Nanoparticle therapeutics: an emerging treatment modality for cancer

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Abstract | Nanoparticles — particles in the size range 1–100 nm — are emerging as a class of therapeutics for cancer. Early clinical results suggest that nanoparticle therapeutics can show enhanced efficacy, while simultaneously reducing side effects, owing to properties such as more targeted localization in tumours and active cellular uptake. Here, we highlight the features of nanoparticle therapeutics that distinguish them from previous anticancer therapies, and describe how these features provide the potential for therapeutic effects that are not achievable with other modalities. While large numbers of preclinical studies have been published, the emphasis here is placed on preclinical and clinical studies that are likely to affect clinical investigations and their implications for advancing the treatment of patients with cancer.

Nanoparticle therapeutics are typically particles comprised of therapeutic entities, such as small-molecule drugs, peptides, proteins and nucleic acids, and components that assemble with the therapeutic entities, such as lipids and polymers, to form nanoparticles (FIG. 1). Such nanoparticles can have enhanced anticancer effects compared with the therapeutic entities they contain. This is owing to more specific targeting to tumour tissues via improved pharmacokinetics and pharmacodynamics, and active intracellular delivery (FIG. 1). These properties depend on the size and surface properties (including the presence of targeting ligands) of the nanoparticles.

In this Review, we first briefly discuss the key properties of nanoparticles and how they differ from other types of cancer drugs. Next, we summarize current clinical uses of first-generation nanoparticle therapeutics and describe how newer, experimental nanoparticle therapeutics differ from first-generation therapeutics. Finally, we discuss what is on the near horizon for cancer therapy that use nanoparticles. Although an enormous amount of research is ongoing in this area, most will not be translatable to the clinic. Some of the main obstacles include the use of immunostimulatory components, the use of components that have barriers to large-scale current good manufacturing practice (cGMP) production and/or hurdles in the development of well-defined chemistry, manufacturing and controls assays. A limited number of nanoparticle systems have reached a clinical application (or are about to reach this status), and information is becoming available to begin to understand some of the issues of moving these experimental systems into humans. Thus, our emphasis here is on issues involving the translation of these experimental nanoparticle therapeutics into the clinic and their application in clinical settings. Although there are several experimental approaches utilizing nanoparticles that can be affected by external stimulation, we focus our attention on systemically administered nanoparticles that do not require external stimuli.

Key properties of anticancer nanoparticles

Nanoparticle size. It is currently thought that the diameter of nanoparticle therapeutics for cancer should be in the range of 10–100 nm. The lower bound is based on the measurement of sieving coefficients for the glomerular capillary wall, as it is estimated that the threshold for first-pass elimination by the kidneys is 10 nm (diameter)[1]. The upper bound on size is not as well defined at this time. The vasculature in tumours is known to be leaky to macromolecules. The lymph system of tumours in mouse models is poorly operational and macromolecules leaking from the blood vessels accumulate — a phenomenon known as "enhanced permeability and retention (EPR) effect"[2]. Numerous lines of evidence suggest that this phenomenon is also operational in humans. It has been shown that entities in the order of hundreds of nanometre in size can leak out of the blood vessels and accumulate within tumours. However, large

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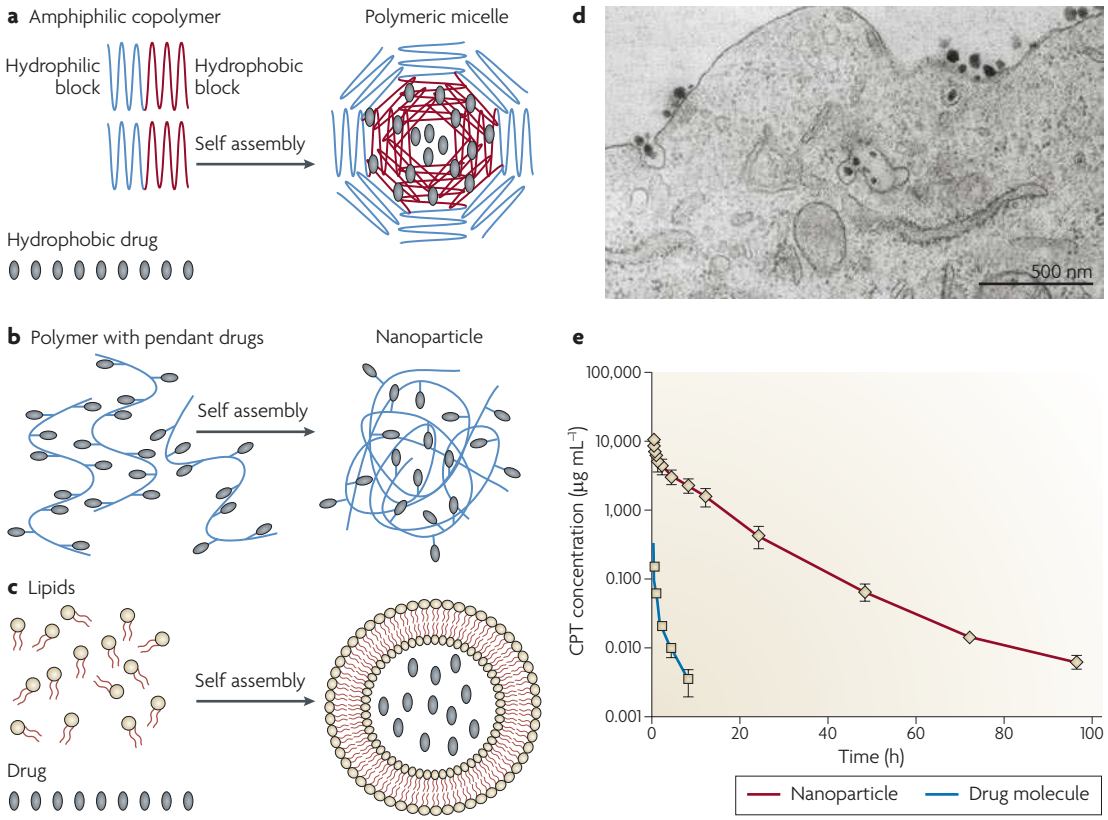

Figure 1 | **Major classes of nanoparticles that are in clinical trials and some of their properties. a** | Nanoparticles formed from therapeutic entities and block copolymers that can form polymeric micelles. **b** | Nanoparticles that form with polymer–drug conjugates. **c** | Nanoparticles formed of liposomes. **d** | Nanoparticles can enter cells via endocytosis. This figure shows a transmission electron micrograph of nanoparticles at the surface of a cancer cell, entering the cell and within endocytic vesicles. **e** | Nanoparticles can have extended pharmacokinetics over the therapeutic entity alone. The data are from a small-molecule drug (CPT) and a nanoparticle containing CPT in rats. Panel (**e**) is adapted with permission from REF. 94 © Springer (2006).

macromolecules or nanoparticles could have limited diffusion in the extracellular space[3]. Experiments from animal models suggest that sub-150 nm, neutral or slightly negatively charged entities can move through tumour tissue[4]. Additionally, recent data show that nanoparticles in the 50–100 nm size range that carry a very slight positive charge can penetrate throughout large tumours following systemic administration[5]. Thus, well-designed nanoparticles in the 10–100 nm size range and with a surface charge either slightly positive or slightly negative should have accessibility to and within disseminated tumours when dosed into the circulatory system. If this size range is correct, then these nanoparticles will be restricted from exiting normal vasculature (that requires sizes less than 1–2 nm); however they will still be able to access the liver, as entities up to 100–150 nm in diameter are able to do so.

Nanoparticle surface properties. Nanoparticles have high surface-to-volume ratios when compared with larger particles, and so control of their surface properties is crucial to their behaviour in humans (for example, see REF. 6). The ultimate fate of nanoparticles within the body can be determined by the interactions of nanoparticles with their local environment, which depends on a combination of size and surface properties. Nanoparticles that are sterically stabilized (for example by polyethylene glycol (PEG) polymers on their surface) and have surface charges that are either slightly negative or slightly positive tend to have minimal self–self and self–non-self interactions. Also, the inside surface of blood vessels and the surface of cells contain many negatively charged components, which would repel negatively charged nanoparticles. As the surface charge becomes larger (either positive or negative), macrophage scavenging is increased and can lead to greater clearance by the reticuloendothelial system. Thus, minimizing nonspecific interactions via steric stabilization and control of surface charge helps to prevent nanoparticle loss to undesired locations. However, the complete removal of nonspecific interactions is not currently possible, and so there is always some particle loss; the key is to minimize these interactions as much as possible.

If nanoparticle loss could be avoided, it would be expected that the distribution of the nanoparticles within a mammal would be uniform if no size restrictions existed on the basis of thermodynamic considerations. However, there are numerous size-restricted locations

within the body that would create non-uniformity. For example, the brain is protected by the blood–brain barrier, which has severe size and surface property limitations for entrance. By understanding the size and surface property requirements for reaching specified sites within the body, localization of nanoparticles to these sites can be accomplished.

Nanoparticle-targeting ligands. The addition of targeting ligands that provide specific nanoparticle–cell surface interactions can play a vital role in the ultimate location of the nanoparticle. For example, nanoparticles can be targeted to cancer cells if their surfaces contain moieties such as small molecules, peptides, proteins or antibodies. These moieties can bind with cancer cell-surface receptor proteins, such as transferrin receptors, that are known to be increased in number on a wide range of cancer cells[7]. These targeting ligands enable nanoparticles to bind to cell-surface receptors and enter cells by receptor-mediated endocytosis (FIG. 1). Recent work comparing non-targeted and targeted nanoparticles (lipid-based[8] or polymer-based[9]) has shown that the primary role of the targeting ligands is to enhance cellular uptake into cancer cells rather than increasing the accumulation in the tumour.

