Nanocarriers as an emerging platform for cancer therapy

Nanotechnology has the potential to revolutionize cancer diagnosis and therapy. Advances in protein engineering and materials science have contributed to novel nanoscale targeting approaches that may bring new hope to cancer patients. Several therapeutic nanocarriers have been approved for clinical use. However, to date, there are only a few clinically approved nanocarriers that incorporate molecules to selectively bind and target cancer cells. This review examines some of the approved formulations and discusses the challenges in translating basic research to the clinic. We detail the arsenal of nanocarriers and molecules available for selective tumour targeting, and emphasize the challenges in cancer treatment.

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Cancer remains one of the world's most devastating diseases, with more than 10 million new cases every year[1]. However, mortality has decreased in the past two years[2] owing to better understanding of tumour biology and improved diagnostic devices and treatments. Current cancer treatments include surgical intervention, radiation and chemotherapeutic drugs, which often also kill healthy cells and cause toxicity to the patient. It would therefore be desirable to develop chemotherapeutics that can either passively or actively target cancerous cells. Passive targeting exploits the characteristic features of tumour biology that allow nanocarriers to accumulate in the tumour by the enhanced permeability and retention (EPR) effect[2]. Passively targeting nanocarriers first reached clinical trials in the mid-1980s, and the first products, based on liposomes and polymer–protein conjugates, were marketed in the mid-1990s. Later, therapeutic nanocarriers based on this strategy were approved for wider use (Table 1) and methods of further enhancing targeting of drugs to cancer cells were investigated. Active approaches achieve this by conjugating nanocarriers containing chemotherapeutics with molecules that bind to overexpressed antigens or receptors on the target cells. Recent reviews provide perspective on the use of nanotechnology as a fundamental tool in cancer research and nanomedicine[3,4]. Here we focus on the potential of nanocarriers and molecules that can selectively target tumours, and highlight the challenges in translating some of the basic research to the clinic.

PASSIVE AND ACTIVE TARGETING

Nanocarriers encounter numerous barriers en route to their target, such as mucosal barriers and non-specific uptake[5,6]. To address the challenges of targeting tumours with nanotechnology, it is necessary to combine the rational design of nanocarriers with the fundamental understanding of tumour biology (Box 1).

General features of tumours include leaky blood vessels and poor lymphatic drainage. Whereas free drugs may diffuse non-specifically, a nanocarrier can extravasate (escape) into the tumour tissues via the leaky vessels by the EPR effect[7] (Fig. 1). The increased permeability of the blood vessels in tumours is characteristic of rapid and defective angiogenesis (formation of new blood vessels from existing ones). Furthermore, the dysfunctional lymphatic drainage in tumours retains the accumulated nanocarriers and allows them to release drugs into the vicinity of the tumour cells. Experiments using liposomes of different mean size suggest that the threshold vesicle size for extravasation into tumours is ~400 nm (ref. 8), but other studies have shown that particles with diameters <200 nm are more effective[5,8–10].

Although passive targeting approaches form the basis of clinical therapy, they suffer from several limitations. Ubiquitously targeting cells within a tumour is not always feasible because some drugs cannot diffuse efficiently and the random nature of the approach makes it difficult to control the process. This lack of control may induce multiple-drug resistance (MDR) — a situation where chemotherapy treatments fail patients owing to resistance of cancer cells towards one or more drugs. MDR occurs because transporter proteins that expel drugs from cells are overexpressed on the surface of cancer cells[4,11,12]. Expelling drugs inevitably lowers the therapeutic effect and cancer cells soon develop resistance to a variety of drugs. The passive strategy is further limited because certain tumours do not exhibit

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Box 1 Rational design of nanocarriers for cancer therapy

Nanocarriers can offer many advantages over free drugs. They:

- protect the drug from premature degradation;
- prevent drugs from prematurely interacting with the biological environment;
- enhance absorption of the drugs into a selected tissue (for example, solid tumour);
- control the pharmacokinetic and drug tissue distribution profile;
- improve intracellular penetration.

For rapid and effective clinical translation, the nanocarrier should:

- be made from a material that is biocompatible, well characterized, and easily functionalized;
- exhibit high differential uptake efficiency in the target cells over normal cells (or tissue);
- be either soluble or colloidal under aqueous conditions for increased effectiveness;
- have an extended circulating half-life, a low rate of aggregation, and a long shelf life.

Table 1 Representative examples of nanocarrier-based drugs on the market

Compound	Commercial name	Nanocarrier	Indications
Styrene maleic anhydride-neocarzinostatin (SMANCS)	Zinostatin/Stimalmer	Polymer–protein conjugate	Hepatocellular carcinoma
PEG-L-asparaginase	Oncaspar	Polymer–protein conjugate	Acute lymphoblastic leukemia
PEG-granulocyte colony-stimulating factor (G-CSF)	Neulasta/PEGfilgrastim	Polymer–protein conjugate	Prevention of chemotherapy-associated neutropenia
IL2 fused to diphtheria toxin	Ontak (Denilelukin diftitox)	Immunotoxin (fusion protein)	Cutaneous T-cell lymphoma
Anti-CD33 antibody conjugated to calicheamicin	Mylotarg	Chemo-immunoconjugate	Acute myelogenous leukemia
Anti-CD20 conjugated to yttrium-90 or indium-111	Zevalin	Radio-immunoconjugate	Relapsed or refractory, low-grade, follicular, or transformed non-Hodgkin's lymphoma
Anti-CD20 conjugated to iodine-131	Bexxar	Radio-immunoconjugate	Relapsed or refractory, low-grade, follicular, or transformed non-Hodgkin's lymphoma
Daunorubicin	DaunoXome	Liposomes	Kaposi's sarcoma
Doxorubicin	Myocet	Liposomes	Combinational therapy of recurrent breast cancer, ovarian cancer, Kaposi's sarcoma
Doxorubicin	Doxil/Caelyx	PEG-liposomes	Refractory Kaposi's sarcoma, recurrent breast cancer, ovarian cancer
Vincristine	Onco TCS	Liposomes	Relapsed aggressive non-Hodgkin's lymphoma (NHL)
Paclitaxel	Abraxane	Albumin-bound paclitaxel nanoparticles	Metastatic breast cancer

the EPR effect, and the permeability of vessels may not be the same throughout a single tumour[13].

