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REVIEW

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Anticancer nanoparticulate polymer-drug conjugate

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Abstract

We review recent progress in polymer-drug conjugate for cancer nanomedicine. Polymer-drug conjugates, including the nanoparticle prepared from these conjugates, are designed to release drug in tumor tissues or cells in order to improve drugs' therapeutic efficacy. We summarize general design principles for the polymer-drug conjugate, including the synthetic strategies, the design of the chemical linkers between the drug and polymer in the conjugate, and the in vivo drug delivery barriers for polymer-drug conjugates. Several new strategies, such as the synthesis of polymer-drug conjugates and supramolecular-drug conjugates, the use of stimulus-responsive delivery, and triggering the change of the nanoparticle physiochemical properties to over delivery barriers, are also highlighted.

KEYWORDS

drug delivery, nanomedicine, nanoparticle, polymer-drug conjugate, stimuli-responsive

1 | INTRODUCTION

In this review we feature various polymer-drug conjugates (PDCs) based nanoparticles (NPs) used to deliver chemotherapeutics. Most of them are designed and fabricated to release drugs in tumor tissues or cells upon the triggering by different stimuli, in order to lower parent drugs' systemic toxicities and improve their therapeutic efficacies.[1] We illustrate some important lessons gleaned from over 60-year development of PDCs, and discuss the promise and outstanding challenges facing the development of PDCs from a perspective of chemistry and materials engineering.

1.1 | Polymeric nanomedicine for cancer therapy

Nanomedicine refers to the application of nanotechnology for the prognosis, diagnosis, prevention, and treatment of clinical conditions.[2] Nanomedicine can enhance therapeutics and diagnostics in many ways, as has been reviewed.[3–9] In cancer chemotherapy the NP enables the preferential delivery of drugs to tumors owing to the enhanced permeability and retention (EPR) effect—NPs are preferentially taken up by the leakier vasculature in tumor beds than small molecules and are retained because of the tortuous lymphatics.[10–12] Several nanoparticulate therapeutics, for example, Doxil™ (~100 nm PEGylated liposome loaded with doxorubicin)[13], Abraxane™ (~130 nm paclitaxel albumin-stabilized NPs)[14,15] and Onivyde™ (nanoliposome loaded with irinotecan),[16] have been approved for use by the FDA, and have shown improved pharmacokinetics and reduced adverse effects compared to their parent drugs. Polymeric drug delivery NPs, one of the major delivery platforms, has actively evolved its paradigm from water-soluble polymeric carriers, to liposome, micelle, dendrimer, polymersome, and other polymeric nanostructures.[17–19]

1.2 | The development of the PDC

PDC is one of the most important and oldest polymeric delivery systems (Figure 1). The conjugation of drugs to macromolecules was initiated about sixty years ago.[20] Early work in 1950–1960s focused on numerous water-soluble PDCs, especially poly(vinylpyrrolidone) conjugates.[21] Mathé et al. pioneered conjugation of drugs to immunoglobulins in 1958, setting the stage for PDCs.[22] In 1975 Ringsdorf presented a clear concept of the use of polymers as targetable drug carriers,[23] which motivates rational design of the first generation of polymer therapeutics candidates (and first-generation PDCs) that later entered clinical testing.[24] Meanwhile Davies and coworkers modified proteins with poly(ethylene glycol) (PEG) to improve protein's circulation half-life, immunogenicity,