Distinguishing features of nanoparticle therapeutics for cancer. Nanoparticles can be tuned to provide long or short circulation times by careful control of size and surface properties. Also, they can be directed to specific cell types within target organs (for example, hepatocytes versus Kupffer cells in the liver[10]). While other types of cancer therapeutics such as molecular conjugates (for example, antibody–drug conjugates) can also meet these minimum specifications, targeted nanoparticles have at least five features that distinguish them from other therapeutic modalities for cancer.

First, nanoparticles can carry a large payload of drug entity and protect it from degradation. For example, a 70 nm nanoparticle can contain approximately 2,000 small interfering RNA (siRNA) molecules[11], whereas antibody conjugates carry fewer than ten[12]. These high payload amounts can also be achieved with other drug types such as small-molecule or peptide drugs. Furthermore, nanoparticle payloads are located within the particle, and their type and number do not affect the pharmacokinetic properties and biodistribution of the nanoparticles. This is unlike molecular conjugates in which the type and number of therapeutic entities conjugated to the targeting ligand (such as an antibody) significantly modifies the overall properties of the conjugate.

Second, the nanoparticles are sufficiently large to contain multiple targeting ligands that can allow multivalent binding to cell-surface receptors[13]. Nanoparticles have two parameters for tuning the binding to target cells: the affinity of the targeting moiety and the density of the targeting moiety. The multivalency effects can lead to high effective affinities when using arrangements of low-affinity ligands[13–15]. Thus, the repertoire of molecules that can be used as targeting agents is greatly expanded as many low-affinity ligands that are not sufficient for use as molecular conjugates can now be attached on nanoparticles to create higher affinity via multivalent binding to cell-surface receptors (FIG. 2).

Third, nanoparticles are sufficiently large to accommodate multiple types of drug molecules. Numerous therapeutic interventions can be simultaneously applied with a nanoparticle in a controlled manner. As mentioned in the first point, the fact that the pharmacokinetic properties of the nanoparticle are not modified by the amount of the therapeutic also holds true with multiple types of the therapeutics being combined together within the nanoparticles.

Fourth, the release kinetics of drug molecules from nanoparticles can be tuned to match the mechanism of action. For example, topoisomerase I inhibitors such as the camptothecin-based chemotherapeutic drugs are reversible binders of the enzyme. So, the mechanism of action for camptothecin-based drugs on the topoisomerase I enzyme suggests enhanced efficacy with prolonged exposure of the drug[16], making a slow release from the nanoparticles most desirable. With siRNA, the gene inhibition kinetics are greatly influenced by cell-cycle times[17,18], and for uses in cancer there may not be a need for slow release of the therapeutic agent.

Fifth, nanoparticles could have the potential to bypass multidrug resistance mechanisms that involve cell-surface protein pumps (for example, glycoprotein P), as they enter cells via endocytosis (FIG. 1 and discussed below). Overall, it seems that controlled combination of these features through nanoparticle design could minimize side effects of anticancer drugs while enhancing efficacy, and clinical results are emerging that suggest that this promise is starting to be realized. Nanoparticle types and results from the clinic are summarized below.

Nanoparticles as anticancer agents

Nanoscaled systems for systemic cancer therapy and their latest stage of development are summarized in TABLE 1. We have included PEG-containing proteins and PEG-conjugated small molecules, which, as single molecules in solution, can be defined as nanoscale therapeutics or as nanoparticles if they have some degree of polymer–polymer interaction to give assembled entities with more than one polymer chain contained within.

Liposomes (~100 nm and larger) carrying chemotherapeutic small-molecule drugs have been approved for cancer since the mid-1990s, and are mainly used to solubilize drugs, leading to biodistributions that favour higher uptake by the tumour than the free drug[19]. However, liposomes do not provide control for the time of drug release, and in most cases do not achieve effective intracellular delivery of the drug molecules[19], therefore limiting their potential to be useful against multidrug-resistant cancers.

A representative example shown in TABLE 1 is Doxil (Ortho Biotech), a PEG-liposome containing the cytotoxic drug doxorubicin. Doxil was originally approved for the treatment of AIDS-related Kaposi's sarcoma and is now approved for use in ovarian cancer and multiple myeloma. This agent circulates in the body as a nanoparticle and has

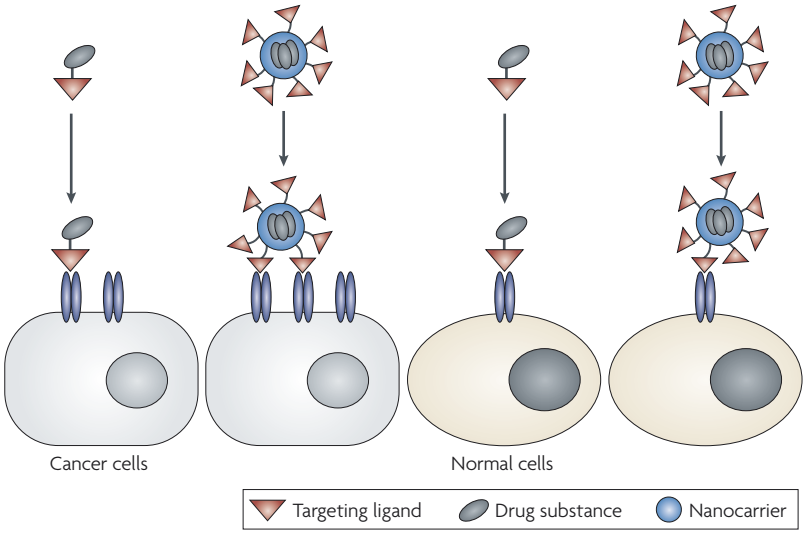

Figure 2 | **Nanoparticles with numerous targeting ligands can provide multivalent binding to the surface of cells with high receptor density.** When the surface density of the receptor is low on normal cells, then a molecular conjugate with a single targeting agent and a targeted nanoparticle can compete equally for the receptor as only one ligand–receptor interaction may occur. However, when there is a high surface density of the receptor on cancer cells (for example, the transferrin receptor), then the targeted nanoparticle can engage numerous receptors simultaneously (multivalency) to provide enhanced interactions over the one ligand–one receptor interaction that would occur with a molecular conjugate.

a half-life ~100-times longer than free doxorubicin (see below). Its primary advantage in the clinic is the reduction in cardiotoxicity over that of doxorubicin[20,21].

However, such nanoscaled systems have also shown that unwanted attributes can manifest themselves. For example, although Doxil has been shown to have reduced cardiotoxicity compared with free doxorubicin, it also has skin toxicity that is not observed with the drug alone[22]. Newer nanoparticle systems, as defined by a higher degree of multifunctionality (incorporating features such as slow release and/or targeting ligands), have enhanced features such as reduced toxicity without the emergence of other toxicities (as with Doxil) compared with the initial approved products, and some of these attributes are described below.

Nanoparticles without targeting ligands

TABLE 2 compares several nanoparticle-based therapeutics with the drug molecules that they carry. The types of particles include liposomes, polymer micelles and polymer-based nanoparticles.

In each case — for example, doxorubicin compared with the doxorubicin-carrying nanoparticles SP1049C, NK911 and Doxil — the nanoparticle alters the pharmacokinetic properties of the drug molecule. Circulation half-lives are listed in TABLE 2, although they are difficult to compare because different models are used for their determination. Clearance is a common pharmacokinetic parameter that is readily available from clinical data, and it is a better indicator of differences in circulation times among the therapeutics. Dramatically reduced clearances have been obtained with nanoparticles such as Doxil, XYOTAX (CT-2103) and IT-101. The longer circulation times of the nanoparticles compared with the free drug alone can improve tumour uptake (for example, see REF. 23). Moreover, polymeric micelles (sub-100 nm) have been shown to accumulate more readily in tumours than the larger liposomes[24]. Additionally, movement of a particle throughout a tumour is also size dependent as described earlier. It is speculated that nanoparticles that are smaller than 100 nm but larger than 10 nm (to avoid renal clearance) will be optimal for tumour penetration. Therefore, careful control of size will be important to the pharmacokinetics, biodistribution, tumour accumulation and tumour penetration of the nanoparticle therapeutic.

Some of the nanoparticles that are now in clinical testing also have mechanisms to control the release of the drug, as discussed below with IT-101. These methodologies are based on cleavage of a chemical bond between the particle and the drug by hydrolysis; by enzymes that are located within and outside cells, for example, lysozymes, esterases; or by enzymes that are located only within cells, for example, cathepsin B.

Clinical trials using non-targeting nanoparticles. Doxil has been used in the clinic for over two decades and it has been discussed extensively elsewhere[25–27]. Numerous other liposomes containing drugs such as irinotecan and SN-38 are currently in clinical trials (ClinicalTrials.gov; see Further information).

