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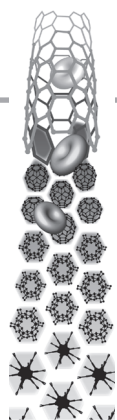
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## The rise and rise of stealth nanocarriers for cancer therapy: passive versus active targeting

Over the past few decades, interest in designing and developing nanosized drug delivery systems (also known as nanocarriers) has undergone considerable explosion. Indeed, these nanocarriers provide potential solutions to improve cancer chemotherapy by over-riding the poor biopharmaceutical properties of drugs, and by altering the pharmacokinetic and biodistribution of conventional cytotoxic molecules [1]. However, the pharmaceutical application of these systems in systemic administration is usually limited, owing to their rapid elimination from the blood circulation, resulting from a nonspecific uptake by the mononuclear phagocyte system (MPS) [2,3]. Consequently, in order to overcome the opsonization of nanocarriers, a number of widely used and effective methods have been investigated to render nanocarriers 'invisible' to the immune system, creating long-circulating nanoparticles (NPs), known as stealth NPs. Interestingly, by coating the nanoparticulate surface with poly(ethylene glycol) (PEG), referred to as a PEGylation process, NPs exhibited decreased levels of uptake by the MPS and, consequently, an increased circulation time in the blood, allowing passive targeting of the tumors [2,4]. Furthermore, surface modification of NPs with PEG moieties has emerged as a platform for the incorporation of active-targeting ligands, thereby providing the drug carriers with specific tumor-targeting properties [5].

In this article, the interest of stealth NPs will be described. Nanocarriers that will be considered include liposomes (vesicles in which an aqueous

volume is entirely surrounded by a bilayer phospholipid membrane), micelles (self-assembly of amphiphiles that form supramolecular core-shell structures in the aqueous environment), polymer NPs (including nanospheres and nanocapsules) and lipid NPs (a biocompatible lipid core that is present under solid matrix [i.e., solid lipid NPs], or a li<sup>4</sup>

further growth and enlargement, tumors need to form new blood vessels, via the angiogenesis process, in order to obtain nutrients and sustain their growth [4]. Tumor vessels are generally abnormal, and have aberrant branching, blind loops of twisted shape, which are characterized by architectural defectiveness, and a high degree of vascular density [10]. When compared with normal vessels, tumor vessels are 'leaky', owing to basement membrane abnormalities and a decreased number of pericytes lining the rapidly proliferating endothelial cells [11]. This results in an enhanced permeability for molecule passage through the microvascular wall into the interstitium surrounding tumor cells [12]. Indeed, the pore size of tumor vessels varies from 100 nm to almost 1  $\mu\text{m}$  in diameter, depending upon the anatomic location of the tumors (e.g., smaller in cranial tumors compared with subcutaneous tumors) and the tumor growth (e.g., smaller in regressing tumors) [13]. In comparison, the tight endothelial junctions of normal vessels are typically of 5–10 nm in size [13].

Moreover, solid tumors are also characterized by an impaired lymphatic network [4]. Lymphatic vessels are widely distributed throughout the body, whose major function is to return the interstitial fluid to the blood circulation. Normally, macromolecules are cleared from tissues via the lymphatic system. However, owing to the lack of lymphatic drainage, the

clearance of macromolecules from solid tumors is eventually decreased and, consequently, they can have extended retention times in the tumor interstitium [14]. This, together with leaky tumor blood vessels, results in the enhanced permeability and retention (EPR) effect [4]. Therefore, passive cancer targeting is obtained through the accumulation of long-circulating nanocarriers, at a higher concentration in the tumors than in the plasma or in other tissues (FIGURE 2).

To achieve selective tumor accumulation via the EPR effect, NPs must reside for long enough in the bloodstream to provide a sufficient level of target accumulation. Unfortunately, following intravenous administration, conventional nanocarriers (first generation) have short circulation half-lives, resulting from their opsonization and interaction with the MPS. The opsonization process consists of the adsorption of protein entities, which interact with specific plasma membrane receptors on monocytes and various subsets of tissue macrophages, thus promoting particle recognition and elimination from the systemic circulation by MPS cells, mainly Kupffer cells of the liver and macrophages of the spleen [2]. This premature elimination prevents first-generation nanocarriers from reaching tumor sites by the EPR effect. Consequently, particulate surfaces must be modified for the purpose of evading the opsonization process, and the uptake by MPS cells [15]. Thus, second generation of long-circulating nanocarriers is based on the physico

discussed by Vonarbourg *et al.* [16]. Briefly, preferentially NPs should be small (<200 nm), composed of biocompatible compounds and present a neutral and hydrophilic surface, with a dense and flexible coating of a polymer or polysaccharide [16]. As shown in TABLE 1, the technology of PEGylation has been successfully applied to various types of NPs. The pioneering publications on PEG liposomes were published at the end of the 1980s by Allen *et al.* [21] and the cell-specific targeting of liposomes even before, in 1980 [22,23].

In this article, our discussions will be limited to the NPs whose surfaces have been modified with PEG, the most widely used material for surface modification because it is nontoxic, non-immunogenic and approved by the US FDA for oral and parenteral applications in humans.