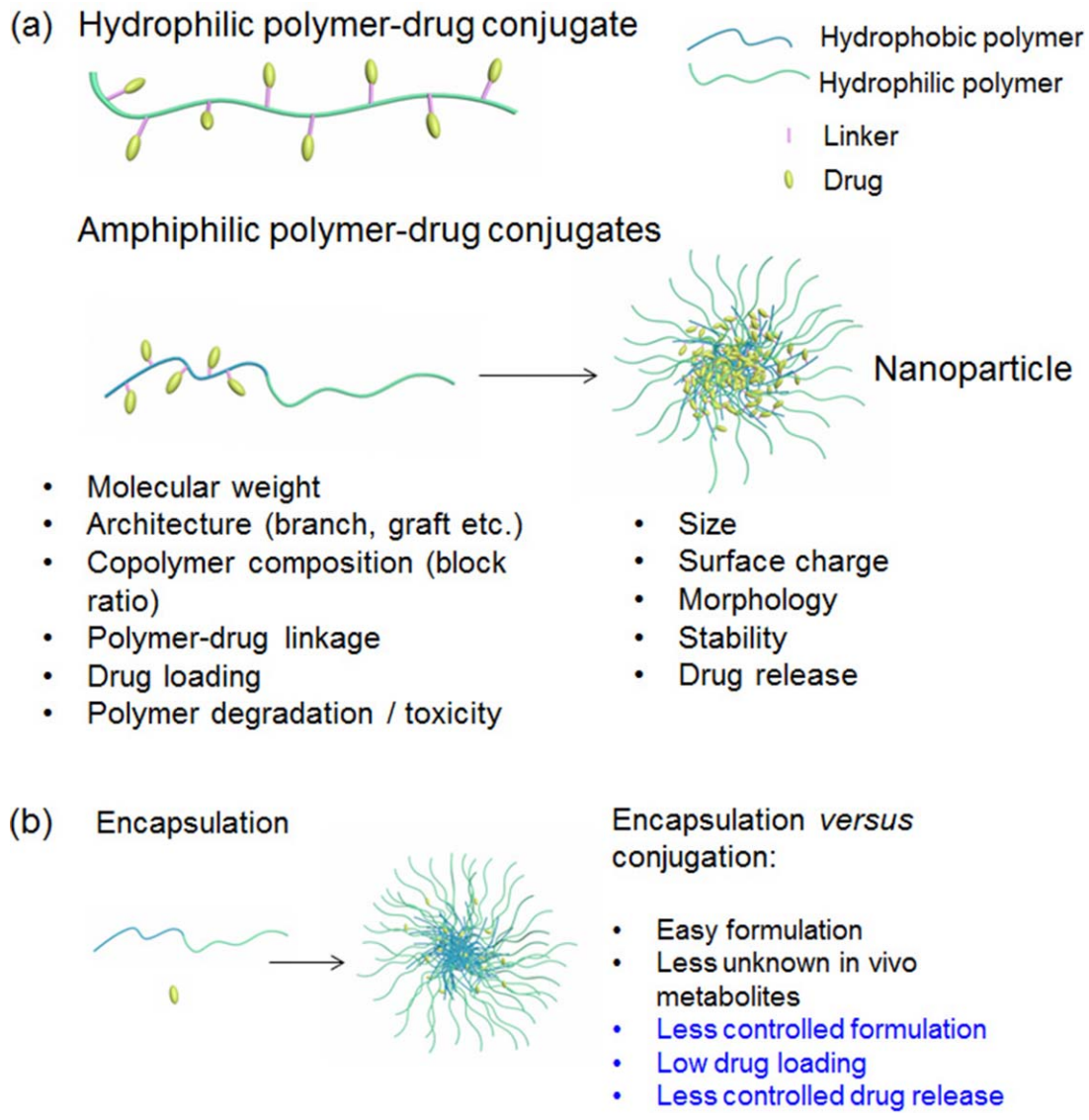

FIGURE 1 (a) Two representative polymer-drug conjugates (PDCs): hydrophilic polymer-drug conjugates, and nanoparticles composed of amphiphilic polymer-drug conjugates. Both the polymer's and nanoparticle's physicochemical properties have to be well characterized for the future translation of PDCs. (b) Scheme of the nanoparticle encapsulating drugs, which is compared to the conjugation strategy

and stability,[25] which leads to the development of therapeutic polymer-protein conjugates. Of note, many of PEGylated protein conjugates have been approved for clinical use (e.g., Oncaspar™, PEG-L-asparaginase, for treating leukemia), and will not be discussed in this review.[26,27] The important first generation PDCs include: poly(*N*-hydroxypropyl methacrylamide) (polyHPMA), which is synthesized by Ulbrich and Kopeček, and later co-developed with Duncan[28–33]; poly(glutamic acid) with paclitaxel (Xyotax™ or Opaxio™) or camptothecin (CT-2106) conjugates by Li and Wallace[34–36]; poly(styrene-maleic anhydride)-neocarzinostatin conjugate (SMANCS, Zinostatin Stimalmer™) by Maeda, which is approved in Japan for the treatment of hepatocellular carcinoma.[37,38] In the late 1980s and early 1990s nanoparticulate drug delivery systems, including PEGylated polymeric micelles and liposomes, were rapidly developed after the discovery of EPR effect.[10] Nanoparticulate form of PDCs in clinical trials also reached the clinic, including: CRLX101 (IT-101) by Davis, a PEG-cyclodextrin-camptothecin polymeric micelle with 30–40 nm size[39,40]; NK-012, NK-911 and NC-6004 all developed by Kataoka, a PEG-polypeptide block copolymer conjugated with SN-38, doxorubicin or cisplatin, respectively (Table 1, Figure 2).[41–45]

