Active targeting with particulate drug carriers in tumor therapy: fundamentals and recent progress

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Drug therapy for the treatment of tumors is often limited by a narrow therapeutic index. One approach that overcomes this limitation is the active targeting of tumors with particulate drug carriers. The derivatization of particulate drug carriers with a ligand leads to the selective targeting of the particulate to selected cells, thereby focusing drug delivery. In addition, particulate drug carriers have a high loading capacity, do not need covalent conjugation of the drug and the formulation protects the entrapped drug from enzymatic inactivation. Despite these favorable properties, their therapeutic efficacy in animal models has been reported only in recent years. The use of internalizing ligands and the targeting of intravascular tumor cells and endothelial cells of tumor blood vessels have been instrumental in demonstrating the clinical effectiveness of particulate drug carriers in animal models. As a result, several actively targeted particulate carriers have now entered, or are about to enter, clinical investigation. Recent findings, for example, the identification of cell-penetrating peptides with restricted cell selectivity, suggest that further improvements in this approach are likely in the near future.

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▼ Active targeting of particulates that carry physically entrapped drugs (hereafter referred to as particulate drug carriers) can achieve drug delivery to target cells *in vivo*, thus maximizing the therapeutic efficacy of the drug and reducing its systemic side-effects. The main application of this approach is to tumor therapy; because of the narrow therapeutic index of most antitumor drugs there is an urgent medical need to achieve focused drug delivery.

To attain active targeting of a drug carrier, particulates are derivatized with ligands that bind to specific receptors expressed on target cells. On binding to the receptors, the ligand–particle complex could be internalized into the cell (internalizing ligand) or remain cell-bound without being internalized (non-internalizing ligand).

Actively targeted particulates are the second generation of particulate drug carriers; the first comprises particles not derivatized with a ligand. Non-targeted particle-drug formulations demonstrate relative tumor selectivity as a consequence of 'passive' targeting, and several have already been approved for clinical use. As a result of the increased permeability of endothelial barriers in tumor blood vessels and the lack of effective lymphatic drainage from the tumor, passive targeting results in the selective extravasation and accumulation of particulates or other macromolecules in tumor tissues [1]. This 'enhanced permeability and retention' (EPR) effect [2] has been confirmed in numerous cases (e.g. [3,4]). However, active targeting is expected to lead to higher intratumoral accumulation and, in the case of targeting with internalizing ligands, to higher intracellular concentrations of the drug. This review covers the fundamentals of active targeting of particulate drug carriers in tumor therapy, as well as the most recent progress in this field.

Classes of particulate drug carriers

Particulate drug carriers and macromolecular conjugates are the two broad classes of drug carriers [5]. In macromolecular conjugates (Figure 1a), the drug is chemically linked to a macromolecule such as a synthetic polymer or an endogenous protein (e.g. human serum albumin). The chemical linker between the drug and the carrier must effect the controlled release of the drug. Therefore, acid-cleavable and/or reduction-sensitive spacers are typically used as chemical linkers [6]. By contrast,

