Expert Opinion

Ligand-based targeted therapy for cancer tissue

Manasi Das, Chandana Mohanty & Sanjeeb K Sahoo†
Institute of Life Sciences, Nalco Square, Chandrasekharpur, Bhubaneswar, Orissa, India

Background: Limited accessibility of drugs to the tumor tissues, the requirement of high doses, intolerable cytotoxicity, the development of multiple drug resistance and non-specific targeting are obstacles to the clinical use of cancer drugs and cancer therapy. *Objective*: Drug delivery through carrier systems to cancerous tissue is no longer simply wrapping up cancer drugs in a new formulation for different routes of delivery, rather the focus is on targeted cancer therapy. *Methods*: This review summarizes the exploitation of drug-loaded nanocarrier conjugates with various targeting moieties for the delivery and targeting of anticancer drugs and describes the current status of and challenges in the field of nanocarrier-aided drug delivery and drug targeting. *Conclusion*: The discovery of targeting ligand to cancer cells and the development of ligand-targeted therapy will help us to improve therapeutic efficacy and reduce side effects. Unlike other forms of therapy, it will allow us to maintain quality of life for patients, while efficiently attacking the cancer tissue. It indicates that ligands have a pivotal role in cancer cell targeting.

Keywords: cancer, ligand, nanocarrier, targeted therapy

Expert Opin. Drug Deliv. (2009) 6(3):285-304

1. Introduction

Cancer is an ever-increasing menace that needs to be curbed. Cancer affects everyone – young and old, rich and poor, men, women, children and represents a tremendous burden on patients, families and societies. Cancer is a term used for diseases in which abnormal cells divide in an uncontrolled manner and are able to invade other tissue. It is a large and complex family of malignancies that is well known for its potentiality to affect every organ in the body. Cancer has become one of the most devastating and life-threatening diseases that causes a large number of deaths worldwide. In 2006, it was reported by the World Health Organization (WHO) that cancer caused about 13% of all deaths. According to the American Cancer Society, 7.6 million people died from cancer in the world during 2007. For many decades it was but a dream to grip such a disease, but research has now come a long way in its journey to fight against cancer.

In order to overpower this life-threatening disease several treatment modalities have been discovered to date. The predominant options for treating cancer are surgery, radiotherapy and chemotherapy. All these approaches may be successful to some extent, but they have their own drawbacks. As conventional therapy has a number of drawbacks such as the limited accessibility of drug to the tumor tissues, the requirement of high doses, intolerable cytotoxicity, the development of multiple drug resistance and non-specific targeting, there is a need to develop new modalities for cancer treatment that can specifically target the cancerous cells [1].

informa
healthcare

10.1517/17425240902780166 © 2009 Informa UK Ltd ISSN 1742-5247

Nanotechnology plays a crucial role in cancer therapy and provides a solution to the problems involved in conventional therapy. It offers the unprecedented and paradigm-changing opportunity to study and interact with normal and cancer cells in real time, at the molecular and cellular scales, and during the earliest stages of the cancer process. Leaving behind the conventional cancer treatment modalities, this technology gives opportunities to make significant advances in cancer diagnosis and imaging and offers smart drug delivery systems for tissue-specific targeting. Nano-materials are at the cutting edge of the rapidly developing era of nanotechnology. The potential of nano-drug carriers in anticancer drug delivery is infinite, with novel new applications. Several decades of biomaterials research have led to a progressively heightened interest in the use of biodegradable and biocompatible nano-vehicles for drug delivery applications [2-6]. Nano-medicine is the medical application of nanotechnology, which helps to overcome the systemic toxicity and adverse effects that result from the inability of most of the current anticancer agents to differentiate between cancerous cells and normal cells. The strength of nano-drug delivery systems lies in their ability to alter the pharmacokinetics and biodistribution of the drugs [7].

Nanotechnology has now changed the definition of drug delivery entirely and evolved it as targeted drug delivery. Drug targeting is defined in the broadest sense, that is to optimize a drug's therapeutic index by strictly localizing its pharmacological activity to the site of action. Targeted medication employs the utilization of homing devices termed as ligands, which can bind to specific molecular signatures expressed on the surface of the neoplastic mass of cells [8]. For pinpoint cancer targeting, several substances such as antibodies, antibodies fragments, aptamers, lectins, peptides, transferrin and vitamins like folic acid can be used as targeting moieties for ligand-based cancer therapy. These ligands recognize and bind specific markers expressed on the targeted tumor cells. The result of such recognition is that active drugs carried by the ligand-conjugated nanocarriers may be delivered only to those cells expressing the appropriate receptor for the targeting moieties with high precision. Normal cells will not be recognized by the targeted nanocarrier, and thus not harmed by them. Overall, it is evident that with a biologically active agent of reasonable activity at hand, targeting to the site of action should be superior to other conventional therapeutic strategies. In this review, we discuss different ligand-conjugated nanocarriers for targeted cancer therapy, mostly emphasizing the ligand-conjugated nanoparticle system.

2. Drug targeting and its significance

Drug targeting is a strategy aimed at the delivery of therapeutic compounds to a particular tissue of the body [8]. The basic approach of targeted drug delivery involves passive targeting and active targeting. A passive targeting strategy takes advantage of anatomical differences between cancerous tissue and normal tissue, whereas an active targeting approach relies on the principle of ligand receptor recognition. Advances in cancer tissue anatomy, drug receptor concept and specific knowledge about the various receptors suggest that this is the direction that should be taken for drug targeting. Achieving effective treatment with a minimum of side effects is the most important goal of a targeted drug delivery strategy. A tremendous amount of work has been concentrated worldwide in the past two decades on the research and development of drugs with improved site-specificity, that is targeted drug delivery systems. Targeted drug delivery has tremendous significance in the field of cancer therapy. The major limiting factors that result in poor prognosis for cancer patients are: low bioavailability, rapid drug clearance, multidrug resistance, significant toxicity, undesirable side effects and unrestricted biodistribution of anticancer drugs [9,10]. A targeted delivery approach helps to overcome some of the above-mentioned limitations to some extent and enhances the activity of the drug [11]. For example in chemotherapy, anticancer drugs reach the tumor tissue with poor specificity and dose-limiting toxicity due to conventional methods including oral and i.v. routes. Oral administration of drugs results in poor pharmacokinetics due to the exposure of these agents to the metabolic pathways of the body [12,13]. This results in low bioavailability of the chemotherapeutic agent due to its degradation. To increase the bioavailability of the drug, larger than necessary doses are administered, which can further cause increased toxicity [14]. The traditional i.v. route often shows harmful effects to healthy tissue due to the non-specific nature of some anticancer drugs. Targeting cancer cells using nanocarriers loaded with anticancer agents are a promising tactic that could help to overcome these challenges associated with low bioavailability and dose-dependent toxicity to some extent, as encapsulating the drug in a carrier system prevents it from unwanted exposure to the body's metabolic pathways [15]. The problem of unrestricted biodistribution of anticancer drugs can be defeated by passively or actively targeting the drug-loaded carrier system to cancerous tissue. If an efficacious compound has a short half-life in circulation, its stability can be increased tremendously by encasing it within nanocarriers [16].

P-glycoprotein (P-gp) transporter and multidrug resistance protein (MRDP) family related multi-drug resistance is another major problem that contributes to the resistance of tumors to anticancer agents. These transporters are *trans*-membrane proteins capable of pumping out many anticancer drugs that diffuse into the plasma membrane. Because these transporters recognize drugs in the plasma membrane, drug-loaded nanocarriers bypass this mechanism and are able to release the drug within the cytoplasm or endosomal vesicles [17,18]. Many first generation MDR modulators, such as verapamil cyclosporin A, curcumin inhibits P-gp-induced drug efflux in cancer, but it requires a near-toxic level to give the desired effect [19-21]. So encapsulating MDR modulator drug

into Nps and targeting could help to overcome P-gp efflux in drug-resistant tumors. Khdair *et al.* demonstrated the success of this strategy in nanoparticle-mediated combination chemotherapy and photodynamic therapy using doxorubicin and methylene blue, which is able to overcome resistance mechanisms and resulted in improved cytotoxicity in drug-resistant tumor cells [22]. Similarly, researchers from Winchip Cancer Institute, Atlanta are actively engaged in the development of tumor-targeted Taxol-delivering nanoparticles (Phase Ib clinical trial) with folate ligand to overcome drug resistance in the treatment of head and neck cancers. Tumor cells can acquire drug resistance by the alteration of pathways involved in the regulation of apoptosis and that failure to activate this pathway in cancer cells may confer resistance to chemotherapy. This can be overcome by gene therapy strategies that specifically target the abnormally functioning apoptic pathways that are not adequately controlling cell growth and differentiation. Targeted delivery of gene to tumor cells through nano-vehicles can helps to mitigate this problem to some extent [23].

3. Nanotechnology and its application in drug delivery and targeting

In cancer therapy, targeting and localized delivery of the drug to the cancer tissues are the key challenges. To wage an effective war against cancer the treatment approach should have the ability to selectively attack the cancer cells, while saving the normal cells from excessive burdens of drug toxicity. However, because many anticancer drugs are designed to simply kill cancer cells, often in a semi-specific fashion, the distribution of anticancer drugs in healthy organs or tissues is especially undesirable due to the potential for severe side effects. Consequently, systemic application of these drugs often causes severe side effects in other tissues which greatly limit the maximum allowable dose of the drug. In addition, rapid elimination and widespread distribution into non-targeted organs and tissues requires the administration of a drug in large quantities, which is often not economical and sometimes complicated due to non-specific toxicity. Although conventional chemotherapy has been considered as one of the main modalities of treatment for cancer patients, its triumph is largely hindered due to inadequate accessibility of antineoplastic agents to tumor tissue, their intolerable concentration-dependent systemic toxicity, requiring high doses, rapid abolition, poor solubility and inconsistent bioavailability [24]. Thus to mitigate the difficulty associated with conventional chemotherapy there is a call for developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects.

Nanotechnology is expected to play a critical role in creating a novel and efficient drug delivery system that can overpower the problems associated with conventional cancer treatment [25]. The application of nanotechnology to drug delivery is widely expected to create novel therapeutics capable of changing the landscape for pharmaceutical and biotechnology industries. Nanotechnology is defined as the science and engineering involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization in at least one dimension is in the nanometer scale, or one billionth of a meter [7,26,27]. Nanotechnology is the science that touches every corner of life starting from nano-scale devices helping in disease diagnosis to nano-particulate drug delivery systems helping in evading life-threatening diseases like cancer. A complete list of the potential applications of nanotechnology is too vast and diverse to discuss in detail, but without doubt one of the greatest values of nanotechnology will be in the development of new and effective medical treatments [26,28-31]. The advent of nanotechnology coupled with a better understanding of various issues involved in drug delivery and targeting such as pharmacokinetics, pharmacodynamics, immunogenicity, biorecognition and efficiency of drugs has opened up new avenues for improving the drug delivery systems. Various type of nano-scale drug delivery systems such as liposome, micelles, dendrimers, nano-tubes and nanoparticles (NPs) are the reward of nanotechnology (**Figure 1**) [11,16,32,33]. Such competent drug carriers are so engineered with the hope that they can act as likely candidates to carry therapeutic agents safely into a specific compartment in an organ, cell or tissue. In the field of drug delivery and cancer therapy, these carrier systems are actively engaged in improving the stability, solubility, absorption and therapeutic action of the drug within the target tissue and permit long-term release of the antineoplastic drug [25,34]. They offer protection and improve the pharmacokinetics of various drugs and help to overcome the systemic toxicity and adverse effects that result from the non-selective nature of most current cancer therapeutic agents. However, despite this versatility, some of the carrier systems are associated with major drawbacks. For example, liposomes show poor control over drug release, low encapsulation efficiency, poor stability during storage, etc [5,35]. But such problems can be conquered to some extent by surface modification of the nanocarrier or by adopting other improved nanocarriers like polymeric nanoparticles. Besides generating novel pharmaceutical delivery systems, nanotechnology also has a dramatic impact on targeted drug delivery. This technology takes advantage of fundamental cancer morphology, molecular principles like ligand receptor interaction, and exploits such strategies in creating a ligand-conjugated nanocarrier system that can specifically target for diagnosis, drug delivery and treatment of the cancerous tissue.

