Expert Opinion

Critical issues in site-specific targeting of solid tumours: the carrier, the tumour barriers and the bioavailable drug

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Background: The concept of passive and active targeting of solid tumours with intravenously administered particulate and macromolecular carriers is an attractive one that has received considerable attention and promising results have emerged from such attempts at the clinical level. Particulate and polymeric drug carriers have the capability to deliver from 2- to 10-times more drug to solid tumours compared with the administered drug in its free form, and it is through the altered pharmacokinetics and pharmacodynamics of the encapsulated/conjugated drugs relative to free drugs that anticancer drug-induced toxicity is dramatically reduced. *Objectives*: It is the intention of this article to examine the role of selected particulate and macromolecular entities as carriers of anticancer drugs and their ability to target different components of solid tumours following the intravenous route of injection, and release their cargo in a bioavailable form at levels that exceed the minimum cytotoxic concentration. *Methods*: The authors of this paper have focused on carrier behaviour (pharmacokinetics of single and multiple injections, and new toxicity issues that may arise from different dosing schedules and dose intensities, as well as from the carrier itself), pathophysiological factors regulating particulate and macromolecular transport into tumours (structural arrangements of tumour vasculature, tumour vascular permeability, interstitial hypertension and interstitial transport), and biochemical and physicochemical factors controlling drug release from extravasated carriers (the bioavailable drug). *Conclusion*: Nanoscale drug carriers can passively target solid tumours, but achieving therapeutic responses involves pathophysiological processes that control carrier transport into tumours and biochemical factors regulating drug release from extravasated carriers and maintaining free drug levels above the minimum cytotoxic concentration. It is conceivable that future sophistication in tumour targeting and the outcome of end results will depend on an improved understanding of tumour biology and biological barriers, as well as advances in carrier design and nanoengineering.

Keywords: cancer chemotherapy, drug carriers, hypersensitivity, liposome, multi-drug resistance, polymer–drug conjugates, polymeric nanoparticles

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1. Introduction

Drug-induced side effects are among the chief complications of cancer chemotherapy, damaging vital sites such as the bone marrow, the gastrointestinal system and the heart. For example, the deleterious effects of cytotoxic drugs (with the exception of bleomycin and vincristine) on the bone marrow are well

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established and may result in anaemia, thrombocytopenia, leucopenia and peripheral haemolysis, which in turn may induce secondary effects of haemorrhage, increased susceptibility to infection and fatigue. Perhaps one of the most important single chemotherapeutic agents available to oncologists is the anthracycline doxorubicin. However, despite its proven efficacy in the treatment of acute leukaemias, lymphomas and a variety of solid tumours, no other class of anticancer agent causes an inexorable cumulative, dose-dependent irreversible cardiomyopathy to the extent of doxorubicin – an effect that is believed mainly to occur through a major disruption of the cardiac-specific programme of gene expression to include the heart- and muscle-specific isoform of ADP/ATP translocase and the ubiquitously expressed electron transport chain component Reiske iron-sulfur protein [1].

A wide range of strategies have been applied to minimise the toxic effects of a plethora of anticancer drugs, including prolonged infusion, local perfusion of an isolated area and changes in drug structure that do not interfere with clinical performance. However, among the most intriguing progress being made in minimising drug-induced toxicity in cancer chemotherapy is drug encapsulation or incorporation into nanoparticulate carriers such as liposomes and polymeric nanoparticles [2-5]. A similar, and complementary, approach is drug conjugation to proteins (e.g., antibodies, albumin) and synthetic macromolecules (e.g., polymers and block copolymers); certain drug-conjugated block copolymers can further assemble into micellar structures [5-7]. It is through the altered pharmacokinetics and pharmacodynamics of the encapsulated/conjugated drugs relative to free drugs that drug-induced toxicity is dramatically reduced.

Following intravenous administration, and providing that rapid detection and sequestration by scavengers of the reticuloendothelial system can be avoided (as with stealth systems), a proportion of circulating drug carriers may escape from the vasculature of solid tumours by extravasation and reach the interstitial spaces [2,8]. Indeed, particulate and polymeric drug carriers have the capability to deliver 2- to 10-times more drug to solid tumours compared with the administered drug in its free form. Through passive accumulation, drug carriers may, therefore, reduce the amount of active agent needed to obtain a therapeutic effect. However, the extent and the amount of carrier deposition in solid tumours are controlled by a complex array of pathophysiological factors (structural arrangements of tumour vasculature, tumour vascular permeability, interstitial hypertension and interstitial transport) [9]. Furthermore, encapsulated or conjugated drugs are not readily bioavailable and, therefore, have no therapeutic activity until they are released from the carrier. Consequently, an understanding of intra-tumoural behaviour that controls drug release from a carrier is of paramount importance, particularly with the view that multi-drug resistance may be overcome to a certain degree following drug release from accumulated carriers (at least in tumours exhibiting a low resistance factor).

Particulate and polymeric carriers are amenable to chemical modification with biological ligands – an approach that might allow these entities to be targeted to tumour neovasculature as well as to tumour cells and components of the interstitial matrix following extravasation [2-6,8,10,11]. These strategies may lead to the design and engineering of multifunctional platforms capable of simultaneously detecting and treating solid tumours.

This article examines the role of selected particulate and macromolecular entities as carriers of anticancer drugs and their ability to target different components of solid tumours following intravenous injection. The authors' attention is focused on carrier behaviour (pharmacokinetics of single and multiple injections, and new toxicity issues that may arise from differing dosing schedules and dose intensities, as well as from the carrier itself), pathophysiological factors regulating particulate and macromolecular transport into tumours (the tumour barriers), and biochemical and physicochemical factors controlling drug release from extravasated carriers (the bioavailable drug).

2. The carrier

Particulate and macromolecular carriers (**Tables 1** and **2**) exert substantial effects on the pharmacokinetics of their entrapped or conjugated cargo, and these issues have been discussed in detail elsewhere [2-6,8,10]. Here the authors focus their discussion on selected cases, particularly licensed clinical formulations and those in clinical trials.

2.1 Particulate agents

Myocet® (Cephalon) is a liposomal formulation of doxorubicin, which is used with cyclophosphamide for the treatment of metastatic breast cancer (60 – 75 mg/m^2 doxorubicin as Myocet, together with cyclophosphamide 600 mg/m^2, usually every 3 weeks). The plasma levels of total doxorubicin in patients receiving Myocet (as a single dose) were shown to be substantially higher compared with conventional doxorubicin, although the peak plasma levels of free (not liposome-encapsulated) doxorubicin were lower with Myocet than with conventional doxorubicin [12]. Myocet releases > 50 % of its associated doxorubicin within a few hours of intravenous administration, and 90% within 24 h [12]. With Myocet the clearance of total doxorubicin and the volume of distribution at steady-state were 9-fold and 25-fold lower, respectively, than that of conventional doxorubicin administration [13]. As a result of these pharmacokinetic parameters the frequency of cardiotoxicity and neutropaenia is lower with Myocet compared with free doxorubicin [13,14]. However, there is still controversy as to whether Myocet exhibits equivalent efficacy to free doxorubicin administration. Liposomes are also susceptible to clearance by macrophages of the reticuloendothelial

Table 1. Examples of regulatory-approved particulate and micellar nanomedicines for the management and treatment of solid tumours.