1.3 | Stimuli-sensitive PDC

Although it is suggested that the EPR effect exist in human tumors,[46,47] it is still questionable whether the EPR effect is sufficient to significantly improve the survival of cancer patients by nanomedicine.[48,49] Several delivery barriers limit the transport of NPs deep into tumors[4,50]; (see Section 2.4) recent advances in biology show that abnormal tumor microenvironments help tumor progress and resist the

TABLE 1 Representative polymer-drug conjugates in clinical trials

Name	Brand name	Polymer composition	Drug	Linker	Status	Molecular weight (kDa)	Loading (wt%)	Size (nm)	Plasma half-life (h)	AUC (h·mg/L)	Cmax (mg/L)	Other	References
PK1, FCE28068		HPMA copolymer	Doxo	GFLG peptide	Phase II (unknown)	30	8.5	7.8	93	N.S.	65	1.3% 24 hrs in head-neck tumor; 50-75% dose undergo renal clearance; 6/62 patients showing partial response	32,33,75
PK2, FCE28069		HPMA copolymer	Doxo/ galactosamine	GFLG peptide	Phase I (completed); Phase II (unknown)	25	7	10.5	28	296	N.S.	16.9% 24 hrs in liver for hepatic tumor, but only 3.3% in the cancerous regions of the liver	31,75
AP5346	ProLindac	HPMA copolymer	DACH-Pt	GGG-carboxylate-Pt coordination	Phase II (unknown)	25	10	N.S.	72.3	136	13		59
PCNU1661 48		HPMA copolymer	Cpt	Ester	Phase I (stopped)	18	10	N.S.	N.S.	N.S.	N.S.		129
CT-2103	Xyotax, Opaxio	Poly(glutamic acid)	Ptxl	ester	Phase II (completed); Phase III (ongoing)	39	36	N.S.	120	1583	N.S.	2/26 NSCLC patients showing partial response; 9/44 patients showing partial response in the combination with cisplatin; 4/12 having complete response in gastric and esophageal cancers in combination with radiation	34,35,263
CT-2106		Poly(glutamic aid)	Cpt	Ester	Phase I (completed)	49	37	N.S.	51	36	14		36
EZ-246	Pegamotecan	PEG	Cpt	Ester	Phase II (terminated)	40	2	N.S.	46	27	0.5		57
AD-70		Dextran	Doxo	Imine	Phase I (completed)	70	N.S.	N.S.	11	N.S.	0.01		70,71
NK911		PEG-*b*)-poly(aspartic acid)	Doxo	Amide	Phase II (unknown)	16	17	40	7.5	3.2	3.9		41,42
NK012		PEG-*b*)-poly(glutamic acid)	SN-38	Ester	Phase II (completed)	19	20	20	137	294	19.1		42,43
NC6004	Nanoplatin	PEG-*b*-poly(glutamic acid)	Cisplatin	Pt-carboxylate coordination	Phase III (recruiting)	26	30	30	129	2836	60.8		42,44,45
NC6300		PEG-*b*-poly(aspartic acid)	Epirubicin	hydrazine	Phase I (unknown)	20	20	60	N.S.	N.S.	N.S.		42
NC4016		PEG-*b*)-poly(glutamic acid)	DACH-Pt	Pt-carboxylate coordination	Phase I (recruiting)	26	30	30	N.S.	N.S.	N.S.		42
CRLX101, IT-101		poly(cyclodextrin)-*co*-PEG	Cpt	glycine	Phase I (completed); Phase II (recruiting)	57	6.8	36	27.9	306	8.3	3/19 patients show partial response; 14/19 having net tumor reduction	39,40

DACH = 1,2-diaminocyclohexane; Doxo = doxorubicin; Ptxl = paclitaxel; Cpt = camptothecin; HPMA = *N*-(2-hydroxypropyl) methacrylamide; N.S. = not stated; AUC = the area under the plasma concentration-time curve; C_{max} = maximum drug concentration.