4. General principle of drug targeting to cancer

The targeting of drugs to tumor tissue offers enormous advantages but is equally challenging. The delivery of the drugs to the target tissue can be achieved primarily in two basic ways: i) passive targeting; and ii) active targeting. A

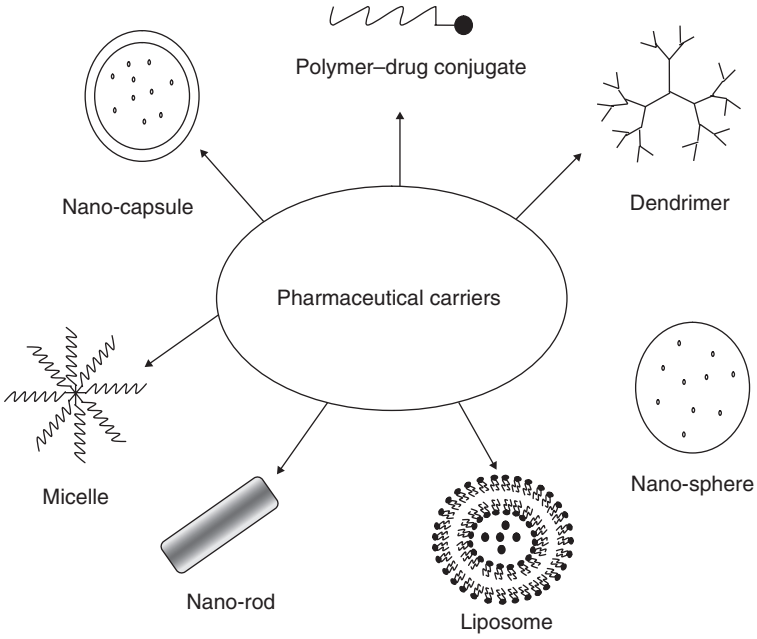


Figure 1. Pharmaceutical carriers for drug targeting.

clear idea regarding the different strategies adopted for drug targeting is given in **Figure 2**.

4.1 Passive targeting

Passive targeting refers to the accumulation of a drug or drug carrier system at a desired site due to physicochemical or pharmacological factors [15,36]. Passive targeting approaches make use of the anatomical and functional differences between the normal and tumor vasculature to deliver the drug to a targeted site, or may include localized delivery [37]. The tumor vasculature is very different from normal tissue. It is greatly heterogeneous in distribution, larger in size, has high vascular density and is more permeable and leaky, unlike the tight endothelium of normal blood vessels [37-39]. The leaky and defective architecture of tumor vasculature may be due to elevated levels of vascular mediators such as bradykinins, nitric oxide, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), prostaglandins, etc [39-43]. This leaky vasculature allows extravasation of circulating nanocarriers within the tumor interstitium. This is called the enhanced permeability effect. This, coupled with the impaired lymphatic drainage of macromolecules in solid tumors, allows an enhanced accumulation and retention of high molecular weight drugs in solid tumors. This is popularly known as the enhanced permeability and retention (EPR) effect (**Figure 3**). The EPR effect can predominantly be used for passive targeting of drugs encapsulated in drug carriers such as polymeric drug conjugates, liposomes, polymeric NPs and the micellar system to solid tumors [39,43]. Because of the EPR effect, the concentration of polymer drug conjugates in tumor tissue reaches a level of 10 – 100 times higher than that which could be achieved with the administration of the same dose of free drug [12].

4.2 Active targeting

Active targeting takes advantage of the difference between cancer cells and normal cells in terms of receptor and antigen expression. Various cell surface receptors like folate receptors and some of the surface bound antigens like prostate-specific membrane antigens (PSMA) are expressed uniquely only in cancer cells or highly in diseased cells as compared to normal cells [15,44]. Active targeting usually employs surface conjugation of the NPs with a targeting moiety called a ligand, having a selective or preferential affinity for surface receptor or antigen on specific cells, tissue or organs in the body [45]. Active components such as ligands for the receptors and antibodies to the surface proteins can be employed extensively to target specific cells (**Figure 4**). Very rarely will antibody (Ab) show cross-reactivity with normal tissue antigens; in general targeted antigens that are not cross-reactive with normal tissue antigens are chosen. Although antibodies have some degree of cross-reactivity with other cell surface molecules, this attribute can be compromised for targeted delivery of anticancer drugs. In addition to ligands, cell surface receptors and antigens also have a dramatic impact on active targeted therapy. For the optimum delivery of drugs, receptor and antigen should be present in abundance on the surface of the tumor tissue [46,47] and there should be a mechanism by which, once the active drug is released into the cell, the receptor or antigen is recycled back onto the surface of the cancer cells. This approach allows preferential accumulation of the drug

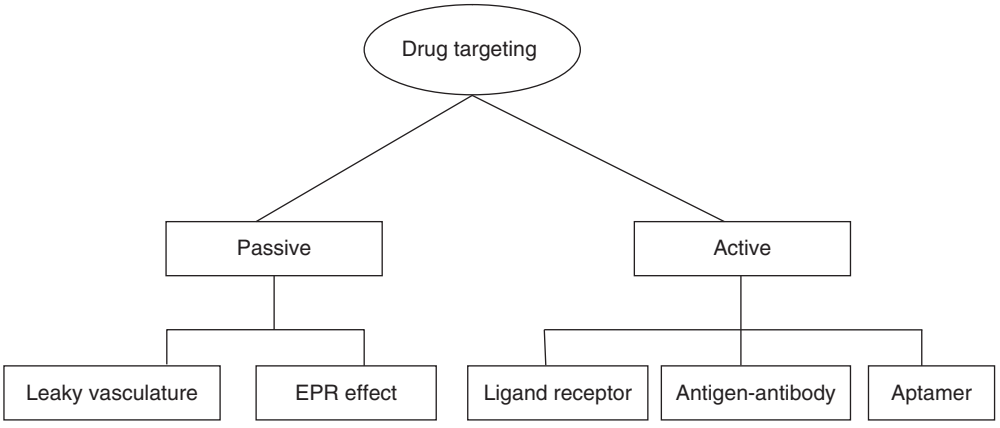
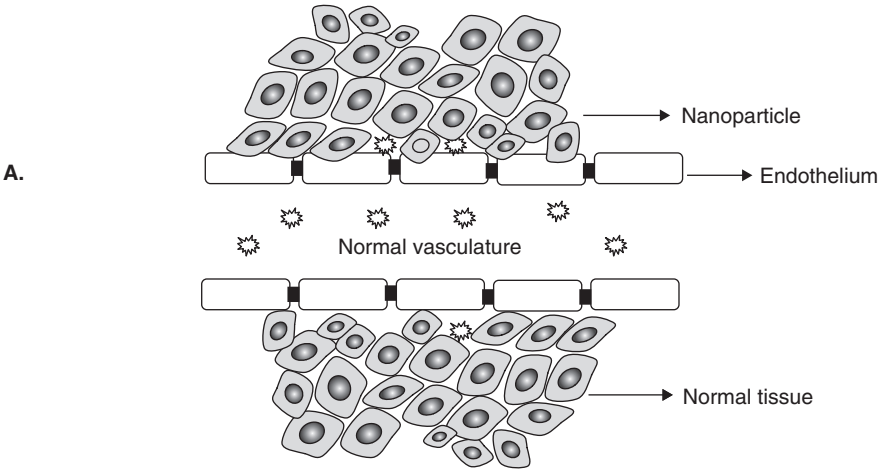


Figure 2. Different drug targeting systems.



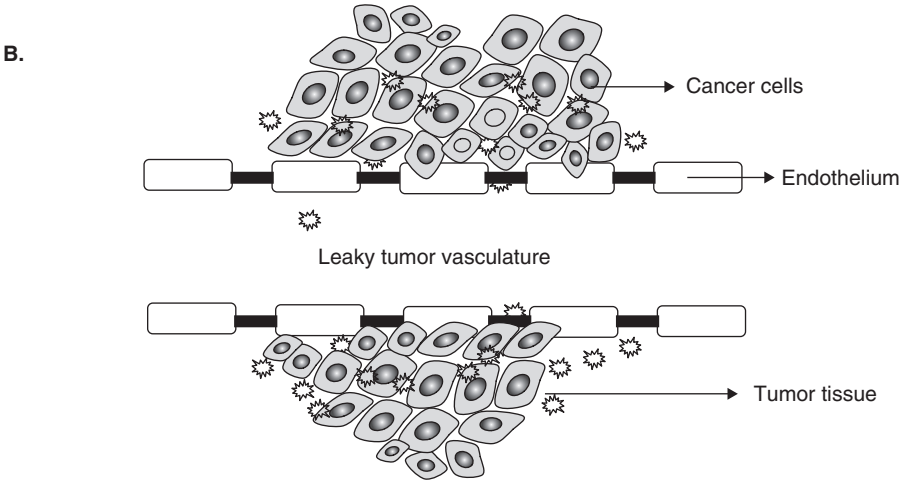


Figure 3. A depicts the normal vasculature, where due to the tight junction, drug-loaded NPs are not able to extravasate, while B depicts an increased accumulation in tumor due to leaky tumor vasculature leading to the enhanced permeability and retention effect.

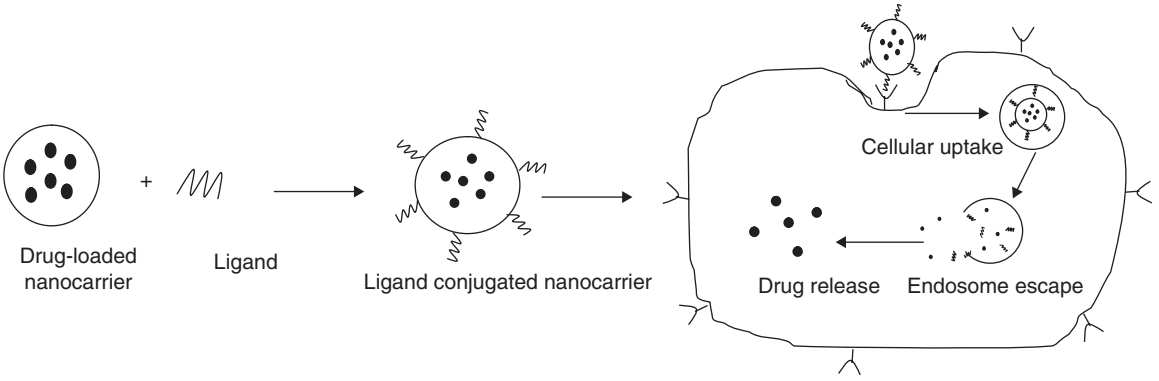


Figure 4. Active targeting mediated by ligands.

in the tumor tissue within individual cancer cells, intracellular organelles or specific molecules in cancer cells [1,3,12].

5. Ligand-based targeting

Medication that can selectively target tumors and at the same time avoid access of the drug to non-target areas employ homing devices termed ligands. These include any molecule that recognizes and binds to target antigen or receptors overexpressed or selectively expressed by particular cells or tissue components. An ideal ligand for targeted delivery would be one with high avidity; specificity of binding to the cell surface receptors; should stimulate the internalization of polymeric particles that need internalization for its intracellular action; should be compatible for chemical modification for conjugation; and can be produced in sufficient quantity [12,48-50]. For effective interaction of ligands with its receptor, the ligand density should be low [51]. Ligands also have a significant impact on drug release kinetics. The release profile of ligand-conjugated nanocarriers is much lower than unconjugated ones. In spite of such lacunae, targeted nanocarriers are more effective than unconjugated nanocarriers, as drug from the former is able to reach its target site more efficiently because of the targeting moiety [52]. The targeted drug delivery is non-toxic and safe to normal tissue as the expression of the receptor to its specific ligand is limited. For example, folate receptor in normal tissue is restricted on the apical membrane of polarized epithelia, unlike the cancer tissue. Numerous nanoparticle-based drug delivery and drug targeting systems have been developed or are currently under development. Their use aims to minimize drug degradation and inactivation upon administration, prevent undesirable side effects, increase drug bioavailability and the fraction of drug delivered in the pathological area. The precise and selective binding of ligand to its receptor makes tumor specificity and limited toxicity possible and may overcome obstacles presented by cytotoxic chemotherapy [11]. The different ligand-based targeting to cancer tissue is illustrated below.