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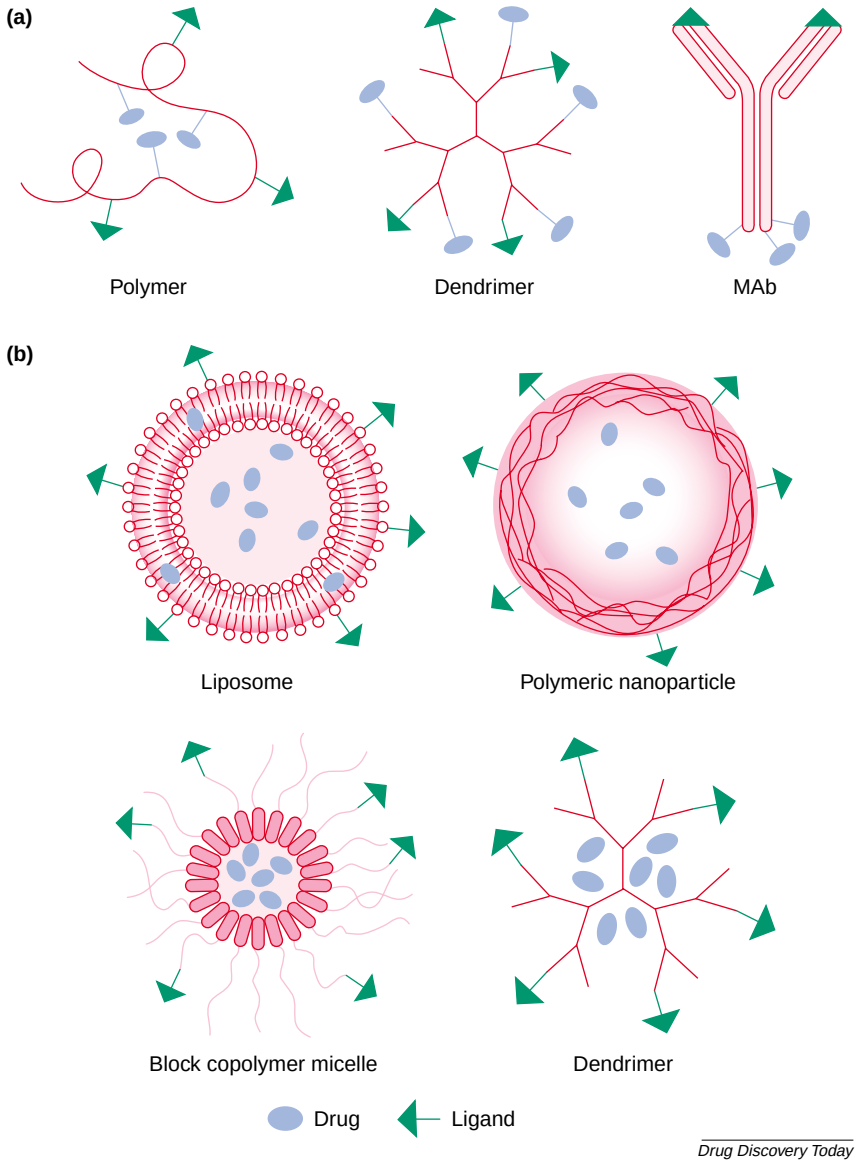

Figure 1. Schematic representation of the two classes of drug carriers **(a)** macromolecular conjugates **(b)** particulate drug carriers. Representatives of each of the two classes are depicted. For active targeting, members of each class are derivatized with a ligand that allows homing of the drug-carrier complex to desired target cells. In macromolecular conjugates, the drug is covalently linked to the carrier, whereas in particulate drug carriers the drug is entrapped in the particle without requiring covalent conjugation to particle components. Abbreviation: MAb, monoclonal antibody.

particulate drug carriers are molecular assemblies (Figure 1b) that entrap the drug in a loading space (generally the core of the particulate), thus isolating the drug from the environment and providing a higher degree of protection from enzymatic inactivation than that afforded by macromolecular conjugates. In addition, covalent conjugation of a drug is not necessary to entrap the drug [7], therefore a single carrier can be used to formulate various drugs, which, when combined with their high loading capacity, illustrates the advantages of using particulate drug carriers.

Several families of molecular assemblies are employed as particulate drug carriers for either passive or active targeting. Liposomes, polymeric nanoparticles and block copolymer micelles (Figure 1b) are colloidal molecular assemblies. Liposomes, the most intensively investigated family, are highly ordered lipid molecules in lamellar arrangement [7] that encapsulate a fraction of the solvent in which they are suspended. Liposomes currently in use as particulate drug carriers are homogeneous unilamellar vesicles that have a size of 50–150 nm. Polymeric nanoparticles comprise biocompatible polymers that generally vary in size from 10 to 1000 nm; the drug is either confined to a cavity surrounded by a polymer membrane (nanocapsules), or uniformly dispersed in a matrix (nanospheres) [8]. Block copolymer micelles are typically spherical, nanosized (10–100 nm) supramolecular assemblies of amphiphilic copolymers [9]. The core of these micelles is a loading space that accommodates hydrophobic drugs and the hydrophilic outer shell facilitates dispersal of the micelles in water.