ABI-007 (Abraxane; Abraxis Bioscience/AstraZeneca), an albumin-bound nanoparticle of paclitaxel (~120 nm in mean diameter) was developed to retain the therapeutic benefits of paclitaxel but eliminate the toxicities associated with the emulsifier Cremophor EL in the paclitaxel formulation (Taxol) and its generic equivalents[28]. The maximally tolerated dose (MTD) of Abraxane was approximately 70–80% higher than that reported for Taxol. In a Phase III study of 454 patients with metatastic breast cancer given Abraxane (260 mg per m^2) or Taxol (175 mg per m^2) intravenously every 3 weeks, response rates were significantly greater in patients treated with Abraxane than those receiving Taxol[29]. Despite the increased dose of paclitaxel in the Abraxane group, the incidence of grade 4 neutropaenia was significantly lower with Abraxane than with Taxol (9% versus 22%; $p = 0.001$). Pharmacokinetic assessments also showed that paclitaxel clearance and volume of distribution were higher for Abraxane than for Taxol. Clearance was 13 litres per hour per m^2 for Abraxane versus 14.76 litres per hour per m^2 for Taxol ($p = 0.048$)[28]. Distribution was 663.8 litres per m^2 for Abraxane versus 433.4 litres per m^2 for Taxol ($p = 0.04$)[28]. These differences in pharmacokinetic properties may be associated with the higher intratumoral concentrations observed with Abraxane compared with the equivalent dose of Taxol. However, it should be noted that it is not clear whether or not the nanoparticles dissolve when infused into the patient, and there are indications that they do (see below in final section). Thus, the clinical benefit from Abraxane is probably not due to its functioning as a nanoparticle but to other factors such as the removal of Cremophor EL from the formulation that causes toxicities of its own[30].

Clearance
This is the volume of blood/plasma cleared of the drug per time. Lower clearances are indicative of higher circulation times.

Neutropaenia
Neutropaenia, usually induced by chemotherapy, is a myelosuppression that involves mainly the neutrophil lineage of white blood cells. Severe (grade 3 or 4) neutropaenia with infection is life-threatening, which should be prevented by treatment with growth factors. For more information see the Common Toxicity Criteria at the US National Cancer Institute web site (see Further information).

Table 1 | **Nanoscaled systems for systemic cancer therapy**

Platform	Latest stage of development	Examples
Liposomes	Approved	DaunoXome, Doxil
Albumin-based particles	Approved	Abraxane
PEGylated proteins	Approved	Oncospar, PEG-Intron, PEGASYS, Neulasta
Biodegradable polymer–drug composites	Clinical trials	Doxorubicin Transdrug
Polymeric micelles	Clinical trials	Genexol-PM*, SP1049C, NK911, NK012, NK105, NC-6004
Polymer–drug conjugate-based particles	Clinical trials	XYOTAX (CT-2103), CT-2106, IT-101, AP5280, AP5346, FCE28068 (PK1), FCE28069 (PK2), PNU166148, PNU166945, MAG-CPT, DE-310, Pegamotecan, NKTR-102, EZN-2208
Dendrimers	Preclinical	Polyamidoamine (PAMAM)
Inorganic or other solid particles	Preclinical (except for gold nanoparticle that is clinical)	Carbon nanotubes, silica particles, gold particles (CYT-6091)

*Approved in South Korea. PEG, polyethylene glycol.

Several nanoscaled therapeutics based on PEGylated proteins have been approved or are in clinical trials. PEGylation has been applied to various proteins including enzymes, cytokines and monoclonal antibody Fab fragments. PEGylation provides a means to increase protein solubility, reduce immunogenicity, prevent rapid renal clearance (due to the increased size of conjugates) and prolong plasma half-life[31]. PEG-L-asparaginase (Oncospar; Enzon) was approved by the US Food and Drug Administration in 1994 to treat acute lymphoblastic leukaemia[32,33]. Although free-drug L-asparaginase depletes asparagine and is active against acute lymphoblastic leukaemia and lymphoma, it frequently induces a hypersensitivity reaction and antibody production that leads to its premature clearance from the circulation. Phase I studies with Oncospar showed an increased plasma half-life compared with the naked enzyme and a reduced frequency of hypersensitivity reaction[34]. A subsequent Phase II study showed a partial response and a reduced hypersensitivity reaction in some Oncospar-treated patients with refractory lymphoma[35,36]. PEG-recombinant arginine deaminase (PEG–rhArg) has been assessed either as a single agent or in combination with 5-fluorouracil[37,38]. Weekly intramuscular injection of PEG–rhArg showed a clinical activity in hepatocellular carcinoma with the achievement of low arginine concentrations of <2 μM[38].

PEG has also been linked to biological response modifiers such as interferon-α (IFN-α) and recombinant granulocyte colony-stimulating factor (G-CSF)[39,40]. Two PEGylated IFN-α conjugates, PEGASYS (Roche) for IFN-α2a and PEGINTRON (Schering) for IFN-α2b, have shown clinically superior antiviral activity compared with free IFN-α and are approved for hepatitis C therapy[41]. These PEG–IFN-α conjugates have been shown to be effective for the treatment of melanoma and renal cell carcinoma[42–44], and are currently being tested in other solid tumours. Because of the prolonged half-lives of PEG–IFN-α conjugates, they can be given by subcutaneous injection once every 12 weeks instead of three-times per week for free IFNs.

Because of the success of PEGylated proteins, it is not surprising that PEG polymers have also been conjugated with small-molecule drugs to create nanoparticles. Pegamotecan is a camptothecin conjugated with linear PEG and has a molecular mass of ~40,000 Daltons. Two Phase I trials were completed with Pegamotecan (weekly dosing[45] and every 3-week dosing[46]) as was a Phase II trial[47]; however Enzon is no longer pursuing this conjugate. Other PEG-conjugated chemotherapeutics are currently in Phase I trials for advanced solid tumours, including PEG–irinotecan (NKTR-102) and PEG–SN-38 (EZN-2208) (ClinicalTrials.gov; see Further information). The PEGylated small molecules have increased circulation times relative to the free drug[45,46], thus providing the potential for greater tumour accumulation via the EPR effects.

In addition to altering the pharmacokinetics of the therapeutic, nanoscaled systems can provide other functions. For example, PG–paclitaxel conjugates that link high loading levels of paclitaxel (37% wt/wt) with a biodegradeable polymer, poly-L-glutamic acid (PG) have been in numerous clinical trials including Phase III, and XYOTAX (also called CT-2103) is clinically the most advanced polymer–small-molecule conjugate used for systemic administration. Paclitaxel is released from the polymer to a small extent by slow hydrolysis (up to 14% over 24 hours), but is released to a greater extent following lysosomal cathepsin B degradation of the polymer backbone after endocytic uptake[48]. EPR-mediated tumour targeting and enhanced efficacy of PG–paclitaxel has been observed in many preclinical tumour models, with improvement of the safety profile due to both decreased exposure to normal tissue and improved drug solubility[49,50]. Phase I/II studies showed a significant number of partial responses or stable disease in patients with non-small-cell lung cancer (NSCLC), renal cell carcinoma, mesothelioma or paclitaxel-resistant ovarian cancer[51]. Severe side effects included neutropaenia and peripheral neuropathy, which are classical paclitaxel-associated toxicities. In a randomized Phase III trial, PG–paclitaxel was compared with gemcitabine or vinorelbine as a first-line therapy for performance status 2 patients with NSCLC[52]. Patients receiving the conjugate showed significantly reduced side effects when compared with control patients, most of whom received gemcitabine, but the nanoparticle conjugate failed to show significance for overall improved survival in comparison with either of the non-nanoparticle drugs. However, there was a greater increase in survival for women treated with PG–paclitaxel compared with men[53]. Such enhanced activities in female patients might correlate with oestrogen levels, as oestrogen has been shown to increase the expression of cathepsin B[54]. A definitive trial is now ongoing to compare PG–paclitaxel and free paclitaxel (175 mg per m^2) as a first-line therapy for women with NSCLC. A PG–camptothecin (CT-2106) nanoparticle has also been tested in Phase I/II clinical trials[45,46,55].

Table 2 | **Comparison of pharmacokinetics (human) of small-molecule drugs with nanoparticle therapeutics**

Name	Formulation	Diameter (nm)	$t_{1/2}$ (h)	Clearance (ml/min•kg)	Comments	Refs
Doxorubicin (DOX)	0.9% NaCl	NA	0.8	14.4	Small-molecule drug	24
SP1049C	Pluronic micelle + DOX	22–27	2.4	12.6	Micelle nanoparticle	24
NK911	PEG–Asp micelle + DOX	40	2.8	6.7	Micelle nanoparticle	24
Doxil	PEG–liposome + DOX	80–90	84.0	0.02	PEGylated liposome nanoparticle with long circulation	24
Taxol (paclitaxel)	Cremophor EL	NA	21.8 (20.5)	3.9 (9.2)	Small-molecule drug	24 (28)
Genexol-PM	PEG–PLA micelle + paclitaxel	20–50	11.0	4.8	Micelle nanoparticle	24
Abraxane	Albumin + paclitaxel	120*	21.6	6.5	Albumin nanoparticle before injection; status *in vivo* unknown	28
XYOTAX	PG + paclitaxel	Unknown	70–120	0.07–0.12	Polymer nanoparticle	23
Camptosar (prodrug of SN-38)	0.9% NaCl	NA	11.7	5.8	Small-molecule prodrug	95
LE-SN-38	Liposome + SN-38	Unknown	7–58	3.5–13.6	Liposome nanoparticle	97
Topotecan (camptothecin analogue)	0.9% NaCl	NA	3.0	13.5	Small-molecule drug	96
CT-2106	PG + camptothecin	Unknown	65–99	0.44	Polymer nanoparticle	98
IT-101	Cyclodextrin-containing polymer + camptothecin	30–40	38	0.03	Polymer nanoparticle with extended circulation times	66

*May dissolve upon exposure to blood. NA, not applicable; PEG, polyethylene glycol; PEG–PLA, block copolymer of PEG and poly(L-lactic acid); PG, polyglutamic acid; SN-38, 7-ethyl-10-hydroxycamptothecin.