5.1 Transferrin-based targeting

Transferrin (Tf) is a β-globulin (β-glycoprotein) which facilitates the transport of ferric ion (Fe^{3+}) through transferrin receptors on the plasma membrane. The intracellular delivery of Fe^{3+} is mediated via receptor-mediated endocytosis. In short, the iron-free form, apotransferrin, binds two Fe^{3+} ions very tightly to form ferrotransferrin. The cells surface transferrin receptors bind avidly to ferrotransferrin at neutral pH, after which the receptor-bound ferrotransferrin is subjected to endocytosis [53]. The unique ability of apotransferrin is to remain bound to the transferrin receptor at the low pH (5.0 – 5.5) of late endosomes. At a pH of less than 6.0, the two bound Fe^{3+} atoms dissociate from ferrotransferrin and are transported from the late endosome vesicle into the cytosol (in an unknown manner). The apotransferrin formed by the dissociation of the iron atoms remains bound to the transferrin receptor and is recycled back to the surface along with the receptor. Remarkably, although apotransferrin binds tightly to its receptor at a pH of 5.0 or 6.0, it does not bind at neutral pH. Hence, the bound apotransferrin dissociates from its receptor when the recycling vesicles fuse with the plasma membrane and the receptor–ligand complex encounters the neutral pH of the extracellular interstitial fluid or growth medium. The surface receptor is then free to bind another molecule of ferrotransferrin. This strategy can be exploited for targeted therapy. Moreover, Tf receptors (Tfr) are overexpressed by 2 – 10-fold in most of the tumor cells, unlike in normal cells [54,55]. It has been reported that Tfr are highly expressed in lung cancer, lymphoma and breast cancer [56]. The average number of Tfr is reported to be 10^5 per cell in RILQ T-lymphoma cells, but less than 5×10^4 per cell in thymus and 5×10^4 per cell for spleen [57]. Enns *et al.* reported the high affinity for binding of Tf to Tfr [58]. Thus, Tf may be used efficiently for targeting drugs to tumor cells expressing Tfr [8,59]. This approach has been specifically investigated for the delivery of therapeutic agents to the brain, as Tf ligand facilitates the transcytosis of conjugated drug carrier systems across the blood–brain barrier (BBB). Another motivation of using Tf as a ligand is its

potential to overcome drug resistance due to pg-glycoprotein. It has easily coupled to the delivery vehicle by its amino group [60]. Transferrin acting as ligand has a significant role in treating different cancers, including breast cancer. Sahoo *et al.* studied solely the molecular mechanism of greater efficacy of paclitaxel (Tx)-loaded, Tf-conjugated NPs in breast cancer cell line. Tf-conjugated NPs at the lowest dose of the drug (1ng/ml) demonstrated a greater and sustained antiproliferative activity when compared with free drug or unconjugated NPs in MCF-7 cells; the greater anti-proliferative activity of the drug with conjugated NPs was due to their greater cellular uptake and reduced exocytosis compared with that of unconjugated NPs [60]. In another study the same authors showed the effectiveness of Tf-conjugated, Tx-loaded NPs in a murine model of prostate cancer [61]. The intracellular uptake of Tf-conjugated NPs was found to be about three times higher than that of unconjugated NPs in PC3 cells. In addition, Tf-conjugated, Tx-loaded NPs show greater anti-proliferative activity as compared to Tx-Solution and Tx-NPs. In a murine model, a single dose (24mg/kg) intra-tumoral injection of Tx-NPs-Tf demonstrated complete tumor regression and a greater survival rate than those that received either Tx-NPs or Tx-Cremophor® (Sigma-Aldrich Co, USA) EL formulation.

Recently, Pulkkinen *et al.* explored three-step tumor targeting of Tx using biotinylated polylactide acid (PLA)-polyethylin glycol (PEG) NPs and avidin–biotin technology. Tx was incorporated both in biotinylated (BP) and non-biotinylated (LP) PEG-PLA nanoparticles. BP nanoparticles targeted to brain tumor (glioma) cells using transferrin as the targeting ligand shows promising results as compared to native Tx and non-targeted BP and LP nanoparticles [62]. Similarly Tf enables targeting of therapeutic proteins and peptides to tumor tissue. Tf-conjugated PEG–protein system improves tumor-targeted delivery of PEG–protein conjugate. A model protein, beta-lactoglobulin B (LG), was modified by the hetero-bifunctional PEG. Tf covalently linked to the distal end of the PEG chains on the PEG–LG (PL) conjugate. Transferrin conjugate, that is Tf-PEG-LG (TPL), can bind specifically to the Tf receptor on the tumor cell surface with affinity similar to that of native Tf. The pharmacokinetics and biodistribution studies in rodents shows that TPL exhibited a significantly delayed blood clearance, the longest tumor residence time and the greatest tumor accumulation, as compared with LG and PL. Such Tf conjugate nanocarriers suggest a promising approach for active targeting of therapeutic proteins and peptides to target tissue [63]. Artemisinin and its derivatives are well known for their anti-malarial activity [64,65]. The cytotoxic effect of artemisinin is specific to cancer cells because most cancer cells express a high concentration of transferrin receptors on the cell surface and have higher iron ion influx than normal cells via transferrin mechanism. Artemisinin tagged to transferrin via carbohydrate chain has been shown to have high potency and specificity against cancer cells. The conjugation facilitates targeted delivery of artemisinin into cancer cells [66]. Selenium-polypyrrole core-shell nanoparticles functionalized with transferrin have been shown to play an active role in the targeting and imaging of human cervical cancer cells. The presence of transferrin molecules on the surface of the core-shell NPs significantly enhances their cellular uptake [67]. Thus transferrin-conjugated core-shell NPs can be potentially used for the targeting and imaging of tissue.

Tf-conjugated nanocarriers are supposed to be more efficient than unconjugated nanocarriers. This is because a major fraction of internalized unconjugated nanocarrier undergoes rapid exocytosis, which may be due to their inefficient escape from the endosomal compartment to the cytoplasmic compartment during their transit through the cell. Tf-conjugated nanocarriers could have a different intracellular sorting pathway following their uptake via Tfr than that of unconjugated nanocarriers via nonspecific endocytosis. This difference in the uptake and sorting pathways of conjugated and unconjugated nanocarriers in turn influences the intracellular retention of nanocarriers as well as the therapeutic efficacy of the encapsulated agent [60]. Thus it is believed that Tf-conjugated nanocarriers are good candidates for targeted therapy.

5.2 Vitamin-based targeting

Many research groups highlighted the utility of using vitamins as a targeting ligand for drug delivery. The vitamins used as targeting moieties may include folate, vitamin B_{12} (VB_{12}), biotin and thiamine. Folic acid (FA) is a vitamin (MW-441 Da) required for the synthesis of purines and pyrimidines and is needed by a variety of tumor cells [68,69]. Folic acid could be exploited as a potential targeting device for cancer tissue, as it is stable in storage and circulation, inexpensive, non-toxic and non-immunogenic compared with proteins such as monoclonal antibodies. Secondly, it can be conjugated easily to the carrier, and has a very high affinity ($K_d \sim 1$nM) for folate receptor (FR) [70-72]. FR is a highly specific tumor marker that is overexpressed in many cancers (ovarian cancer, choriocarcinoma, uterine sarcomas, osteocarcinomas) [8]. It has acquired considerable attention for drug targeting purposes since it is absent in most normal tissue, with the exception of the placenta, choroids plexus and low levels in the lungs and kidney [73]. The FR has two glycosyl phosphatidylinositol (GPI)-anchored isoforms, α and β. FR-α expression is frequently amplified in epithelial cancers, whereas FR-β expression is found in myeloid leukemia and activated macrophages associated with chronic inflammatory diseases. Nanocarriers conjugated with FA can be taken up by cancer cells via receptor-mediated endocytosis, thus providing a mechanism for targeted delivery to FR-positive cells.

Recently, it has been reported that doxorubicin (Dox)-loaded folate-decorated poly(lactide-co-glycolide) (PLGA)–vitamin-E TPGS NPs can act as a potential candidate for targeted

chemotherapy of tumors overexpressing FR [12]. The cellular uptake and cell viability of the drug-encapsulated NPs were found to depend on the content of targeting TPGS-FA conjugate. It has been shown that the cellular uptake of PLGA-TPGS-FA NPs was 1.5 and 1.7 times higher in MCF-7 and C-6 cells, respectively, as compared with NPs with no TPGS-FA component. Moreover PLGA-TPGS-FA NPs also resulted in higher cytotoxicity. In another study, Pasut *et al.* studied the antitumoral activity of PEG–gemcitabine prodrugs targeted by FA. The antiproliferative activity of unconjugated PEG–gemcitabine was evaluated on HL60, HeLa, HT-29 and MCF-7 cell lines, while PEG-gemcitabine–folate conjugates were tested in KB-3-1 cells that overexpress the FA receptor. The targeted conjugates showed a higher antiproliferative activity and a higher selectivity than the non-targeted ones in KB-3-1 cells. Although conjugated NPs show decreased cytotoxicity in HT-29 cells, they did not show any decline in cytotoxicity going from HT-29 to KB-3-1, as in the case of free gemcitabine and unconjugated NPs. This indicates that targeted conjugates need a receptor-mediated endocytosis mechanism for cell penetration and are more selective than unconjugated particles as well as free gemcitabine in KB-3-1 cells [74]. Docetaxel (DTX) NPs conjugated with FA is another approach in folate receptor-targeted anticancer therapy. The FA-targeted NPs showed an increased level of intracellular uptake in FR positive cancer cells (SKOV3) in comparison with the non-targeted NPs, indicating that the FR-mediated endocytosis mechanism could have a role in the cellular uptake of NPs. Thus FA-targeted DTX NPs could be a potentially useful delivery system for FR positive cancer cells [75].

The role of VB_{12} in cancer treatment is a topic of current interest. Cancer cells have a need for methionine in protein production, for which they crave VB_{12} that converts homocysteine to methionine. To satisfy their cellular need for VB_{12}, tumor cells produce excessive amounts of the receptor for VB_{12}. Thus VB_{12} and its receptor interaction can be exploited for targeted therapy. The oral uptake of VB_{12} takes place via receptor-mediated endocytosis. In this mechanism VB_{12} is first bound to intrinsic factor (IF), a VB_{12}-binding protein present in the small intestine. The VB_{12}-IF complex then binds to an IF receptor located on the surface of intestinal epithelial cells and VB_{12} is subsequently transported across the cell. It can also be transported through transcobalamin II (TcII), another VB_{12}-binding protein, in an IF-independent fashion. Recently, it has been shown that the uptake mechanism for VB_{12} can be exploited to enhance the oral uptake of various peptides and proteins. Chalasani *et al.* considered VB_{12} as a model molecule for targeting the peptide or protein encapsulated NPs across caco-2 cells. In their study they have shown that VB_{12}-modified NPs showed a higher level of uptake, whereas a smaller degree of binding, uptake and transport of uncoated NPs takes place by caco-2 cells. The addition of IF enhanced the cellular uptake of VB_{12}-modified NPs but no such increase occurred in uncoated particles. The result shows that enhanced cellular uptake takes place in VB_{12}-modified NPs through IF-dependent or IF-independent mechanisms and the addition of IF to VB_{12}-modified NPs increases the uptake to a smaller extent. The potential efficacy of a peptide and protein tagged VB_{12} carrier system for oral delivery has been further investigated [76,77].

5.3 Lectin-based targeting

The gastrointestinal route is the most convenient and attractive method for systemic delivery of drugs. It affords ease of administration, high acceptability, compliance, painless administration and is cost effective. From a technological point of view, however, it is one of the most challenging routes of administration because of numerous barriers present on the gastrointestinal surface that counteract successful drug delivery. The interplay between digestive fluids and peristalsis dilute greatly interfere with the residence time of drugs and drug carriers at the site of attachment, leading to poor absorption. Moreover, the acidity of the gastric juice, luminal enzymes and brush border hydrolases can degrade the drug, peptide and protein. All in all this can result in poor bioavailability [78].

Cytoadhesive ligands that show higher affinity for carbohydrate moiety can be explored to mediate an adhesive interaction between the particle and biological surface. Lectin presents a second generation bioadhesive that is capable of recognizing cell mucosal surface receptors. Lectins are proteins or glycoproteins, moderately specific, having high binding capacity with their sugar moieties and are appropriate for targeting drugs. Several lectins have been found to possess anticancer properties *in vitro*, *in vivo* and in human case studies. They are used as therapeutic agents by preferentially binding to cancer cell membranes or their receptors causing cytotoxicity, apoptosis and inhibition of tumor growth. Thus lectin–carbohydrate interaction can be explored for the development of lectin-conjugated nanocarriers [79]. The selection of suitable lectins allows cell-specific targeting. For example the high affinity of wheat germ agglutinin (WGA) for the brain surface helps to target conjugated nanocarriers without disruption to the barrier properties of the brain endothelia [80,81]. Asialoglycoprotein receptor expressed on the surface of hepatocytes is another example of endogenous lectin that can be exploited for drug targeting by galactose-bearing drug carrier systems [82].