Dendrimers are another family of particulate carriers that is creating great interest [10]. Dendrimers are synthetic, highly branched, monodispersed macromolecules. As their molecular size increases, they adopt a spherical shape that has a vacant inner core that can encapsulate drug molecules, and a highly functionalized outer surface that can be derivatized with a ligand.

Importantly, molecular assemblies acting as particulate drug carriers have the potential to carry additional drugs through a chemical linker to one of their molecular components. The resulting complexes have properties that class them as macromolecular conjugates.

The composition and size of each class of particulate drug carrier can be manipulated to obtain particulates of desired physicochemical properties. These properties influence the stability of the particulates in biological fluids and, consequently, the rate of drug release [11,12]. Size is an

important parameter for the extravasation of particulates into the interstitium of solid tumors. The endothelium of blood vessels in the majority of healthy tissues has a pore size of 2 nm, and 6 nm pores are found in postcapillary venules. However, in the discontinuous tumor vasculature, pores vary in size from 100 to 780 nm. Therefore, particulate drug carriers of a particle size in the 50–150 nm range are of the optimum size to avoid extravasation into healthy tissues, but are small enough to extravasate from most tumor blood vessels into the tumor interstitium [11]. Furthermore, the grafting of hydrophilic polymers [e.g. poly(ethylene glycol) (PEG)] onto the particle surface inhibits the uptake of particulate drug carriers by the reticuloendothelial system (RES), thus increasing the circulation time and the potential of the particulate drug carrier to reach the selected target, the tumor [1].

Conjugation of ligands to particulate drug carriers

The choice of synthetic strategy for the coupling of a ligand to a particulate drug carrier principally depends on whether coupling is performed before or after assembly of the carrier. The obvious goal of each approach is to achieve high coupling efficiency, but with the ligand retaining full binding affinity for its receptor. The coupling of a ligand after the carrier has been assembled involves the introduction of suitable, activated functional groups onto the terminus of one of the carrier components. Activated functional groups must be compatible with the process of carrier assembly and available on the carrier surface for efficient chemical ligation of the ligand. This strategy has proven to be particularly successful for the coupling of large ligands, for example, monoclonal antibodies (MAb), but has also been applied to the coupling of other classes of ligands [13,14]. As a strategy, the coupling of a ligand to a carrier component before assembly of the carrier is chemically less complicated. However, this approach has the disadvantage that following final assembly of the carrier, a fraction of the conjugated ligand remains entrapped in the particle interior and is not available for receptor binding [15]. In the case of liposomes, methods were developed to insert a ligand–lipid conjugate into the outer monolayer of preformed, drug-loaded liposomes [16].

Several particulate drug carriers incorporate a component that prolongs their *in vivo* half-life, for example, the formulation of sterically stabilized liposomes (SSL) incorporates PEG. The ligand must be conjugated to the termini of the stabilizing component, either before or after the assembly of the particulate carrier [17–21], because, otherwise, the shielding effect of the stabilizing moiety would lead to a drastic reduction in the efficiency of the interaction between the ligand and its receptor.

Classes of targets and ligands used for active targeting of particulate drug carriers

Targets that are employed to achieve tumor-selective localization of particulate drug carriers can be divided into the following classes: (i) targets that are preferentially expressed on endothelial cells of tumor blood vessels (e.g. integrin-$\alpha_v\beta_3$ and negatively charged phospholipids) [21,22], (ii) targets that are overexpressed on tumor cells [e.g. HER2 and disialoganglioside (GD_2)] [13,23], and (iii) lineage-specific targets that are expressed at the same level on tumor cells and on normal cells (e.g. CD19) [24].