Haematological toxicity
This includes suppression of red blood cells, white blood cells or platelet counts, and is usually induced by chemotherapeutic agents. Grade 4 toxicity, including severe anaemia, leucopaneia or thrombocytopaenia, requires immediate intervention to prevent life-threatening conditions. For more information see the Common Toxicity Criteria at the US National Cancer Institute web site (see Further information).

Cardiotoxicity
Cardiotoxicity is a toxicity that affects the heart functions. It includes arrhythmia, cardiac pumping dysfunction and eventually heart failure when it develops in severity. Grade 4 (severe) cardiotoxicity is associated with the life-threatening condition of arrhythmia or heart failure. For more information see the Common Toxicity Criteria at the US National Cancer Institute web site (see Further information).

Other polymer–small-molecule conjugates have completed several clinical programmes. For example, *N*-(2-hydroxypropyl)methacrylamide (HPMA) polymer conjugates demonstrated EPR-mediated tumour targeting[56], and improved antitumour efficacy in animal studies[57]. HPMA-polymer-Gly-Phe-Leu-Gly-doxorubicin (PK1; FCE28068) entered Phase I studies with a dosing schedule of once every 3 weeks at increasing doses up to a MTD of 320 mg per m^2 (doxorubicin equivalent), which is fourfold to fivefold higher than the usual dose of doxorubicin[58]. The dose-limiting toxicities were neutropaenia and mucositis, but the cumulative doses reached 1,680 mg per m^2 without observation of cardiotoxicity. Antitumour activity was observed in anthracycline-resistant breast cancer, NSCLC and colorectal cancer. A clinical pharmacokinetic study showed prolonged plasma circulation[58,59]. The HPMA conjugates of doxorubicin (PK1), paclitaxel (PNU166945), camptothecin (MAG-CPT) and platinum-containing drugs (AP5280 and AP5346) have been tested in the clinic, but at this time only the platinum-containing conjugate AP5346 remains in clinical development. The other conjugates have either shown unacceptable toxicity or disappointing efficacy[60].

NK911, a polymeric micelle nanoparticle, was designed for the enhanced delivery of doxorubicin. NK911 was given intravenously to patients with solid tumours every 3 weeks in a Phase I trial[61]. The starting dose was 6 mg per m^2 (doxorubicin equivalent) with dose escalation. A total of 23 patients were enrolled and a MTD of 67 mg per m^2 and a dose-limiting toxicity of neutropaenia were observed. Among these 23 patients, a partial response was observed in a patient with pancreatic cancer. The recommended Phase II dose was determined to be 50 mg per m^2 every 3 weeks.

SP1049C, a novel anticancer agent containing doxorubicin and two non-ionic pluronic block co-polymers, was designed to increase efficacy compared with doxorubicin. In a Phase I study, the pharmacokinetic profile of SP1049C showed a slower clearance than has been reported for free doxorubicin[62]. A subsequent Phase II study was conducted with SP1049C. Among 19 eligible patients with adenocarcinoma of the oesophagus, nine partial responses (47%) and eight stable diseases (42%) were achieved[63]. Haematological toxicities (grade 3 to 4) included neutropaenia (62%) and anaemia (5%), resulting in nine patients (43%) having the dose reduced to 55mg per m^2. Grade 1 cardiotoxicity was seen in four (19%) patients. The investigators concluded that SP1049C appears to be active in monotherapy in this group of patients and combination studies with other active agents are recommended.

Genexol-PM[64,65], a polymeric micelle containing paclitaxel, has just received approval in South Korea and represents the first such nanoparticle therapeutic to

be approved for the treatment of cancer. Genexol-PM is currently in Phase II trials in the United States. In a completed Phase I trial, of the 21 patients treated there were three partial responses (14%) and two of these patients were refractory to prior taxane therapy[64]. Similar to Abraxane, Genexol-PM does not require the use of pre-medications that are normally required with Taxol. A Phase II trial with patients with metastatic breast cancer has been reported for Genexol-PM administered at 300 mg per m^2 every 3 weeks[65]. Of the 39 patients that were evaluated, there were 5 complete responses (13%), 19 partial responses (49%), 13 stable diseases (33%) and 2 with progressive diseases (5%). However, because of grade 3 neuropathy, 17 patients had to have dose reductions.

Some of the newer multifunctional nanoparticles, such as IT-101, a conjugate of camptothecin and a cyclodextrin-based polymer, can have greatly extended circulation times (shown both in animals and in humans[66]), enter tumour cells and allow slow release of the drug[67]. Initial results from a Phase I clinical trial showed that several patients receiving IT-101, at doses compatible with a high quality of life, had long-term stable disease (approximately 1 year and more)[66]. These results and others are suggestive that agents shown to be active against tumours that are resistant to the drug via multidrug-resistant-mediated pump mechanisms[67] in animal experiments, may also be active in drug-resistant tumours in humans (discussed below).

Multifunctional nanoparticles such at IT-101 can provide for significantly reduced side effects without the generation of new toxicities compared with the drug that is contained within them. Because of the low side-effect profile of IT-101, it will be tested in a Phase II clinical trial in ovarian cancer as maintenance therapy (ClinicalTrials.gov; see Further information). IT-101 will be dosed in women who normally 'wait and watch' for disease progression to occur after chemotherapy to test whether or not the time to disease progression can be prolonged. If successful, this will open a new paradigm for nanoparticle therapeutics based on their potential to provide low toxicity and high efficacy.

Nanoparticles with targeting ligands

The nanoparticles listed in TABLE 2 utilize passive targeting to reach tumours. That is, it is thought that the leaky vasculature of tumours allows the nanoparticles to extravasate,whereas the normal vasculature does not (this property is involved in the altered biodistribution of nanoparticles compared with the drug molecule). Ultimately, active targeting via the inclusion of a targeting ligand on the nanoparticles is envisioned to provide the most effective therapy. TABLE 3 lists the few ligand-targeted therapeutics that are either approved or in (was in) the clinic.

PK2 (FCE28069), a HPMA-polymer-Gly-Phe-Leu-Gly-doxorubicin conjugate that also contains the sugar galactosamine, was the first ligand-targeted nanoparticle to reach the clinic. The galactose-based ligand was used to target the asialoglycoprotein receptor (ASGPR), which is expressed on hepatocytes, in the hope that its high expression is retained on primary liver cancer cells. However, as ASGPR is also expressed on healthy hepatocytes, the targeted nanoparticles accumulated in normal liver cells as well as in the tumour. In a Phase I trial, PK2 had a MTD of 160 mg per m^2 (doxorubicin equivalent dose), with dose-limiting toxicities that are typical of anthracyclines[69]. Of the 23 patients with primary hepatocellular carcinoma, two had progressive diseases, lasting 26 and 47 months, a third showed a reduction of tumour volume, and 11 had stable diseases[70]. Concentrations of drug in liver were 15–20% of the administered dose after 24 hours[70] and the concentrations in the tumour were 12–50-fold higher than would have been achieved through free doxorubicin.

At present, the only targeted nanoparticles in the clinic are MBP-426, which contains the cytotoxic platinum-based drug oxaliplatin in a liposome; SGT-53, a liposome containing a plasmid coding for the tumour suppressor p53; and CALAA-01, a polymer–siRNA composite. MCC-465, a targeted doxorubicin-containing liposome, does not appear to be currently in use. These nanoparticles all target the transferrin receptor, which is known to be upregulated in many types of cancer[7].

Additionally, targeted nanoparticles can have active mechanisms for the intracellular release of the therapeutic agent. For example, CALAA-01 is a targeted nanoparticle that has high drug (siRNA) payload per targeting ligand, proven multivalent binding to cancer cell surfaces and an active drug (siRNA) release mechanism that is triggered upon the recognition of intracellular localization by pH decline below a value of 6.0 (which occurs in the endocytic pathway)[5,9,11,71].

As mentioned above, recent work comparing non-targeted and targeted nanoparticles (lipid-based[8] or polymer-based[9] nanoparticles) has shown that the primary role of the targeting ligands is to enhance cellular uptake into cancer cells and to minimize the accumulation in normal tissue. This behaviour suggests that the colloidal properties of nanoparticles will determine their biodistribution, whereas the targeting ligand serves to increase the intracellular uptake in the target tumour. If this turns out to be the case in general, then the targeting ligand affinity and surface density on the nanoparticle will determine cellular uptake. Recently, Zhou *et al.* showed that the affinity–density relationships for nanoparticles can be determined[72]. The density of single-chain Fv antibody fragments was important for nanoparticle uptake and high affinities were not necessary if high densities were used. The results clearly demonstrated that high-density, low-affinity antibody fragments can provide uptake into cancer cells that is not increased when the affinity is increased. Although the specific affinities and densities are probably a function of cell-surface receptor densities, this work illustrates the importance of multivalency of the targeted nanoparticles discussed here.

Targeting efflux-pump-mediated resistance?