Lectin-conjugated PLGA NPs, loaded with thymopentin (TP5) for oral delivery, were studied by Yin *et al.* in 2006 where they encapsulated an aqueous soluble TP5 into WGA-conjugated PLGA nanoparticle. The *in vitro* release profiles of TP5 shows a higher release in WGA-conjugated NPs as compared to non-conjugated NPs, which may be due to the hydrophilic attributes of WGA. The *in vivo* pharmacodynamic studies in immune-suppressed rat by FACScan flow cytometry indicate that WGA-TP5-NPs can improve the oral absorption of TP5 compared with conventional

TP5-NPs and TP5 solution. The enhanced uptake was due to increased content of WGA on NPs. The content of WGA in NPs not only affects the dissolution of TP5 from NPs, but also has an important influence on the oral uptake of TP5 [83]. Thus, lectin-conjugated NPs offer an effective and promising oral delivery system to improve the absorption and bioavailability of poorly absorbable drugs.

In order to improve the absorption of peptides and protein drugs in the brain surface, engineered NPs with lectins have opened a novel pathway. Gao *et al.* conjugate WGA to the surface of PEG-PLA NPs for delivering drugs to brain cells. The brain cell uptake of WGA-functionalized NPs was found to be about twofold higher compared to unmodified NPs. Thus the approach offers a novel, effective system for brain drug delivery [84]. Another study by Mo *et al.* developed a novel lectin-conjugated isopropyl myristate (IPM) incorporating PLGA NPs to potentiate the anticancer activity of paclitaxel. These NPs had superior *in vitro* cytotoxicity against malignant AS-49 and H1299 cells as compared with Tx-loaded NPs without IPM or WGA or Tx-loaded NPs with only IPM or WGA. These NPs exhibit a stronger cell-killing effect due to more efficient cellular uptake via WGA receptor-mediated endocytosis and IPM-facilitated release of TX from the NPs [85].

Lectin-based targeted systems have several advantages. For example the cytoadhesive properties of the lectin and the protective effects of the nanocarrier can be combined by the conjugation of lectin to the surface of nanocarriers, which might result in improved bioavailability of the formulation [86]. Lectins possess anticancer properties, which is an additional quality that enhances the quality of cancer therapy. In summary, a lectin-based drug delivery approach opens a new door for the better absorption of colloidal carriers incorporating non-replicating antigen for oral vaccination and drug molecule, peptide, protein, therapeutic DNA, that are either susceptible to gastrointestinal degradation or those which are poorly absorbed.

Despite its merits, lectin-based targeting has some lacunae, in that it possesses some degree of toxicity. About 0.5 – 5% of lectin in red kidney beans causes diarrhea and growth reduction. However the amount of lectin necessary for glycotargeting of colloidal carrier systems is in the microgram range, so that toxic effects should not be provoked. So toxicity associated with lectin could be negated to achieve targeted therapy. Lectin also elicits an immune response. Oral administration of tomato lectin can provoke the formation of specific antibodies in mice and humans [87,88]. But WGA, red kidney bean lectin elicits low or no specific immune response [89]. Glycotargeting with lectins may be affected by food-derived glycans. It is likely that glucose or galactose-targeted drug delivery systems are neutralized by food stuffs, but a formulation grafted with WGA or lectins specific for complex glycan are not expected to be influenced [78]. Although lectin possesses some limitations, the hurdles associated with targeted therapy may be overcome by using WGA or other glycan-specific lectins to a certain degree. A targeting approach mediated by lectin is a promising mode of oral delivery of drugs, but many scientific problems and technical issues associated with it needed to be solved.

5.4 Epidermal growth factor-based targeting

Epidermal growth factor (EGF) is a small mitogenic protein that is thought to be involved in mechanisms such as cell growth, transcription, proliferation, oncogenesis and wound healing. Epidermal growth factor receptors (EGFR) are highly expressed – that is 100-fold greater than normal cells – in a variety of human cancers such as lung, prostate and breast and hence they provide a rational target for anticancer drugs [90,91]. EGFR are the proteins found on the surface of some cancer cells and to which epidermal growth factor binds with high affinity, causing the cells to divide. The EGFR family comprises four members: EGFR, HER2, HER3 and HER4 [92]. The binding of EGF to EGFR activates intrinsic protein–tyrosine kinase activity and activation of tyrosine kinase promotes cell growth. The drug carrier systems decorated with EGF as ligand can act as smart vehicle for targeting. For example several EGF-conjugated liposome-based formulations have been investigated to overcome problems such as lower degree specificity and low intracellular drug accumulation [93-96]. It has also been reported that upon binding of EGF to the binding domain of cell surface EGFR, dimerization and clustering of the EGF–EGFR complexes occurs rapidly at the cell surface. The EGF–EGFR complexes are then internalized into the cell [97,98]. This receptor–ligand mediated internalization has been shown to effectively allow for the delivery of radiopharmaceuticals and liposome-encapsulated agents into EGFR-overexpressing cells [96,99]. A few groups have demonstrated that the cell surface EGFR is able to translocate into the nucleus via a specific endocytotic pathway following stimulation of the cells with EGF [99-101]. This is an advantage over other receptors that are not capable of nuclear translocation. Moreover, the internalization of the EGF is reported to occur faster than that of the anti-EGFR antibody in EGFR-overexpressing cells, so it provides effective treatment over antibody-mediated targeted therapy [102].

In a recent study Zeng *et al.* developed an EGF-conjugated poly(ethylene glycol)-block–poly(S-Valerolactone) copolymer micelles to EGFR overexpressing cancer cells. An enhanced intracellular uptake of targeted micelles containing CM-Dil (hydrophobic fluorescent probe) occurs in EGFR overexpressing MDA-MB-468 breast cancer cells, while no detectable cell uptake was observed for the non-targeted micelles. The results suggest that EGF-conjugated copolymer micelles have the potential to act as vehicles for targeting hydrophobic drugs to EGFR overexpressing cancer cells [103]. Tseng *et al.* exploited the merits of using EGF as a targeting moiety and studied the targeting efficiency and biodistribution of biotinylated-avidin-EGF-conjugated gelatin NPs (GP-Av-bEGF). In order to develop an effective drug delivery vehicle for

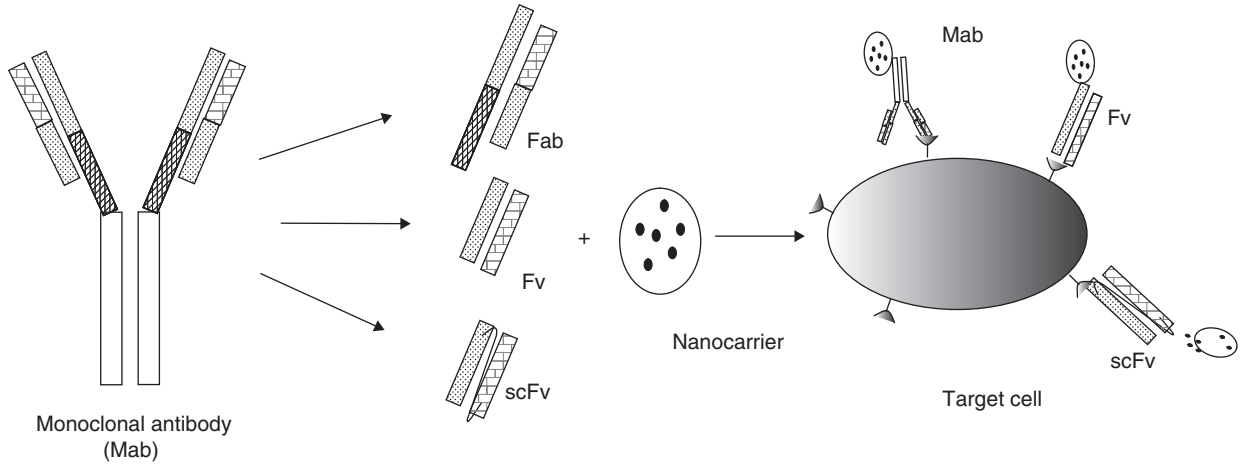


Figure 5. Parts of an antibody acting as targeting ligands.

lung cancer therapy, they prepared gelatin nanoparticles (GPs) modified with biotinylated growth factor. The distribution profile of the GP-Av-bEGF in mice model represents a 3.6-fold increased accumulation of GP-Av-bEGF in cancerous lung compared to normal lung [104]. In another study GP-Av-bEGF was used to detect lung adenocarcinoma and found that A549 cells treated with GP-Av-bEGF shows higher internalization of conjugated NPs with homogenous distribution in cytoplasm, implying that GP-Av-bEGF undergoes receptor-mediated endocytosis [105]. Human epidermal growth factor acts as a vital ligand to target cancer cells overexpressing epidermal growth factor receptor. Even if EGF is a bonafide ligand for targeting EGFR overexpressing cells, it does have some limitations. For example the binding affinity of EGF to its receptor is extremely sensitive to its conformation. To preserve the conformation of EGF, which is necessary for active targeting, one has to carry out the EGF-copolymer coupling reaction in the absence of any organic solvent [103].

5.5 Antibody-based targeting

Immunotherapy has been explored with the discovery of the structure of antibodies and the development of hybridoma technology. These advances allowed for the specific targeting of tumors both *in vitro* and *in vivo* [106]. Modern antibody technology involves design and preparation of fragments of antibodies F (ab)2, Fab, Fv, and single chain Fv (Sc Fv) against almost any tumor antigen (**Figure 5**). Due to their transformed nature, cancer cells overexpress many known protein biomarkers or express some unique proteins that have been named as tumor-associated antigens (TAA) [106,107]. These TAAs provide important insights for antibody-mediated targeting. Of all the coupled homing devices, antibody-coupled nanocarriers can be regarded as a very attractive drug targeting system due to such advantageous properties as specificity and stability towards the biological system [108]. A number of targeted cancer treatments using antibodies for specific cancer types have been approved by the FDA and are summarized in **Table 1**.

Receptors have gained a lot of attention for antibody-mediated targeting for cancer therapy. Growth factor receptors are predominantly overexpressed in cancer cells. Vascular endothelial growth factor (VEGF) is a key mediator of tumor angiogenesis. Blocking angiogenesis is an effective strategy for treating cancer in humans. VEGF receptors (VEGFR) are expressed by variety of leukemias and other hematological malignancies, indicating that inhibitors of VEGF or VEGFR signaling might play a role in the treatment of such conditions [109]. Bevacizumab is a tumor angiogenesis inhibitor that binds to VEGF and was approved for treating colorectal cancer in 2004. Bevacizumab conjugated to polymeric microspheres loaded with Tx has been successfully trialed in mice bearing prostate tumors for systemic targeting to angiogenic sites, which has proved to be an effective way of administering drugs to target cancer tissue [110].

Receptor HER2 is a tyrosine kinase found in excessive quantities in 25 – 30% of patients with breast cancer and is associated with the aggressive growth of tumor cells [111]. Trastuzumab is an anti-HER2 monoclonal antibody which inhibits the proliferation of tumor cells. It specifically targets HER2, which binds to HER2 receptor and was approved by the FDA for treating breast cancer [112]. Liu *et al.* confirmed that Herceptin® (Genentech, Inc., USA, [brand name of Trastuzumab]) conjugated nanoparticle for specific binding to breast cancer cells and achieved targeted ultrasound imaging due to its novel target specificity properties. Immunocytochemistry and flow cytometry study shows the targeting specificity and the resultant ultrasound enhancement in receptor HER2 -positive cells, while minimal staining was found in receptor HER2-negative cells, indicating receptor-specific binding of the conjugated PLA nanoparticles. This

Table 1. FDA approved Monoclonal antibodies used to target cancer.

Cancer therapeutic antibodies	Manufacturer and approval date	Brand name	Type	Target and treatment
Rituximab	IDEC Pharmaceuticals,1997 San Diego, California,USA	Rituxan®, Mabthera	Chimeric	Anti-CD20 antibody or relapsed/refractory CD-20 positive B-call non-Hodgkin's lymphoma and low-grade or follicular-type lymphoma
Trastuzumab	Genentech, Inc.,1998 South San Francisco, CA, USA,	Herceptin®	humanized	Blocks HER2 receptor for HER-2 positive metastatic breast cancer
Gemtuzumab ozogamicin	Wyeth, 2005, 5 Giralda Farms Madison, NJ , USA	Mylotarg®	Humanized	humanized anti-CD33 antibody-targeted myeloid leukemia
Alemtuzumab	Berlex aboratories, 2001,California, USA	Campath®	Humanized	Anti-CD52 antibody for B-cell chronic lymphocytic leukemia
Ibritumomab tiuxetan	ZevalinR IDEC Pharmaceuticals, 2002, San Diego, CA	Zevalin®	Murine	Anti-CD20 antibody for Rituximab-failed non-Hodgkins lymphoma
Tositumomab	GlaxoSmithKline, 2003, ResearcThr iangle Park, NC, USA	Bexxar®	Murine	Anti-CD20 antibody for Non-Hodgkin lymphoma
Gefitinib	AstraZeneca, 2003 USA	Iressa®	Humanized	Blocks epidermal growth factor receptors and tyrosine kinase activity for advanced non-small cell lung cancer
Cetuximab	Canopus BioPharma, Inc., 2004,USA	Erbitux®	Chimeric	Targeting the epidermal growth factor receptor (EGFR)
Bevacizumab	Genentech, Inc., 2004 South San Francisco,CA, USA	Avastin®	Humanized	Targeting the vascular endothelial growth factor receptor (VEGF)
Panitumumab	Amgen Europe B.V,2007, Thousand OAKS, CA, USA	Vectibix®	Humanized	Targeting the epidermal growth factor receptor (EGFR) Drug for Colorectal Cancer

is a promising approach to target cancer biomarkers for site-specific ultrasound imaging and therapeutics [112].