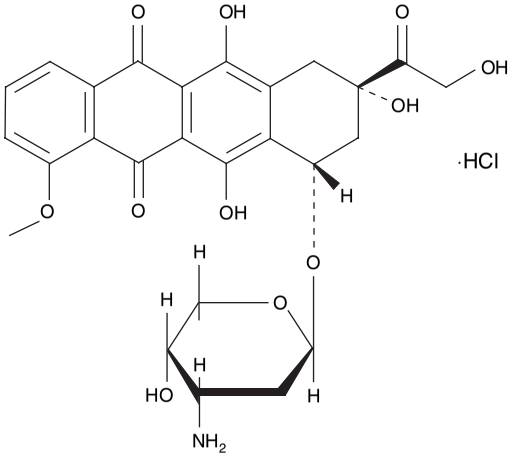
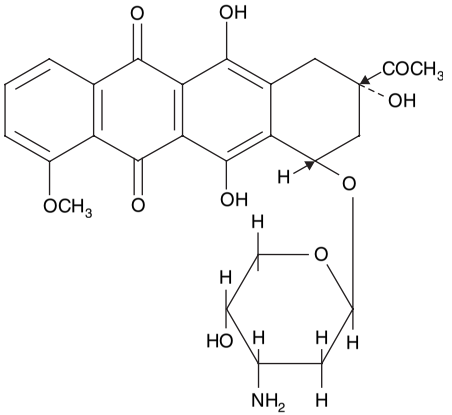
Brand	Carrier type	Encapsulated drug	Indication
Myocet®	Liposome	Doxorubicin HCl (as doxorubicin–citrate complex) 	Metastatic breast cancer with cyclophosphamide
Doxil®	PEGylated liposome	Doxorubicin HCl	HIV-related Kaposi's sarcoma in patients with low CD4 count and extensive mucocutaneous or visceral disease; advanced ovarian cancer when platinum-based chemotherapy has failed; monotherapy for metastatic breast cancer with increased cardiac risk
DaunoXome®	Liposome	Danuorubicin citrate 	Advanced AIDS-related Kaposi's sarcoma

Table 1. Examples of regulatory-approved particulate and micellar nanomedicines for the management and treatment of solid tumours (continued).

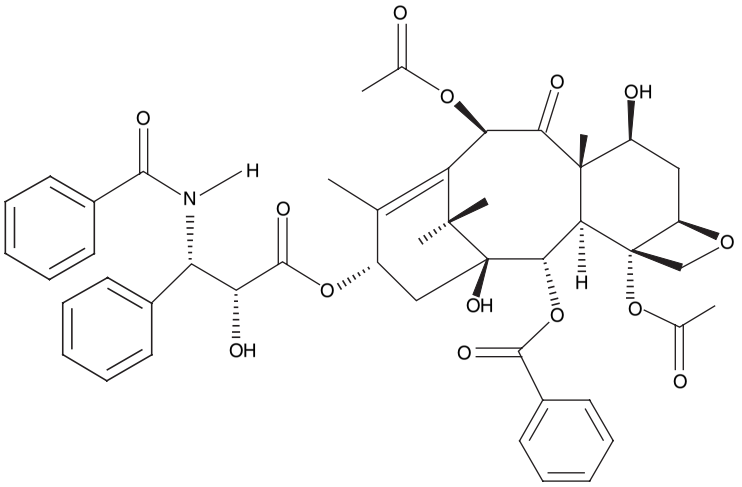
Brand	Carrier type	Encapsulated drug	Indication
Taxol®	Micelle	Paclitaxel 	Advanced or metastatic breast cancer (in combination with trastuzumab) when an anthracycline not appropriate; metastatic breast cancer where standard anthracycline-containing therapy has failed or inappropriate; primary ovarian cancer (advanced or residual disease following laparotomy) in combination with cisplatin; ovarian cancer where standard platinum-containing therapy has failed; non-small cell lung cancer (in combination with cisplatin) when surgery or radiotherapy not appropriate
Abraxane®	Albumin nanoparticle	Paclitaxel	Metastatic breast cancer after failure of combination chemotherapy or relapse after adjuvant therapy within 6 months (approved in US); metastatic breast cancer (approved in Canada)

Table 2. Selected examples of polymer–drug conjugates for cancer drug delivery.

Conjugate	Linker	Status	Indication
Styren-maleic anhydride copolymer–neocarzinostatin (SMANC)	Amine (non-biodegradable)	Approved (Japan 1990)	Hepatocellular carcinoma
Poly(ethyleneglycol)–camptothecin (Prothecan®)	Ester	Phase II	Various solid tumours
N-(2-hydroxypropyl)methacrylamide copolymer (HPMA)–doxorubicin (PK1)	Amide	Phase II	Various cancers (including lung and breast cancers)
HPMA–doxorubicin–galactosamine (PK2)	Amide	Phase I/II	Hepatocellular carcinoma
Polyglutamate–paclitaxel (Xyotax™)	Ester	Phase III	Various cancers (including non-small cell lung carcinoma and ovarian cancer)
Carboxymethyldextran polyalcohol–doxorubicin (DE310)	Amide	Phase I	Various solid tumours and lymphomas

system, notably the hepatic Kupffer cells [2,8]. Hepatic deposition of Myocet could also lead to gradual release of the cytotoxic agent back to the systemic circulation (a macrophage depot system), as well as induction of Kupffer cell apoptosis. The latter case may be of some concern – following apoptosis, the restoration of Kupffer cells may take up to 2 weeks [8]. A potentially harmful effect is the occurrence of bacteremia during the period of Kupffer cell deficiency.

Macrophage deposition of intravenously administered particulate drug carriers can be markedly minimised by surface modification with polymers such as methoxypoly(ethyleneglycol) (mPEG), thus yielding stealth systems [8]. One example is Doxil® (also known as Caelyx® Johnson & Johnson), a stealth liposome with encapsulated doxorubicin that has a biphasic circulation half-life (84 min and 46 h) in humans [15,16]. Doxil also has a high drug loading capacity, as doxorubicin is actively loaded by an ammonium sulfate gradient (as doxorubicin sulfate) yielding vesicles with a high content of doxorubicin aggregates [16]. These vesicles remain highly stable within the systemic circulation with minimum drug loss. The area under the curve after a dose of 50 mg/m^2 doxorubicin as Doxil is ~ 300-fold greater than that of free doxorubicin. Volume of distribution and clearance are also reduced by at least 60-fold and 250-fold, respectively [16].