#### ■ Anticancer drugs loaded in stealth NPs: from animal studies to clinical trials in humans

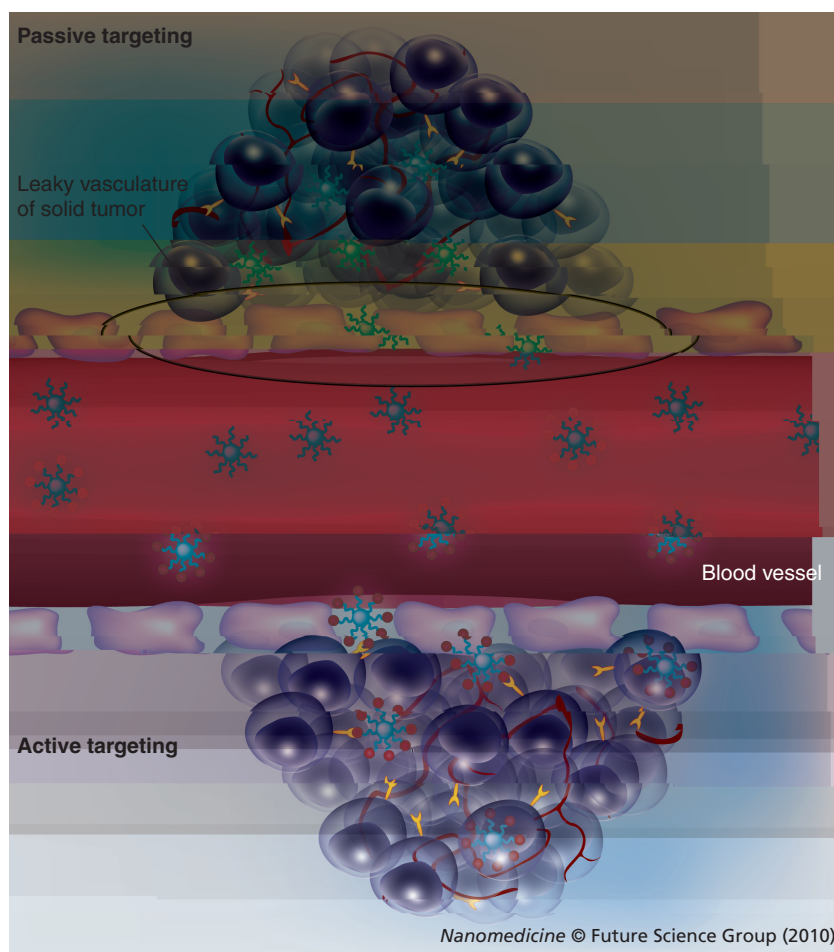
According to the properties of anticancer drugs, the sites of interest, and the scientific and economic constraints (e.g., patents, cost of materials and final products, and scaling-up of the production processes), appropriate NPs have to be optimized. Many *in vivo* studies of stealth NPs for cancer therapy have been performed in this way, and are summarized in TABLE 2. Among them, several formulations of drug-loaded stealth NPs were approved by the FDA for clinical studies, but few of them are currently on the market (TABLE 3). The following chapter focuses on some of the clinical studies using PEGylated NPs loaded with various anticancer agents, classified by function of their drug family. Moreover, passive and active targeting strategies were grouped in TABLES 2 & 3 in order to facilitate their comparison.

#### Taxanes

One of the most successful cancer drugs, paclitaxel (PTX), has shown its potency against a broad spectrum of cancers, including breast, ovarian, and small-cell and non-small-cell lung cancers [24]. Another used taxane anticancer agent is docetaxel (DTX). Owing to their high hydrophobicity (aqueous solubility is 0.7 and 6–7 µg/ml for PTX and DTX, respectively), special formulations were prepared for intravenous administration. PTX formulations (e.g., Taxol<sup>®</sup> and Paxen<sup>®</sup>) consist of a mixture of absolute alcohol and Cremophor EL<sup>®</sup>; DTX (Taxotere<sup>®</sup>) is present as a concentrated viscous solution in polysorbate 80 (Tween<sup>®</sup> 80). However,

toxicological side effects of these current commercial formulations of taxanes were observed, owing to the presence of these excipients [25,26]. Consequently, the clinical use of both formulations is recommended, in association with premedication with corticosteroids and H1/H2 antihistamines (for PTX) to minimize the side effects [24].

Therefore, many strategies to design alternative formulations, for both PTX and DTX, have been under investigation. Among potential approaches to achieve a desired formulation by various methods (e.g., cosolvency, emulsification, micellization, local drug delivery devices [e.g., pastes and implants]) [27], nanotechnology offers a promising solution for chemotherapy with taxanes. Recently, Abraxane<sup>®</sup>, an albumin-bound NP formulation of PTX, was granted FDA approval in January 2005. Such drug-conjugated albumin formulations are also considered as an attractive approach for selective drug targeting by improving the pharmacokinetic profile of anticancer agents, owing to the long



**Figure 2. Passive targeting and active targeting for drug delivery to solid tumors.**

Table 1. Various coatings used for the preparation of stealth nanoparticles.

Group	Coating	Type of NP	Ref.
PEG	PEG		[137]
PEG–lipid conjugates	DSPE–PEG	Liposomes	[51,60,61,138–141]
		LNCs	[6,142,143]
	DPPE–PEG	Liposomes	[144]
		SLNs	[145]
Acid stearic PEG	SLNs	[91,145–147]	
	LNCs	[148]	
Block copolymers	PEG–PLA	Polymer NPs	[149–153]
		Micelles	[30,154]
	PEG–PCL (poly- $\epsilon$ -caprolactone)	Polymer NPs	[155]
	PEG–PLGA	Polymer NPs	[155–162]
	PEG–poly(hexadecylcyanoacrylate)	Polymer NPs	[163–168]
	PCL–PEG–PCL	Polymer NPs	[169–171]
	PLA–PEG–PLA	Polymer NPs	[172–174]
	PEG–PLA–PEG	Polymer NPs	[78,152,174]
Other polymers	Poloxamer®/Poloxamine®	Polymer micelles	[44]
		Polymer NPs	[150,151,175]
	Poly(amino acid)		[88,176]
Polysaccharides	Oxazoline-derived polymers		[177]
	Polyvinyl pyrrolidone		[178,179]
	Chitosan		[77,180,181]
Polysaccharides	Dextran		[72,182,183]
	Heparin		[72,181,183]

DPPE: Dipalmitoyl-phosphatidylethanol-amine; DSPE: Distearoylphosphatidylethanolamine; LNC: Lipid nanocapsules; NP: Nanoparticle; PCL: Polycaprolactone; PEG: Polyethylene glycol; PLA: Polylactic acid; SLN: Solid lipid nanoparticles.

half-life of albumin in the body [28,29]. However, we do not discuss this in detail because their surfaces were not coated with PEG to confer the stealth properties.

Another example of a Cremophor-free formulation of PTX is Genexol®-PM, a polymer micellar system prepared with methoxy-PEG–poly(lactide) (mPEG–PLA)-block copolymers. *In vivo* studies on mice, following intravenous injection, demonstrated considerable accumulation of Genexol-PM in subcutaneously implanted B16 melanoma tumor, and a significant antitumor effect against SKOV-3 human ovarian cancer and MX-1 human breast cancer when compared with Taxol [30]. In a Phase I study, in which patients received Genexol-PM intravenously without premedication for 3 h once every 3 weeks, the maximum tolerated dosage (MTD) of 390 mg/m<sup>2</sup> was obtained [31]. The main dose-limiting toxicities of Genexol-PM were neuropathy, myalgia and neutropenia, with an absence of acute hypersensitivity reactions. The Phase II trials showed that Genexol-PM was tolerated and effective in patients with advanced pancreatic cancer [32], metastatic breast cancer [33], and also in patients with advanced non-small-cell cancer who were administered a combination of Genexol-PM

and cisplatin [34]. Genexol-PM is now approved in South Korea [35], and is undergoing Phase III and IV trials in patients with recurrent or metastatic breast cancer [301].