Epidermal growth factor receptor (EGFR) overexpress in a variety of cancers such as head, neck, renal, breast, colorectal, prostate, pancreatic,c etc and monoclonal antibody(Mab) Cetuximab and Panitumumab have the affinity to bind with EGFR [113,114]. This approach was carried out with gold nanoparticle, loaded with gemcitabine, conjugated with anti-EGFR antibody Cetuximab (as a targeting agent) against three pancreatic cancer cell lines (PANC-1, AsPC-1 and MIA Paca2) with variable EGFR expression. This targeted delivery of cytotoxic drug as a gold immunoconjugate can effectively inhibit *in vitro* pancreatic tumor cell proliferation and *in vivo* orthotropic pancreatic tumor growth [115]. Another recent experiment shows selective cancer cell targeting by biodegradable poly(lactic acid) (PLA) nanoparticles in SKOV-3 (HER2 positive) ovarian cancer and Daudi (CD20 positive) lymphoma cells. In this experiment, cancer targeting therapeutics were mediated by anti-HER2 (Trastuzumab; Herceptin) and anti-CD20 (Rituximab IDEC Pharmaceutical, USA) Mab. Here PLA-NPs were surface coated with 1-pyrenebutanol (PB) and the resulting PB-NP was coupled with the two anti-HER2 and anti-CD20 monoclonal antibodies (mAbs) using a bifunctional cross-linker. The effective targeting of cells by mAb-PB-NP was shown by flow cytometry analysis. Clearly anti-HER2-PB-NP specifically bound to the SKOV-3(HER2 positive) cells and not to the Daudi cells, while anti-CD20-PB-NPs bound to Daudi cells (CD20 positive) but not to SKOV-3 cells. Here NPs covalently coupled with antibodies can specifically and efficiently bind to cancer cells, suggesting that immuno-nanocarrier conjugates are useful for tumor targeting [116]. The majority of antibodies are in a pipeline of clinical trials, which has recently developed a nucleosome-specific tumor-targeted 2c5 anticancer antibody coupled with liposome. These targeted liposomes have been successfully trialed in mice, showing best imaging [117]. Similarly, Dox-loaded prostate cell-specific Mab (Mab5D4) conjugated with liposomes has given interesting results in enhancing cytotoxicity towards prostate cancer cell lines compared with the non-targeted Dox liposome *in vitro* [118].

Compared with whole monoclonal antibodies, the use of antibody fragments as a targeting moiety can reduce immunogenicity and improve the pharmacokinetic profiles of nanocarriers [111]. For example, liposomes coupled with mAb fragments instead of whole antibodies showed decreased clearance rates and increased circulation half-lifes, allowing the liposomes sufficient time to be distributed and bind to the targeted cells. This strategy improved the therapeutic efficacy of immunoliposomal–DOX targeted against CD19 on human B lymphoma cells in animal models [119]. Paclitaxel-loaded PLGA nanoparticles coated with cationic SM5-1 single-chain antibody (scFv) containing a polylysine (SMFv-polylys) were raised. SM5-1 scFv (SMFv) is derived from SM5-1 monoclonal antibody, which binds to a 230 kDa membrane protein specifically expressed on myeloma, hepatocellular carcinoma and breast cancer cells. SMFv-polylys was fixed to paclitaxel-loaded PLGA nanoparticles to form paclitaxel-loaded PLGA nanoparticles coated with SMFv-polylys (Ptx-NP-S). Ptx-NP-S significantly enhanced *in vitro* cytotoxicity against neoplastic cells as compared with non-targeted paclitaxel-loaded PLGA nanoparticles [120].

Although antibody-mediated targeting has achieved significant attention, there are a number of obstacles to successful targeted therapy with monoclonal antibodies, such as the heterogeneity of antigens on malignant cells, high interstitial pressure within the tumor can prevent the passive Mab from binding and binding of Mab to a free-floating antigen instead of to tumor cell [121]. These hurdles are not only associated with targeted therapy, but also with targeted delivery with penetration especially in solid tumors [106]. To overcome this obstacle, significant progress in virology may come to help: researchers are currently studying novel gene delivery methods and in this context recombinant adeno-associated viruses may serve as low immunogenic and cell- or tissue-specific vectors. Already the proof of the principle has been delivered in experiments with adeno-associated viruses which penetrate solid tumor tissue [122] and it is to be expected that these tailored viruses can be used as a platform for advanced drug and imaging nanotechnology [123]. Furthermore, double action can be achieved by combining oncolytic viruses with preferential replication in tumor cells with drug containing nanoparticles [124].

5.6 Aptamer-mediated targeting

Aptamers are RNA or DNA oligonucleotides that fold by intra-molecular interaction into unique three-dimensional confirmations capable of binding to target antigens [125]. In the field of cancer nanotechnology, aptamers have the potential to act as targeting molecules, directing the delivery of nanoparticles to tumor antigens present on the surface of cancer cells [126]. The unique property that labels these as the new generation of targeted drug delivery molecules is their ability to bind to various molecular targets such as small molecules, proteins, nucleic acids and even cells, tissues and organisms [127]. Aptamers offer advantages over antibodies as they can be engineered completely in a test tube, are rapidly produced by chemical synthesis, possess desirable storage properties, are easy to characterize and modify, elicit little or no immunogenicity in therapeutic applications and exhibit a high specificity and affinity for their target antigen [125,128]. Furthermore, due to their small size and similarity with endogenous molecules, aptamers exhibit superior tissue penetration [129]. Aptamers are excellent potential candidates for the targeted delivery of an active chemotherapeutic drug, either through direct conjugation to an aptamer or through its encapsulation in an aptamer-coated vesicle such as a liposome. Aptamers should be directed for the receptor, the molecular signature that is preferentially or exclusively expressed on the plasma membrane

of the cancer cell, alternatively they may be delivered to the extracellular matrix molecules that are expressed preferentially in tumors. To date many aptamers have been isolated for cancer therapeutics, as can been seen listed in **Table 2**.

Aptamers generated by a method called SELEX (systematic evolution of ligands by exponential enrichment) have been widely used in various biomedical applications. The newly developed Cell-SELEX (cell based-SELEX), targeting whole living cells, has raised great expectations for cancer biology, therapy and regenerative medicine. Combining nano-biotechnology with aptamers, this technology opens the way to more sophisticated applications in molecular diagnosis. The first aptamer approved for use in humans is an RNA-based molecule (Macugen, Pegaptanib) that is administered locally to treat age-related macular degeneration by targeting VEGF. Pegaptanib is a PEGylated anti-VEGF aptamer, a single-stranded nucleic acid that binds with high specificity to a particular target. Pegaptanib specifically binds to VEGF 165, a protein that plays a critical role in angiogenesis and in increased permeability, two of the primary pathological processes responsible for the vision loss associated with age-related macular degeneration (AMD) which was approved by the FDA in December 2004 [130].

Aptamers are small molecules, with a half-life of minutes to hours due to nuclease degradation; they can be rapidly cleaned from the bloodstream by the kidneys. This rapid clearance is an advantage in some applications such as *in vivo* diagnostic imaging, for example a tenascin-binding aptamer under development for cancer imaging [131]. For clinical therapeutics several modifications are available, such as 2'-fluorinesubstituted pyrimidines and polyethylene glycol (PEG) linkage (both of which are used in Macugen), that can increase the half-life of aptamers easily to a day or even a week [131]. Applying nanotechnology to the aptamer field, Smith *et al.* have successfully constructed an aptamer-conjugated nanoparticle for the collection and detection of multiple cancer cells [126]. Neutral polymers such as PLA, PLGA or those with more negative charge such as polyanhydride may be the most suitable for conjugation to aptamers. Cationic nanoparticles are particularly useful for gene delivery application and thus may enable efficient targeted gene delivery. Farokhzad *et al.* formulated PLA-PEG-COOH nanoparticles encapsulating rhodamine-labeled dextran (a model drug) bioconjugate with A10 PSMA aptamer. This was efficiently targeted and taken up by the prostate LNCaP epithelial cell expressing the prostate-specific membrane antigen protein [132,133]. In cancer therapy, the most advanced aptamer and the first to enter humans was AS1411, formerly developed as AGRO100 by a US biotech company named Aptamera. AS1411 is a 26-mer unmodified guanosine-rich oligonucleotide which induces growth inhibition *in vitro* and has shown activity against human tumor xenografts *in vivo*. The mechanism underlying its antiproliferative effects in cancer cells seems to involve initial binding to cell surface nucleolin and internalization leading to an inhibition of DNA [134]. The company Antisoma is evaluating this aptamer in Phase I clinical trials. The therapeutic benefit of AS1411 is attributed to the disruption of the nuclear factor-kappa B (NF-κB) signaling inside the cell. It was shown that AS1411 is used as a capping ligand to directly synthesize highly fluorescent nano-crystal quantum dots (NQD) in aqueous solution. The DNA aptamer retains its unique secondary structure on the NQD surface, which is necessary for selective and specific binding to its target protein nucleolin of MCF-7 human breast cancer cells [135].

In cancer tissue the activated T cells produce a molecule called cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4). This molecule then produces a signal that tells the T cells to stop their attack. This T-cell inhibition prevents normal cells from being harmed by an immune response, but it may also prevent the immune system from destroying malignant tumors. Researchers hope that blocking CTLA-4's inhibitory signal will lead to a more robust immune response against tumors. The development of NP-RNA aptamers that bind CTLA-4 with high affinity and specificity may solve some of this problem. These aptamers inhibit CTLA-4 function *in vitro* and enhance tumor immunity in mice [136]. Scientists have now constructed Au-Ag nano-rods (NRs) as a nano-platform for multivalent binding for multiple aptamers on the rod to increase both the signal and binding strengths of these aptamers in cancer cell recognition. Up to 80 fluorophore-labeled aptamers can be attached on a 12 × 56 nm NR, resulting in a much stronger fluorescence signal than that of an individual dye-labeled aptamer probe. The molecular assembly of aptamers on the NR surfaces also significantly improves the binding affinity with cancer cells through simultaneous multivalent interactions with the cell membrane receptors. This leads to an affinity at least 26-fold higher than the intrinsic affinity of the original aptamer probe [137].

Fluorescent semiconductor quantum dots (QDs) hold great potential for molecular imaging [138]. Novel quantum dot–aptamer (Apt)–doxorubicin (Dox) conjugate (QD-Apt [Dox]) acts as a targeted cancer imaging therapy and sensing system. It functionalizes the surface of fluorescent QDs with the A10 RNA aptamer, which recognizes the extracellular domain of the prostate-specific membrane antigen (PSMA) for pinpoint drug targeting and imaging [139]. Recently, Javier *et al.* formulated PSMA aptamer-based gold nanoparticles using aptamers as targeting agents and gold nanoparticles as imaging agents. The aptamer–gold conjugates approach creates a contrast agent designed for the detection of PSMA, obtaining reflectance images of PSMA (+ve) and PSMA (-ve) cell lines treated with the anti-PSMA aptamer–gold conjugates [140].

5.7 Peptide-mediated targeting

A large number of peptide receptors are highly expressed in large quantities in certain tumor cell such as somatostatin analog, vasoactive intestinal peptide, gastrine-related peptide,

Table 2. List of cancer targeting aptamers.