One way to overcome these limitations is to programme the nanocarriers so they actively bind to specific cells after extravasation. This binding may be achieved by attaching targeting agents such as ligands — molecules that bind to specific receptors on the cell surface — to the surface of the nanocarrier by a variety of conjugation chemistries[9]. Nanocarriers will recognize and bind to target cells through ligand–receptor interactions, and bound carriers are internalized before the drug is released inside the cell (Fig 1). In general, when using a targeting agent to deliver nanocarriers to cancer cells, it is imperative that the agent binds with high selectivity to molecules that are uniquely expressed on the cell surface. Other important considerations are outlined below.

To maximize specificity, a surface marker (antigen or receptor) should be overexpressed on target cells relative to normal cells. For example, to efficiently deliver liposomes to B-cell receptors using the anti-CD19 monoclonal antibody (mAb), the density of receptors should be in the range of 10^4–10^5 copies per cell. Those with lower density are less effectively targeted[14]. In a breast cancer model, a receptor density of 10^5 copies of ErbB2 receptors per cell was necessary to improve the therapeutic efficacy of an anti-ErbB2-targeted liposomal doxorubicin relative to its non-targeted counterpart[15].

The binding of certain ligands to their receptors may cause receptor-mediated internalization, which is often necessary if nanocarriers are to release drugs inside the cell[16–18]. For example, a more significant therapeutic outcome was achieved when immunoliposomes targeted to human blood cancer (B-cell lymphoma) were labelled with an internalizing anti-CD19 ligand rather than a non-internalizing anti-CD20 ligand[19]. In contrast, targeting nanocarriers to non-internalizing receptors may sometimes be advantageous in solid tumours owing to the bystander effect, where cells lacking the target receptor can be killed through drug release at the surface of the neighbouring cells, where carriers can bind[20].

It is generally known that higher binding affinity increases targeting efficacy. However, for solid tumours, there is evidence that high binding affinity can decrease penetration of nanocarriers due to a 'binding-site barrier', where the nanocarrier binds to its target so strongly that penetration into the tissue is prevented[16,21]. In addition to enhanced affinity, multivalent binding effects (or avidity) may also be used to improve targeting. The collective binding in a multivalent interaction is much stronger than monovalent binding. For example, dendrimer nanocarriers conjugated to 3–15 folate molecules showed a 2,500–170,000-fold enhancement in dissociation constants (K_D) over free folate when attaching to folate-binding proteins immobilized on a surface. This was attributed to the avidity of the multiple folic acid groups on the periphery of the dendrimers[22].

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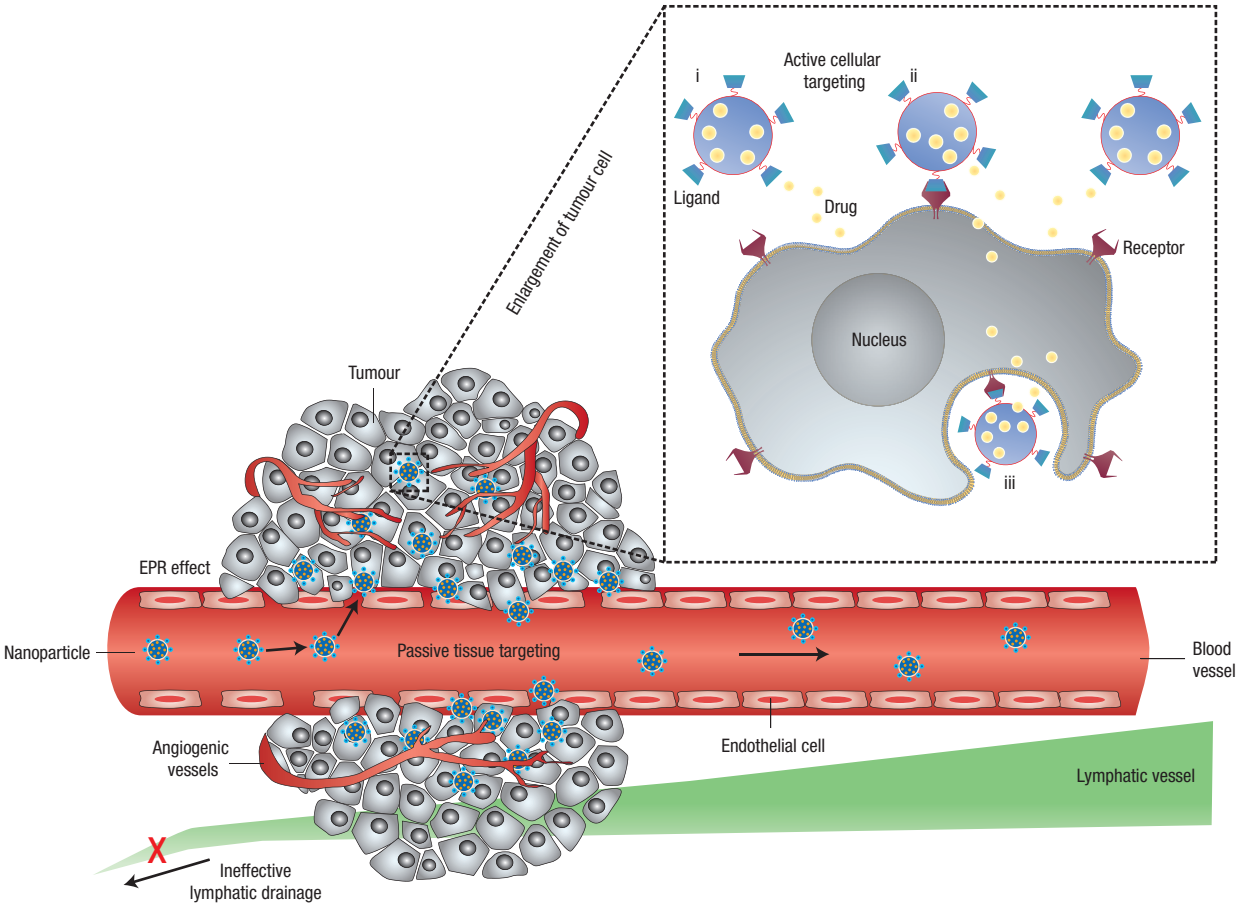