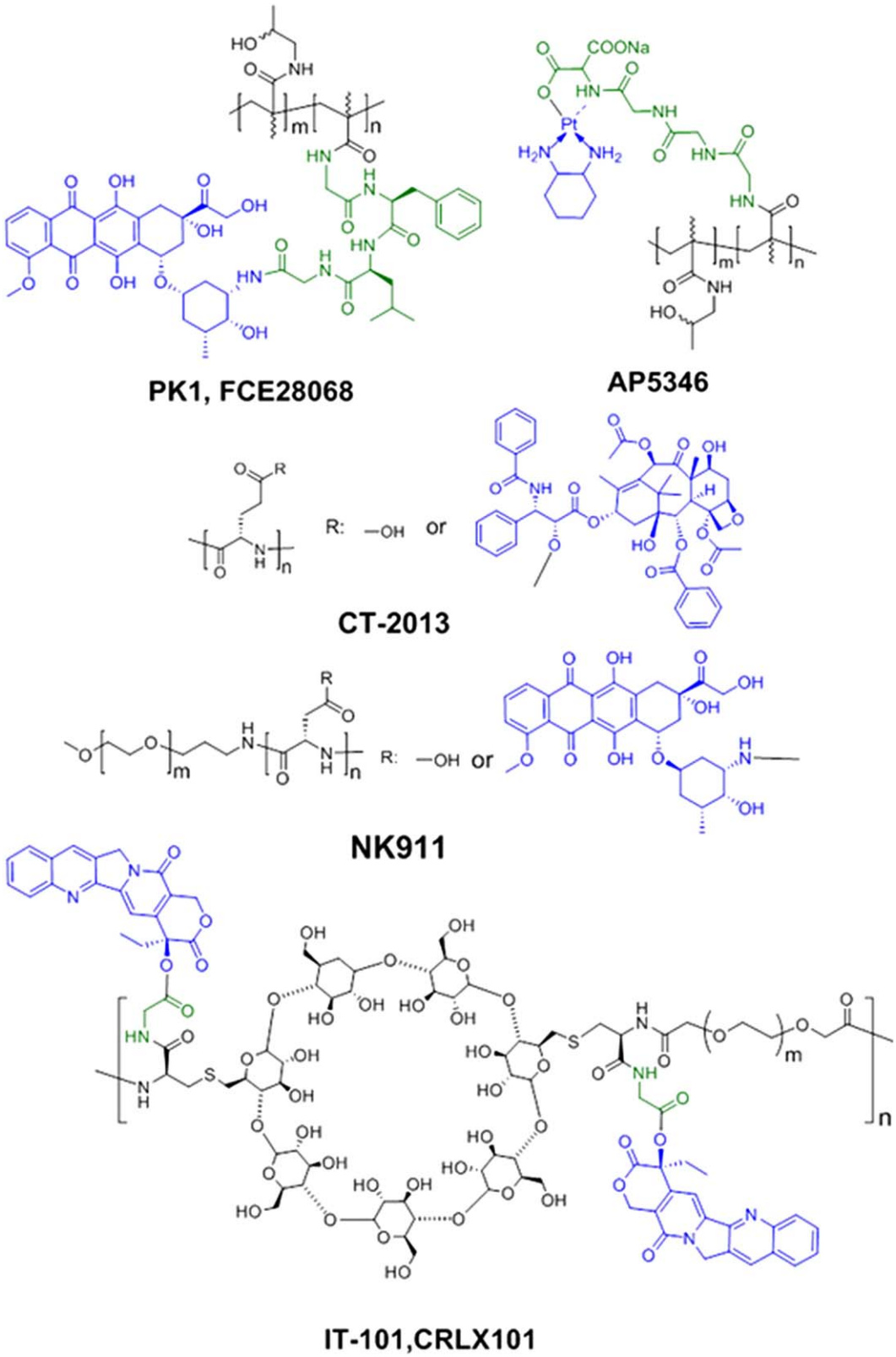

FIGURE 2 Chemical structures of some polymer-drug conjugates in the clinical trials. The drugs are highlighted in blue, the linkers in green