#### Anthracyclines: doxorubicin/daunorubicin

Anthracyclines, including doxorubicin (DOX) and daunorubicin, are among the most active agents available for the treatment of breast cancer, and are a key component of adjuvant therapy regimens. However, the clinical use of these agents is always limited, particularly in patients receiving adjuvant treatment with anthracyclines during the early phases of breast cancer, owing to their cumulative dose-related cardiotoxicity [36]. Therefore, it is necessary to exploit drug delivery systems to confer altered biodistribution and drug-release properties to minimize cardiotoxicity and maximize drug delivery to tumor tissues.

One striking success of DOX-loaded stealth NPs is the case of Doxil® (in the USA) or Caelyx® (in Europe) in 1995, which was the first nanocarrier approved by the US FDA and the European Medicines Agency (EMA) for cancer therapeutics. This is a DOX-containing formulation based on stealth liposomes, which,

Table 2. Preclinical studies using stealth nanoparticles in cancer chemotherapy.

Stealth NPs	Targeting type	Animal	Findings	Ref.
<b>Paclitaxel</b>				
DSPE-mPEG2000-liposomes	Passive	Rat, nude mouse	$T_{1/2\beta} = 17.8$ vs 5.05 h for uncoated liposomes, and 1.65 h for Taxol® in rats. Enhanced antitumor effect due to high accumulation in MDA-MB-231 human breast cancer bearing nude mice. High PTX payload with an efficiency level up to 70%	[139]
Anti-HER2- $S_{100}$ PC:CHOL:mPEG2000-DSPE:Mal-PEG2000-DSPE liposomes	Active	Nude mouse	Higher ratio of tumor to plasma drug concentrations (T/P ratio) than nontargeted liposomes in HER2-overexpressing breast carcinoma model (BT-474) linked to a higher antitumor efficacy. Weak T/P ratio in low HER2-expressing model (MDA-MB-231)	[184]
DPPE-mPEG5000 liposomes	Passive	Mouse	Long circulation time ( $T_{1/2\beta} = 48.6$ h vs 9.27 h for uncoated liposomes). Considerable decrease in drug uptake in MPS-containing organs (liver and spleen) at 0.5 and 3 h after injection as compared with the conventional liposomes	[144]
mPEG-PLA NPs	Passive	Rat	Higher half-life ( $18.80 \pm 3.14$ h) and AUC than Taxol (2.75- and 3.09-times, respectively) at the same dose of 10 mg/kg. Drug encapsulation efficiency up to 87%	[153]
PLA-PEG-RGD micelles	Active	Nude mouse	Higher tumor accumulation than nontargeted micelles in a model of MDA-MB-435 breast tumor linked to a tumor regression with PTX-targeted micelles	[185]
PEG-PLGA NPs	Passive	Mouse	Remarkable tumor growth inhibition and increased survival rate of transplantable liver tumor-bearing mice compared with Taxol	[160]
<b>Docetaxel</b>				
PEG-PLGA NPs	Passive	Mouse	Drug biological half-life increased and tumor accumulation five-times higher than Taxotere® on C26 tumor-bearing mice	[161]
DSPE-mPEG2000 LNCs	Passive	Mouse	Long circulation time (half-life: 1.4 vs 0.3 h for Taxotere)	[143]
<b>Doxorubicin</b>				
Anti-HER2-DSPE-COOH-PEG2000 liposomes	Active	Rat, nude mouse	Long circulation time of immunoliposomes and PEGylated liposomes in normal rats. Increased antitumor efficacy and reduced systemic toxicity with DOX anti-HER2 immunoliposomes. Similar levels of tumor tissue accumulation in a nude mouse model of HER2-overexpressing breast cancer (BT-474) for both targeted and nontargeted liposomes	[106,107]
DSPE-PEG-maleimide-RGD liposomes	Active	Mouse	Specific interaction with tumor vasculature (intravital microscopy), not observed for nontargeted PEGylated liposomes. Inhibition of tumor growth in a doxorubicin-insensitive murine C26 colon carcinoma model by DOX RGD-immunoliposomes. No growth deceleration with DOX nontargeted liposomes	[121]
SPC:CHOL:DSPE-PEG:DSPE-PEG-RGD liposomes	Active	Nude mouse	Similar drug accumulation in tumors in a model of murine B16 melanoma between targeted and nontargeted liposomes. Higher antitumor effect for DOX immunoliposomes than nontargeted liposomes	[186,187]
Folate-targeted PEGylated liposomes	Active	Mouse	Similar tumor accumulation between targeted and nontargeted liposomes in BALB/c mice with high folate receptor-expressing tumors (mouse M109, human KB carcinomas)	[188]
mAb 2C5-modified PEGylated liposomes (mAb 2C5-modified Doxil®)	Active	Nude mouse	Significant therapeutic benefit showing substantial decrease of tumor size and doubling of survival time provided by 2C5-immunoliposomes in intracranial U-87 MG brain tumors in nude mice over control formulations	[103]
PEG-PLGA NPs	Passive	Mouse	Remaining 40% injected dose in the serum of BALB/c mice 24 h postinjection. Decrease in cardiotoxicity	[156]
PCL-PEG-PCL NPs	Passive	Mouse	Significant antitumor effect on a subcutaneous C-26 tumor	[171]
Folate-PLGA-PEG micelles	Active	Nude mouse	Higher drug concentration in tumor and higher antitumor effect than nontargeted micelles in a model of KB human squamous cell carcinoma	[189]
<p>AUC: Area under the concentration-time curve; DOX: Doxorubicin; DPPE: Dipalmitoyl-phosphatidylethanol-amine; DSPE: Distearoylphosphatidylethanolamine; mAb: Monoclonal antibody; mPEG: Methoxy polyethylene glycol; MPS: Mononuclear phagocyte system; NP: Nanoparticle; PCL-LA-PEG-PCL-LA: Poly(caprolactone-co-lactide)-b-PEG-b-poly(caprolactone-co-lactide); PLA: Poly(lactide); PLGA: Poly(lactic-co-glycolic acid); PTX: paclitaxel; RGD: Arginine-glycine-aspartate; <math>S_{100}</math>PC:CHOL:mPEG2000-DSPE:Mal-PEG2000-DSPE: Soybean phosphatidylcholine:cholesterol:1,2-distearoyl-sn-glycero-3-phospho-ethanolamine [methoxy(polyethyleneglycol)-2000]:maleimide-derivatized PEG2000-DSPE.</p>				

Table 2. Preclinical studies using stealth nanoparticles in cancer chemotherapy.