Figure 1 Schematic representation of different mechanisms by which nanocarriers can deliver drugs to tumours. Polymeric nanoparticles are shown as representative nanocarriers (circles). Passive tissue targeting is achieved by extravasation of nanoparticles through increased permeability of the tumour vasculature and ineffective lymphatic drainage (EPR effect). Active cellular targeting (inset) can be achieved by functionalizing the surface of nanoparticles with ligands that promote cell-specific recognition and binding. The nanoparticles can (i) release their contents in close proximity to the target cells; (ii) attach to the membrane of the cell and act as an extracellular sustained-release drug depot; or (iii) internalize into the cell.

TYPES OF TARGETING AGENTS

Targeting agents can be broadly classified as proteins (mainly antibodies and their fragments), nucleic acids (aptamers), or other receptor ligands (peptides, vitamins, and carbohydrates).

Targeting cancer with a mAb was described by Milstein in 1981[23]. Over the past two decades, the feasibility of antibody-based tissue targeting has been clinically demonstrated (reviewed in refs 24,25) with 17 different mAbs approved by the US Food and Drug Administration (FDA)[26]. The mAb rituximab (Rituxan) was approved in 1997 for treatment of patients with non-Hodgkin's lymphoma — a type of cancer that originates in lymphocytes[27]. A year later, Trastuzumab (Herceptin), an anti-HER2 mAb that binds to ErbB2 receptors, was approved for the treatment of breast cancer[28]. The first angiogenesis inhibitor for treating colorectal cancer, Bevacizumab (Avastin), an anti-VEGF mAb that inhibits the factor responsible for the growth of new blood vessels, was approved in 2004[29]. Today, over 200 delivery systems based on antibodies or their fragments are in preclinical and clinical trials[16,30]. Recent developments in the field of antibody engineering have resulted in the production of antibodies that contain animal and human origins such chimeric mAbs, humanized mAbs (those with a greater human contribution), and antibody fragments.

Antibodies may be used in their native state or as fragments for targeting (Fig. 2a). However, use of whole mAbs is advantageous because the presence of two binding sites (within a single antibody) gives rise to a higher binding avidity. Furthermore, when immune cells bind to the Fc portion of the antibody, a signalling cascade is initiated to kill the cancer cells. However, the Fc domain of an intact mAb can also bind to the Fc receptors on normal cells, as occurs with macrophages. This may lead to increased immunogenicity — the ability to evoke an immune response — and liver and spleen uptake of the nanocarrier. An additional advantage of whole/intact antibodies is their ability to maintain stability during long-term storage. Although antibody fragments including antigen-binding fragments (Fab), dimers of antigen-binding fragments ($F(ab')_2$), single-chain fragment variables (scFv) and other engineered fragments are less stable than whole antibodies, they are considered safer when injected systemically owing to reduced non-specific binding[16,30]. To rapidly select antibodies or their fragments that bind to and internalize within cancer cells, phage display libraries that involve a high throughput approach may be used[31,32]. This method generates a multitude of potentially useful antibodies that bind to the same target cells but to different epitopes (a part of a macromolecule that is recognized by antibodies; one receptor may have several epitopes that will be recognized by multiple antibodies). For example, through a selective

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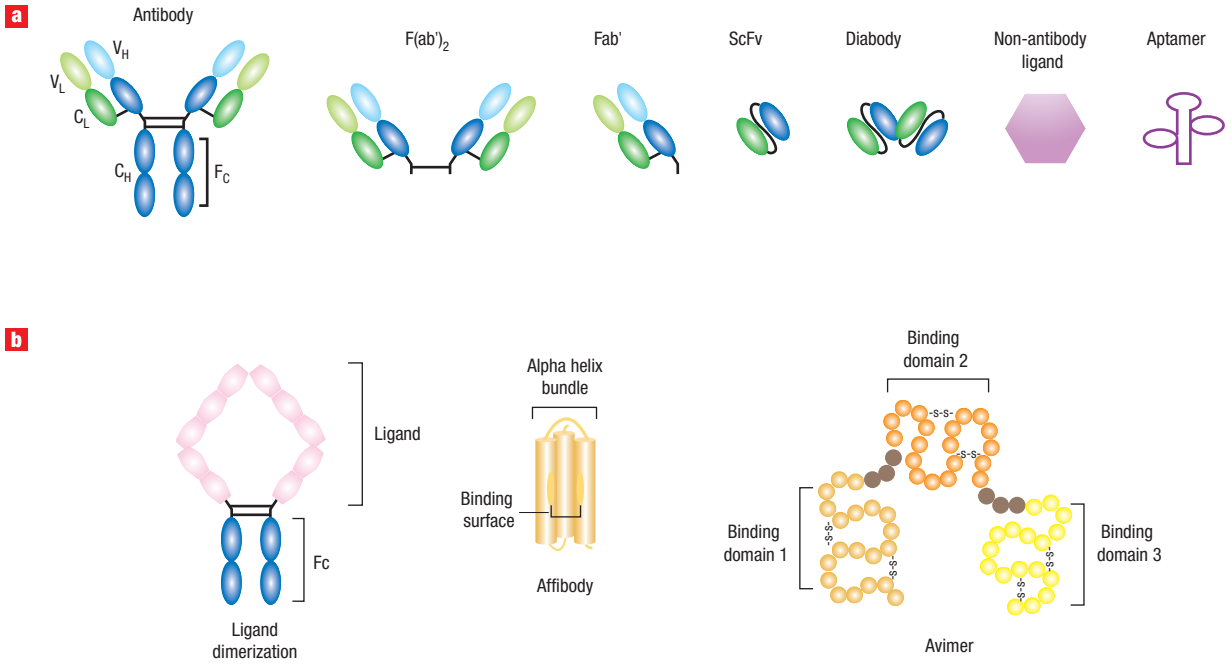