Limited information is available for differing dosing schedules and dose intensities with drug encapsulated stealth nanoparticles. This is of particular importance where patients are expected to receive multiple injections. The effect of dosing schedule on Doxil pharmacokinetics was recently investigated following multiple intravenous injections into mice (four injections of liposomes at a dose of 9 mg/kg and dose intervals of either 1, 2 or 4 weeks) [17]. These studies concluded that the plasma pharmacokinetics of doxorubicin in each injection cycle was independent of the next cycle [17]. In contrast to these observations, it was demonstrated that intravenous injection of a dose of drug-free PEGylated liposomes in rats could elicit the production of PEG-specific IgM antibodies in the spleen [18,19]. This led to rapid hepatic elimination of a subsequent dose of PEGylated liposomes (as a result of subsequent vesicle opsonisation by complement proteins) when given 5 days later. However, increasing the time interval between the two injections weakened the effect and by the third dose, hardly any effect on pharmacokinetics relative to the first injection was seen. The altered pharmacokinetics was also dose dependent: the second liposome dose circulated for prolonged periods of time when the quantity of the first liposome dose exceeded 5 µmol phospholipid/kg body weight [20]. Unlike the case of drug-free PEGylated liposomes, intravenous injection of a dose of doxorubicin-encapsulated PEGylated vesicles did not elevate the plasma levels of PEG-specific IgM antibodies sufficiently enough to accelerate macrophage sequestration and clearance of a second liposome dose [20]. This may presumably be due to doxorubicin-mediated macrophage death and inhibition of B-cell proliferation and/or killing of proliferating B cells. The nature of the liposome 'coat' also seems to play a key role in controlling the pharmacokinetics of intravenously injected vesicles following repeated administration. For example, similar to PEGylated liposomes, drug-free poly(hydroxyl-L-asparaginase)-grafted vesicles also exhibit prolonged circulation times in the blood. However, these vesicles are less susceptible to accelerated hepato-splenic clearance following repeated intravenous administration into rats [21].

Due to their extended circulations times, encapsulated drug stealth systems may induce toxic reactions. For instance, the most notable dose-limiting toxicity associated with the continuous and repeated infusion of Doxil is doxorubicin release from accumulated vesicles in the skin, resulting in palmar–plantar erythrodysesthesis (PPE) [16]. The incidence of PPE is greatest in patients receiving Doxil (doxorubicin equivalent) > 10 – 12 mg/m^2/week, but can be lowered substantially by reducing the dose intensity as well as the interval between subsequent dosing. For example, recent

studies in mice have demonstrated that the skin pharmacokinetics of one injection cycle of Doxil was independent of the next injection cycle when the dose interval was 4 weeks, with little evidence of symptoms of PPE [17].

2.2 Selected macromolecular and related systems

Natural and synthetic polymers have also received much attention as carriers of cytotoxic drugs. Examples include linear polysaccharides (e.g., dextran), carboxymethyldextran polyalcohol, polyglutamate, *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers and PEG covalently linked to cytotoxic agents (**Table 2**) [6]. A plethora of block copolymers capable of assembling into micellar structures have also been employed for drug delivery to solid tumours [7,22]. Here, the drug is either solubilised in the micellar core or conjugated to copolymers. These polymeric micelles usually exhibit far lower critical micelle concentrations than conventional surfactant micelles, and as a result they usually sustain their structural integrity in the blood and exhibit prolonged circulation times.

Progress with some of the polymeric systems have been rather limited due to poor drug loading, inappropriate linkage chemistry (e.g., leading to lack of or premature drug release), inadequate pharmacokinetics (short half-life) and polymer-related toxicity (e.g., immunogenicity or other related immunotoxicity reactions). Furthermore, classical cytotoxic drug-induced side effects may still occur; this is usually a reflection of linker hydrolysis either in the blood or during the renal elimination process leading to rapid drug release and exposure to sensitive sites. Indeed, early trials with doxorubicin bound to oxidised dextran (M_r = 70,000 g/mol) induced thrombocytopenia and severe hepatotoxicity [23]. Dose-limiting side effects including thrombocytopenia, neutropenia and reversible hepatotoxicity have also been shown following 3 h infusion of camptothecin analogue DX-8951 bound to carboxymethyldextran polyalcohol (M_r = 340,000 g/mol, 6.6 wt% drug) every 4 weeks and up to 9 mg DX-8951 equivalent/m^2 [24]. A Phase I evaluation of HPMA copolymer-Gly-Phe-Leu-Gly-doxorubicin, given as a short intravenous infusion every 3 weeks at gradually increasing doses up to a maximum-tolerated dose of 320 mg doxorubicin equivalent/m^2 (which is 5-times higher than the usual doxorubicin dose), also reported typical anthracyline dose-limiting toxicities (neutropenia and mucositis), but despite cumulative doses up to 1680 mg doxorubicin equivalent/m^2, no cardiotoxicity was observed [25].

2.3 Pseudoallergic responses to drug carriers

Acute allergic reactions have been reported to occur in some patients within minutes of liposome or polymeric nanoparticle infusion [26]. For instance, the frequency of allergic reactions to liposomes shows large variation of 3 – 45% and some of the observed symptoms are similar to those of IgE-mediated type I allergy [26]. These include symptoms of cardiopulmonary distress such as dyspnoea, tachypnoea, hypertension/hypotension and chest and back pain. Contrary to type I allergy, the response arises at the first exposure without prior sensitisation, and the symptoms may lessen or disappear on later treatments. Indeed, the liposome-induced haemodynamic changes are highly reproducible in the porcine model following intravenous injection of minute amounts (5 – 10 mg total lipid) and include a massive rise in pulmonary arterial pressure, and a decline in systemic arterial pressure, cardiac output and left ventricular end-diastolic pressure [26,27]. Haemodynamic changes in the porcine model are also associated with massive but transient ECG alterations, including tachycardia, bradycardia, ST-segment depression and T-wave changes, ventricular fibrillation or cardiac arrest. These pseudoallergic reactions to liposomes are strongly correlated with complement activation [26,27], but the extent of complement activation by liposomes (via all three pathways of complement activation) depends on vesicular lipid composition, bilayer packing, surface characteristics, electric charge, morphology and size, and these are reviewed in detail elsewhere [28]. Complement activation leads to the rapid production of anaphylatoxins C3a and C5a, and the subsequent release of thromboxane A2 and other anaphylatoxin-derived mediators. Inhibitors of complement activation such as soluble complement receptor type 1 and anti-C5a monoclonal antibody can dramatically suppress liposome-induced cardiopulmonary changes in the porcine model [27].