Stealth NPs	Targeting type	Animal	Findings	Ref.
<b>Doxorubicin (cont.)</b>				
Stearic acid-PEG2000 SLNs	Passive	Rat, rabbit	Higher half-life time and plasma AUC, decrease of the DOX heart concentration compared with free-drug commercial solution. Highest concentration of DOX in the blood obtained with the highest concentration of stealth agent	[91,146]
Glycol chitosan NPs	Passive	Mouse	Amount of NPs gradually increased in tumor as blood circulation time increased. Concentration in blood 14% of dose at 1 day, and 8% 3 days after intravenous injection. DOX-glycol chitosan NPs exhibited lower toxicity but comparable antitumor activity to free DOX	[190]
<b>Cisplatin</b>				
mPEG-PLGA NPs	Passive	Mouse	Decrease of tumor volume and higher survival rate in HT-29 tumor-bearing SCID mice than free drug, and good tolerance by normal BALB/c mice	[155]
<b>Camptothecin analogs</b>				
10-Hydroxycamptothecin-loaded PCL-LA-PEG-PCL-LA NPs	Passive	Mouse	Superior antitumor effect and extended retention time in the blood of S-180 (murine sarcoma)-bearing mice (>22 ng/ml detected at 24 h postinjection, whereas free drug injection was undetectable)	[169,170]
Topotecan loaded anti-HER2 and anti-EGF receptor liposomes	Active	Mouse	Improved antitumor activity against HER2-overexpressing human breast cancer (BT474) xenografts	[191]
AUC: Area under the concentration–time curve; DOX: Doxorubicin; DPPE: Dipalmitoyl-phosphatidylethanol-amine; DSPE: Distearoylphosphatidylethanolamine; mAb: Monoclonal antibody; mPEG: Methoxy polyethylene glycol; MPS: Mononuclear phagocyte system; NP: Nanoparticle; PCL-LA-PEG-PCL-LA: Poly(caprolactone-co-lactide)-b-PEG-b-poly(caprolactone-co-lactide); PLA: Poly(lactide); PLGA: Poly(lactic-co-glycolic acid); PTX: paclitaxel; RGD: Arginine-glycine-aspartate; S <sub>100</sub> PC:CHOL:mPEG2000-DSPE:Mal-PEG2000-DSPE: Soybean phosphatidylcholine:cholesterol:1,2-distearoyl-sn-glycero-3-phospho-ethanolamine [methoxy(polyethyleneglycol)-2000]:maleimide-derivatized PEG2000-DSPE.				

consequently, has been approved for AIDS-related Kaposi's sarcoma, and is now commercially available for the treatment of metastatic breast cancer, advanced ovarian cancer and multiple myeloma [37,38]. Preclinical and clinical studies have shown that Doxil preferentially accumulated in tumor xenografts and human tumors, resulting in an enhancement of drug concentration in the tumor compared with free DOX [39]. The plasma area under the concentration–time curve (AUC) was at least 60-fold higher in animals, and approximately 300-fold higher in humans, than free DOX. Doxil toxicity is characterized by dominant and dose-limiting mucocutaneous toxicities (stomatitis, hand-foot syndrome or palmar-plantar erythrodysesthesia [40]), mild myelosuppression and minimal alopecia, but no apparent cardiac toxicity [41]. The reduced cardiotoxicity allows a larger cumulative dose than that acceptable for free DOX. Therefore, Doxil can be effectively and safely substituted for conventional DOX, allowing retreatment with an anthracycline in the metastatic setting [42].

Other nanoparticulate systems have also been investigated to encapsulate DOX. SP1049C, a micellar formulation containing DOX and two nonionic block copolymers, Pluronic® L-61 and

Pluronic® F-127, can be used for the treatment of primary and relapsed tumors. First, in preclinical studies, SP1049C (micellar DOX) demonstrated higher antitumor activity than free DOX in DOX-resistant tumors and in multidrug-resistance (MDR) cells [43,44]. The authors explained this higher activity of micellar DOX, in comparison with free DOX, by mechanistic hypotheses: the micellar formulation increases the drug uptake; Pluronic L-61 (one of the two copolymers used in the formulation) inhibits P-glycoprotein efflux, which normally limits the free-drug uptake and, consequently, micellar formulation increases the drug uptake; and, finally, the micellar formulation changes the intracellular drug trafficking. Then, in the Phase I study, the pharmacokinetic profile of SP1049C showed a slower clearance than free DOX [45]. The principal dose-limiting toxicity was neutropenia, and the MTD was 70 mg/m<sup>2</sup>, whereas the MTD of free DOX was 50 mg/m<sup>2</sup> [46]. Moreover, recent results from a Phase II study in patients with advanced adenocarcinoma of the esophagus and gastro-esophageal junction, demonstrated that SP1049C has a notable single-agent activity. The objective response was 47% for the evaluate patients with the micellar formulation, whereas it was 43% in the

Table 3. Examples of anticancer drugs loaded in stealth nanocarriers in clinical trials or approved for cancer therapy.