The list of ligands that are used to derivatize particulates for active targeting includes a wide range of synthetic and natural compounds of different chemical classes. Polypeptides, including MAbs and other proteins (e.g. fibrinogen) [25] have been widely employed. MAbs, either whole MAbs [26] or MAb fragments, including Fab′ [27] and single-chain Fv fragments [13], are among the most frequently used ligands. Because MAb fragments lack the Fc domain that binds to Fc receptors on phagocytic cells (resulting in the RES scavenging up particles bearing whole MAbs), particulates derivatized with MAb fragments have increased circulation times in the blood compared to particulates derivatized with whole MAbs [28]. The frequent use of MAbs or MAb fragments as ligands for particulate drug carriers is the result of a well established and relatively facile conjugation chemistry that affords derivatives that retain full binding activity [29]. MAb fragments have the additional advantage that they can be expressed as recombinant proteins in prokaryotic cells, a procedure that facilitates a more cost-effective large-scale production than the production of whole MAbs in eukaryotic cells [30].

The use of phage display peptide libraries to identify novel, site-specific ligands has significantly contributed to the increased use of peptides acting as ligands [31–33]. More recently, the use of the closely related phage display DNA libraries led to the identification of several peptides that display characteristics of cell-penetrating peptides (CPP) and, in contrast to all other previously described CPPs, demonstrate a restricted cell recognition pattern [34,35]. Two of these peptides have been used for the active targeting *in vivo* of tumor blood vessels and tumor lymphatic vessels, with the site of localization determined by the specificity of the peptide used [36]. As well as peptides identified through phage display libraries, natural peptides are also used as ligands [37]. In addition, an organic, low molecular weight (M_r) peptidomimetic has been used for the active targeting of tumor blood vessels with nanoparticles. The nanoparticles carried a gene coding for a protein that on expression interfered with the

angiogenesis of the tumor blood vessels. Significant tumor regressions were observed *in vivo* with this gene therapy approach [21].

Folic acid, a natural, non-peptidic, low M_r compound has also been employed as a targeting moiety [38]. Initial *in vitro* results using liposomes derivatized with this ligand were promising [38,39]. However, recent results obtained *in vivo* were disappointing because no enhanced uptake by tumor cells overexpressing folate receptors could be demonstrated [39].

Cationic liposomes represent an alternative approach to active targeting. Cationic liposomes comprise a lipid component that creates an overall positive surface charge and no specific ligand is used as a targeting moiety. It is the positively charged component of the liposome itself that binds through electrostatic interactions to negatively charged phospholipid headgroups, which are preferentially expressed on tumor endothelial cells [22,40–42].

Targeting ligands with and without pharmacological activity

An important issue concerning ligands is whether ligands only participate in targeting (which we will refer to as 'neutral ligands'), or whether ligands are pharmacologically active (which we will refer to as 'active ligands'). In the majority of studies, ligands are intended to act only as targeting moieties (i.e. neutral ligands). However, research indicates that ligands can contribute to the pharmacological activity of the drug-carrier complex [30,32,43,44]. Furthermore, the two pharmacologically active moieties in the formulation (the ligand and the drug) can have additive or synergistic therapeutic effects, an aspect that as yet has not been studied extensively, but could be of considerable pharmacological interest.

Increased affinity of particle-conjugated ligands through multivalent presentation

Conjugation of a ligand to a particle leads to multivalent presentation of the ligand, which facilitates the formation of simultaneous interactions between several ligands and (several) cell-surface receptors. This mode of interaction results in a significant increase in the binding affinity of the system, which is referred to as avidity, and maximizes the stability of the interaction between the particulate drug carrier and its receptor [25,45-47]. The potential of artificially created, multivalent display platforms in modern pharmacology lies far beyond the targeting of particulate drug carriers and is creating great interest for alternative applications, for example, the generation of agonists or antagonists having affinities that are orders of magnitude higher their monovalent counterparts [48].