A major clinical obstacle that limits the efficacy of cancer therapeutics is the resistance of cancer cells to a multitude of chemotherapeutic and biological agents, known as multidrug resistance (MDR)[73,74]. MDR can

Neuropathy
Neuropathy is a disorder of the nervous system that includes dysfunction of cranial, motor and sensory nerves. When grade 3 or 4 neuropathy is developed, it can significantly jeopardize normal functions. Severe neuropathy includes paralysis, paraesthesia and disabling cognitive impairments. For more information see the Common Toxicity Criteria at the US National Cancer Institute web site (see Further information).

Table 3 | **Examples of ligand-targeted therapeutic agents**

Name	Targeting agent	Therapeutic agent	Status	Comments	Refs
Gemtuzumab ozogamicin (Mylotarg; UCB/Wyeth)	Humanized anti-CD33 antibody	Calicheamicin	Approved	Antibody–drug conjugate	99
Denileukin diftitox (Ontak; Ligand Pharmaceuticals/Eisai)	Interleukin 2	Diphtheria toxin fragment	Approved	Fusion protein of targeting agent and therapeutic protein	100
Ibritumomab tiuxetan (Zevalin; Cell Therapeutics)	Mouse anti-CD20 antibody	^{90}Yttrium	Approved	Antibody–radioactive element conjugate	101
Tositumomab (Bexxar; GlaxoSmithKline)	Mouse anti-CD20 antibody	^{131}Iodine	Approved	Antibody–radioactive element conjugate	101
FCE28069 (PK2)	Galactose	Doxorubicin	Phase I (stopped)	Small-molecule targeting agent conjugated to polymer nanoparticle	102
MCC-465	F(ab')$_2$ fragment of human antibody GAH	Doxorubicin	Phase I	Liposome nanoparticle containing antibody fragment targeting agent	103
MBP-426	Transferrin	Oxaliplatin	Phase I	Liposome nanoparticle containing human transferrin protein targeting agent	104,105
SGT-53	Antibody fragment to transferrin receptor	Plasmid DNA with p53 gene	Phase I	Liposome nanoparticle containing antibody fragment targeting agent	106
CALAA-01	Transferrin	Small interfering RNA	Phase I	Polymer-based nanoparticle containing human transferrin protein targeting agent	71,107

be caused by physiological barriers (non-cellular-based mechanisms), or by alterations in the biological and biochemical characteristics of cancer cells (cellular mechanisms). In the first case, non-cellular drug resistance mechanisms can be due to poorly vascularized tumour regions that greatly reduce drug access to the tumour tissues and thus protect cancerous cells from drug-induced cytotoxicity. Furthermore, high interstitial pressure and low microvascular pressure may also impede extravasation of drug molecules. In the second case, resistance of tumours to therapeutic intervention can be due to cellular mechanisms, including alteration of specific enzyme systems for drug metabolism, reduction of apoptotic activity, induction of the cellular repair system, mutation of the drug target, or increasing drug efflux in tumour cells.

Among these mechanisms, changes in the drug-efflux pump are the best known and most extensively investigated. The discovery that P-glycoprotein (P-gp) mediates active efflux of chemotherapeutic drugs from tumour cells initiated this line of reasoning[75,76]. P-gp is a product of the *MDR1* gene and is a 170-kDa transmembrane glycoprotein that functions as a transporter or efflux pump (it is one of the ATP-binding cassette (ABC) proteins) that removes drug out of cells. To date, numerous inhibitors of ABC transporters (including P-gp) have been investigated as potential anticancer agents[77]; however, the results have been disappointing.

Alternative strategies for overcoming drug resistance could be based on systems that allow selective drug accumulation in tumour tissues, tumour cells or even compartments of tumour cells without increased systemic toxicity. This might be provided by nanoparticle-based drugs because they enter cells by endocytosis (FIGS 1,3). Also, by choosing an appropriate nanoparticle formulation, it is possible to protect a drug from the acidic (or other degrading entities such as nucleases for oligonucleotides) microenvironment it encounters before entering into tumour cells. For example, doxorubicin-loaded poly(alkyl cyanoacrylate) (PACA) nanoparticles are able to penetrate cells without being recognized by P-gp[78,79]. Using PACA nanoparticles, it has been demonstrated that the MDR of P388 leukaemia cells in culture was partially overcome. PEG-coated PACA nanoparticles were prepared from a poly(PEGcyanoacrylate-co-hexadecyl cyanoacrylate) co-polymer[79]. In murine cancer models, these nanoparticles circulated longer in the blood stream, whereas their uptake by the liver was reduced. An increased accumulation of the drug in tumour tissue was observed when the drug was administered in the form of PEG-coated PACA nanoparticles[80]. Additionally, Schluep *et al.* showed that IT-101 administered systemically in mice can overcome P-gp resistance in mouse tumour xenografts[67]. This was further evaluated in mice bearing six different xenografts — LS 174T and HT29 colorectal cancer; H1299 NSCLC;

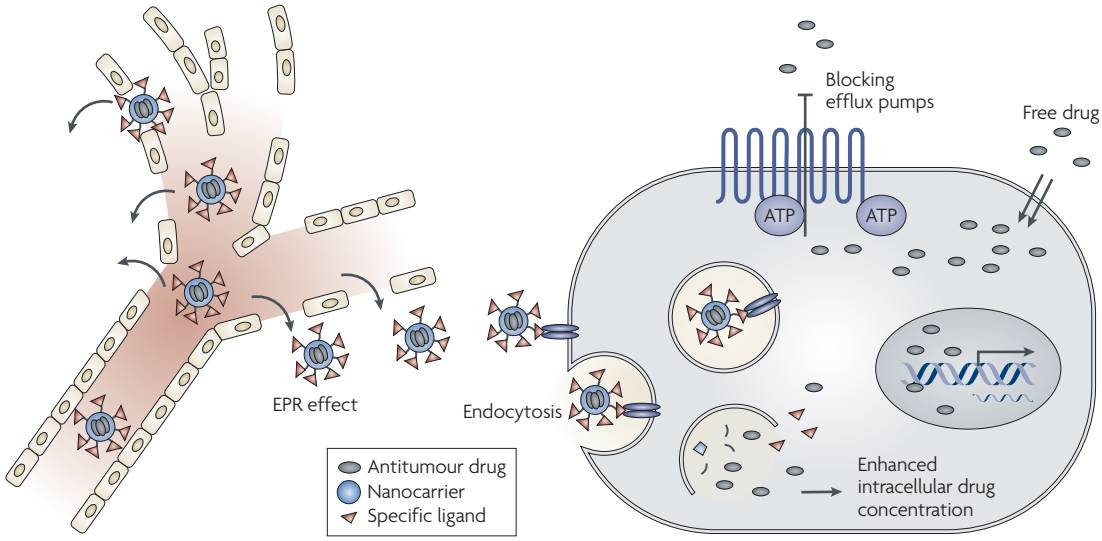

Figure 3 | **Nanoparticles can overcome surface efflux pump mediated drug resistance.** Efflux-pump mediated resistance is based on the rapid elimination of free drug that enters the cell. Drugs that inhibit efflux pumps are in development, but have had limited success to date. Nanoparticle agents are designed to utilize the enhanced permeability and retention (EPR) effect to exit blood vessels in the tumour, to target surface receptors on tumour cells, and to enter tumour cells by endocytosis before releasing their drug payloads. This method of delivery allows for high intracellular drug concentrations that can overcome efflux-pump mediated drug resistance.

H69 small-cell lung cancer; Panc-1 pancreatic cancer; MDA-MB-231 breast cancer — and one disseminated xenograft (TC71-luc Ewing's sarcoma). In all cases, a single treatment cycle of three weekly doses of IT-101 resulted in significant antitumour effects. Complete tumour regression was observed in some of the animals bearing H1299 tumours, and in the majority of the animals with disseminated Ewing's sarcoma[67]. Furthermore, IT-101 was shown to be effective in a number of tumours (for example, HT29 colorectal tumours), which are resistant to treatment with irinotecan[67]. This is consistent with the hypothesis that polymeric drug conjugates may be able to overcome certain kinds of MDR (FIG. 3).

As strategies using targeted nanoparticles take advantage of their binding to cell-surface receptors that are then endocytosed, this approach has also been applied to overcome drug resistance. Folate-receptor-targeted, pH-sensitive polymeric micelles containing doxorubicin[81], transferrin-conjugated paclitaxel nanoparticles[82] and transferrin-ligated liposomes containing oxaliplatin[83] all exhibited greater antitumour activity than the respective free drugs in drug-resistant mouse models. Overall, these studies show that nanoparticles of different types that contain small-molecule drugs and targeting moieties can outperform non-targeted nanoparticles.

There is no conclusive proof that nanoparticles have bypassed surface pump mediated resistance in humans, although clinical results that report partial responses in patients[65,68,84] who had previously failed drug therapies are suggestive of this possibility. For example, patients who were refractory to prior taxane therapy (for example, NSCLC with paclitaxel/carboplatin and ovarian cancer with paclitaxel/carboplatin and paclitaxel regimens) experienced objective responses after treatments with Genexol-PM, a polymeric micelle nanoparticle containing paclitaxel, or XYOTAX (CT-2103), a PG–paclitaxel conjugate[64,68].

Achievements and future challenges

Nanoparticles provide opportunities for designing and tuning properties that are not possible with other types of therapeutics, and as more clinical data become available (see also reviews on polymer–drug conjugates[85] and polymeric micelles[86] for further clinical data), the nanoparticle approach should improve further as the optimal properties are elucidated. As illustrated by the agents in TABLES 1–3, nanoparticle-based therapeutics are evolving, and newer, more sophisticated multifunctional nanoparticles are reaching the clinic. Results from these trials are already fuelling enthusiasm for this type of therapeutic modality.