treatment.[51] Therefore the stimuli-responsive NPs are designed to overcome the delivery barriers in tumor microenvironment to improve the therapeutic efficacy.[52] In fact many PDCs contain stimuli-sensitive linkers positioned between the drug molecule and the polymer; the drug remains on the PDC in circulation and can be locally triggered release, by either endogenous stimuli in tumor microenvironment such as pH or enzyme, or by applied endogenous stimuli on tumor, such as light or heat source (see detailed discussion in Sections 2.3, 3.3–3.5).

1.4 | Loading drugs in NPs: encapsulation versus conjugation

The method of drug loading imposes numerous design constraints on the delivery platform. The drug-encapsulated delivery platform has continuous drug release during the circulation, which making it difficult to achieve therapeutically effective concentration at tumor and could cause systemic side effects in normal tissues (Figure 1b).[53,54] The covalent linkage between the drug and polymer in the PDC offers opportunities for triggered release only at the tumor tissue or cell. In addition, a high drug loading can be achieved relatively easily in PDCs compared with drug-encapsulated NPs. Higher drug loading of delivery vehicles is desirable for optimal therapeutic effect, to enhance the potency of NPs that reach the tumors.[55] However, one obvious shortcoming for PDCs is that not all of the drugs have chemical functional groups for covalent conjugation. Fortunately, many prevalent chemotherapeutics, including paclitaxel (Ptxl), docetaxel (Dtxl), doxorubicin (Doxo), gemcitabine, irinotecan, and camptothecin (Cpt), can be conjugated to polymers. In addition, PDCs may require tremendous synthetic efforts compared with encapsulation. Furthermore, the in vivo characterization of PDC's stability, release, metabolism, excretion and toxicity can be demanding: a PDC is viewed as a new drug by FDA, and its' metabolites' toxicity and pharmacokinetics require detailed examination.[55]

Overcoming these challenges requires (a) the judicious chemistry design to ensure tumor-specific drug release; and (b) qualitative preclinical in vivo characterization of PDCs' pharmacokinetics, drug release and metabolism for better understanding. Here we mainly focus on the stimuli-sensitive PDCs in this review. We first summarize some design principles of PDCs based on the preclinical studies (Section 2), including the polymer and conjugation linker's chemistry, NP's physicochemical properties, and the in vivo delivery barriers requiring design consideration. We then highlight recent strategies in the development of PDCs (Section 3), aiming to address challenges in chemistry, materials and in vivo application of PDCs.

2 | THE DESIGN PRINCIPLES OF PDC FOR CANCER NANOMEDICINE

There are a number of overarching designing principles in the delivery of PDCs to tumor sites, which recur throughout this review. Most of these are based on the preclinical findings in animal models. Some of these are common to many other delivery carriers where NPs are of significant interest, while others are unique to the polymeric chemistry and materials in PDCs.

2.1 | Polymer

Polymers that have functional groups for the incorporation and release of drugs in PDCs must be well characterized (Figure 1a). All in vivo metabolic products of PDCs should be nontoxic and nonantigenic. Polymers in PDCs should be either biodegradable or completely eliminated from the body. In this review, various polymers are discussed, including: hydrophilic polymers used in first-generation PDCs for clinical trials, such as polyHPMA, and PEG[1,56–59]; copolymers, especially block copolymers that can be formulate to nanostructures such as micelle[60] or polymersome[61,62]; dendrimers[63] and hyperbranched polymers,[64,65] and natural macromolecules such as polysaccharides (dextran, cellulose, chitosan) and polypeptides.[66–71]

2.1.1 | Polymer molecular weight

The polymer molecular weight affects the in vivo circulation of hydrophilic polymers. In general the higher the molecular weight, the longer the intravascular half-life and the slower the elimination of hydrophilic polymer based conjugates from the body. Such trend has been shown in the studies of polyHPMA,[72–75] dextran[76,77] and dendrimers, etc.[78] The half-life of polyHPMA-Doxo conjugate (molecular weight 1230 kDa) in blood was up to 28 times longer, and the elimination rate from the tumor was 25 times slower than that of free Doxo.[72]