Aptamer	Target on cancer cell	Binding site	Ref.
MUC-1	Muc-1 protein in epithelial malignant cell	Cancer cell surface	[120]
PSMA APTAMER (A9, A10)	Prostate specific membrane antigen	Cancer cell surface	[138,139]
A30	Human EGFR-3	Cancer cell surface	[134]
AS-1411	Nucleolin	Cancer cell surface	[134]
CTLA-4 APTAMER	Cytotoxic T cell antigen-4	T cell	[136]
CLONE 5	Sialyl Lewis x	Cancer cell surface	[134]
TTA 1	Fibrogen-like domain of tenascin-c	Extracellular matrix protein	[130]
PDGF-r APTAMER	Platelet-derived growth factor receptor	Microvasculature	[134]
III.1	Pigpen	Microvasculature	[134]
PEGAPTANIB	VEGF of ocular cell	Cancer cell surface	[131]

cholecystokine, luteinizing hormone releasing hormone localized on tumor cells [141]. Peptide analog chemiconjugated to a drug carrier system to allow tumor-specific targeting of cytotoxic agents, following interaction with peptide receptors [142]. Peptides are becoming the alternative to other targeting moieties because of their small size, lower immunogenicity, higher stability and ease to manufacture [143].

The α_v β_3 expressed in angiogenic vessels and isoDGR-containing peptides acts as ligands for targeted delivery of drugs to tumor neovasculature. Curnis *et al.* generated isoaspartate-glycine-arginine (isoDGR), a new α_v β_3 integrin-binding motif. They reported that cyclic peptide coupled to fluorescent nanoparticles (QDs) binds α_v β_3 integrin and co-localizes with antibody in human renal cell carcinoma tissue sections [144]. Similarly, peptides such as arginine-glycine-aspartic acid (RGD) peptide are also being developed as an alternative to antibodies. Cilengitide® is a cyclic (RGD) peptide that binds to the integrins and is currently in Phase II clinical trials for the treatment of non-small cell lung cancer and pancreatic cancer. Robust angiogenesis underlies the aggressive growth of tumors. Therefore, one of the mechanisms to inhibit angiogenesis is to starve tumor cells. The synthetic peptide-bearing RGD sequence is known to specifically bind to the α_v β_3 integrin expressed on cancer cells in the angiogenic blood vessels. The studies of Dijkgraaf *et al* showed that the tetrameric RGD-dendrimer had better tumor-targeting properties and they found that the radio-labeled multimeric dendrimers showed specifically enhanced uptake in α_v β_3 integrin-expressing tumors *in vivo* [145].

Peptide-like VEGF, vasoactive intestinal peptide (VIP), luteinizing hormone-releasing hormone (LHRH), somatostatin, etc have been intensively studied for targeting to the specific receptors which are expressed abundantly in different disease states [141]. Tumor targeting requires very specific compounds and the peptide analogs like somatostatin, neurotensin or bombesin to target G-coupled receptors, which are overexpressed on tumor cells. However, many of those analogs are rapidly degraded in the plasma and are cytotoxic [146,147]. Due to the limited efficiency and high toxicity of conventional chemotherapy, different strategies have been developed for non-cytotoxic cancer treatment and cancer localization [148]. Somatostatin peptides (somatostatin 28 or 14) which are known for their high binding affinity to G-coupled peptide receptors, overexpressed are in tumor cells. Using this binding property Graff *et al.* developed a new type of functional peptide nanoparticles based on somatostatin for tumor targeting and drug delivery. Somatostatin 28 and 14 were genetically incorporated into the nanoparticles and radio-labeled with radionuclides for drug delivery and imaging [149].

Vasoactive intestinal peptide (VIP) is a 28-amino acid peptide, isolated from intestinal extracts and shown to be a potent vasodilator. Certain tumors arising from the pancreatic islets or nervous tissue secrete excessive quantities of VIP. Recently it was suggested that VIP-grafted sterically stabilized phospholipids mixed nano-micelles (VIP-SSMM), a novel nano-sized targeted drug delivery carrier for MCF-7 breast cancer cells [150]. The experimental and clinical evidence shows that bombesin peptides (markers for lung cancer, gastric cancer and neuroblastoma) play an important role in cancer targeting. Montet *et al.* conducted experiments on mice with pancreatic tumors by injecting nanoparticles with the bombesin peptide into the mice tail veins. The relative signal coming from normal pancreas tissue was lowered and tumors with the bound nanoparticles showed up selective bright objects in subsequent magnetic resonance images [151].

In brain cancer difficulties arise due to low permeability of delivery systems in the passage of the desired drug from the blood to the brain parenchyma [152]. Nanoparticles can cross the BBB and allow the drugs to exert their pharmacological activity in the CNS. Recently the biocompatible polyester poly(d,l-lactide-co-glycolide) (PLGA) with the peptide H(2)

N-Gly-l-Phe-d-Thr-Gly-l-Phe-l-Leu-l-Ser(O-beta-d-Glucose)-CONH(2) was used to prepare g7-Np. This g7-Np contains loperamide (drug) and with Rhodamine-123 (marker) for biodistribution and imaging in different brain macro-areas. Fluorescent studies showed that these NPs were able to cross the BBB due to the linked sequence of the peptidic moiety present on their surface [153]. Glucose-regulated protein 78 (GRP78) is overexpressed on the cell surface of human cancer cells. In a reported xenograft mouse model, GRP78-Pep42-conjugated QDs have the ability to selectively enrich in tumor tissue by clathrin-mediated endocytosis. These results suggest that the highly specific GRP78-Pep42 interaction can be utilized for the generation of Pep42–drug conjugates as a powerful anticancer drug delivery system that could substantially enhance the efficacy of chemotherapy by increasing the drug–tumor specificity, thus minimizing the adverse side effects associated with conventional cancer therapeutics [154].

6. Conclusion

The discovery of targeting ligand to cancer cells and the development of ligand-targeted therapy will help us to improve therapeutic efficacy and reduce side effects. Unlike other forms of therapy it will allow us to maintain quality of life of patients, while efficiently attacking the cancer tissue. Nanocarrier-based delivery systems have greater potential for many applications, including anti-tumor therapy, gene therapy, AIDS treatment and radiotherapy in the delivery of protein, antibiotics, virostatics, vaccines and as vesicles to cross the BBB. To date numerous attempts have been made for targeting, including the use of antibodies, aptamers, small molecules, etc, which we have highlighted in this review. Research activities are aimed towards the development of novel molecularly targeted anticancer agents. If such a tumor-specific agent can be efficiently conjugated to fit the model of biodegradable nanocarrier, then targeted therapy will become increasingly favored and specific.

7. Expert opinion

In summary, there exist multiple important achievements in the development of nanocarriers loaded with anticancer drugs and targeted to tumors by different specific ligands. To date, scientists have achieved significant improvements in the therapeutic index of a targeted ligand–drug conjugated over a free drug, which could have occurred from both a decrease in toxicity and an increase in drug effectiveness. Research activities are aimed towards achieving specific and targeted delivery of cancer drugs with new methods of wrapping, delivery and targeting formulation. Currently it is not yet apparent whether some of the cancer targeting therapeutics methodology discussed in this review has been successful in a clinical setting, having shown encouraging results in an animal model.

There remains the challenge ahead to choose the perfect ligand and select the dosing that delivers consistent pharmacokinetics. Some of the present limitations of ligand-conjugated anticancer drugs appear to be their inability to access, initiating secondary complication and penetrating solid tumors ineffectually [155]. Aberrant methylation, primarily hypermethylation of certain genes, including tumor suppressors, has been implicated in cancer development. Vitamin B12 is essential for methyl group metabolism and thus is involved in an increased risk of cancer [156]. A better understanding and new ideas on the chemistry of binding between ligands, nanocarriers and cancer cell receptors will likely help with the development of more potent agent, as selectivity is also enhanced with other technological advances. So further studies in nanocarrier–ligand-targeted drugs are warranted. From a clinical point of view, studies of molecular mechanisms that drive invasive growth and tumor cell metastatization should provide a valuable direction in the development of targeted therapies. Further understanding of molecular pathways that comprise the cell cycle, apoptosis, angiogenesis and invasion will provide novel targets in cancer therapy.

New techniques of targeted drug delivery have tremendous significance in the field of cancer therapy. Various radio-immuno-pharmaceuticals, immunotoxins and immuno-conjugate-targeted delivery systems are already on the market. Antibody-directed enzyme prodrug therapies and gene-directed enzyme prodrug therapy are in clinical development. In the field of drug delivery and cancer targeting, this technique is actively engaged in improving the stability [6], solubility [39], absorption and therapeutic action of the drug within the target tissue [39] and permitting the long-term release of the drugs. The future of such targeted technology will be highly dependent on the development of safe, non-toxic and non-immunogenic nanocarriers. There are still safety concerns associated with the introduction of nanoparticles to the human body. These will require further studies before some of the products can be approved. Carbon nanoparticles for instance have been shown to induce lipid peroxidation in the brain cells of fish and pulmonary inflammation in rats [157]. With cancer metastasis or spread to sites remote from the primary tumor to a new target site, treatment become more difficult and in many case ineffectual. Antibodies in combination with chemotherapy is a well-known methodology for the treatment of metastatic cancer [158]. Few reports on nanocarrier systems were noted: Li *et al* described that gene silencing in metastatic tumors can be achieved by si-RNA formulated in surface-modified nanoparticles [159]. This is an interesting upcoming field and further studies are required. A great number of questions and issues remain unresolved in targeted cancer therapies. However, if the uncertainties can be addressed, this technology will have a resounding impact on the targeted drug delivery field related to cancer.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. George P, Richard K. Site-specific (targeted) drug delivery in cancer therapy. Nat Biotechnol 1983;1:869-78
2. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science 2004;303:1818-22
3. Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. Adv Drug Deliv Rev 2004;56:1649-59
4. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. Adv Drug Deliv Rev 2002;54:631-51
5. Fahmy T, Fong P, Goyal A, et al. Targeted for drug delivery. Mater Today 2005;8:18-26
6. LaVan DA, McGuire T, Langer R. Small-scale systems for in vivo drug delivery. Nat Biotechnol 2003;21:1184-91
7. Sahoo S, Labhasetwar V. Nanotech approach to drug delivery and imaging. Drug discovery today 2003;8:1112-20

•• **This is a fundamental review that contains detail description of the different nanotechmology based drug delivery and imaging approaches in pharmaceutical and biomedical industries.**

8. Vasir JK, Labhasetwar V. Targeted drug delivery in cancer therapy. Technol Cancer Res Treat 2005;4:363-74
9. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008;8:592-603
10. Han B, Zhang JT. Multidrug resistance in cancer chemotherapy and xenobiotic protection mediated by the half ATP-binding cassette transporter ABCG2. Curr Med Chem Anticancer Agents 2004;4:31-42
11. Torchilin VP. Targeted pharmaceutical nanocarriers for cancer therapy and imaging. AAPS J 2007;9:E128-47

• **This review addressed the different ways to target tumors via specific ligand and via stimuli sensitivity of the carrier for cancer therapy and imaging.**

12. Sinha R, Kim GJ, Nie S, et al. Nanotechnology in cancer therapeutics: bioconjugated nanoparticles for drug delivery. Mol Cancer Ther 2006;5:1909-17
13. Williams J, Lansdown R, Sweitzer R, et al. Nanoparticle drug delivery system for intravenous delivery of topoisomerase inhibitors. J Control Release 2003;91:167-72
14. Leroux JC, Allémann E, Jaeghere FD, et al. Biodegradable nanoparticles –From sustained release formulations to improved site specific drug delivery. J of Control Release 1996;39:339-50
15. Parveen S, Sahoo SK. Polymeric nanoparticles for cancer therapy. J Drug Target 2008;16:108-23
16. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 2005;4:145-60
17. Huwyler J, Cerletti A, Fricker G, et al. By-passing of P-glycoprotein using immunoliposomes. J Drug Target 2002;10:73-9
18. Mamot C, Drummond DC, Hong K, et al. Liposome-based approaches to overcome anticancer drug resistance. Drug Resist Updat 2003;6:271-9
19. Ferry DR, Traunecker H, Kerr DJ. Clinical trials of P-glycoprotein reversal in solid tumours. Eur J Cancer 1996;32A:1070-81
20. Kellen JA. The reversal of multidrug resistance: an update. J Exp Ther Oncol 2003;3:5-13
21. Thomas H, Coley HM. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. Cancer Control 2003;10:159-65
22. Khdair A, Handa H, Mao G, et al. Nanoparticle-mediated combination chemotherapy and photodynamic therapy overcomes tumor drug resistance in vitro. Eur J Pharm Biopharm 2009;71:214-22
23. Piche A, Rancourt C. Gene therapy to overcome drug resistance in cancer: targeting key regulators of the apoptotic pathway. Curr Gene Ther 2001;1:317-24
24. Lipinski CA, Lombardo F, Dominy BW, et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001;46:3-26
25. Farokhzad OC. Nanotechnology for drug delivery: the perfect partnership. Expert Opin Drug Deliv 2008;5:927-29
26. Emerich DF, Thanos CG. Nanotechnology and medicine. Expert Opin Biol Ther 2003;3:655-63
27. Sahoo SK, Parveen S, Panda JJ. The present and future of nanotechnology in human health care. Nanomedicine 2007;3:20-31
28. Emerich D. Nanomedicine – prospective therapeutic and diagnostic applications. Expert Opin Biol Ther 2005;(5):1-5
29. Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. Faseb J 2005;19:311-30
30. Shaffer C. Nanomedicine transforms drug delivery. Drug Discov Today 2005;10:1581-2
31. Yoshikawa T, Tsutsumi Y, Nakagawa S. Development of nanomedicine using intracellular DDS. Nippon Rinsho 2006;64:247-52
32. Torchilin VP. Liposomes as targetable drug carriers. Crit Rev Ther Drug Carrier Syst 1985;2:65-115
33. Torchilin VP. Targeted polymeric micelles for delivery of poorly soluble drugs. Cell Mol Life Sci 2004;61:2549-59
34. Gref R, Minamitake Y, Peracchia MT, et al. Biodegradable long-circulating polymeric nanospheres. Science 1994;263:1600-3
35. Hans M, Lowman A. Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid State Mater Sci 2002;6:319-27
36. Garnett MC. Targeted drug conjugates: principles and progress. Adv Drug Deliv Rev 2001;53:171-216
37. Au JL, Jang SH, Wientjes MG. Clinical aspects of drug delivery to tumors. J Control Release 2002;78:81-95
38. Maeda H. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv Enzyme Regul 2001;41:189-207
39. Maeda H, Wu J, Sawa T, et al. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 2000;65:271-84
40. Kaul G, Lee-Parsons C, Amiji M. Poly(ethylene glycol)-modified gelatin nanoparticles for intracellular delivery. Pharma Eng–J Int Soc Pharm Eng 2003;23:108-14
41. Monsky WL, Fukumura D, Gohongi T, et al. Augmentation of transvascular transport of macromolecules and nanoparticles in tumors using vascular endothelial growth factor. Cancer Res 1999;59:4129-35