An infusion of PEGylated liposomes such as Doxil also induces cardiopulmonary distress in a substantial percentage of human subjects [16,29,30]. For example, a recent investigation has reported acute allergic reactions to Doxil infusion in 13 out of 29 cancer patients [30]. Doxil was further shown to induce complement activation in 21 out of those 29 patients (72%), as reflected by significant elevation of SC5b-9 (the terminal complex activation marker) levels in plasma within 10 to 30 min of infusion. This is rather remarkable, as surface modification with mPEG is generally thought to dramatically suppress complement activation and blood opsonisation events [8,31] (**Figure 1**). Remarkably, among the 13 sensitive patients, 12 (92%) had elevated plasma SC5b-9 levels, whereas among the 16 non-responding individuals significant rises in plasma SC5b-9 level occurred in 9 (56%) [30]. Similarly, in a study involving radiolabelled PEGylated liposomes for scintigraphic detection of bowel inflammation, pseudoallergy was observed in three out of nine patients, and in the case of one sensitive patient the plasma levels of complement proteins (notably C3, C4 and factor B) decreased substantially [32]. Therefore, it appears that PEGylated liposome-induced cardiopulmonary distress may be a manifestation of complement activation-related pseudoallergy. In this context, it is worth mentioning that complement has been known to be involved in the effector arm of antibody-mediated type II and type III

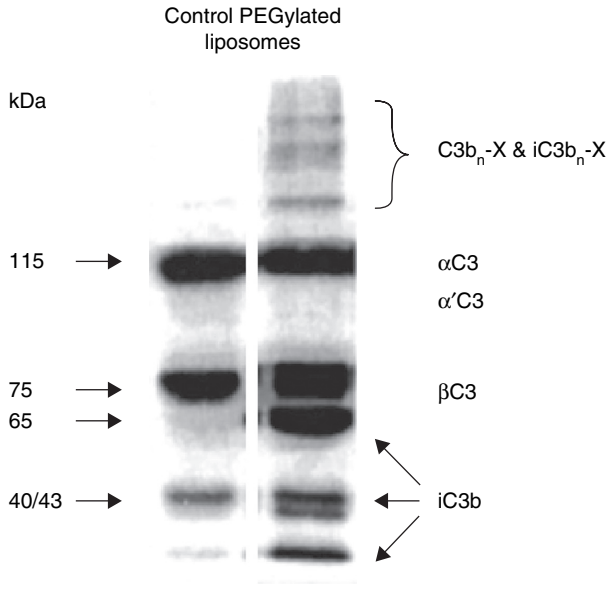


Figure 1. SDS-PAGE analysis of Doxil-mediated complement activation in human serum (20% v/v) supplemented with ^{125}I-labelled C3. Generation of the scission products of iC3b (40/43 kDa bands) together with the lack of α′C3 band implies efficient inactivation of C3b to iC3b by factors H and I. Also, the presence of C3b$_n$-X/iC3b$_n$-X complexes suggests that C3 is activated in a manner similar to complement activation by immune aggregates [31].

allergic reactions. However, the elevated plasma SC5b-9 levels in non-responders (as in the Doxil study) suggest that complement activation *per se* cannot solely account for liposome-induced pseudoallergy, and that other contributing factors therefore must be considered. The differences between sensitive and non-responding individuals, for instance, may depend on the extent of activation of other plasma cascades such as the kallikrein-kinin system that results in the generation of a co-stimulus for mast cell activation. Alternatively, the mast cells and basophils of sensitive individuals may have a lower than normal threshold for degranulation following the binding of anaphylatoxins to their G protein coupled receptors. It is also plausible that the risk of complement activation-related pseudoallergic cardiopulmonary reactions may be highest in a complement-responder with atopic constitution.

Recent studies have shed light on the possible mechanisms of complement activation by PEGylated liposomes [33]. These studies indicated the importance of a spatial relationship between the headgroup of zwitterionic phospholipids in the liposomal bilayer and the net anionic charge on the phosphate moiety of phospholipid–mPEG conjugate in controlling antibody binding and its orientation into a complement activating posture [33]. In addition, a direct role for C1q binding to PEGylated vesicles and subsequent complement activation was speculated, where C1q may interact with the ether oxygen groups of the projected mPEG chains via hydrophobic interactions and/or hydrogen bonding as well as with the anionic phosphate oxygen of the phospholipid–mPEG through the top of its basic head [33]. If complement activation is a precondition to liposome-mediated hypersensitivity, then it is highly desirable to engineer liposomes and related structures that circumvent complement activation; this is despite the fact that slow intravenous liposome infusion together with high-dose steroid and antihistamine premedication substantially reduces the risk of hypersensitivity. Some progress has now been made towards the design of non-complement activating PEGylated liposomes, which includes zwitterionic dipalmitoylphosphatidylcholine vesicles with incorporated non-ionic lipid–mPEG conjugates [33].

The occurrence of hypersensitivity reactions to Taxol® (paclitaxel solubilised in Cremophor EL® [BASF]/ethanol micelles; Bristol-Myers Squibb Company; **Figure 2**) is also well documented [34,35]. These occur in ~ 2 – 7% of cancer patients despite premedication with high-dose dexamethasone, antihistamines and H2-receptor antagonists, and are severe and life-threatening in some 2% of the recipients. Hypersensitivity to Taxol has been referred to in some studies as a type I allergic reaction, primarily on the basis of its clinical course and symptoms, but conclusive evidence for a role of IgE in Taxol-mediated hypersensitivity has never been established. In addition, a direct effect of paclitaxel on basophils and/or mast cells has never been proven. Similar to liposomes, most reactions to Taxol occur during the first or second treatment cycle. However, the reactions may spontaneously disappear on slowing the infusion rate and may be associated with transient pulmonary infiltration, hypertension and major cardiac arrhythmia [26]. These symptoms are consistent with complement-activation-related pseudoallergy. Indeed, recent *in vitro* studies have shown that, at clinically relevant concentrations, both Taxol and its vehicle (Cremophor EL/ethanol micelles) can equally induce complement activation in human sera via both calcium-sensitive and alternative pathways, thus suggesting that the vehicle, and not paclitaxel, is a complement-activating agent [36]. The exact mechanisms of complement activation by Cremophor EL remain unknown, but the available data suggests that, in serum, Cremophor EL can substantially decrease the electrophoretic mobility of both high- and low-density lipoprotein, resulting in *de novo* formation of larger molecular size complexes with no evidence of lipoprotein dissociation into smaller fragments [37]. These alterations were speculated to arise from incorporation of some of the hydrophobic components of Cremophor EL into lipoproteins [38]. The components of Cremophor EL that did not associate with lipoproteins were suggested to form large droplets (100 – 300 nm; **Figure 2**), capable of assembling C3 convertases, presumably as a result of direct C3 binding to the surface-exposed hydroxyl and ethylene oxide residues [38]. Nevertheless, Taxol or