Immunogenicity of ligand-conjugated particulates

The presentation of peptidic ligands on a particulate surface maximizes their immunogenic potential through phagocytosis of the particulate and subsequent presentation of antigenic peptides by antigen-presenting cells (APC). Research indicates that SSLs significantly enhance the immunogenicity of the ligand and induce rapid clearance of the particulates from the bloodstream on repeated administrations, which are accompanied by a progressive increase of antibody titers against the ligand [49–51]. These results might seem surprising because PEG, a flexible and hydrophilic polymer, is thought to prolong the circulation time of particulate drug carriers by forming a hydrophilic barrier around the particulate that excludes proteins and other macromolecules from the particulate surface, thus preventing opsonization and subsequent phagocytosis of the particulate [11,52]. However, PEG does not completely prevent uptake of the particulate drug carrier by APCs, but does reduce the rate of uptake. Therefore, it is reasonable to assume that it is the residual uptake of particulates by APCs that leads to highly efficient antigen presentation.

Although PEG-grafting does not result in a decrease of the immunogenicity of ligand-conjugated particulates, it has been argued that, through the barrier effect, it inhibits the binding of antibodies raised against the ligand. Research indicates that PEG-grafting leads to a substantial reduction of antibody binding to an antigenic, liposome-conjugated ligand (biotin), but that complete protection is achieved only when the biotin concentration on the liposome surface is low (0.1%) and when liposomes containing distearoyl-phosphoethanolamine-PEG or dipalmitoyl-phosphoethanolamine-PEG, but not dimyristoyl-phosphoethanolamine-PEG, are used [53]. These data suggest that ligand-conjugated particulates can be protected from antibody-mediated elimination under carefully controlled conditions. However, data on this subject generally suggest that the immunogenicity of particle-conjugated ligands is a significant problem illustrating the necessity of using ligands of the lowest possible immunogenicity.

Intracellular delivery of the particulates and/or of the drug

For the majority of actively targeted particulates, the physically entrapped drug must be internalized into a cell to effect pharmacological activity. There are two main approaches whereby this can be achieved (Table 1):

(i) Internalization of the particulate and the drug

Internalization of the particulate as well as the drug is effected through particle-conjugated ligands that are endocytosed upon interaction with their cell-surface receptors.

Table 1. Methods available to achieve intracellular delivery of drugs from actively targeted particulate carriers

Component internalized	Method of internalization	Refs
Internalization of the particle-encapsulated drug	Receptor-mediated endocytosis:	
	(i) breakdown of the particulate and release of the drug in endosomes (use of particulate components to accelerate particle breakdown and endosomal escape of the drug);	[54–57]
	(ii) breakdown of the particulate and release of the drug in lysosomes (few drugs resist the harsh conditions in this environment)	[89]
	Direct delivery into the cytoplasm using CPPs: probably requires devices that accelerate intracellular breakdown of the particulate and release of the drug (e.g. reduction-sensitive particulates)	[34–36,59,60]
Internalization of the drug alone	Constitutive release of the drug in the tumor interstitium	[61]
	Use of particulate components that allow accelerated release of the drug	[62]
	Release of the drug on accelerated breakdown of the particulate through the use of thermo- or pH-sensitive particulate components	[64,65]
	Transfer of a lipophilic prodrug from the particulate to the cell	[66,67]

The internalized particle–drug complex accumulates in endosomes and, eventually, in lysosomes. To exert pharmacological activity, a drug must exit these organelles to reach the cytosol or the nucleus, which are the sites of action of intracellularly active drugs. In lysosomes, this is effected by enzyme- and/or pH-dependent disintegration of the particle and subsequent diffusion of the drug out of the lysosomes. However, there are few drugs (e.g. doxorubicin) that resist the harsh conditions of this milieu and are discharged intact from the lysosomes. Alternatively, it can be desirable for the drug to diffuse out of the endosomal compartment, because the microenvironmental conditions do not allow rapid disintegration of the particles here and, consequently, release of the drug. Several approaches to optimize the efficiency of endosomal escape have been investigated, including the use of lytic peptides (either conjugated to the particle surface [54] or encapsulated with the drug within the particles [55]), pH-sensitive polymers [56] and swellable dendritic polymers [57].