Compound	Formulation (carrier/size)	Company	Indication	Targeting strategy	Route of administration	Status	Ref.
<b>Taxanes</b>							
Paclitaxel	Genexol®-PM: polymer micelles mPEG-PLA; 20–50 nm	Samyang, Daejeon City, Korea	Advanced pancreatic cancer, metastatic breast cancer	Passive	Intravenous	Approved in South Korea, Phase III/IV	[31–35,301]
	Nanoxel®: polymer micelles poly(vinyl pyrrolidone)-b-poly( <i>N</i> -isopropyl acrylamide); 80–100 nm	Fresenius Kabi Oncology Ltd, Haryana, India	Advanced breast cancer	Passive	Intravenous	Approved in India Phase I	[192,193]
	NK105 polymer micelles; 85 nm	Nippon Kayaku Co.,Ltd, Tokyo, Japan	Colon and gastric cancer	Passive	Intravenous infusion	Phase I	[194]
Docetaxel	Docetaxel-polymer nanoparticles; 20–50 nm	Samyang, Daejeon City, Korea	Advanced solid malignancies	Passive	Intravenous	Phase I	[195,302]
<b>Anthracyclines and analogs</b>							
Doxorubicin	Doxil®/Caelyx®: PEGylated liposomes; 80–90 nm	Ortho Biotech, PA, USA; Schering-Plough, QC, Canada	Kaposi's sarcoma, refractory breast cancer and refractory ovarian cancer	Passive	Intravenous/intramuscular	Approved	[196,197]
	SP1049C: polymer micelles Pluronic® block copolymers; 22–27 nm	Supratek Pharma, QC, Canada	Esophageal carcinoma	Passive	Intravenous	Phase II/III	[45,47]
	NK911: polymer micelles from a copolymer of PEG and DOX-conjugated poly(aspartic acid); 40 nm	Supralife International, CA, USA	Solid tumors	Passive	Intravenous infusion	Phase I	[198]
	MCC465: PEGylated antibody-liposomes; 143 nm	Mitsubishi Pharma Corporation, Osaka, Japan	Metastatic stomach and colorectal cancer	Active	Intravenous infusion	Phase I	[199,200]
Mitoxantrone (anthracenedione)	ΔpH MTO-liposomes: PEGylated liposomes; 123 nm	University Hospital of Zurich, Switzerland	Advanced breast cancer	Passive	Intravenous	Phase I	[201]
<b>Metal-based drugs</b>							
Cisplatin	SPI-077®: mPEG liposomes; 110 nm	Alza Corporation, CA, USA	Head and neck cancer, lung cancer	Passive	Intravenous	Phase II	[52–58,202]
	Lipoplatin®: PEGylated liposomes; 110 nm	Regulon Inc., CA, USA	Multiple cancer indications	Passive	Intravenous	Phase II/III	[203,204]
	Nanoplatin® (NC-6004): polymer micelles; 30 nm	NanoCarrier Co., Ltd, Kashiwa City, Japan	Multiple cancer indications	Passive	Intravenous infusion	Phase I/II	[205]
Oxaliplatin	MBP-426: Transferrin-liposomes; 100 nm	Mebiopharm Co., Ltd, Kanagawa, Japan	Advanced or metastatic solid tumors, colorectal, gastric and esophageal cancer	Active	Intravenous infusion	Phase II	[303]
<b>Camptothecin analogs</b>							
CKD602	S-CKD602: PEGylated liposome; 100 nm	Alza Corporation, CA, USA	Multiple cancer indications (advanced solid tumors)	Passive	Intravenous	Phase I/II	[62,206]
SN38	NK012: polymer micelles PEG–poly(glutamic acid) block copolymer; 20 nm	Nippon Kayaku Co.,Ltd, Tokyo, Japan	Triple-negative breast cancer and colorectal cancer	Passive	Intravenous	Phase II	[207,208]

DOX: Doxorubicin; mPEG: Methoxy-polyethylene glycol; PEG: Polyethylene glycol; PLA: Poly(lactide).



intent-to-treat population. The overall median survival and the progression-free survival were 10.0 and 6.6 months, respectively [47], while median survival of free DOX was not available. Nevertheless, with conventional therapy, which combines 5-fluorouracil with cisplatin, the median survival was 7 months, with epirubicin, cisplatin and 5-fluorouracil (ECF), the median survival was 8.9 months [48], and with 5-fluorouracil, doxorubicin and methotrexate, the median survival was 5.7 months [49].

### Metal-based drugs

Cisplatin is one of the leading metal-based drugs used for the treatment of many malignancies. However, its expansion in clinical application is limited, owing to significant side effects, such as acute nephrotoxicity and chronic neurotoxicity [50]. Therefore, extensive efforts have been devoted to the specific delivery of cisplatin to tumor tissues. SPI-077 is a formulation of cisplatin, which is encapsulated in mPEG liposomes for the purpose of specific drug delivery and decreased cisplatin-related toxicity. Preclinical studies in mice demonstrated that SPI-077 exhibited increased efficacy against Lewis carcinomas and C26 colon carcinomas in BALB/c mice, and was well tolerated with high cumulative doses [51]. Unfortunately, results from different Phase I and II studies of SPI-077 demonstrated the lack of efficacy of this formulation, since no response rates, or only modest response rates, were observed in patients with non-small-cell lung cancer or advanced head and neck cancer [52–58].

### Camptothecin analogs

A camptothecin analog, 7-[2-(*N*-isopropylamino)ethyl]-(2*S*)-camptothecin, or CKD-602, was synthesized, and proven to be more potent than topotecan in 14 out of 26 human cancer cell lines tested [59]. CKD-602-loaded stealth liposomes (S-CKD-602) demonstrated prolonged circulation time in the plasma, resulting in threefold longer drug exposure in tumors, and improved antitumor efficacy compared with free drugs in nude mice bearing human tumor xenografts [60,61]. Recently, this stealth liposomal formulation of CKD-602 has entered a Phase I study in patients with advanced malignancies [62]. It was well tolerated, with a MTD of 2.1 mg/m<sup>2</sup>. The overall incidence of grade 3 or 4 toxicity was comparable to other camptothecins. Therefore, the Phase II studies of S-CKD-602 at a dose of 2.1 mg/m<sup>2</sup>, administered intravenously once every 3 weeks, have been planned [62].