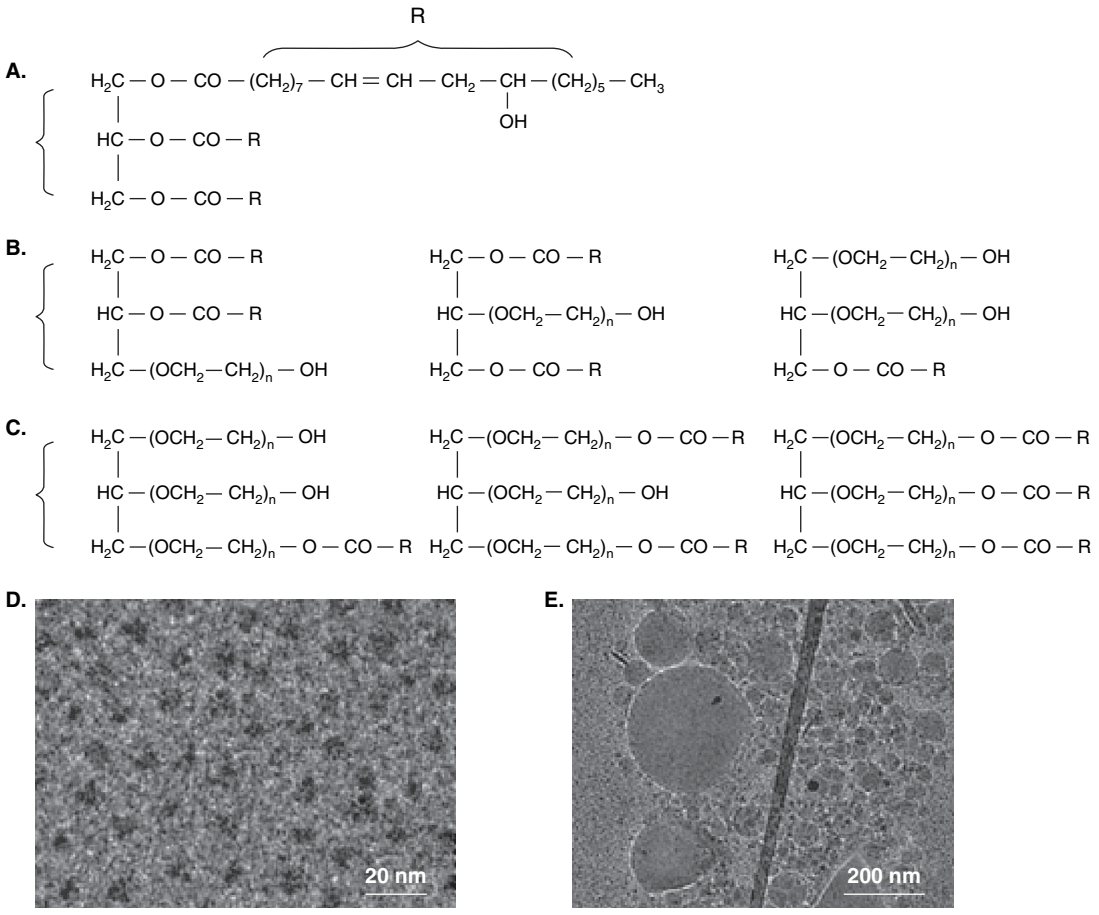


Figure 2. Cremophor EL composition, micelles and its transformation in human plasma. Cremophor EL is a non-ionic solubiliser and emulsifier obtained by reacting castor oil with ethylene oxide in a molar ratio of 1:35. The product consists of a complex mixture of unmodified castor oil (the most abundant triglyceride in castor oil, in which the glycerol is esterified by ricinoleic acid, is shown in **A.**, ricinoleic acid mono- and di-esters with non-esterified OH moiety of glycerol bound to poly(oxyethylene), shown in **B.**, polyethoxylated glycerol with one, two or all polyethylene residues esterified by ricinoleic acid, shown in **C.**, and free PEG chains and ethoxylated glycerol (not shown). In aqueous solution, and at a concentration > 60 µg/ml, Cremophor EL can form 'star-shaped' micelles of 10 – 25 nm in size. Cremophor EL formulations may contain ethanol as a co-solvent to further aid drug solubilisation and micelle formation. **D** shows cryogenic temperature transmission electron micrograph of Cremophor EL (the sample was taken from a Taxol-equivalent Cremophor EL/ethanol mixture and diluted 10-fold in phosphate-buffered saline to mimic the conditions during patient infusion). **E** represents cryogenic temperature transmission electron micrograph of Taxol in human plasma (the injection concentrate was first diluted 15-fold in phosphate-buffered saline and then 5-fold in plasma) showing formation of large (100 – 300 nm) droplets. Micrographs **D.** and **E.** are courtesy of J Szebeni.

Cremophor EL/ethanol-mediated complement activation in human serum can be inhibited by soluble complement receptor type 1 and human intravenous immunoglobulin [36,38]. With regard to polymeric systems of relevance to cancer drug delivery, the present the present authors have recently demonstrated complement activation by a number of poloxamer and poloxamine block copolymers, both at submicellar concentrations (e.g., poloxamer 188, poloxamine 908), as well as in micellar forms (e.g., poloxamer 407) [31,33,39].

Recently, intriguing progress was made in cancer drug delivery with the development of Abraxane® (ABI-007, *nab*-paclitaxel; Abraxis BioScience), which is an albumin nanoparticle-based paclitaxel formulation. Abraxane is readily reconstituted in saline at concentrations of 2 – 10 mg/ml (compared with 0.25 – 1.25 mg/ml for paclitaxel alone), thereby overcoming the poor solubility of paclitaxel in the blood and the allowing administration of more paclitaxel per dose over a shorter period of time [40]. Apparently, hypersensitivity reactions to Abraxane are considerably fewer when compared with Taxol, despite the fact that it is given to patients without steroid and antihistamine premedication at a 50% higher dose and with a shorter infusion time [40]. Rapid disassembly of Abraxane into drug-bound albumin molecules in the systemic circulation may account for poor complement activation and related consequences.