Recent results indicate that an approach using CPPs might achieve active targeting and internalization of particulate drug carriers without relying on receptor-mediated endocytosis. CPPs do not interact with classical cell-surface receptors and are internalized directly into the cytosol. Their internalization is neither saturable nor limited by receptor downregulation. The molecular mechanism of the internalization of these peptides is still unclear and is probably specific to a particular CPP [58]. A study has demonstrated the feasibility of using CPPs to internalize particles for liposomes conjugated with the 7-amino acid residue CPP Tat [14]. However, the key disadvantage with this approach was that all known CPPs internalized with no cell selectivity. More recently, several peptides have been described that have characteristics of CPPs (non-saturable, receptor-independent internalization), but also demonstrate restricted cell selectivity [34,35]. Although these peptides show great potential for active targeting with particulates, a problem with using CPPs for drug delivery was described recently [59]. Two CPPs (penetratin and Tat) were used to facilitate internalization of doxorubicin-loaded liposomes. Although their efficacy in facilitating internalization was confirmed, as a result of the inefficient escape of the drug from the liposomes, the improved uptake of liposomal doxorubicin was not reflected by cytotoxicity *in vitro* or tumor control *in vivo*. Thus, an additional approach to facilitate intracytosolic release of the encapsulated drug, for example, the use of liposomes that undergo accelerated disintegration under reducing conditions, should circumvent this problem [60].

(ii) Internalization of the drug without internalization of the particulate

Intracellular delivery of particulate-incorporated drugs can also be effected through internalization of the drug without concomitant internalization of the particulate. It is known that particulates degrade to a greater extent in the tumor interstitium compared to normal tissues or biological fluids, possibly because of the atypical conditions of the tumor microenvironment, for example acidic pH, presence of lipases, enzymes and oxidizing agents [61]. This preferential degradation results in the accelerated release of the drug, which then diffuses into tumor cells, either through passive diffusion or active transport.

Other approaches have been pursued to further improve the rate of drug release from the particulates and the transfer of the drug to the tumor cell. Research indicates that a drug diffuses more quickly from a liposome comprising a low-phase transition phospholipid component [62]. Moreover, the atypical conditions of the tumor microenvironment, and other conditions, for example, local temperature increase in inflamed organs [63,64], can be exploited to effect accelerated drug release at a specific target by synthesizing particulates that are sensitive to these microenvironmental conditions. Thus, particulates incorporating pH- or thermo-sensitive components have been synthesized to preferentially disintegrate at acidic pH or increased temperature, respectively [60,65].

Another approach involves the transfer of a prodrug from the particulate to the cell. For example, the use of MAb-targeted liposomes, which are specific for colon adenocarcinoma cells, comprising the amphiphilic dipalmitoyl derivative of 5-fluorodeoxyuridine (FUdR-dP) has been investigated [66,67]. The prodrug (FUdR-dP) was efficiently hydrolyzed intracellularly to yield 5-fluorodeoxyuridine (FUdR), the active drug. Furthermore, FUdR-dP incorporated in MAb-targeted liposomes effected a 13-fold stronger inhibition of cell growth than FUdR-dP in non-targeted liposomes. The potential advantage of this approach is to minimize systemic dispersion and, consequently, toxicity of the drug.

In vivo studies with actively targeted particulate drug carriers: a critical appraisal

Systemic toxicity of actively targeted particulate drug carriers

With the exception of cationic particulates, no significant toxic effects have been reported for the *in vivo* administration of particulate drug carriers. In the case of cationic particulates, severe adverse side-effects were observed on systemic application [68,69]. The level of toxic effects observed was found to be dependent on the particle size (most pronounced for particles of 300–400 nm diameter). Furthermore, a study on positively charged dextran derivatives [70] identified a correlation between the degree of toxic effects observed and the substitution ratio of positively charged diethylaminoethyl groups; derivatives with the highest substitution ratio led to fatal toxicities in experimental animals.

Studies with cationic particulates suggest that systemic toxic effects are a consequence of the formation of large aggregates that obstruct capillaries and/or of intravascular activation of the coagulation cascade [69,71]. However, it should be considered that the majority of studies reporting severe toxic effects on administration of cationic particulates were performed on cationic particulate–DNA complexes, and no effective controls were set up to establish whether the toxic effects were caused by the cationic particulate, the DNA component or the complex. One report identified that toxic effects observed on systemic administration of a cationic liposome and DNA formulation were the result of a synergistic effect between the two components [72]. This adverse reaction is to be expected because DNA itself induces significant proinflammatory effects that can contribute to the toxic effects of polycationic particulate–DNA complexes [73].