Some of the important features of nanoparticles observed in preclinical studies have been confirmed in humans, such as extended pharmacokinetic data with sub-100 nm particles[66]. As discussed above, there are also suggestions that pump-mediated MDR might be overcome, and side effects significantly reduced (without the emergence of new side effects) while providing improved efficacy. These combined features may allow for new therapeutic strategies such as maintenance therapy.

Although there are numerous positive features of nanoparticle therapeutics for cancer, there are also issues of concern. First, while size can provide useful features such as large payloads and accommodation of multiple targeting ligands, it also can be a detriment.

At present, it remains unknown how nanoparticles move through tumour tissue once they have localized into the tumour area. Tumour penetration is important and especially so when the nanoparticles are designed to carry the drug molecules into the cancer cells before release. Much further work is required to understand how nanoparticles function in humans as early claims of some nanoparticle function are now being called into question. For example, with Abraxane there is evidence to suggest that the proposed nanoparticle delivery may not be the true mechanism leading to enhanced amounts of drug in tumours[87].

Second, there are valid concerns about nanoparticle toxicity, as little is known about how nanoscale entities behave in humans. The size and surface properties of nanoparticles can give them access to locations that are not available to larger particles. Surface properties also affect biodistribution through mechanisms such as nonspecific binding to proteins in the blood, removal by macrophages and by causing local disturbances in barriers that would otherwise limit their access. An example of the latter phenomenon was recently published: in this study neutral and slightly negatively charged nanoparticles did not alter the integrity of the blood–brain barrier in rats, whereas highly charged nanoparticles did regardless of whether they were positively or negatively charged[88]. Studies of this type suggest that further work is necessary in order to fully define the biocompatibility of nanoparticles in humans. The careful analysis of toxicities of nanostructures in animal models revealed no detrimental effects for some (for example, silica coated magnetic 50 nm particles[89]) but toxicity for others (for example, carbon nanotubes[90]). As expected, the size and surface properties of the nanoparticles dictate their behaviour and more data are necessary in order to develop understanding of their structure–property relationships. Nevertheless, some nanoparticles described in this article have passed rigorous toxicity testing for regulatory approvals and have years of experience in humans. Although each new nanostructure will need to be tested, there is good reason to believe that nanoparticles can ultimately be used in humans as effective systemic medicines and imaging agents[91,92]. As more biocompatibility data become available, further understanding of what is needed to tune the size and surface properties of nanoparticles to provide safety will aid the creation of new, more effective nanomedicines for systemic use[93].

Third, there are important commercial and regulatory challenges to be tackled with the emerging generation of more complex nanoparticles, in part owing to their multicomponent nature. Such nanoparticles are likely to be difficult and expensive to manufacture at large scale with appropriate quality. However, some highly complex nanoparticles have reached the clinic. For example, CALAA-01 is a four-component system that assembles into a highly multifunctional, targeted nanoparticle that contains siRNA. This multicomponent system is now in clinical studies and this example shows that complex nanoparticles can be manufactured at cGMP and satisfy regulatory requirements, at least for the initiation of Phase I trials. It remains to be seen whether nanoparticles of this complexity can reach the market. In addition to supporting the cost of development, intellectual property costs could be higher because so many components are needed to create the nanoparticle and each might have multiple intellectual property licenses for use. Given these barriers to commercialization, it seems that lack of sufficient financial support could be an issue, and in addition it is likely that approved products will be expensive because of these issues.

There are also numerous efforts focused on combining imaging and therapeutic agents within the same particle. Although there are situations in which this combination might be useful, there are numerous others were this would not. For example, imaging is not necessary every time therapy is administered, especially if this is daily or even more frequently. To carry along an expensive imaging agent and not use it is not a particularly good idea with the rising costs of medicines. Additionally, there are significant regulatory and developmental issues that make the concept of commercializing a combined agent daunting. An alternative, appropriate methodology for these situations is to create individual nanoparticles for imaging and therapy in which the size and surface properties are essentially the same between the two nanoparticle types. The closest analogue to this combination is antibodies possessing a radionucleotide for imaging and another for therapy. As the size and surface properties define the biodistribution, the imaging agent should localize similarly to the therapeutic agent. This is a general strategy that is achievable using nanoparticles that can use numerous types of therapeutics and imaging modalities. One can imagine nanoparticle imaging agents that provide information on intracellular targets. The molecular target of the disease could be verified to exist in a patient before treatment, and as the observation was made via a nanoparticle with the same size and surface properties of the therapeutic particle, the therapy would be expected to reach the target. This combination will allow personalized medicine in the sense that treatment does not have to occur until the target is known to exist in the patient. Also, follow-up imaging can be performed to verify that the target has been reached and that the therapy is working.

There is no doubt that nanoparticle therapeutics with increasing multifunctionality will exist in the future. As newer and more complex nanoparticle systems appear, better methodologies to define biocompatibility will need to be created, especially those that can assess intracellular biocompatibility. While the details of issues regarding scale-up and cGMP production are not often discussed, sophisticated nanoparticles such as CALAA-01 show that efforts towards overcoming cGMP and regulatory hurdles is progressing. Although many challenges exist for the translation of nanoparticles that are currently research tools into approved products for patients, their potential advantages should drive their successful development, and the continuing emergence of a new class of anticancer therapies.