3. Tumour barriers (and opportunities for targeting)

Tumour blood vessels are highly irregular and show gross architectural changes that differ from the vasculature of normal organs and the newly formed blood vessels (as in wound healing) [9,41]. One consistent abnormality of tumour blood vessels is their permeability to macromolecules and particulate entities, arising from irregularly shaped and loosely interconnected endothelial cells and less frequent and intimate association of these endothelial cells with pericytes and the vascular basement membrane [9,41,42]. These features are particularly controlled by the multi-functional cytokine vascular permeability factor/vascular endothelial growth factor secreted by tumour cells [43,44]. Detailed morphological investigations have convincingly demonstrated heterogeneity in pore sizes along a typical tumour blood vessel: tumours that were grown subcutaneously exhibited a characteristic pore cutoff size ranging 200 – 1200 nm, with the majority ranging 380 – 780 nm [42]. Despite these features, the movement of macromolecules and particulate matter out of the tumour blood vessels and into the extravascular compartment is remarkably limited, which is the result of a higher-than-expected interstitial pressure and a lack of functional lymphatic drainage, coupled with lower intravascular pressure [45]. However, these pathophysiological barriers are not fully developed in micrometastases and also pose a lesser problem with respect to particulate/macromolecule extravasation into well-perfused, and low-pressure regions in larger tumours.

It should be emphasised that not all tumour blood vessels are leaky: there is marked variability in endothelial permeability among different tumours, different vessels within the same tumours and during tumour growth, regression and relapse [41]. For instance, the pore cutoff size is reduced when tumours are grown in the cranium [42]. Also, in androgen-dependent mouse mammary tumour, testosterone withdrawal reduced the pore cutoff size from 200 nm to < 7 nm within 48 h [42].

Distribution, organisation and relative levels of extracellular components such as collagen, fibronectin, laminin, decorin and hyaluronan vary with tumour types, and the anatomical locations and these are expected to impede the diffusion of extravasated macromolecules and particulate entities in tumours [9,46]. Indeed, it has also been shown that following extravasation into solid tumours, stealth liposomes and nanoparticles often distribute heterogeneously in perivascular clusters that do not move significantly and poorly interact with cancer cells [47]. Extravasated polymer–drug conjugates may become internalised by tumour cells (particularly those at the periphery of the tumour mass) through either fluid-phase or receptor-mediated pinocytosis [6].

The vascular barrier of the solid tumour is also its Achilles' heel: the nutritionally demanding tumour cells are entirely dependent on a functional vasculature. Attention has, therefore, been focused on tumour vasculature as a target for tumour-endothelial cell-specific ligand-tagged macromolecular and particulate agents – albeit with rather poor efficiency – and these have been repeatedly reviewed [2-6,8,10,11].

The connective tissue in and around malignancies contains a meshwork of fibrin clots, which presumably arises as a result of vascular epidermal growth factor-induced leakage of Factor VII(a) and fibrinogen, and the subsequent initiation of the coagulation cascade by tissue factor expressed by tumour cells and tumour vascular endothelial cells. Recently, it was demonstrated that fibrin clots in the tumour micro-environment could serve as a target for nanoparticles that are surface-decorated with peptides that have an affinity for fibrin [48]. Following binding to tumour blood clots, these nanoparticles induced further local clotting, thereby producing new binding sites for more particles (self-amplified homing). The molecular basis of nanoparticle-mediated clot formation remains unknown, but the procoagulant microenvironment of tumours and the expression of ancillary molecules (such as phosphatidylserine) on the tumour endothelium may be among the major factors contributing to tumour specificity of the coagulation cascade. Also the binding of clotting products to engineered nanoparticles may shift the balance of clot formation–clot dissolution in the direction of clot formation, and that the presence of this activity at the nanoparticle surface may facilitate contact-dependent coagulation. The described system seems to overcome the poor efficiency usually encountered in targeting components of the vascular system with polymeric and nanoparticulate entities [48]. This is a promising beginning for the design of different self-amplifying homing-enabled particulate nano-medicine for simultaneous monitoring and treatment of experimental malignancies. However, these particles achieved a 20% occlusion rate in tumour vessels, which is insufficient to starve the tumour mass by local embolism [48]. Further optimisation in terms of surface ligand density and orientation or utilisation of particulate systems of different shapes (e.g., rod, oblate ellipsoidal and dendrameric) may change that. Nevertheless, these approaches must be viewed cautiously, because, on release within tumour vessels, the thrombo-emboli might cause a sudden vascular occlusion at other sites, such as lung capillaries.

4. The bioavailable drug

Drug entrapped in a particulate carrier or covalently linked to a macromolecule is not bioavailable and has no therapeutic activity until it is released in free form. The free drug levels at the target site must also exceed the minimum cytotoxic concentration, otherwise no appreciable therapeutic response will be seen. The rate of drug release from a carrier can have a substantial effect on the therapeutic outcome – a process that not only depends on the composition of the interstitial fluid surrounding tumours, as well as

intracellular degradation, but also on the drug type and encapsulation/conjugation procedures.

4.1 Drug release from particulate systems

The pharmacokinetics, body distribution and therapeutic activity of three different formulations of PEGylated liposomal doxorubicin (where doxorubicin was actively loaded) having different doxorubicin release rates were studied recently in a murine mammary carcinoma model [49]. The extent of drug release was controlled by altering the fluidity of the liposomal bilayer through changing the fatty acyl chain length and/or degree of saturation of the phospholipid. The plasma pharmacokinetics of the liposomal lipid was similar for all three formulations, and the plasma pharmacokintics of doxorubicin component was dependent on the liposome type [49]. For all three formulations, liposomal lipid accumulated to similar levels in tumour and cutaneous tissues. Interestingly, liposomes with the most rapid release rates expressed the lowest tumour response; liposomes with the slowest release rates had the best therapeutic activity and those with intermediate release rates produced unexpected cardiotoxicity that was not related to the lipid content of the formulation [49]. Thus, drug leakage rates are important in determining the therapeutic activity as well as the toxicity of liposomal formulations. These observations are also consistent with the therapeutic activity of extravasated Doxil in solid tumours, where the encapsulated doxorubicin is released in a slow manner. For example, the bioavailable doxorubicin levels for mice receiving Doxil are 43-fold higher than those seen in animals receiving free drug [50]. The mechanism of doxorubicin release from Doxil is not clear, but it has been hypothesised that non-specific chemical disruption or collapse of the liposomal pH gradient, which is used to actively load liposomes with doxorubicin, may trigger doxorubicin release from extravasated vesicles [16]. If this notion is true, then some of the antitumour effects of liposomal doxorubicin may be ascribed to a possible antiangiogenic effect of bioavailable doxorubicin (slow and prolonged doxorubicin release with no extended rest periods for tumour endothelial cells). The importance of the mode of drug loading in liposomes on subsequent drug release is further highlighted by the observation that extravasated long-circulating cisplatin-containing liposomes (SPI-077, where cisplatin is loaded passively) lack antitumour activity both in animal models and humans, despite the observation that more total liposomal cisplatin distribution to tumours occur than free cisplatin [51,52]. It appears that with SPI-077, the concentration of free cisplatin does not reach a minimum cytotoxic concentration (the release rate is presumably too slow) and, therefore, no appreciable therapeutic response is seen. Consequently, the development of SPI-077 was stopped in Phase II clinical trials.