Figure 2 Common targeting agents and ways to improve their affinity and selectivity. **a**, The panel shows a variety of targeting molecules such as a monoclonal antibody or antibodies' fragments, non-antibody ligands, and aptamers. The antibody fragments F(ab')$_2$ and Fab' are generated by enzymatic cleavage whereas the Fab', scFv, and bivalent scFv (diabody) fragments are created by molecular biology techniques. V_H: variable heavy chain; V_L: variable light chain; C_H: constant heavy chain; C_L: constant light chain. Non-antibody ligands include vitamins, carbohydrates, peptides, and other proteins. Aptamers can be composed of either DNA or RNA. **b**, Affinity and selectivity can be increased through ligand dimerization or by screening for conformational-sensitive targeting agents such as affibodies, avimers and nanobodies, as well as intact antibodies and their fragments.

process, scFv antibodies have been identified for superior binding and internalization properties for prostate cancer cells[33].

It is possible to increase the efficacy of antibodies by conjugating a therapeutic agent directly to it for targeted delivery. For example, in 2000, the chemotherapeutic drug, calicheamicin, which is conjugated with the anti-CD33 antibody (marketed under the trade name Mylotarg), was the first clinically approved formulation that targets cancerous cells. Others include Zevalin and Bexxar, which use anti-CD20 antibodies to target radioisotopes to cancer cells (Table 1). Although the efficacy of these therapies has been proven, lethal side effects have been observed, likely due to non-specific binding[34] between the targeting agent and non-target moieties on the cell surface. Another reason could be the interaction of the targeting agent with its target expressed on non-cancerous cells. For example, BR96-doxorubicin — an immunoconjugate linked with doxorubicin and comprising an antibody that targets and binds to the Lewis-Y antigen (expressed on 75% of all breast cancers) — demonstrated significant anti-tumour activity in mouse tumour models. BR96-doxorubicin showed lower toxicity than that resulting from doxorubicin alone and it was efficacious in these animal models[35]. However, in dogs, an acute enteropathy (pathology of the intestine) was observed presumably due to binding of the conjugate to Lewis-Y-related antigens expressed by non-targeted gastrointestinal epithelial cells. In Phase II human clinical studies, BR96-doxorubicin immunoconjugates had limited anti-tumour activity and caused severe gastrointestinal toxicity, leading to termination of the study[36].

Although using genomics and proteomics technology to choose appropriate targets is an active area of research, to date no clinically effective targets have been identified. Creating new technologies to enhance selectivity and targeting efficacy with existing targets seem more promising. For example, fusion proteins can be created by combining two or more genes to produce a new protein with desired properties. Antibodies can be engineered so they bind to their target with high affinity, and using molecular biology techniques, it is possible to design protein-based ligand mimetics based on the structure of a receptor. Dimerization of proteins or peptides can increase ligand affinity through divalency — two simultaneous binding events, usually involving concurrent binding of a protein or a peptide to the two Fc domains of an antibody (Fig 2b). For example, dimerization of a low-affinity scFv (also known as diabody) against the ErbB2, led to enhanced tumour localization in a mouse tumour model[37].

It is also possible to increase binding affinity and selectivity to cell surface targets by engineering proteins that detect a specific conformation of a target receptor. In a recent *in vivo* study using a fusion protein consisting of an scFv antibody fragment to target and deliver small interfering RNA (siRNA) to lymphocytes — a type of white blood cell — a 10,000-fold increased affinity for the target receptor, integrin LFA-1, was observed[18]. Integrin LFA-1 is usually present in a low-affinity non-adhesive form on naïve leukocytes (white blood cells that are not activated by cancer cells or pathogens that enter the body), but converts to the high-affinity adhesive form through conformational changes on activation of the immune system. Therefore, targeting the high-affinity form of LFA-1 enables drugs to be selectively delivered to the activated and adhesive leukocytes. New classes of targeting molecules can be engineered to target specific conformations. These include small protein domains, known as affibodies, that can be engineered to bind specifically to different target proteins in a conformational-sensitive manner. Other small proteins that act like antibodies — called avimers — are used to bind selectively to target receptors through multivalent effects. Nanobodies, which are heavy-chain antibodies engineered to one tenth of the size of an intact antibody with a missing light chain, have been used to

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Table 2 Examples of nano-based platforms and their current stage of development for use in cancer therapy