1. Venturoli, D. & Rippe, B. Ficoll and dextran vs. globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge and deformability. *Am. J. Physiol.* **288**, F605–F613 (2005).
2. Matsumura, Y. & Maeda, H. A new concept of macromolecular therapies in cancer chemotherapy: mechanism of tumortropic accumulation of proteins and the antitumor agent SMANCS. *Cancer Res.* **6**, 6387–6392 (1986).
3. Dreher, M. R. *et al.* Tumor vascular permeability, accumulation, and penetration of macromolecular drug carriers. *J. Natl Cancer Inst.* **98**, 335–344 (2006).
4. Nomura, T., Koreeda, N., Yamashita, F., Takakura, Y. & Hashida, M. Effect of particle size and charge on the disposition of lipid carriers after intratumoral injection into tissue-isolated tumors. *Pharm Res.* **15**, 128–132 (1998).
5. Hu-Lieskovan, S., Heidel, J. D., Bartlett, D. W., Davis, M. E. & Triche, T. J. Sequence-specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma. *Cancer Res.* **65**, 8984–8992 (2005).
6. Chen, M. Y. *et al.* Surface properties, more than size, limiting convective distribution of virus-sized particles and viruses in the central nervous system. *J. Neurosurg.* **103**, 311–319 (2005).
7. Gatter, K. C. *et al.* Transferrin receptors in human tissues: their distribution and possible clinical relevance. *J. Clin. Pathol.* **36**, 539–545 (1983).
8. Kirpotin, D. B. *et al.* Antibody targeting of long-circulating lipidic particles does not increase tumor localization but does increase internalization in animal models. *Cancer Res.* **66**, 6732–6740 (2006).
9. Bartlett, D. W., Su, H., Hildebrandt, I. J., Weber, W. A. & Davis, M. E. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality *in vivo* imaging. *Proc. Natl Acad. Sci. USA* **104**, 15549–15554 (2007).
10. Popielarski, S. R., Hu-Lieskovan, S., French, S. W., Triche, T. J. & Davis, M. E. A nanoparticle-based model delivery system to guide the rational design of gene delivery to the liver. 2. *In vitro* and *in vivo* uptake results. *Bioconjug. Chem.* **16**, 1071–1080 (2005).
11. Bartlett, D. W. & Davis, M. E. Physicochemical and biological characterization of targeted, nucleic acid-containing nanoparticles. *Bioconjug. Chem.* **18**, 456–468 (2007).
12. Song, E. *et al.* Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. *Nature Biotech.* **23**, 709–717 (2005).
13. Hong, S. *et al.* The binding avidity of a nanoparticle-based multivalent targeted drug delivery platform. *Chem. Biol.* **14**, 107–115 (2007).
14. Montet, X., Funovics, M., Montet-Abou, K., Weissleder, R. & Josephson, L. Multivalent effects of RGD peptides obtained by nanoparticle display. *J. Med. Chem.* **49**, 6087–6093 (2006).
15. Carlson, C. B., Mowery, P., Owen, R. M., Dykhuizen, E. C. & Kiessling, L. L. Selective tumor cell targeting using low-affinity, multivalent interactions. *ACS Chem. Biol.* **2**, 119–127 (2007).
16. Pommier, Y. Camptothecins and topoisomerase I: a foot in the door. Targeting the genome beyond topoisomerase I with camptothecins and novel anticancer drugs: importance of DNA replication, repair and cell cycle check points. *Curr. Med. Chem. Anticancer Agents* **4**, 429–434 (2004).
17. Bartlett, D. W. & Davis, M. E. Insights into the kinetics of siRNA-mediated gene silencing from live-cell and live-animal bioluminescent imaging. *Nucl. Acid Res.* **34**, 322–333 (2006).
18. Bartlett, D. W. & Davis, M. E. Effect of siRNA nuclease stability on the *in vitro* and *in vivo* kinetics of siRNA-mediated gene silencing. *Biotech. Bioeng.* **97**, 909–921 (2007).
19. Zamboni, W. C. Liposomal, nanoparticle, and conjugated formulation of anticancer agents. *Clin. Cancer Res.* **11**, 8230–8234 (2005).
20. Rahman, A. M., Yusuf S. W. & Ewer, M. S. Anthracycline-induced cardiotoxicity and the cardiac-sparing effect of liposomal formulation. *Int. J. Nanomedicine* **2**, 567–583 (2007).
21. Batist, G. Cardiac safety of liposomal anthracyclines. *Cardiovasc. Toxicol.* **7**, 72–74 (2007).
22. Uziely, B. *et al.* Liposomal doxorubicin: antitumor activity and unique toxicities during two complementary Phase I studies. *J. Clin. Oncol.* **13**, 1777–1785 (1995).
23. Boddy, A. V. *et al.* A phase I and pharmacokinetic study of paclitaxel poliglumex (XYOTAX), investigating both 3-weekly and 2-weekly schedules. *Clin. Cancer Res.* **11**, 7834–7840 (2005).
24. Sutton, D., Nasongkla, N., Blanco, E. & Gao, J. Functionalized micellar systems for cancer targeted drug delivery. *Pharm. Res.* **24**, 1029–1046 (2007).
25. O'Brien, M. E. Single-agent treatment with pegylated liposomal doxorubicin for metastatic breast cancer. *Anticancer Drugs* **19**, 1–7 (2008).
26. Green, A. E. & Rose P. G. Pegylated liposomal doxorubicin in ovarian cancer. *Int. J. Nanomedicine* **1**, 229–239 (2006).
27. Chanan-Khan, A. A. & Lee, K. Pegylated liposomal doxorubicin and immunomodulatory drug combinations in multiple myeloma: rationale and clinical experience. *Clin. Lymphoma Myeloma* **7** (Suppl. 4), S163–S169 (2007).
28. Sparreboom, A. *et al.* Comparative preclinical and clinical pharmacokinetics of a Cremophor-free, nanoparticle albumin-bound paclitaxel (ABI-007) and paclitaxel formulated in Cremophor (Taxol). *Clin. Cancer Res.* **11**, 4136–4143 (2005).
29. Gradishar, W. J. *et al.* Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. *J. Clin Oncol.* **23**, 7794–7803 (2005).
30. Henderson, I. C. & Bhatia, V. Nab-paclitaxel for breast cancer: a new formulation with an improved safety profile and greater efficacy. *Expert Rev. Anticancer Ther.* **7**, 919–943 (2007).
31. Harris, J. M. & Chess, R. B. Effect of peglyation on pharmaceuticals. *Nature Rev. Drug Discov.* **2**, 214–221 (2003).
32. Fuertges, F. & Abuchowski, A. The clinical efficacy of poly(ethylene glycol) modified proteins. *J. Control. Release* **11**, 139–148 (1990).
33. Graham, M. L. Pegaspargase: a review of clinical studies. *Adv. Drug Deliv. Rev.* **55**. 1293–1302 (2003).
34. Ho, D. H. *et al.* Clinical pharmacology of polyethylene glycol–asparaginase. *Drug Metab. Disposit.* **14**, 349–352 (1986).
35. Kurtzberg, J., Moore, J. O. Scudiery, D. & Franklin, A. A phase II study of polyethylene glycol (PEG) conjugated L-asparaginase in patients with refractory acute leukaemias. *Proc. Am. Assoc. Cancer Res.* **29**, 213 (1988).
36. Abshire, T. C., Pollock, B. H., Billett, A. L., Bradley, P. & Buchanan, G. R. Weekly polyethylene glycol conjugated-asparaginase compared with biweekly dosing produces superior induction remission rates in childhood relapsed acute lymphoblastic leukemia: a Pediatric Oncology Group Study. *Blood* **96**, 1709–1715 (2000).
37. Cheng, P. N. *et al.* Pegylated recombinant human Arginase (rhArg-peg 5000Mw) has *in vitro* and *in vitro* anti-proliferative potential and apoptotic activities in human hepatocellular carcinoma (HCC). *J. Clin. Oncol.* **26** (Suppl. 16), 3179 (2005).
38. Delman, K. A. *et al.* Phase I/II trial of pegylated arginine deiminase (ADI-PEG20) in unresectable hepatocellular carcinoma. *J. Clin. Oncol.* **26** (Suppl. 16), 4139 (2005).
39. Molineux, G. The design and development of pegfilgrastim (PEG-rmetHuG-CSF, Neulasta). *Curr. Pharm. Des.* **10**, 1235–1244 (2004).
40. Tanaka, H. Satake-Ishikawa, R., Ishikawa M., Matsuki, S. & Asano, K. Pharmacokinetics of recombined human granulocyte colony-stimulating factor conjugated to polyethylene glycol in rats. *Cancer Res.* **51**, 3710–3714 (1991).
41. Wang, Y. S. *et al.* Structural and biological characterisation of pegylated recombinant interferon α2b and its therapeutic implications. *Adv. Drug Del. Rev.* **54**, 547–570 (2002).
42. Fiaherty, L., Heilbrun, L., Marsack, C. & Vaishampayan U. N. Phase II trial of pegylated interferon (Peg-Intron) and thalidomide (Thal) in pretreated metatastic malignant melanomal. *J. Clin. Oncol.* **23** (Suppl. 16), 7562 (2005).
43. Bukowski, R. *et al.* Pegylated interferon α-2b treatment for patients with solid tumors: a phase I/II study. *J. Clin. Oncol.* **20**, 3841–3849 (2002).
44. Holmes, F. A. *et al.* Comparable efficacy and safety profiles of once-per-cycle pegfilgrastim and daily injection filgrastim in chemotherapy-induced neutropenia: a multicenter dose-finding study in women with breast cancer. *Ann. Oncol.* **13**, 903–909 (2002).
45. Posey, J. A. 3rd *et al.* Phase I study of weekly polyethylene glycol-camptothecin in patients with advanced solid tumors and lymphoma. *Clin. Cancer Res.* **11**, 7866–7871 (2005).
46. Rowinsky, E. K. *et al.* A phase I and pharmacokinetic study of pegylated camptothecin as a 1-hour infusion every 3 weeks in patients with advanced solid malignancies. *J. Clin. Oncol.* **21**, 148–157 (2003).
47. Scott, L. C. *et al.* A phase II study of pegylated-camptothecin (pegamotecan) in the treatment of locally advanced and metastatic gastric and gastro-oesophageal junction adnenocarcinoma. *Cancer Chemother. Pharmacol.* 9 April 2008 (doi:10.1007/s00280-008-0746-2).
48. Shaffer, S. A. *et al.* Proteolysis of Xyotax™ by lysosomal cathepsin B; metabolic profiling in tumor cells using LC-MS. *Eur. J. Cancer* **38** (Suppl. 7), S129 (2002).
49. Singer, J. W. *et al.* Poly-(L)-glutamic acid-paclitaxel (CT-2103) [XYOTAX], a biodegradable polymeric drug conjugate: characterization, preclinical pharmacology, and preliminary clinical data. *Adv. Exp. Med. Biol.* **519**, 81–99 (2003).
50. Singer, J. W. *et al.* Paclitaxel poliglumex (XYOTAX; CT-2103) [XYOTAX™]: an intracellularly targeted taxane. *Anticancer Drugs* **16**, 243–254 (2005).
51. Todd, R. *et al.* Phase I and pharmacological study of CT-2103, a poly(L-glutamic acid)-paclitaxel conjugate. *Proc. AACR-NCI-EORTC 12th Int. Conf. Mol. Targets Cancer Therapeut. Discov. Develop. Clin. Valid.* Abstract 439 (2001).
52. Langer, C. J. *et al.* Paclitaxel poliglumex (PPX)/carboplatin vs. Paclitaxel/carboplatin for the treatment of PS2 patients with chemotherapy-naive advanced non-small cell lung cancer (NSCLC): a phase III study. *J. Clin. Oncol.* **23** (Suppl. 16), LBA7011 (2005).
53. Socinski, M. & Ramalingham, S. XYOTAX in NSCLC and other solid tumors. Emerging evidence on biological differences between men and women: is gender-specific therapy warranted? *Chemotherapy Foundation web site* [online], <http://www.chemotherapyfoundationsymposium.org/meeting_archives/meetingarchives_tcf2005_main.html> (2005).
54. Kremer, M., Judd, J., Rifkin, B., Auszmann, J. & Oursler, M. J. Estrogen modulation of osteoclast lysosomal-enzyme secretion. *J. Cell. Biochem.* **57**, 271–279 (1995).
55. Homsi, J. *et al.* Phase I trial of poly-L-glutamate camptothecin (CT-2106) administered weekly in patient with advanced solid malignancies. *Clin. Cancer Res.* **13**, 5855–5861 (2007).
56. Seymour, L. W. *et al.* Tumouritropism and anticancer efficacy of polymer-based doxorubicin prodrugs in the treatment of subcutaneous murine B16F10 melanoma. *Br. J. Cancer* **70**, 636–641 (1994).
57. Duncan, R. *et al.* Preclinical evaluation of polymer-bound doxorubicin. *J. Control. Release* **19**, 331–346 (1992).
58. Vasey, P. *et al.* Phase I clinical and pharmacokinetic study of PKI (HPMA copolymer doxorubicin) first member of a new class of chemotherapeutics agents: drug–polymer conjugates. *Clin. Cancer Res.* **5**, 83–94 (1999).
59. Thomson, A. H. *et al.* Population pharmacokinetics in phase I drug development: a phase I study of PK1 in patients with solid tumors. *Br. J. Cancer* **81**, 99–107 (1999).
60. Duncan, R. Designing polymer conjugates as lysomsomotropic nanomedicines. *Biochem. Soc. Trans.* **35**, 56–60 (2007).
61. Matsumura, Y. *et al.* Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. *Br. J. Cancer.* **91**, 1775–1781 (2004).
62. Danson, S. *et al.* Phase I dose escalation and pharmacokinetic study of pluronic polymer-bound doxorubicin (SP1049C) in patients with advanced cancer. *Br. J. Cancer.* **11**, 2085–2091 (2004).
63. Armstrong, A. *et al.* SP1049C as first-line therapy in advanced (inoperable or metatastic) adenocarcinoma of the oesophagus: a phase II window study. *J. Clin. Oncol.* **24**, 4080 (2006).
64. Kim, T. Y. *et al.* Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. *Clin. Cancer Res.* **10**, 3708–3716 (2004).
65. Lee K. S. *et al.* Multicenter phase II study of a cremophor-free polymeric micelle-formulated paclitaxel in patients with metastatic breast cancer. *J. Clin. Oncol.* **24**, (Suppl. 18), 10520 (2006).