Mixed therapeutic responses have also been reported following the intravenous administration of long-circulating polymeric nanoparticles, which again may be related to inadequate drug release. For instance, doxorubicin-loaded long-circulating PEG-coated poly(alkyl cyanoacrylate) nanospheres were shown to accumulate in the brain of a rodent cerebral gliosarcoma model, where particle accumulation was mainly confined to the implanted tumour [53]. However, no improvement in antitumour efficacy was observed, and despite the notion that the maximum tolerated dose of doxorubicin is higher when the drug is incorporated into nanospheres compared with its free form. Contrary to these observations, the *in vivo* antitumour activity of recombinant TNF-α was improved following incorporation into long-circulating poly(alkyl cyanoacrylate) nanospheres [54].

4.2 Drug release from polymeric conjugates

Mixed therapeutic responses have been observed with polymer–drug conjugates and these are listed elsewhere [6]. The lack or poor antitumour activity of polymer–drug conjugates often arises from rapid drug release within the systemic circulation and rapid hepatic elimination of the conjugate, as well as from poor to modest conjugate extravasation to solid tumours. For the majority of extravasated polymer–drug conjugates, the process of drug release is mostly intracellular (e.g., following fluid-phase or receptor-mediated pinocytic uptake) and is limited to cells at the periphery of the tumour mass. Thus, the rate-limiting intratumoural steps include the extent of conjugate uptake and its access to the lysosomal compartments, the nature of polymer–drug linkage (e.g., peptidyl, ester, pH-sensitive *cis*-aconityl, acetal) and the activity of the lysosomal enzymes for subsequent drug release (e.g., cathepsin B). Finally, rapid exocytic elimination of some polymer–drug conjugates from cancer cells may further contribute to the lack of therapeutic activity.

4.3 Multi-drug resistance

The intrinsic and acquired resistance of many cancer cells to cytotoxic drugs is a major problem in cancer chemotherapy. Some tumours exhibit a high degree of multi-drug resistance, which is due to overexpression of energy-dependent efflux pumps such as the P-glycoprotein and multi-drug resistance related protein (MRP) [55-57]. Furthermore, the expression of some of these efflux pumps may change during chemotherapy. For example, MRP-1 expression in tumour cells is further upregulated after challenge with cytotoxic agents such as doxorubicin, whereas MRP-2, which is initially not expressed in untreated small lung cancer cells, becomes overexpressed during the course of chemotherapy [58].

Multi-drug resistance, particularly in tumours exhibiting a low resistance factor, may be overcome to some extent following the gradual release of cytotoxic drugs from extravasated particulate carriers. With tumours exhibiting higher resistance levels, novel strategies have been sought. One approach is the use of long-circulating temperature-sensitive liposomes or polymeric micelles (with and without targeting ligands) in conjugation with hyperthermia or

pulsed high-intensity focused ultrasound [22,59-61], but this has limited applicability for visceral and widespread malignancies. However, rapid drug release during hyperthermia could also allow the drug to shutdown tumour blood flow, with little effect on normal microcirculation [62]. Others have elaborated on biochemical triggers such as cleaveable PEG–phospholipid conjugates to generate fusion competent vesicles [63], enzyme-mediated liposome destabilisation [64-66] and pore formation [67]. For instance, prodrug ether liposomes (e.g., vesicles containing phospholipids with a non-hydrolysable ether bond in the 1 position) are susceptible to degradation by secretory phospholipases, and the level of these enzymes (such as the secretory phospholipase A2) is dramatically elevated within the interstitium of various tumours [64-66]. Secretory phospholiapse A2 not only acts as a trigger resulting in the release of encapsulated cytotoxic drugs from prodrug ether liposomes, but also generates highly cytotoxic lysolipids that destabilise the plasma membrane of tumour cells, thereby enhancing their permeability to cytotoxic drugs. Another interesting attempt was tumour infection, first with the anaerobic bacterium *Clostridium novyi-NT*, followed by intravenous administration of liposome-encapsulated cytotoxic drugs [68]. *C. novyi-NT* not only infects hypoxic regions within experimental tumours, but also possesses a lipase, which has little phospholipase activity, but has a lipid-binding domain capable of directly disrupting lipid bilayers. It was shown that the treatment of mice bearing large, established tumours with *C. novyi-NT* plus a single dose of Doxil led to eradication of the tumours [68]. This approach also proved successful in animals bearing small and large tumours resistant to irinotecan. When liposomal irinotecan was delivered in combination with *C. novyi-NT* spores, resistant tumours regressed and > 60% of the animals survived for at least 3 months.

Polymeric nanoparticles may also restore cancer cell sensitivity to anticancer drugs by a number of mechanisms depending on their composition and surface characteristics [4]. One interesting example is the exclusive ability of doxorubicin-loaded poly(alkyl cyanoacrylate) nanoparticles to overcome doxorubicin resistance in P-glycoprotein overexpressed cancer cells (doxorubicin resistance was not overcome when the drug was incorporated into alginate or poly(lactide-*co*-glycolic acid) nanoparticles) [69]. The mechanism of action of poly(alkyl cyanoacrylate) nanoparticles was related to their simultaneous adherence to cancer cell plasma membrane, and rapid degradation. This initiated the formation of a complex between the positively charged doxorubicin and the negatively charged cyanoacrylic acid (derived from nanoparticle degradation) that could diffuse easily into the cells. It would appear that by masking the cationic charge of the amino sugar of doxorubicin the P-glycoprotein-mediated multi-drug resistance could be overcome without compromising the cytotoxic effect of doxorubicin. Other interesting approaches with poly(alkyl cyanoacrylate) nanoparticles have included preparations that contained doxorubicin within the particle core, and ciclosporin, an inhibitor of P-glycoprotein, at the surface [70].