Type of carrier and mean diameter (nm)	Drug entrapped or linked	Current stage of development	Type of cancer (for clinical trials)	References
Polymer–drug conjugates (6–15)	Doxorubicin, Paclitaxel, Camptothecin, Platinate, TNP-470	12 products under clinical trials (Phases I–III) and *in vivo*	Various tumours	Reviewed in 3, 61
Liposomes (both PEG and non-PEG coated) (85–100)	Lurtotecan, platinum compounds, Annamycin	Several products in clinical trials (Phases I–III) and *in vivo*	Solid tumours, renal cell carcinoma, mesothelioma, ovarian and acute lymphoblastic leukaemia	Reviewed in 9
Polymeric nanoparticles (50–200)	Doxorubicin, Paclitaxel, platinum-based drugs, Docetaxel	Several products are in clinical trials (Phases I–III) and *in vivo*	Adenocarcinoma of the oesophagus, metastatic breast cancer and acute lymphoblastic leukemia	5, 91, 100, 101
Polymersomes (~100)	Doxorubicin, Paclitaxel	*In vivo*		73, 74
Micelles (lipid based and polymeric) (5–100)	Doxorubicin	Clinical trials (Phase I)	Metastatic or recurrent solid tumours refractory to conventional chemotherapy	77, 92, 102
	Paclitaxel	Clinical trials (Phase I)	Pancreatic, bile duct, gastric and colonic cancers	
	Platinum-based drugs (carboplatin/cisplatin), Camptothecin, Tamoxifen, Epirubicin	*In vivo* and *in vitro*		Reviewed in 75
Nanoshells (Gold-silica) (~130)	No drug (for photothermal therapy)	*In vivo*		37, 103
Gold nanoparticles (10–40)	No drug (for photothermal ablation)	*In vivo*		104
Nanocages (30–40)	No drug	Chemistry, structural analysis and *in vitro*		90, 105
Dendrimers (~ 5)	Methotrexate	*In vitro / in vivo*		46, 86
Immuno-PEG-liposomes (100)	Doxorubicin	Clinical trials (Phase I)	Metastatic stomach cancer	76
Immunoliposomes (100–150)	Doxorubicin, platinum-based drugs, Vinblastin, Vincristin, Topotecan, Paclitaxel	*In vivo*		Reviewed in 9, 106
Immunotoxins, Immunopolymers, and fusion proteins (3–15)	Various drugs, toxins	Clinical trials (Phases I–III)	Various types of cancer	Reviewed in 16, 17, 61

bind to carcinoembryonic antigen (CEA), a protein used as a tumour marker[38–40] (Fig. 2b).

In addition to the rational design of antibodies, high-throughput approaches have been used to generate targeting agents such as aptamers, which are short single-stranded DNA or RNA oligonucleotides selected *in vitro* from a large number of random sequences (~10^{14}–10^{15}). Aptamers are selected to bind to a wide variety of targets, including intracellular proteins, transmembrane proteins, soluble proteins, carbohydrates, and small molecule drugs. Several aptamers have also been developed to bind specifically to receptors on cancer cells, and thus may be suitable for nanoparticle-aptamer conjugate therapy[41]. For example, docetaxel (Dtxl)-encapsulated nanoparticles whose surface is modified with an aptamer that targets the antigen on the surface of prostate cancer cells, were delivered with high selectivity and efficacy *in vivo*[42].

Growth factor or vitamin interactions with cancer cells represent a commonly used targeting strategy, as cancer cells often overexpress the receptors for nutrition to maintain their fast-growing metabolism. Epidermal growth factor (EGF) has been shown to block and reduce tumour expression of the EGF receptor, which is overexpressed in a variety of tumour cells such as breast and tongue cancer[43]. Additionally, based on the same idea, the vitamin folic acid (folate) has also been used for cancer targeting because folate receptors (FRs) are frequently overexpressed in a range of tumour cells including ovarian, endometrial and kidney cancer[44]. Transferrin (Tf) interacts with Tf receptors (TfRs), which are overexpressed on a variety of tumour cells (including pancreatic, colon, lung, and bladder cancer) owing to increased metabolic rates[45]. Direct coupling of these targeting agents to nanocarriers containing chemotherapies such as drugs has improved intracellular delivery and therapeutic outcome in animal tumour models[46–48]. One challenge with targeting receptors whose expression correlates with metabolic rate, such as folate and Tf, is that these receptors are also expressed in fast-growing healthy cells such as fibroblasts, epithelial and endothelial cells. This could lead to non-specific targeting and subsequently decrease the effectiveness of the drug and increase toxicity[49].

The use of peptides as targeting agents — including arginine–glycine–aspartic acid (RGD), which is the ligand of the cell adhesion integrin $\alpha_v\beta_3$ on endothelial cells — results in increased intracellular drug delivery in different murine tumour models[50,51]. However, RGD also binds to other integrins such as $\alpha_5\beta_1$ and $\alpha_4\beta_1$ and therefore is not specific to cancer cells, which may limit its use. In addition to cell surface antigens, extracellular matrices (ECMs) overexpressed in tumours, such as heparin sulphate, chondroitin sulphate, and hyaluronan (HA), may also serve as effective targets for specific ECM receptors[52,53]. Coating liposomes with HA improves circulation time and enhances targeting to HA receptor-expressing tumours *in vivo*[54,55].

THE ARSENAL OF NANOCARRIERS

Nanocarriers are nanosized materials (diameter 1–100 nm) that can carry multiple drugs and/or imaging agents. Owing to their high surface-area-to-volume ratio, it is possible to achieve high ligand density on the surface for targeting purposes. Nanocarriers can also be used to increase local drug concentration by carrying the drug within and control-releasing it when bound to the targets. Currently, natural and synthetic polymers and lipids are typically used as drug delivery vectors; clinically approved formulations are listed in Table 1. The family of nanocarriers includes polymer