66. Yen, Y. *et al.* First-in-human phase I trial of a cyclodextrin-containing polymer-camptothecin nanoparticle in patients with various solid tumors. *J. Clin. Oncol.* **25** (Suppl. 18), 14078 (2007).
67. Schluep, T. *et al.* Preclinical efficacy of the camptothecin–polymer conjugate IT-101 in multiple cancer models. *Clin. Cancer Res.* **12**, 1606–1614 (2006).
68. Nemunaitis, J. *et al.* Phase I study of CT-2103, a polymer-conjugated paclitaxel, and carboplatin in patients with advanced solid tumors. *Cancer Invest.* **23**, 671–676 (2005).
69. Seymour, L. W. *et al.* Hepatic drug targeting: Phase I evaluation of polymer bound doxorubicin. *J. Clin. Oncol.* **20**, 1668–1676 (2002).
70. Julyan, P. J. *et al.* Preliminary clinical study of the distribution of HPMA copolymer-doxorubicin bearing galactosamine. *J. Control. Release* **57**, 281–290 (1999).
71. Heidel, J. D. *et al.* Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. *Proc. Natl Acad. Sci. USA* **104**, 5715–5721 (2007).
72. Zhou, Y. *et al.* Impact of single-chain Fv antibody fragment affinity on nanoparticle targeting of epidermal growth factor receptor-expressing tumor cells. *J. Mol. Biol.* **371**, 934–947 (2007).
73. Gottesman, M. M. Mechanisms of cancer drug resistance. *Annu. Rev. Med.* **53**, 615–627 (2002).
74. Gottesman, M. M., Fojo, T. & Bates, S. E. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nature Rev. Cancer* **2**, 48–58 (2002).
75. Modok, S., Mellor, H. R. & Callaghan, R. Modulation of multidrug resistance efflux pump activity to overcome chemoresistance in cancer. *Curr. Opin. Pharmacol.* **6**, 350–354 (2006).
76. Nobili, S., Landini, I., Giglioni, B. & Mini, E. Pharmacological strategies for overcoming multidrug resistance. *Curr. Drug Targets* **7**, 861–879 (2006).
77. Thomas, H. & Coley, H. M. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. *Cancer Control* **10**, 159–165 (2003).
78. Pepin, X. *et al.* On the use of ion-pair chromatography to elucidate doxorubicin release mechanism from polyalkylcyanoacrylate nanoparticles at the cellular level. *J. Chromatogr. B Biomed. Sci. Appl.* **702**, 181–191 (1997).
79. Vauthier, C., Dubernet, C., Chauvierre, C., Brigger, I. & Couvreur, P. Drug delivery to resistant tumors: the potential of poly(alkyl cyanoacrylate) nanoparticles. *J. Control. Release* **93**, 151–160 (2003).
80. Peracchia, M. T. *et al.* Stealth PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting. *J. Control. Release* **60**, 121–128 (1999).
81. Lee, E. S., Na, K. & Bae, Y. H. Doxorubicin loaded pH-sensitive polymeric micelles for reversal of resistant MCF-7 tumor. *J. Control. Release* **103**, 405–418 (2005).
82. Sahoo, S. K., Ma, W. & Labhasetwar, V. Efficacy of transferrin-conjugated paclitaxel-loaded nanoparticles in a murine model of prostate cancer. *Int. J. Cancer* **112**, 335–340 (2004).
83. Suzuki, R. *et al.* Effective anti-tumor activity of oxaliplatin encapsulated in transferrin-PEG-liposome. *Int. J. Pharm.* **346**, 143–150 (2008).
84. Northfelt, D. W. *et al.* Efficacy of pegylated-liposomal doxorubicin in the treatment of AIDS-related Kaposi's sarcoma after failure of standard chemotherapy. *J. Clin. Oncol.* **15**, 653–659 (1997).
85. Li, C. & Wallace, S. Polymer–drug conjugates: recent development in clinical oncology. *Adv. Drug Del. Rev.* **60**, 886–898 (2008).
86. Matsumura, Y. Poly(amino acid) micelle nanocarriers in preclinical and clinical studies. *Adv. Drug Del. Rev.* **60**, 899–914 (2008).
87. Gardner, E. R. *et. al.* Randomized crossover pharmacokinetic study of solvent-based paclitaxel and nab-paclitaxel. *Clin. Cancer Res.* **14**, 4200–4205 (2008).
88. Lockman, P. R., Koziara, J. M., Mumper, R. J. & Allen, D. D. Nanoparticle surface charges alter blood–brain barrier integrity and permeability. *J. Drug Target.* **12**, 635–641 (2004).
89. Kim, J. S. *et al.* Toxicity and tissue distribution of magnetic nanoparticles in mice. *Toxicol. Sci.* **89**, 338–347 (2006).
90. Salvador-Morales, C. Complement activation and protein adsorption by carbon nanotubes. *Mol. Immunol.* **43**, 193–201 (2006).
91. Wang, X., Yang, L., Chen, Z. G. & Shin, D. M. Application of nanotechnology in cancer therapy and imaging. *CA Cancer J. Clin.* **58**, 97–110 (2008).
92. Qian, X. *et al. In vivo* tumor targeting and spectroscopic detection with surface-enhanced Raman nanoparticle tags. *Nature Biotech.* **26**, 83–90 (2008).
93. Cho, K., Wang X., Nie, S., Chen Z.G. & Shin, D. M. Therapeutic nanoparticles for drug delivery in cancer. *Clin. Cancer Res.* **14**, 1310–1316 (2008).
94. Schluep, T. *et al.* Pharmacokinetics and biodistribution of the camptohecin-polymer conjugate IT-101 in rats and tumor-bearing mice. *Cancer Chemother. Pharmacol.* **57**, 654–662 (2006).
95. Pfizer. CAMPTOSAR U.S. Physician Prescribing Information. *Pfizer web site* [online], <www.pfizer.com/files/products/uspi_camptosar.pdf> (2008).
96. Herben, V. M., ten Bokkel Huinink, W. W. & Beijnen, J. H. Clinical pharmacokinetics of topotecan. *Clin. Pharmacokinet.* **31**, 85–102 (1996).
97. Kraut, E. H. *et al.* Final results of a phase I study of liposome encapsulated SN-38 (LE-SN38): safety, pharmacogenomics, pharmacokinetics, and tumor response. *J. Clin. Oncol.* **23** (Suppl. 16), 2017 (2005).
98. Daud, A. *et al.* Phase I trial of CT-2106 (polyglutamated camptothecin) administered weekly in patients with advanced solid tumor malignancies. *J. Clin. Oncol.* **24** (Suppl. 18), 2015 (2006).
99. Bross, P. F. *et al.* Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. *Clin. Cancer Res.* **7**, 1490–1496 (2001).
100. Kawakami, K., Nakajima, O., Morishita, R. & Nagai, R. Targeted anticancer immunotoxins and cytotoxic agents with direct killing moieties. *ScientificWorldJournal* **6**, 781–790 (2006).
101. Allen, T. M. Ligand-targeted therapeutics in anticancer therapy. *Nature Rev. Cancer* **2**, 750–763 (2002).
102. Duncan, R. Polymer conjugates as anticancer nanomedicines. *Nature Rev. Cancer* **6**, 688–701 (2006).
103. Matsumura, M. *et al.* Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer. *Ann. Oncol.* **15**, 517–525 (2004).
104. MedBiopharm Co., Ltd. Safety study of MBP-426 (liposomal oxaliplatin suspension for injection) to treat advanced or metastatic solid tumors. *ClinicalTrials.gov web site* [online], <http://www.clinicaltrials.gov/ct/show/NCT00355888> (2008).
105. Phan, A. *et al.* Open label phase I study of MBP-426, a novel formulation of oxalipatin, in patients with advanced or metastatic solid tumors. *Proc. AACR-NCI-EORTC Int. Conf. Mol. Targets Cancer Therapeut. Discov. Develop. Clin. Valid.* Abstract C115 (2007).
106. SynerGene Therapeutics, Inc. Safety Study of Infusion of SGT-53 to Treat Solid Tumors. *ClinicalTrials.gov web site* [online], <http://www.clinicaltrials.gov/ct2/show/NCT00470613> (2008).
107. Calando Pharmaceuticals. Safety Study of CALAA-01 to Treat Solid Tumor Cancers. *ClinicalTrials.gov web site* [online], <http://www.clinicaltrials.gov/ct/show/NCT00689065> (2008).

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