### From passive to active targeting

#### ■ Drawbacks of passive targeting

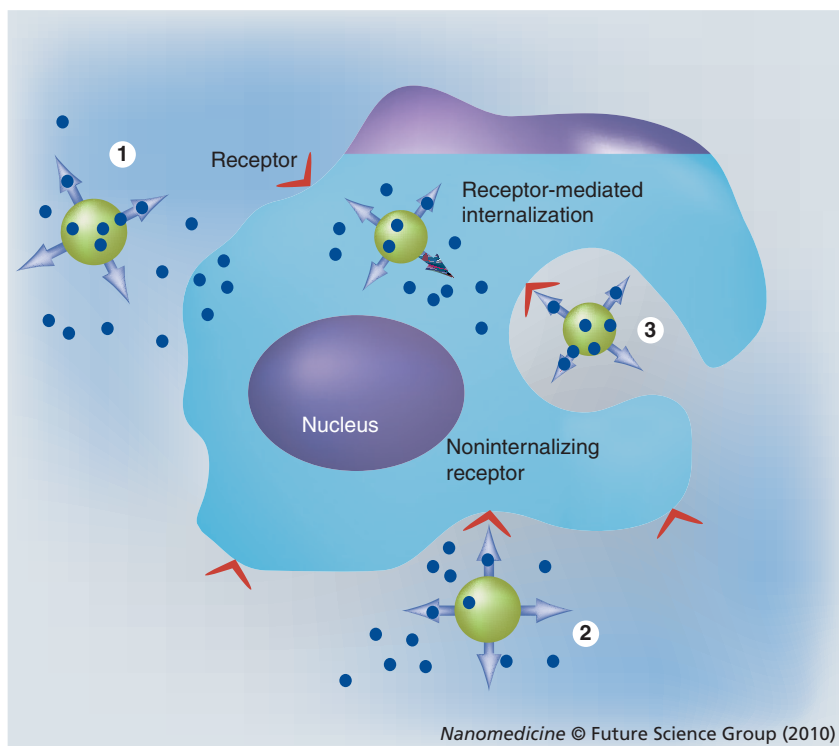
Most stealth NPs are expected to accumulate in the tumor site owing to the passive targeting. This passive targeting is, hence, highly dependent on the degree of tumor vascularization and angiogenesis [63,64]. Unfortunately, certain

be nontoxic, and readily excreted by the kidneys, it is a nonbiodegradable polymer. It cannot be excluded that, after cellular uptake, PEG affects cell functioning at the long-term follow-up after internalization in the macrophages [73–75], as well as in liver, spleen, kidney and lung cells, where a considerable fraction of NPs can be found [76–78]. For example, some polymers may elicit immune response or allergic reactions, which are more likely following subsequent injections, although they may be controlled by corticosteroids and antihistamines. There were previous reports on the so-called ‘macromolecular syndrome’ or, more lately, on the infusion-related reaction, which is observed at first usage, even with antibody drugs or liposomal drugs, and is not observed in the second administration [64].

On the other hand, Ishida *et al.* reported that PEGylated liposomes lost their long-circulating property when they were administered twice in the same animal, and accumulated extensively in the liver [79]. This is referred to as the ‘accelerated blood clearance’ (ABC), the mechanism of which is related to the abundant production of anti-PEG IgM from the spleen in response to the first injection of PEGylated liposomes [80]. The IgM can selectively bind to the PEG chains on a second injection, administered several days later, and subsequently activates the complement system and enhances uptake of the second PEGylated liposome injection by the Kupffer cells [81,82]. Consequently, this ABC phenomenon may decrease the therapeutic effect of the loaded drug, and potentiate the adverse effects or even induce the morbidity [83,84]. This was also reported with other PEGylated nanocarriers, for example PEG–PLA NPs [85,86] and polymer micelles [87]. Therefore, further evidence is required to elucidate whether the ABC mechanism is induced by all kinds of PEGylated NPs, and if it can occur in humans. Recently, it was observed that the ABC phenomenon could be prevented by changing the properties of the PEG lipid derivatives or by replacing the coating by other polymers (i.e., poly[amino acid]) [88]. Judge *et al.* [89] and Semple *et al.* [83] demonstrated the lack of this phenomenon by using PEG-conjugated ceramides (PEG–Cer), with a small C14 lipid anchor in modified liposomes. Xu *et al.* demonstrated that the use of other cleavable PEG–lipid derivatives could also prevent ABC [90]. Indeed, repeated injection of conventional distearoylphosphatidylethanolamine (DSPE) PEG liposomes resulted in the enhanced elimination of liposomes, accompanied by

increased accumulation in the liver. By contrast, the pharmacokinetic and biodistribution profiles after two injections of cholesteryl hemisuccinate (CHEMS) PEG liposomes were comparable to a single injection.

Finally, modifying the physicochemical properties of the NP surface, in order to render them as stealth NPs, in parallel can bring important drawbacks. For example, increasing the concentration of PEG is known to influence the surface polymer conformation and reduce the zeta potential of the nanocarriers in an interesting manner, but also increases their average diameter [6,91], which can negatively influence the biodistribution of NPs [16]. More specifically, it can also limit cellular uptake by the endocytotic route [92]. As mentioned, a high density of PEG on the NP surface is necessary to improve *in vivo* blood circulation of the NPs, but the abundant presence of PEG may hinder the binding and uptake of nanocarriers by cancer cells by preventing nanocarrier/tumor cell contact [93]. Indeed, although PEGylated NPs frequently show superior *in vivo* antitumor activity, they are generally less cytotoxic *in vitro* than conventional NPs [94,95], owing to lack of interactions. Moreover, since most cytotoxic anticancer agents act intracellularly, the release of entrapped drugs from NPs must occur inside the tumor cells. Rapid leakage of the drug payload in the blood circulation may lead to a decrease in drug efficacy and potentiate an undesirable effect. Once they have arrived at the target tumor site, nanocarriers can release their content in close proximity to the target cells (i.e., in the case of passive targeting), attach to the membrane of the cell via nonspecific receptors and act as an extracellular sustained-release drug depot, or internalize into the cell before the drug is released owing to specific receptor-mediated internalization (FIGURE 3). When biopharmaceutical agents are not membrane permeable, such as proteins, peptides or nucleic acids, the extracellular release of the drug is not expected, and it is required that carriers are internalized to the target tumor cell in order to deliver the encapsulated drug to the appropriate location inside the cell. Therefore, the enhanced intracellular delivery by using PEGylated NPs without loss of drug activity (i.e., the improved bioavailability of drug), is a key step in drug delivery, which still remains a scientific challenge. As a consequence, in order to increase tumor localization, as well as tumor cell internalization, third-generation nanocarriers based on specific targeting have been developed.