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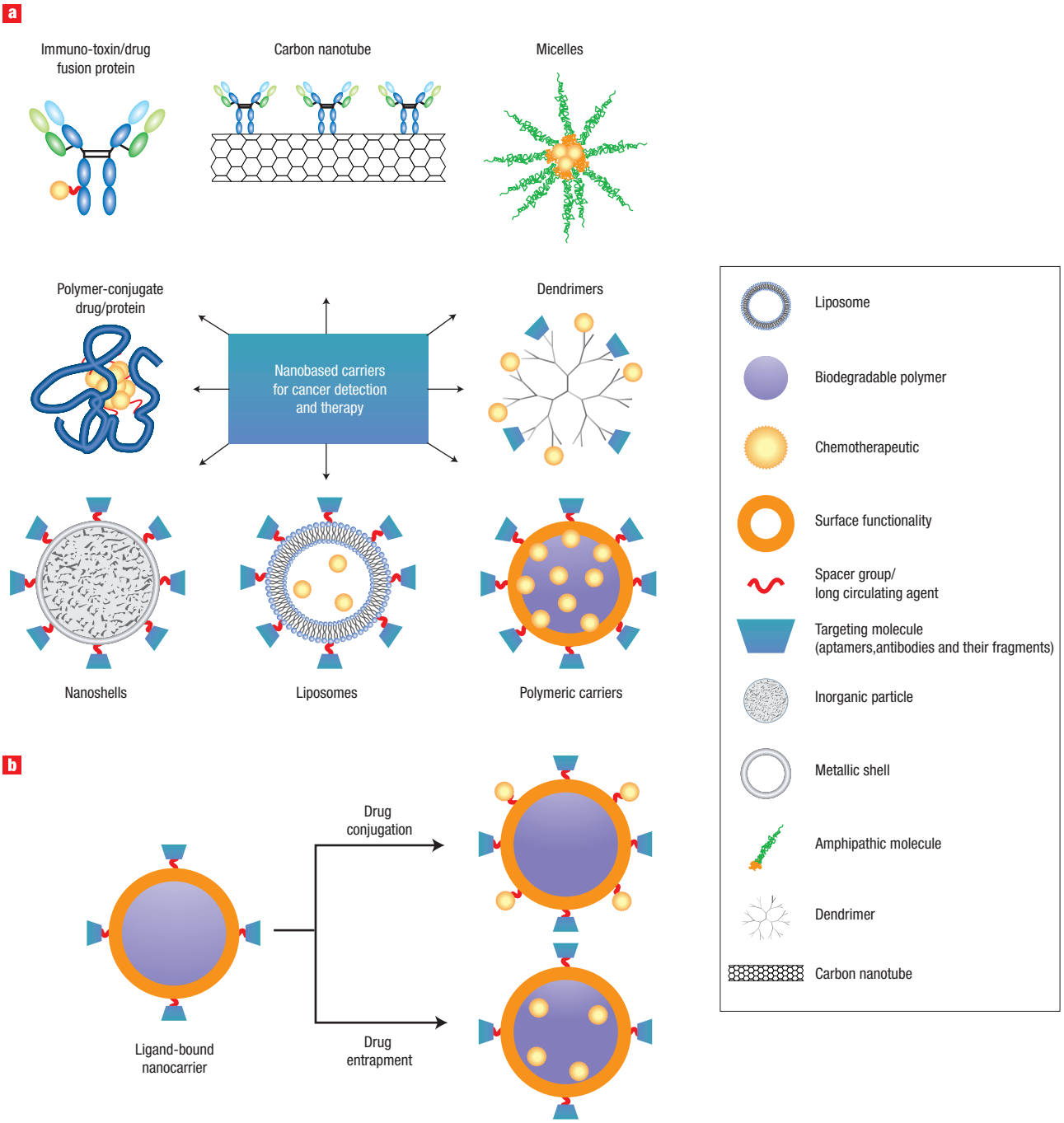

Figure 3 Examples of nanocarriers for targeting cancer. **a**, A whole range of delivery agents are possible but the main components typically include a nanocarrier, a targeting moiety conjugated to the nanocarrier, and a cargo (such as the desired chemotherapeutic drugs). **b**, Schematic diagram of the drug conjugation and entrapment processes. The chemotherapeutics could be bound to the nanocarrier, as in the use of polymer–drug conjugates, dendrimers and some particulate carriers, or they could be entrapped inside the nanocarrier.

conjugates, polymeric nanoparticles, lipid-based carriers such as liposomes and micelles, dendrimers, carbon nanotubes, and gold nanoparticles, including nanoshells and nanocages (Fig. 3a). These nanocarriers have been explored for a variety of applications such as drug delivery, imaging, photothermal ablation of tumours, radiation sensitizers, detection of apoptosis, and sentinel lymph-node mapping[3,4,56] (Table 2).

To date, at least 12 polymer–drug conjugates have entered Phase I and II clinical trials (Table 2 and Fig. 3a) and are especially useful for targeting blood vessels in tumours. Examples include anti-endothelial immunoconjugates, fusion proteins[57–59], and caplostatin, the first polymer-angiogenesis inhibitor conjugates[60]. Polymers that are chemically conjugated with drugs are often considered new chemical entities (NCEs) owing to a distinct pharmacokinetic profile from

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that of the parent drug. Despite the variety of novel drug targets and sophisticated chemistries available, only four drugs (doxorubicin, camptothecin, paclitaxel, and platinate) and four polymers (N-(2-hydroxylpropyl)methacrylamide (HPMA) copolymer, poly-*L*-glutamic acid, poly(ethylene glycol) (PEG), and Dextran) have been repeatedly used to develop polymer–drug conjugates[3,61].

Polymers are the most commonly explored materials for constructing nanoparticle-based drug carriers. One of the earliest reports of their use for cancer therapy dates back to 1979[62] when adsorption of anticancer drugs to polyalkylcyanoacrylate nanoparticles was described. Couvreur *et al.* revealed the release mechanism of the drugs from the polymer in calf serum, followed by tissue distribution and efficacy studies in a tumour model[63]. This work laid the foundation for the development of doxorubicin-loaded nanoparticles that were tested in clinical trials in the mid-1980s[64]. Polymeric nanoparticles can be made from synthetic polymers, including poly(lactic acid) (PLA) and poly(lactic co-glycolic acid)[65], or from natural polymers such as chitosan[66] and collagen[67] and may be used to encapsulate drugs without chemical modification. The drugs can be released in a controlled manner through surface or bulk erosion, diffusion through the polymer matrix, swelling followed by diffusion, or in response to the local environment. Several multifunctional polymeric nanoparticles are now in various stages of pre-clinical and clinical development[4,56,68,69]. Concerns arising from the use of polymer-based nanocarriers include the inherent structural heterogeneity of polymers, reflected, for example, in a high polydispersity index (the ratio of the weight-and-number-average molecular weight (M_w/M_n)). There are, however, a few examples of polymeric nanoparticles that show near-homogenous size distribution[70].