Therapeutic effects of actively targeted particulate drug carriers

A significant number of animal studies with actively targeted particulate drug carriers have been published over the past decade. An overview of several studies published during the past four years is shown in Table 2. Positive results were obtained in preclinical studies of various experimental tumor treatments, but active targeting performed better than other therapies tested in parallel, a result that has been affirmed by recent research [74–78]. However, it has not always proven straightforward to demonstrate this superior efficacy *in vivo*. The rapid clearance and short circulation time of particulates derivatized with whole MAbs can be overcome by using MAb fragments as ligands, thus preventing scavenging of the particles by the RES. Most studies using particulate drug carriers have investigated the treatment of solid tumors with particulates derivatized with ligands that target tumor cells, which implies that extravasation must occur for the particulates to reach their targets. In spite of the EPR effect that favors extravasation, there are opposing forces that inhibit penetration of the particulates into the tumor nodules, for example, the increasing interstitial pressure from the periphery to the center of the tumor [79]. Furthermore, the binding of actively targeted particulates to tumor cells at the periphery of a tumor nodule prevents further penetration into the interior of the tumor, a phenomenon referred to as 'binding site barrier' [80]. Based on these considerations and results obtained *in vivo* [23,43,81], it has been proposed that actively targeted particulate drug carriers are suitable only for the prevention of early metastatic spread and not for effecting regression of established tumors [82]. However, particulates that target cells circulating in the intravascular compartment are not expected to be restricted by these limitations. Indeed, a doxorubicin-containing MAb-liposome formulation targeting CD19 on lymphoma cells has demonstrated superior therapeutic efficacy [24,83]. However, there is an animal model for solid tumors in which a drug-particulate complex targeting tumor cells (anti-HER2 doxorubicin-loaded liposomes) not only demonstrated superior

Table 2. Overview of recent *in vivo* studies with actively targeted particulate drug carriers

Product	Cellular target	Therapeutic outcome	Comparison with other therapeutic approaches	Refs
Doxo in anti-GD_2 SSLs	Human neuroblastoma cell xenograft	Prevention of metastatic growth (up to 100% long-term survivors)	Better than anti-GD_2, anti-GD_2-SSLs (without drug), free doxo, doxo in in SSLs	[23]
Doxo in anti-HER2 SSLs	Human HER2 overexpressing breast cancer cell xenograft	Regression of established tumors, frequent cures	Better than free doxo, doxo in SSLs, anti-HER2	[13]
Doxo in anti-rat Neu PEG virosomes	Rat Neu-positive breast cancer cells	Inhibition of progression in established tumors; prevention of tumor formation	Better than free doxo, doxo in virosomes, anti-rat Neu virosomes (without drug)	[43]
Doxo in anti-β_1 integrin SSLs	Human lung tumor cells in SCID mice	Inhibition of progression in established tumors, prevention of metastatic spread, prolongation of MST	Better than free doxo, doxo in SSLs, anti-β_1 SSLs without doxo	[81]
Doxo in pH-sensitive anti CD19-SSLs	β-lymphoma cells in SCID mice	Prolongation of MST	Better than free doxo, doxo in non-targeted SSLs, doxo in targeted, non-pH-sensitive SSLs	[24]
Paclitaxel in cationic liposomes	Endothelial cells of melanoma blood vessels	Retardation of growth of primary tumor and metastases; retardation of growth of established tumor	Better than paclitaxel alone and cationic liposomes alone	[44,42]
DPP-CNDAC in anti-angiogenic liposomes	Endothelial cells of sarcoma blood vessels	Inhibition of tumor progression and prolongation of MST	Better than DPP-CNDAC in non-targeted liposomes	[32]

