tumors, Annals of oncology, 21, 2009, 382-388.

Source of Support: Nil, **Conflict of Interest:** None.