Lipid-based carriers have attractive biological properties, including general biocompatibility, biodegradability, isolation of drugs from the surrounding environment, and the ability to entrap both hydrophilic and hydrophobic drugs. Through the addition of agents to the lipid membrane or by the alteration of the surface chemistry, properties of lipid-based carriers, such as their size, charge, and surface functionality, can easily be modified. Liposomes, polymersomes, and micelles represent a class of amphiphile-based particles. Liposomes are spherical, self-closed structures formed by one or several concentric lipid bilayers with inner aqueous phases. Today, liposomes are approved by regulatory agencies to carry a range of chemotherapeutics[26,71,72] (Table 1).

Polymersomes have an architecture similar to that of liposomes, but they are composed of synthetic polymer amphiphiles, including PLA-based copolymers[73,74] (Table 2). However, as with polymer therapeutics, there are still no clinically approved strategies that use active cellular targeting for lipid-based carriers.

Micelles, which are self-assembling closed lipid monolayers with a hydrophobic core and hydrophilic shell, have been successfully used as pharmaceutical carriers for water-insoluble drugs (Table 2)[75]. They belong to a group of amphiphilic colloids that can be formed spontaneously under certain concentrations and temperatures from amphiphilic or surface-active agents (surfactants) (Fig. 3a). An example of a polymeric micelle under clinical evaluation is NK911, which is a block copolymer of PEG and poly(aspartic acid). NK911, which consists of a bound doxorubicin fraction (~45%) (Fig. 3b) and a free drug[76], was evaluated for metastatic pancreatic cancer treatment. Another carrier is NK105, a micelle containing paclitaxel, was evaluated for pancreatic, colonic and gastric tumour treatment[77].

Lipid-based carriers pose several challenges, which represent general issues in the use of other targeted nanocarriers such as polymeric nanoparticles. For example, upon intravenous injection, particles are rapidly cleared from the bloodstream by the reticuloendothelial defence mechanism, regardless of particle composition[78,79]. Moreover, instability of the carrier and burst drug release, as well as non-specific uptake by the mononuclear phagocytic system (MPS), provides additional challenges for translating these carriers to the clinic.

Given their long history, liposome-based carriers serve as a classic example of the challenges encountered in the development of nanocarriers and the solutions that have been attempted. For example, PEG has been used to improve circulation time by stabilizing and protecting micelles and liposomes from opsonization — a plasma protein deposition process that signals Kupffer cells in the liver to remove the carriers from circulation[75,80]. However, Daunosome and Myocet are examples of clinically used liposomes (80–90 nm in diameter) without PEG coating that have been reported to exhibit enhanced circulation times, although to a lesser degree than PEGylated liposomes such as Doxil/Caelyx (Table 1).

In addition to rapid clearance, another challenge is the fast burst release of the chemotherapeutic drugs from the liposomes. To overcome this phenomenon, doxorubicin, for example, may be encapsulated in the liposomal aqueous phase by an ammonium sulphate gradient[81]. This method achieves a stable drug entrapment with negligible drug leakage during circulation, even after prolonged residence in the blood stream[82]. In clinical practice, liposomal systems have shown preferential accumulation in tumours, via the EPR effect, and reduced toxicity of their cargo (Tables 1 and 2). However, long-circulating liposomes may lead to extravasation of the drug in unexpected sites. The most commonly experienced clinical toxic effect from the PEGylated liposomal doxorubicin is palmar-plantar erythrodysesthesia (PPE), also called the hand-foot syndrome. PPE — a dermatologic toxicity reaction seen with high doses of many types of chemotherapy — can be addressed by changing the dosing and scheduling of the treatment[83]. Other challenges facing the use of liposomes in the clinic include the high production cost, fast oxidation of some phospholipids, and lack of controlled-release properties of encapsulated drugs.

To achieve temporal release of two drugs, polymers and phospholipids can be combined as a single delivery agent (polymer core/lipid shell). After locating at a tumour site through the EPR effect, the outer phospholipid shell releases an anti-angiogenesis agent, and the inner polymeric nanoparticle subsequently releases a chemotherapy agent in response to local hypoxia — shortage of oxygen. This strategy led to reduced toxicity and enhanced anti-metastatic effects in two different mouse tumour models, emphasizing the advantages of a mechanism-based design for targeted nanocarriers[84].

Organic nanoparticles include dendrimers, viral capsids and nanostructures made from biological building blocks such as proteins. Abraxane is an albumin-bound paclitaxel nanoparticle formulation approved by the FDA in 2005 as a second-line treatment for metastatic breast cancer. Abraxane was designed to address insolubility problems encountered with paclitaxel. Its use eliminates the need for toxic solvents like Cremophor EL (polyoxyethylated castor oil), which has been shown to limit the dose of Taxol that can be administered[85].

Dendrimers are synthetic, branched macromolecules that form a tree-like structure whose synthesis represents a relatively new field in polymer chemistry. Polyamidoamine dendrimers have shown promise for biomedical applications because they (1) can be easily conjugated with targeting molecules, imaging agents, and drugs, (2) have high water solubility and well-defined chemical structures, (3) are biocompatible, and (4) are rapidly cleared from the blood through the kidneys, made possible by their small size (<5 nm), which eliminates the need for biodegradability. *In vivo* delivery of dendrimer–methotrexate conjugates using multivalent targeting results in a tenfold reduction in tumour size compared with that achieved with the same molar concentration of free systemic methotrexate[22,46]. This work provided motivation for further pre-clinical development, and a variety of dendrimers are now under investigation for cancer treatment and are extensively reviewed elsewhere[86,87]. Although

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promising, dendrimers are more expensive than other nanoparticles and require many repetitive steps for synthesis, posing a challenge for large-scale production.

Inorganic nanoparticles are primarily metal based and have the potential to be produced with near monodispersity. Inorganic materials have been extensively studied for magnetic resonance imaging and high-resolution superconducting quantum interference devices[88]. Inorganic particles may also be functionalized to introduce targeting molecules and drugs. Specific types of recently developed inorganic nanoparticles include nanoshells and gold nanoparticles.