2.1.2 | Polymer architecture

Hydrophilic polymer architecture has an important impact on the in vivo activity of the PDCs. Ulbrich's group studied in detail the relationship between the architecture of HPMA copolymers—linear conjugates, branched conjugates, grafted conjugates, self-assembled micellar conjugates, and grafted dendritic star conjugates—and their activity.[79] Other studies showed the impact of the polymer architecture (conformation, flexibility, branching, and hydrodynamic volume) on the fate of the circulation of polymers in vivo. The polymer architecture has a serious impact on the clearance of polymers through the kidney.[80] Large-sized hydrophilic polymers with decreased flexibility, and an increased number of polymer chain ends, help prevent elimination of the polymer by the kidneys and can improve blood circulation time. However, the polymer architecture has much smaller effect on the extravasation of the polymer into the tumor.[80]

2.1.3 | Block copolymer's composition

The relative ratio of the hydrophobic to hydrophilic block length profoundly affects the NP's morphology.[81–83] Typically the morphology of prepared amphiphilic block copolymer NP is spherical, particularly if the molecular weight of the hydrophilic block exceeds that of the hydrophobic block (so-called star micelles). However, if the copolymer is asymmetric in its relative block lengths (i.e., the hydrophobic block is considerably longer than the hydrophilic block) during the self-assembly process, varying morphologies can be obtained.[84,85] In addition, the copolymer's concentration in water-miscible solvent affects the final NPs' size.[86] The use of triblock polymers could improve the NP's stability.[54] Nevertheless, there lacks systemic studies on the block copolymer ratio or composition on the in vivo circulation and stability of the NPs' morphology, presumably due to the technical difficulty to monitor the sub-100 nm polymeric NPs in vivo. Recent in vivo pharmacokinetic studies using dual-radiolabeling of lipid and drug in liposomes could provide a valuable example for the study the biodistribution of copolymer-based PDCs in vivo.[87]

Notably, recent studies have shown that the zwitterionic copolymers (i.e., polymers containing both cationic and anionic groups) are

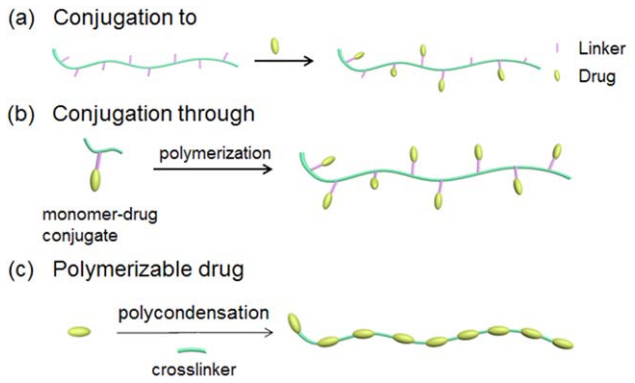

FIGURE 3 Three synthetic strategies of polymer-drug conjugates

super-hydrophilic, which can prolong the circulation of NPs in the way similar to PEG.[88]

2.2 | Synthetic strategy of PDC

There are three strategies for drug-polymer conjugates (Figure 3).[89] The first is conjugating a drug to a pre-synthesized polymer, named as "conjugation to." The second is to conjugate a drug to a monomer prior to polymerization, namely "conjugation through." The last is the polymerization of drugs to prepare PDCs, where drugs are directly used in the polymerization as monomers or initiator.[90] The last two strategies have been recently developed to prepare PDCs, aiming to overcome the non-controlled drug conjugation problem in the "conjugation to" strategy (see Sections 3.1 and 3.2).

2.3 | Drug release and the conjugation linker

An ideal PDC for cancer treatment should be able to release the drug in tumor tissues or cells, but not to the normal tissues or cells. Two types of linker can be positioned between drug and polymer: cleavable linker and non-cleavable linker. Non-cleavable linkers, such as thioether linkers, have been seen in the antibody-drug conjugates (ADCs).[91] The release of the drug from these ADCs requires complete hydrolysis of the polypeptide backbone of the antibody in cell lysosomes.[92] One example is T-DM1 (Kadcyla™), an ADC to treat metastatic breast cancer, which has the thioether linker, and exhibited better antitumor efficacy than the same ADC but with disulfide linker.[93] However, the use of non-cleavable linker of degrading the delivery platform after cell uptake might not be feasible in more complex NP systems.