42. Dellian M, Witwer BP, Salehi HA, et al. Quantitation and physiological characterization of angiogenic vessels in mice: effect of basic fibroblast growth factor, vascular endothelial growth factor/vascular permeability factor, and host microenvironment. Am J Pathol 1996;149:59-71

43. Muggia FM. Doxorubicin-polymer conjugates: further demonstration of the concept of enhanced permeability and retention. Clin Cancer Res 1999;5:7-8

44. Gu F, Zhang L, Teply B, et al. Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. PNAS 2008;105:2586-91

45. Nishioka Y, Yoshino H. Lymphatic targeting with nanoparticulate system. Adv Drug Deliv Rev 2001;47:55-64

46. Kummer U, Thierfelder S, Mysliwietz J. Antigen density on target cells determines the immunosuppressive potential of rat IgG2b monoclonal antibodies. Eur J Immunol 1990;20:107-12

47. Yousaf N, Howard JC, Williams BD. Targeting behavior of rat monoclonal IgG antibodies in vivo: role of antibody isotype, specificity and the target cell antigen density. Eur J Immunol 1991;21:943-50

48. Lanza GM, Yu X, Winter PM, et al. Targeted antiproliferative drug delivery to vascular smooth muscle cells with a magnetic resonance imaging nanoparticle contrast agent: implications for rational therapy of restenosis. Circulation 2002;106:2842-7

49. Murphy EA, Majeti BK, Barnes LA, et al. Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis. Proc Natl Acad Sci USA 2008;105:9343-8

50. Yukio K, Takeshi S, Takashi K, et al. Kinetic analysis of receptor-mediated endocytosis (RME) of proteins and peptides: use of RME as a drug delivery system. J of Control Release 1996;39:191-200

51. Eniola AO, Hammer DA. Artificial polymeric cells for targeted drug delivery. J Control Release 2003;87:15-22

52. Keegan M, Falcone J, Leung T, et al. Biodegradable microspheres with enhanced capacity for covalently bound surface ligands. Macromol 2004;37:9779-84

53. Pun SH, Tack F, Bellocq NC, et al. Targeted delivery of RNA-cleaving DNA enzyme (DNAzyme) to tumor tissue by transferrin-modified, cyclodextrin-based particles. Cancer Biol Ther 2004;3:641-50

54. Anabousi S, Bakowsky U, Schneider M, et al. In vitro assessment of transferrin-conjugated liposomes as drug delivery systems for inhalation therapy of lung cancer. Eur J Pharm Sci 2006;29:367-74

55. Vandewalle B, Granier AM, Peyrat JP, et al. Transferrin receptors in cultured breast cancer cells. J Cancer Res Clin Oncol 1985;110:71-6

56. Dowlati A, Loo M, Bury T, et al. Soluble and cell-associated transferrin receptor in lung cancer. Br J Cancer 1997;75:1802-6

57. Agarwal A, Saraf S, Asthana A, et al. Ligand based dendritic systems for tumor targeting. Int J Pharm 2008;350:3-13

58. Enns CA, Shindelman JE, Tonik SE, et al. Radioimmunochemical measurement of the transferrin receptor in human trophoblast and reticulocyte membranes with a specific anti-receptor antibody. Proc Natl Acad Sci USA 1981;78:4222-25

59. Daniels T, Delgado T, Helguera G, et al. The transferrin receptor part II: targeted delivery of therapeutic agents into cancer cells. Clin Immunol 2006;121:159-76

60. Sahoo SK, Labhasetwar V. Enhanced antiproliferative activity of transferrin-conjugated paclitaxel-loaded nanoparticles is mediated via sustained intracellular drug retention. Mol Pharm 2005;2:373-83

61. Sahoo SK, Ma W, Labhasetwar V. Efficacy of transferrin-conjugated paclitaxel-loaded nanoparticles in a murine model of prostate cancer. Int J Cancer 2004;112:335-40

•• **An interesting comparison between the commercial formulation and surface modified nanoparticle.**

62. Pulkkinen M, Pikkarainen J, Wirth T, et al. Three-step tumor targeting of paclitaxel using biotinylated PLA-PEG nanoparticles and avidin–biotin technology: formulation development and in vitro anticancer activity. Eur J Pharm Biopharm 2008;70:66-74

63. Jiang Y, Tang G, Hong M, et al. Active tumor-targeted delivery of PEG-protein via transferrin-transferrin-receptor system. J Drug Target 2007;15:672-83

64. Hou J, Wang D, Zhang R, et al. Experimental therapy of hepatoma with artemisinin and its derivatives: in vitro and in vivo activity, chemosensitization, and mechanisms of action. Clin Cancer Res 2008;14:5519-30

65. Nam W, Tak J, Ryu JK, et al. Effects of artemisinin and its derivatives on growth inhibition and apoptosis of oral cancer cells. Head Neck 2007;29:335-40

66. Nakase I, Lai H, Singh NP, et al. Anticancer properties of artemisinin derivatives and their targeted delivery by transferrin conjugation. Int J Pharm 2008;354:28-33

•• **A solid review discussing anticancer activities of artemisinins an antimalaria drug and the efficacy of transferrin conjugated artemisinins against cancer cell.**

67. Li JL, Liu XY. Fabrication and biofunctionalization of selenium-polypyrrole core-shell nanoparticles for targeting and imaging of cancer cells. J Nanosci Nanotechnol 2008;8:2488-91

68. Kukowska-Latallo JF, Candido KA, Cao Z, et al. Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. Cancer Res 2005;65:5317-24

69. Kukowska-Latallo J, Candido K, Cao Z, et al. Nanoparticle Targeting of Anticancer Drug Improves Therapeutic Response in Animal Model of Human Epithelial Cancer. Cancer Res 2005;65:5317-5324

70. Low PS, Antony AC. Folate receptor-targeted drugs for cancer and inflammatory diseases. Adv Drug Deliv Rev 2004;56:1055-8

71. Shmeeda H, Mak L, Tzemach D, et al. Intracellular uptake and intracavitary targeting of folate-conjugated liposomes in a mouse lymphoma model with up-regulated folate receptors. Mol Cancer Ther 2006;5:818-24

72. Stella B, Arpicco S, Peracchia MT, et al. Design of folic acid-conjugated nanoparticles for drug targeting. J Pharm Sci 2000;89:1452-64

73. Sudimack J, Lee RJ. Targeted drug delivery via the folate receptor. Adv Drug Deliv Rev 2000;41:147-62

74. Pasut G, Canal F, Dalla Via L, et al. Antitumoral activity of PEG-gemcitabine prodrugs targeted by folic acid. J Control Release 2008;127:239-48

75. Esmaeili F, Ghahremani M, Ostad S, et al. Folate-receptor-targeted delivery of docetaxel nanoparticles prepared by

PLGA-PEG-folate conjugate. J Drug Target 2008;16:415-23

76. Chalasani K, Russell-jones G, Jain A, et al. Effective oral delivery of insulin in animal models using vitamin B12-coated dextran nanoparticles. J Control Release 2007;122:141-50

77. Chalasani KB, Russell-Jones GJ, Yandrapu SK, et al. A novel vitamin B12-nanosphere conjugate carrier system for peroral delivery of insulin. J Control Release 2007;117:421-9

78. Gabor F, Bogner E, Weissenboeck A, et al. The lectin–cell interaction and its implications to intestinal lectin-mediated drug delivery. Advanced Drug Delivery Reviews 2004;56:459-80

79. De Mejia V, Prisecaru V. Lectins as bioactive plant proteins: a potential in cancer treatment. Crit Rev Food Sci Nutr 2005;45:425-45

80. Banks W, Kastin A. Characterization of lectin-mediated brain uptake of HIV-1 GP120. J Neurosci Res 1998;54:522-9

81. Fischer D, Kissel T. Histochemical characterization of primary capillary endothelial cells from porcine brains using monoclonal antibodies and fluorescein isothiocyanate-labelled lectins: implications for drug delivery. Eur J Pharm Biopharm 2001;52:1-11

82. Bies C, Lehr C, Woodley J. Lectin-mediated drug targeting: history and applications. Adv Drug Deliv Rev 2004;56:425-35

83. Yin Y, Chen D, Qiao M, et al. Preparation and evaluation of lectin-conjugated PLGA nanoparticles for oral delivery of thymopentin. J Control Release 2006;116:337-45

84. Gao X, Tao W, Lu W, et al. Lectin-conjugated PEG-PLA nanoparticles: preparation and brain delivery after intranasal administration. Biomater 2006;27:3482-90

• **An important article explaining a novel effective non-invasive WGA conjugated PEG-PLA nanosystem for brain drug delivery.**

85. Mo Y, Lim LY. Preparation and in vitro anticancer activity of wheat germ agglutinin (WGA)-conjugated PLGA nanoparticles loaded with paclitaxel and isopropyl myristate. J Control Release 2005;107:30-42

86. Weissenboeck A, Bogner E, Wirth M, et al. Binding and uptake of wheat germ agglutinin-grafted PLGA-nanospheres by caco-2 monolayers. Pharm Res 2004;21:1917-23

87. Naisbett B, Woodley J. The potential use of tomato lectin for oral drug delivery: 4. Immunol consequences Int J of Pharm 1995;120:247-54

88. Woodley JF. Lectins for gastrointestinal targeting – 15 years on. J Drug Target 2000;7:325-33

89. Lavelle EC, Grant G, Pusztai A, et al. Mucosal immunogenicity of plant lectins in mice. Immunol 2000;99:30-7

90. Salomon DS, Brandt R, Ciardiello F, et al. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995;19:183-232

91. Schwechheimer K, Huang S, Cavenee WK. EGFR gene amplification-rearrangement in human glioblastomas. Int J Cancer 1995;62:145-8

92. Zwick E, Bange J, Ullrich A. Receptor tyrosine kinase signalling as a target for cancer intervention strategies. Endocr-Relat Cancer 2001;8:161–73

93. Bohl Kullberg E, Bergstrand N, Carlsson J, et al. Development of EGF-conjugated liposomes for targeted delivery of boronated DNA-binding agents. Bioconjug Chem 2002;13:737-43

94. Bohl Kullberg E, Carlsson J, Edwards K, et al. Introductory experiments on ligand liposomes as delivery agents for boron neutron capture therapy. Int J Oncol 2003;23:461-7

95. Carlsson J, Kullberg EB, Capala J, et al. Ligand liposomes and boron neutron capture therapy. J Neurooncol 2003;62:47-59

96. Kullberg EB, Nestor M, Gedda L. Tumor-cell targeted epidermal growth factor liposomes loaded with boronated acridine: uptake and processing. Pharm Res 2003;20:229-36

97. Wang Y, Pennock S, Chen X, et al. Endosomal signaling of epidermal growth factor receptor stimulates signal transduction pathways leading to cell survival. Mol Cell Biol 2002;22:7279-90