Nanoshells (100–200 nm) may use the same carrier for both imaging and therapy (Table 2). They are composed of a silica core and a metallic outer layer. Nanoshells have optical resonances that can be adjusted to absorb or scatter essentially anywhere in the electromagnetic spectrum, including the near infrared region (NIR, 820 nm, 4 W cm^{-2}), where transmission of light through tissue is optimal. Absorbing nanoshells are suitable for hyperthermia-based therapeutics, where the nanoshells absorb radiation and heat up the surrounding cancer tissue. Scattering nanoshells, on the other hand, are desirable as contrast agents for imaging applications. Recently, a cancer therapy was developed based on absorption of NIR light by nanoshells, resulting in rapid localized heating to selectively kill tumours implanted in mice. Tissues heated above the thermal damage threshold displayed coagulation, cell shrinkage and loss of nuclear staining, which are indicators of irreversible thermal damage, whereas control tissues appeared undamaged[37,89].

A similar approach involves gold nanocages which are smaller (<50 nm) than the nanoshells. These gold nanocages (Table 2) can be constructed to generate heat in response to NIR light and thus may also be useful in hyperthermia-based therapeutics[90]. Unlike nanoshells and nanocages, pure gold nanoparticles (Table 2) are relatively easy to synthesize and manipulate. Non-specific interactions that cause toxicity in healthy tissues may impede the use of many types of nanoparticles, but using inorganic particles for photo-ablation significantly limits non-specific toxicity because light is locally directed. However, inorganic particles may not provide advantages over other types of nanoparticles for systemic targeting of individual cancer cells because they are not biodegradable or small enough to be cleared easily, resulting in potential accumulation in the body, which may cause long-term toxicity.

THE CHALLENGES OF MULTIDRUG RESISTANCE

The delivery of drugs through targeted nanocarriers that are internalized by cells provides an alternative route to diffusion of drugs into cells. This approach may allow targeted carriers to bypass the activity of integral membrane proteins, known as MDR transporters, which transport a variety of anticancer drugs out of the cancer cell and produce resistance against chemotherapy[11]. The molecular basis of cancer drug resistance is complex and has been correlated to elevated levels of enzymes that can neutralize chemotherapeutic drugs. More often, however, it is due to the overexpression of MDR transporters that actively pump chemotherapeutic drugs out of the cell and reduce the intracellular drug doses below lethal threshold levels. Because not all cancer cells express the MDR transporters, chemotherapy will kill only drug-sensitive cells that do not or only mildly express MDR transporters, while leaving behind a small population of drug-resistant cells that highly express MDR transporters. With tumour recurrence, chemotherapy may fail because residual drug-resistant cells dominate the tumour population.

Among the MDR transporters, the most widely investigated proteins are: P-glycoprotein (also referred to as MDR1 or ABCB1); the multidrug resistance associated proteins (MRPs), of which the most studied is the MRP1 (or ABCC1); and the breast cancer resistance protein (ABCG2). These proteins have different structures, but they share a similar function of expelling chemotherapy drugs from the cells[12]. Several studies have demonstrated the possibility of using nanocarriers to bypass the MDR transporters. SP1049C is a non-ionic (pluronic or also known as poloxamer) block copolymer composed of a hydrophobic core and hydrophilic tail that contains doxorubicin. SP1049C has been shown to circumvent p-glycoprotein-mediated drug resistance in a mouse model of leukaemia and is now under clinical evaluation[91,92]. Folate receptor-mediated cell uptake of doxorubicin–loaded liposomes into an MDR cell line was shown to be unaffected by P-glycoprotein (Pgp)-mediated drug efflux, in contrast to the uptake of free doxorubicin[93]. In an attempt to reverse MDR, vincristine-loaded lipid nanoparticles conjugated to an anti-Pgp mAb (MRK-16), showed greater cytotoxicity in resistant human myelogenous leukaemia cell lines than control non-targeted particles — a response attributed to the inhibition of the Pgp-mediated efflux of vincristine by MRK-16[94]. Additional reports have addressed the challenge of MDR using polymer therapeutics[95], polymeric nanoparticles[96], lipid nanocapsules[97] and micelles[98] within cell lines or in mouse tumour models. Combination treatments with targeted nanocarriers for selective delivery of drugs and MDR pump inhibitors will likely address some of the problems posed by resistant tumours.

INTO THE FUTURE

The choice of an appropriate nanocarrier is not obvious, and the few existing comparative studies are difficult to interpret because several factors may simultaneously affect biodistribution and targeting. In addition, developing suitable screening methodologies for determining optimal characteristics of nanocarriers remains elusive. Therefore, successful targeting strategies must be determined experimentally on a case-by-case basis, which is laborious. In addition, systemic therapies using nanocarriers require methods that can overcome non-specific uptake by mononuclear phagocytic cells and by non-targeted cells. It is also not clear to what extent this is possible without substantially increasing the complexity of the nanocarrier and without influencing commercial scale-up. Improved therapeutic efficacy of targeted nanocarriers has been established in multiple animal models of cancer, and currently more than 120 clinical trials are underway with various antibody-containing nanocarrier formulations[99]. For the clinician, in addition to enhancing confidence through the ability to image the type and location of the tumour, it is imperative to construct appropriate therapeutic regimens. When targeting cell surface markers presents a significant challenge, as in the case for solid tumours, targeting tumour vasculature or the extracellular matrix surrounding the tumour microenvironment may be necessary. In the case of circulating cancer cells, as in leukaemia and lymphoma, a therapy that targets surface antigens with high affinity and includes a carrier with a long circulating half-life may be the most efficacious. Similar to combination drug strategies that may be personalized to optimize treatment regimens, oncologists in the near future may be presented with the ability to choose specific nanocarrier/targeting molecule combinations which could lead to improved therapeutic outcomes and reduced costs.

Although we are still far from Nobel Prize winner Paul Ehrlich's 'magic bullet', many believe that we will soon enter an era in which nanocarrier-based approaches will represent an important modality within therapeutic and diagnostic oncology.

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Competing financial interests

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/naturenanotechnology.

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