Enormous synthetic efforts have been devoted to design stimulus-sensitive cleavable linkers to trigger drug release (Figure 4). During the NP's extravasation, local tumor microenvironmental factors, such as pH (6.7–7.0),[94] redox state (hypoxic tumor microenvironment[95] and elevated reactive oxygen species generated by tumor cells[96]) and specific molecules overexpressed in tumor (e.g., matrix metalloproteinases [MMP]),[97] can be utilized to disrupt PDCs' structures to release loaded drugs, or induce NPs size or morphology change for enhanced penetration (see Section 3.5). Besides the endogenous stimuli, the external stimuli—such as magnetic field, temperature, light, and ultrasound—can be applied in a spatiotemporal manner to control drug release.[98–102]

More importantly, cleavable linkers have to result in direct release of the drug from the remaining linker fragment upon the cleavage, that is, no prodrug released. For systemically delivered PDCs, these linkers should be stable in circulation to avoid the side effects from the free drug and/or the decreased drug accumulation in tumors. We mainly discuss each type of stimulus-sensitive linker, focusing on the general chemistry, in vivo stability and some preclinical successful examples.

2.3.1 | pH sensitive linker

The mildly acidic pH in tumor tissues (pH ~ 6.7–7.0)[94] as well as in the endosomal intracellular compartments (pH ~ 4.5–6.5)[103,104] can trigger drug release from pH-sensitive PDCs upon their retention at tumor sites. Many pH-sensitive PDCs have been developed including *cis*-aconityl amide, hydrazone, imine, oxime, acetal/ketal/orthoester,[105] or other groups like trityl, *N*-ethoxybenzylimidazoles and thiopropionate,[106] and silyl ether etc (Figure 4).[101,105,107]

For Doxo PDCs, the acid sensitive hydrazone linker is often used to conjugate polymers to the ketone group in Doxo. However, the acid-labile hydrazone linker is relatively unstable in vivo, with half-lives in plasma of 48–72 hrs, less than that of the antibody moiety.[108] In some cases, some hydrazone linker could induce the cyclic reaction and release less active Doxo prodrug, instead of free Doxo.[109] Some other Doxo conjugates containing pH-sensitive cis-aconityl spacer were prepared by the reaction of amino group of Doxo with *cis*-aconitic anhydride forming α,β-unsaturated amide.[110–114]

2.3.2 | Redox sensitive linker

The difference in redox potential between normal and tumor tissues, and between the intracellular and extracellular environment, can be exploited for triggered drug delivery.[115] In the nanomedicine field, it is generally believed that the concentration of glutathione, a reducing tripeptide with thiol group, in cancer cells is 100- to 1,000-fold higher than in the blood, and in a tumor mass the glutathione concentration is also markedly (100-fold) higher than the extracellular level of glutathione in normal tissue.[116] However, studies showed that in mice model a total fourfold higher level of glutathione in tumor tissues compared with normal tissues, and there exists significant heterogeneity of redox status in the tumor tissue.[117] In human cancer patients, glutathione levels tend to be elevated in breast, ovarian, head and neck, and lung cancers compared with disease-free peritumoral or healthy tissue; conversely, brain and liver tumors patients exhibit lower tissue level of glutathione in tumor compared with that in healthy tissue.[118] In addition, two studies concluded that glutathione levels did not differ between parenchymal tissue sampled from healthy patients and uninvolved parenchymal tissue from lungs with tumors.[119,120] Therefore, right preclinical models and tumor types have to be rationally chosen when applying redox-sensitive PDCs.