**Figure 3. Mechanism of the drug release when reaching the tumor site (1–3).**

#### ■ Active ligand-conjugated stealth NPs

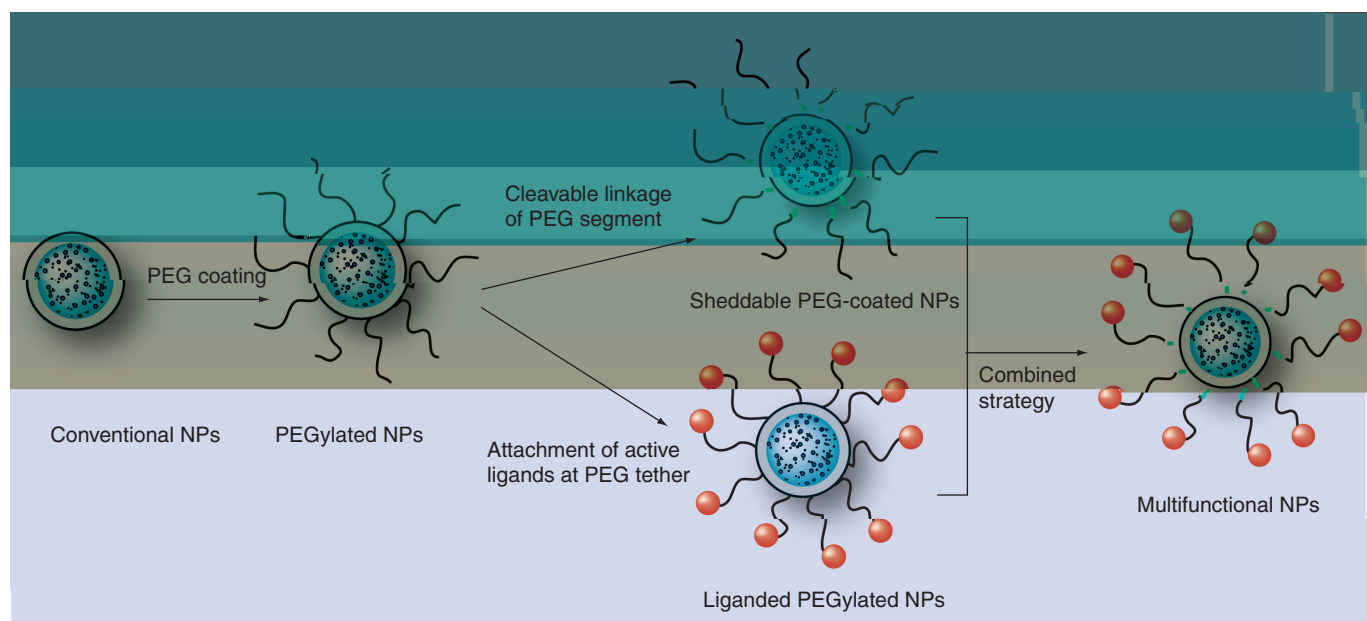
Active targeting involves the use of peripherally conjugated targeting moiety (or ligand) for enhanced selective cellular binding due to specific interactions with receptors overexpressed on tumor cells. In some cases, it can be followed by the internalization of NPs through receptor-mediated endocytosis [96]. The targeting moieties are generally attached onto the surface of the colloidal particles, preferably at the end of the PEG molecules, since the targeted colloidal particles will be much more efficient if they are also sterically stabilized and, of course, considered stealth NPs [97]. Various targeting moieties have been employed for drug delivery systems, for example antibody fragments, peptides, sequences identified by phage display, small molecules or aptamers (FIGURE 4) [98,99]. Detailed descriptions of some active targeted NPs are depicted to illustrate the description of the ligands below.

#### ■ Antibodies

Monoclonal antibodies (mAbs) or whole mAb fragments, including Fab' and single-chain Fv (scFv) fragments, have been widely used as ligands to target receptor-expressing cancer cells [100]. For example, an *in vitro* study by Kocbek *et al.* demonstrated that poly(lactico-glycolic acid) (PLGA) NPs conjugated

with mAbs were localized directly into MCF-10A neoT cells, whereas noncoated NPs were distributed randomly [101]. Elsewhere, mAb 2C5-modified PEGylated liposomes, demonstrated three- to eight-fold increase in binding and internalization by various cancer cell lines of diverse origins, higher cytotoxicity towards various cancer cells and a significant therapeutic benefit over control (liposomes modified with a nonspecific IgG) [102,103]. Nevertheless, antibody fragments Fab' and scFv, rather than intact mAbs, were frequently used to minimize immunogenicity, and to circumvent clearance via Fc receptor-mediated mechanisms [98,104]. Kou *et al.* developed PLGA NPs coated with SM5-1 monoclonal antibody (scFv), which enhanced *in vitro* cytotoxicity against human hepatocellular carcinoma cell lines, and significantly inhibited tumor development and induced tumor regression [105]. Herceptin® is another therapeutic antibody targeting human EGF receptor-2 (HER2), which is overexpressed on breast cancer cell surfaces [100]. Studies using anti-HER2 immunoliposomes, by conjugating anti-HER2 antibody fragments to PEGylated liposomes, demonstrated that anti-HER2 immunoliposomes achieved intracellular drug delivery via mAb-mediated endocytosis, whereas nontargeted liposomes were predominantly found in extracellular stroma or within macrophages [106]. DOX-loaded anti-HER2 immunoliposomes also showed significant antitumor effect compared with nontargeted liposomes [107]. However, although the authors demonstrated a high uptake of these immunoliposomes, the role of the active targeting is questionable. Indeed, in the same paper [108], the authors showed similar high levels of tumor tissue accumulation in HER2-overexpressing breast cancer xenografts (BT-474) between anti-HER2 immunoliposomes and nontargeted liposomes. Thus, in this case, targeted nanocarriers did not augment the tumor accumulation, as compared with nontargeted ones. The first explanation on the lack of accumulation is the type of model used in this paper. The study was performed with tumor cells, and not with tumor endothelial cells, which are the target. Another explanation is the high density of ligands on the NP surface. Indeed, the presence at a high density of active ligands can prevent the long-circulating properties of PEG, and lead to an accelerated elimination of NPs from the blood circulation [108]. Moreover, in order to use antibodies as a targeting moiety, different factors should be taken into consideration [96,109–112]:

- The method of conjugation to incorporate antibodies to nanocarriers (i.e., conjugation could modify the activity of the antibody and its receptor affinity);
- The consequence of circulating free antibody;
- The immunogenicity of these entities, since most of them are derived from animals and



**Figure 5. Different strategies for surface modifications from the conventional nanoparticles.**

NP: Nanoparticle; PEG: Poly(ethylene glycol).

in many cancer cells that require folic acid for their rapid proliferation [124]. Folate-targeted liposomes have been shown to be effective carriers for intracellular delivery of nucleic acids and anticancer drugs to *in vitro* tumor cells [124]. Similarly, cell surface membrane lectins are also overexpressed at the surface of numerous cancer cells and able to specifically internalize sugar molecules via receptor-mediated endocytosis mechanisms, such as the mannose or fructose receptor of macrophages, and the asialoglycoprotein receptors of hepatocytes [125,126].