Pinocytic and endocytic modes of delivery also bypass the mechanism of resistance associated with membrane efflux pumps [6]. Indeed, this mode of delivery may explain partial responses seen with polymer–drug conjugates, where following internalisation the drug is released from the polymer backbone in the endolysosomal compartment. Subsequently free drug molecules may reach the cytoplasm either by diffusion or by specific transporters, depending on the physicochemical nature of the drug. To further enhance pinocytic/endocytic delivery and bypass multi-drug resistance, active targeting has been sought. This strategy uses a long-circulating drug carrier linked to tumour-specific monoclonal antibodies or their internalising epitopes or ligands for receptors over-expressed by cancer cells (e.g., folate, transferrin), and in some cases therapeutic responses have been observed [2-6,8,10,22]. However, with such approaches, the acidic conditions of endolysosomes may not favour rapid drug release from certain type of carriers. For instance, doxorubicin release in lysosomes from the ammonium sulfate gradient-loaded doxorubicin liposomes is a slow process; doxorubicin reaches lysosomes as the sulfate salt and the concentration of doxorubicin in the liposome aqueous spaces is far above the drug solubility product. For more profound effects in resistant tumours pH-sensitive carriers may be employed [10,22]. One recent example is poly(L-histidine)–PEG diblock copolymer micelles loaded with doxorubicin and surface decorated with folate [71]. Here, poly(L-histidine) chains become ionised at early endosomes, resulting in micellar destabilisation and subsequent disruption of the endosomal membrane.

5. Conclusions

Particulate and macromolecular drug carriers provide a range of new opportunities to increase the targeting of therapeutic agents to the elements of solid tumours and promising results have come through such attempts at clinical levels. Indeed, several formulations have already reached the market and several more are in advanced clinical trials. Drug carriers exert substantial effects on the pharmacokinetics and pharmacodynamics of their entrapped or conjugated cargo relative to free drugs, and could minimise drug-induced toxicity. Nanoscale drug carriers can passively target solid tumours, but effective therapeutic responses are still confronted by pathophysiological processes that control carrier transport into tumours and biochemical factors regulating drug release from extravasated carriers and maintaining free drug levels above the minimum cytotoxic concentration. Active targeting also holds great promise for the battle against cancer; successful attempts have been recorded with active targeting of angiogenic tumour vasculature (particulary in delocalised tumours and those in the early stages of their development), tumour blood clots and tumour cells with

ligand-decorated carriers. It is conceivable that future sophistication in tumour targeting and the outcome of end results will depend on a better understanding of tumour biology and biological barriers, as well as advances in carrier design and nanoengineering.

6. Expert opinion

Although promising therapeutic responses have come from attempts to administer and direct cytotoxic agents to solid tumours by using particulate and macromolecular carriers, a better understanding of the major barriers hindering optimal carrier transport into tumours and subsequent drug release is still necessary. Already compromised by abnormal hydrostatic pressure gradients, compressive mechanical forces generated by tumour cell proliferation may further cause intra-tumoural vessels to compress and collapse; this creates further barriers for passive targeting [72]. Attempts are increasingly being directed towards agents that can lower the interstitial fluid pressure in tumours, thereby allowing more efficient delivery and deeper penetration by decompressing these same vessels [9,72]. However, this enhanced perfusion could provide a route for metastasis.

The heterogeneity of genetic abnormalities present in the tumour cell population has been well recognised. This means that drug combinations are most likely to improve long-term patient prognosis. Here future attempts may lead to the development of a 'single vial' fixed-dose combination product with an enabling technology (e.g., liposome) to facilitate the delivery of cytotoxic agents to the tumour site at concentrations and/or drug ratios that are synergistic [73]. The concept of the active targeting of tumour cells with ligand-decorated carriers is also an attractive one, which may bypass a mechanism of resistance associated with membrane efflux pumps. However, such attempts may show no therapeutic activity in tumours that also overexpress antiapoptotic proteins such as BCL2. Multiple strategies are therefore needed and attempts have been initiated for the simultaneous intracellular delivery of cytotoxic drugs and agents that could halt the action of BCL2 function (e.g., antisense oligonucleotides) [58]. Nevertheless, with such approaches the delivery part is still passive and relies on carrier extravasation.

As a result of altered pharmacokinetics, differing dosing schedules and dose intensities of drug encapsulated/conjugated carriers may induce unexpected side effects, as exemplified with certain stealth liposomes and polymer–drug conjugates. Still, the carrier itself or some of its components may initiate untoward immunological responses (e.g., hypersensitivity, immunogenicity). A clear understanding of the molecular basis of these events is essential for the further development of safer carriers for the transport of cytotoxic drugs. Indeed, a recent breakthrough in this respect is the development of Abraxane. Since Abraxane disassembles rapidly into drug-bound albumin molecules in the systemic circulation, these nanoparticles are beginning to be applied for metronomic chemotherapy at experimental levels [74]. In addition, not much is known with respect to the intracellular fate (both in cancer and immune cells) of non-degradable components of a carrier [75,76]. Can these components interact with cellular machineries and induce secondary effects (e.g., altered gene expression, mitochondrially mediated apoptosis) or simply undergo exocytic elimination? Some evidence presently suggests that HPMA copolymer–doxorubicin may exert part of its therapeutic effect through immunostimulation and, in addition to this, can further modulate the expression of genes involved in apoptosis signalling [77,78]. Also, polycations such as poly(ethylenimine) and poly(L-lysine), which are used in cellular transfections, are capable of inducing mitochondrially mediated apoptosis and may be beneficial in tumour destruction [76,79,80].

Finally, advances in nanotechnology are even beginning to have an impact in experimental oncology, providing new and complementary strategies to combat tumour growth [81]. One well-explored example is composite metal nanoshells, which are entities consisting of a spherical dielectric core of 20 – 80 nm (e.g., made from silica) surrounded by a thin metal shell of 5 – 20 nm (e.g., made from gold). The principal operation of a nanoshell is based on the plasmon excitation process and the type of plasmon that exists on the surface of a metallic shell is directly related to its shape and curvature. By controlling the relative thickness of the core and shell layers, the plasmon resonance and resultant optical absorption properties can be adjusted from near-UV to mid-infrared. Drugs may be incorporated within the nanoshell core [82], and the surface of a nanoshell is further amenable for decoration with biological ligands and polymers for site-specific targeting. Indeed, the plasmon resonance property of near-infrared-responsive gold nanoshells has recently been exploited for the irreversible photothermal ablation of cancer cells *in vivo* under magnetic resonance guidance [81]. Here, the temperature change was induced only in nanoshell-associated regions with an external infrared light, which is transmitted through tissue at depths of a few cm with relatively little attenuation and no local damage. Nanoshells are presently undergoing clinical testing for the treatment of glioblastoma and astrocytoma. Nevertheless, detailed investigations are necessary to assess the short- and long-term safety of such nanotechnology-derived entities. Other recent novel approaches include an electrostatic layer-by-layer self-assembly procedure to encapsulate solid core paclitaxel nanoparticles within a polymeric nanometer-scale shell for controlled drug release [83].

It is, therefore, clear that there is no shortage of possibilities for combinations of carriers and nanotechnology-based 'warheads' in attempting to fashion the ultimate weapon against tumours and their microvasculature. It is now a question of correctly matching the components to achieve specificity and therapeutic efficacy.

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