98. Wang Z, Zhang L, Yeung TK, et al. Endocytosis deficiency of epidermal growth factor (EGF) receptor-ErbB2 heterodimers in response to EGF stimulation. Mol Biol Cell 1999;10:1621-36

99. Wang J, Chen P, Su ZF, et al. Amplified delivery of indium-111 to EGFR-positive human breast cancer cells. Nucl Med Biol 2001;28:895-902

100. Lin SY, Makino K, Xia W, et al. Nuclear localization of EGF receptor and its potential new role as a transcription factor. Nat Cell Biol 2001;3:802-8

101. Torrisi MR, Lotti LV, Belleudi F, et al. Eps15 is recruited to the plasma membrane upon epidermal growth factor receptor activation and localizes to components of the endocytic pathway during receptor internalization. Mol Biol Cell 1999;10:417-34

102. Fan Z, Lu Y, Wu X, et al. Antibody-induced epidermal growth factor receptor dimerization mediates inhibition of autocrine proliferation of A431 squamous carcinoma cells. J Biol Chem 1994;269:27595-602

103. Zeng F, Lee H, Allen C. Epidermal growth factor-conjugated poly(ethylene glycol)-block- poly(delta-valerolactone) copolymer micelles for targeted delivery of chemotherapeutics. Bioconjug Chem 2006;17:399-409

•• **This article depicts the role of organic solvent with respect to conformation of epidermal growth factor.**

104. Tseng CL, Wu SY, Wang WH, et al. Targeting efficiency and biodistribution of biotinylated-EGF-conjugated gelatin nanoparticles administered via aerosol delivery in nude mice with lung cancer. Biomater 2008;29:3014-22

105. Tseng CL, Wang TW, Dong GC, et al. Development of gelatin nanoparticles with biotinylated EGF conjugation for lung cancer targeting. Biomater 2007;28:3996-4005

106. Muller D, Kohler G, Ohlinger R. Staging procedures in primary breast cancer. Anticancer Res 2008;28:2397-400

107. Reiter Y. Recombinant immunotoxins in targeted cancer cell therapy. Adv Cancer Res 2001;81:93-124

108. Chen L, Liu J, Yu X, et al. The biocompatibility of quantum dot probes used for the targeted imaging of hepatocellular carcinoma metastasis. Biomater 2008;29:4170-6

109. Bozec A, Gros F, Penault-Llorca F, et al. Vertical VEGF targeting: A combination of ligand blockade with receptor tyrosine kinase inhibition. Eur J Cancer 2008;44:1922-30

110. Lu J, Jackson JK, Gleave ME, et al. The preparation and characterization of anti-VEGFR2 conjugated, paclitaxel-loaded PLLA or PLGA microspheres for the systemic targeting of human prostate tumors. Cancer Chemother Pharmacol 2008;61:997-1005

111. Fischgrabe J, Wulfing P. Targeted therapies in breast cancer: established drugs and recent developments. Curr Clin Pharmacol 2008;3:85-98

112. Liu J, Li J, Rosol TJ, et al. Biodegradable nanoparticles for targeted ultrasound imaging of breast cancer cells in vitro. Phys Med Biol 2007;52:4739-47

113. Bernier J. Drug Insight: cetuximab in the treatment of recurrent and metastatic squamous cell carcinoma of the head and neck. Nat Clin Pract Oncol 2008;

114. Nakashima K, Yamazaki K, Boku N. Molecular target therapy and chemotherapy for advanced colorectal cancer. Gan To Kagaku Ryoho 2008;35:725-30

115. Patra CR, Bhattacharya R, Wang E, et al. Targeted delivery of gemcitabine to pancreatic adenocarcinoma using cetuximab as a targeting agent. Cancer Res 2008;68:1970-8

116. Nobs L, Buchegger F, Gurny R, et al. Biodegradable nanoparticles for direct or two-step tumor immunotargeting. Bioconjug Chem 2006;17:139-45

117. Elbayoumi T, VP T. Enhanced accumulation of long-circulating liposomes modified with the nucleosome-specific monoclonal antibody 2C5 in various tumours in mice: gamma-imaging studies. Eur J Nucl Med Mol Imaging 2006;33:1196-205

118. Sawant RM, Cohen MB, Torchilin VP, et al. Prostate cancer-specific monoclonal antibody 5D4 significantly enhances the cytotoxicity of doxorubicin-loaded liposomes against target cells in vitro. J Drug Target 2008;16:601-4

•• **An illustrative comparison between drug loaded antibodies conjugated liposome with non-targeted drug loaded liposome.**

119. Wang X, Yang L, Chen ZG, et al. Application of nanotechnology in cancer therapy and imaging. CA Cancer J Clin 2008;58:97-110

120. Kou G, Gao J, Wang H, et al. Preparation and Characterization of Paclitaxel-loaded PLGA nanoparticles coated with cationic SM5-1 single-chain antibody. J Biochem Mol Biol 2007;40:731-9

121. Sanz L, Qiao J, Vile RG, et al. Antibody engineering, virus retargeting and cellular immunotherapy: one ring to rule them all? Curr Gene Ther 2005;5:63-70

122. Wu Z, Asokan A, Samulski RJ. Adeno-associated virus serotypes: vector toolkit for human gene therapy. Mol Ther 2006;14:316-27

123. Thorsen F, Afione S, Huszthy PC, et al. Adeno-associated virus (AAV) serotypes 2, 4 and 5 display similar transduction profiles and penetrate solid tumor tissue in models of human glioma. J Gene Med 2006;8:1131-40

124. Hoffmann D, Wildner O. Efficient generation of double heterologous promoter controlled oncolytic adenovirus vectors by a single homologous recombination step in Escherichia coli. BMC Biotechnol 2006;6:36

125. Pestourie C, Tavitian B, Duconge F. Aptamers against extracellular targets for in vivo applications. Biochimie 2005;87:921-30

126. Smith JE, Medley CD, Tang Z, et al. Aptamer-conjugated nanoparticles for the collection and detection of multiple cancer cells. Anal Chem 2007;79:3075-82

127. Burgstaller P, Jenne A, Blind M. Aptamers and aptazymes: accelerating small molecule drug discovery. Curr Opin Drug Discov Devel 2002;5:690-700

128. Drolet DW, Nelson J, Tucker CE, et al. Pharmacokinetics and safety of an anti-vascular endothelial growth factor aptamer (NX1838) following injection into the vitreous humor of rhesus monkeys. Pharm Res 2000;17:1503-10

129. Hicke BJ, Stephens AW. Escort aptamers: a delivery service for diagnosis and therapy. J Clin Invest 2000;106:923-8

130. Kompella UB. Drug delivery to the back of the eye. Arch Soc Esp Oftalmol 2007;82:667-8;69-70

131. Ulrich H, Martins AH, Pesquero JB. RNA and DNA aptamers in cytomics analysis. Curr Protoc Cytom 2005; Chapter 7: Unit 7 28

•• **Provides the protocols for the development of fluorescent-tagged RNA or DNA aptamers for any cell surface protein in cytomics analysis.**

132. Farokhzad O, Jon S, Khademhosseini A, et al. Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res 2004;64:7668-72

133. Farokhzad OC, Karp JM, Langer R. Nanoparticle-aptamer bioconjugates for cancer targeting. Expert Opin Drug Deliv 2006;3:311-24

•• **Fundamental review contains detail description of the structure and properties of aptamer as well as the criteria for constructing effective conjugates.**

134. Ireson CR, Kelland LR. Discovery and development of anticancer aptamers. Mol Cancer Ther 2006;5:2957-62

135. Soundararajan S, Chen W, Spicer EK, et al. The nucleolin targeting aptamer AS1411 destabilizes Bcl-2 messenger RNA in human breast cancer cells. Cancer Res 2008;68:2358-65

136. Santulli-Marotto S, Nair SK, Rusconi C, et al. Multivalent RNA aptamers that inhibit CTLA-4 and enhance tumor immunity. Cancer Res 2003;63:7483-9

137. Huang Y, Chang H, Tan W. Cancer cell targeting using multiple aptamers conjugated on nanorods. Anal Chem 2008;80:567-72

138. Xing Y, So MK, Koh AL, et al. Improved QD-BRET conjugates for detection and imaging. Biochem Biophys Res Commun 2008;372:388-94

139. Bagalkot V, Zhang L, Levy-Nissenbaum E, et al. Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. Nano Lett 2007;7:3065 -70

140. Javier DJ, Nitin N, Levy M, et al. Aptamer-targeted gold nanoparticles as molecular-specific contrast agents for reflectance imaging. Bioconjug Chem 2008;19:1309-12

141. Li G, Wong AJ. EGF receptor variant III as a target antigen for tumor immunotherapy. Expert Rev Vaccines 2008;7:977-85

142. Minko T, Dharap SS, Pakunlu RI, et al. Molecular targeting of drug delivery systems to cancer. Curr Drug Targets 2004;5:389-406

143. Gu FX, Karnik R, Wang AZ, et al. Targeted nanoparticles for cancer therapy. nano today 2007;2:14-21

144. Curnis F, Sacchi A, Gasparri A, et al. Isoaspartate-glycine-arginine: a new tumor vasculature-targeting motif. Cancer Res 2008;68:7073-82

145. Dijkgraaf I, Rijnders AY, Soede A, et al. Synthesis of DOTA-conjugated multivalent cyclic-RGD peptide dendrimers via 1,3-dipolar cycloaddition and their biological evaluation: implications for tumor targeting and tumor imaging purposes. Org Biomol Chem 2007;5:935-44

•• **This article high lights that tetrameric RGD dendrimer has better tumor targeting properties than its dimeric and monomeric congeners.**

146. Rubinstein I SI, Onyuksel H. Intracellular delivery of VIP-grafted sterically stabilized phospholipid mixed nanomicelles in human breast cancer cells. Chem Biol Interact 2008;171:190-4

147. Tkachenko AG, Xie H, Liu Y, et al. Cellular trajectories of peptide-modified gold particle complexes: comparison of nuclear localization signals and peptide transduction domains. Bioconjug Chem 2004;15:482-90

148. Kakar SS, Jin H, Hong B, et al. LHRH receptor targeted therapy for breast cancer. Adv Exp Med Biol 2008;614:285-96

149. Graff A, Tropel D, Raman S, et al. Peptidic nanoparticles: for cancer diagnosis and therapy. NanoBioTechnol 2005;1:293-94

150. Surti N, Naik S, Bagchi T, et al. Intracellular delivery of nanoparticles of an antiasthmatic drug. AAPS PharmSciTech 2008;9:217-23

151. Montet X, Weissleder R, Josephson L. Imaging pancreatic cancer with a peptide-nanoparticle conjugate targeted to normal pancreas. Bioconjug Chem 2006;17:905-11

152. Reimold I, Domke D, Bender J, et al. Delivery of nanoparticles to the brain detected by fluorescence microscopy. Eur J Pharm Biopharm 2008;70:627-32

153. Tosi G, Costantino L, Rivasi F, et al. Targeting the central nervous system: in vivo experiments with peptide-derivatized nanoparticles loaded with Loperamide and Rhodamine-123. J Control Release 2007;122:1-9

154. Liu Y, Steiniger SC, Kim Y, et al. Mechanistic studies of a peptidic GRP78 ligand for cancer cell-specific drug delivery. Mol Pharm 2007;4:435-47

155. Brumlik M, Daniel B, Waehler R, et al. Trends in immunoconjugate and ligand-receptor based targeting development for cancer therapy. Expert Opin Drug Deliv 2008;5:87-103

156. Hultdin J, Van Guelpen B, Bergh A, et al. Plasma folate, vitamin B12, and homocysteine and prostate cancer risk: a prospective study. Int J Cancer 2005;113:819-24

157. Stern ST, McNeil SE. Nanotechnology safety concerns revisited. Toxicol Sci 2008;101:4-21

158. Harichand-Herdt S, Ramalingam SS. Targeted therapy for the treatment of non-small cell lung cancer: focus on inhibition of epidermal growth factor receptor. Semin Thorac Cardiovasc Surg 2008;20:217-23

159. Li SD, Chono S, Huang L. Efficient gene silencing in metastatic tumor by siRNA formulated in surface-modified nanoparticles. J Control Release 2008;126:77-84

Affiliation

Manasi Das, Chandana Mohanty, Sanjeeb K Sahoo† PhD
Author for correspondence
Laboratory for Nanomedicine,
Institute of Life Sciences,
Nalco Square, Chandrasekharpur,
Bhubaneswar, Orissa, India
Tel: +91 674 230 2094;
Fax: +91 674 230 0728;
E-mail: sanjeebsahoo2005@gmail.com