In summary, it is noteworthy that active targeting using stealth NPs functionalized with ligands that have high-affinity levels and high specificity promises to be a powerful approach, leading to effective drug delivery into tumor cells for the treatment of cancer.

### Conclusion & future perspective

After intravenous administration, pharmacological activity of anticancer drugs is limited by various physiological barriers. The first barrier met by nanocarriers is their rapid elimination from the blood circulation, resulting from a nonspecific uptake by the MPS. Then, prior to reaching tumor cells, the second barrier is their extravasation out of the blood vessel. Finally, they need to bind and release the entrapped drug, generally inside the tumor cells.

To overcome the rapid elimination from systemic circulation, stealth NPs were developed, and already show promising results (e.g., commercial formulations and new clinical trials in

process). When stealth, both targeted and non-targeted NPs are generally able to arrive at the tumor vicinity via the EPR effect. However, to date, in the majority of studies, the use of active targeted nanocarriers has not provided evidence of increased tumor accumulation after intravenous injection (TABLE 2) [106,127], in spite of the presence of a specific ligand, except in the case of targeting specific cell types within the vasculature [128] (e.g., in order to reach the brain) [112]. However, there is an undeniable increase of therapeutic effect when the drug is loaded in active targeted NPs, owing to the enhancement of tumor cell internalization due to ligand–receptor interactions [106,129]. These active targeted NPs actually represent a very interesting therapeutic modality for the management of localized tumor when intratumoral injection is possible [118].

However, administration by intravenous route, despite a few encouraging results, still causes important problems. First, conjugation of active ligands may lead to an accelerated elimination of NPs by preventing their stealth properties, which, as described in this article, constitute the indispensable prerequisite. Thus, to add an active targeting agent, different parameters have to be considered (i.e., choice of ligands [130], method of coupling, concentration of ligands and control of ligand activity after coupling), as well as their influence on stealth properties. With passive targeted NPs, however, cell internalization could be prevented by a PEG layer, whereas active targeted counterparts can allow an increase in cellular uptake, which is an interesting advantage.

Accordingly, active targeting increases drug efficacy, and decreases systemic toxicity owing to drug release in normal cells. However, arrival at the drug's target site is not guaranteed, and drug efficacy is greatly dependent on intracellular trafficking and subsequent transport.

Actually, in spite of the different advantages expected with active targeting, currently no active targeted NPs are on the market, and only a few formulations are subjected to clinical experiments (TABLE 3). Thus, many papers in the literature describe the procedure and the *in vitro* characterization of formulation; nevertheless, few articles on activity after *in vivo* study have been published. Is it linked to the complexity of the formulation processes of these active targeted NPs, the expensive price of the ligands and effective scaling-up production, the difficulty to formulate these systems in large quantities for *in vivo* studies, or quite simply, to the lack of activity? Consequently, since passive targeted NPs are easier to produce, and owing to the low benefit supplied with active targeting in comparison with passive targeting, passive targeting NPs have been promoted [131] and, nowadays, constitute an interesting and promising field in nanotechnology development.

As a consequence, attempts to improve the effectiveness and safety of chemotherapy have brought about several tumor-targeted drug delivery strategies. From the benefits of PEG coating,

the engineering of drug delivery nanoscale systems is moving in the direction of 'smart' devices, which are specially homed to, and responsive in, their target environment only [132,133]. For example, stealth NPs can possess a PEG hydrophilic surface, which is able to vanish upon arrival at the site of interest by the cleavage of a sensitive linkage via chemical stimuli, such as the presence of low pH or reducing agents, or enzymatic stimuli, such as proteases [134,135]. Nanocarriers could then be transformed to a more cell-interactive form, by restoring the possible interaction of the targeting moieties with the cell membrane receptors, once the tumor site is attained, and improving drug bioavailability [136]. For instance, Romberg *et al.* prepared long-circulating liposomes with an enzymatically degradable coating polymer that triggers cellular uptake of liposomes and/or release of the entrapped biologically active agent [88]. Such promising strategies offer not only a great increase in the intrinsic pharmacologic effect of drug-loaded in NPs for therapeutic success, but also prevention of interactions at nonspecific sites. In the near future, more safe and effective nanotechnology anticancer products for cancer therapy are expected, especially nanocarriers with multivalent properties (e.g., stealth, specific targeting, internalization and drug controlled release) (FIGURE 3 & 5).

## Executive summary

### Background

- Stealth nanoparticles still represent a promising generation of nanocarriers for cancer therapy.

### Passive targeting

- Surface PEGylation has been successfully applied to various types of nanoparticles, such as liposomes, polymer nanoparticles, lipid nanoparticles and micelles. Polyethylene glycol coating confers to nanoparticles the stealth properties that limit nanoparticle elimination by the mononuclear phagocyte system in the bloodstream and allow tumor accumulation by the enhanced permeability and retention effect.
- Polyethylene glycol nanoparticles loaded with various anticancer drugs (e.g., taxanes, anthracyclines, metal-based drugs and camptothecin analogs) have shown promising results. These nanocarriers have entered clinical trials in humans and some are approved by the US FDA.
- Various parameters have to be taken into consideration, such as PEGylation process versus physicochemical characteristics of nanoparticles, tumor vasculature versus passive accumulation of nanoparticles, and safety versus efficacy of anticancer-loaded nanoparticles.

### Active targeting

- Active targeted nanoparticles can be prepared with active ligands that confer selective cellular binding. Ligands can be antibodies, nucleic acid aptamers, peptides and small molecules, such as folic acid and sugar molecules.
- There is an undeniable increase of therapeutic effect when drug is loaded in active targeted nanoparticles thanks to the enhancement of tumor cell internalization due to the ligand/receptor interactions.
- In order to keep stealth properties with active targeted nanoparticles, various parameters have to be taken into consideration (e.g., choice of ligand, method of coupling and density of ligands). The control of ligand activity after coupling must be checked.

### Conclusion

- Stealth nanocarriers can be designed to optimize chemotherapy after intravenous administration thanks to passive accumulation into tumor.
- Active strategy promises to be a powerful approach leading to effective drug delivery into tumor cells for the treatment of cancer, provided that stealth properties are kept.
- Beside the stealth and active properties, it would be interesting to design nanocarriers of new generation, being able to control drug release in the specific target. Thus, the multifunctional 'smart' devices represent the future nanomedicines.

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