Abbreviations: doxo, doxorubicin; DPP-CNDAC, 5′-O-dipalmitoylphosphatidyl 2′-C-cyano-2′-deoxy-1-β-D-arabino-pentofuranosylcytosine; GD_2, disialoganglioside; MST, mean survival time; PEG, poly(ethylene) glycol; SCID, severe combined immunodeficiency; SSL, sterically stabilized liposomes.

therapeutic efficacy, but was also shown to eradicate established tumors in a significant proportion of animals treated [13]. The superior therapeutic efficacy of the actively targeted, compared with the passively targeted, liposomal formulation was attributed to efficient intracellular drug delivery on binding and internalization of the particulates through receptor-mediated endocytosis [84], and not higher intratumoral accumulation of the actively targeted drug carrier. Other research supports the finding that only particulates derivatized with internalizing ligands demonstrate improved therapeutic efficacy [85]. Taken together, these results suggest that active targeting does not enhance tumor localization of a particulate drug carrier when compared to passive targeting and that internalizing ligands are essential to achieve superior therapeutic effects. However, the assumption that actively targeted particulates do not achieve higher levels of intratumoral accumulation does not seem to be generally applicable. Research performed by Dagor *et al.* [37] indicated that vasoactive intestinal peptide-targeted liposomes achieve significantly higher intratumoral accumulation than non-targeted counterparts, suggesting that levels of intratumoral accumulation of actively targeted particulates are influenced by the choice of the ligand and/or by the microenvironment of the individual tumor type. It remains to be established whether non-internalizing ligands will also yield improved therapeutic efficacy compared to passively targeted particulates under conditions of higher intratumoral accumulation.

In spite of the limitations in targeting tumor cells in solid tumors, one actively targeted particulate drug carrier has entered clinical trials. MCC465 is a SSL incorporating doxorubicin that selectively targets stomach cancer cells using a $F(ab')_2$ human IgG1 MAb fragment and is currently in Phase I clinical trials [74]. MCC465 demonstrated clinical efficacy and considerably reduced side-effects relative to other therapies. Furthermore, an anti-HER2, doxorubicin-incorporating SSL formulation is about to enter clinical development [13,86].

Targeting of the endothelial cells of tumor blood vessels is another approach that is being intensively investigated. Active targeting of particulate drug carriers to these cells has the potential to effect destruction of the tumor microvasculature, which will subsequently result in tumor cell death. The limitations that typically accompany the active targeting of tumor cells do not affect this approach; tumor endothelial cells are directly accessible from the bloodstream and particles do not need to extravasate to reach their target. Thus, the efficacy of cationic liposomal

formulations that target endothelial cells with enhanced surface expression of negatively charged phosphatidylserine residues (resulting from the stress conditions in the tumor environment) is under investigation [22,87]. However, enhanced expression of negative surface charges was observed in only 15–40% of tumor blood vessels; it remains to be established whether this is sufficient to achieve efficient tumor debulking. Initial reports on this approach in animal models have been encouraging [42,44,88]. Favorable preclinical results of a cationic liposome formulation composed of 1,2-dioleoyl-3-trimethylammonium-propane, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine and the antitumor drug paclitaxel, resulted in this compound entering Phase I clinical trials for the treatment of patients with metastatic or advanced, unresectable colon cancer [42,44]. Disease control (objective response or stable disease) was observed in 13% of evaluated patients and the treatment was well-tolerated, only low levels of mild side-effects were observed.

Conclusions

The entry of actively targeted particulate drug delivery systems into clinical trials suggests that this approach could become a viable therapeutic option over the next decade. Although several hurdles to the *in vivo* application of these systems have been overcome, there are some aspects that still need clarification. Based on present knowledge, two issues stand out in terms of their practical relevance. First, the potential to achieve higher intratumoral accumulation with actively than with passively targeted particulates. Second, the practicality of achieving tumor regression in addition to preventing of tumor growth or metastatic spread. In spite of several current limitations, new findings, including the use of CPPs with restricted cell selectivity, suggest that there is the possibility of further improvements in intracellular drug delivery and, therefore, in the therapeutic efficacy of these actively targeted drug delivery devices.

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