The reducing materials in vivo could facilitate the cleavage of redox-sensitive bonds such as disulfide bond and diselenide bond.[121] For example Kopeček and coworkers conjugated the photosensitizer mesochlorin e6 to HPMA copolymer via a disulfide bond, which showed a time-dependent release of Mce6 and concomitant increase in the photodynamic efficacy when exposing to DTT.[122] However, the disulfide-based linker showed relatively short in vivo stability less than

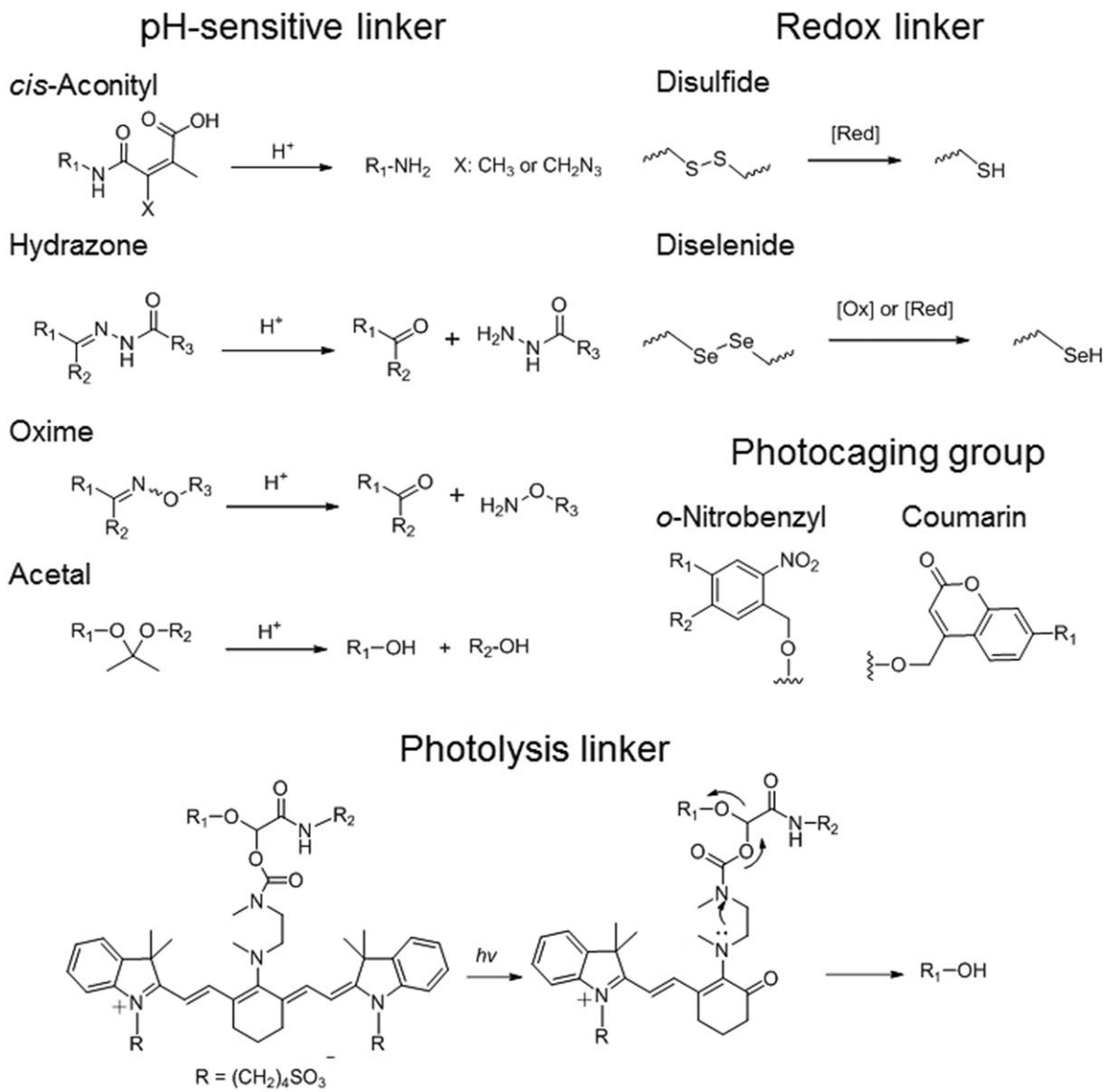

FIGURE 4 Schemes of various stimuli-responsive linkers used in polymer-drug conjugates, including pH-sensitive linkers, redox-sensitive linkers, photocaging groups, and photolysis linkers

1 day in ADCs, respectively, which is much shorter than the parent antibody moieties.[108] The in vivo circulation stability PDCs containing redox-sensitive linker should be evaluated carefully in future.

2.3.3 | Enzyme sensitive linker

The increased expression of certain local enzymes in cancer, such as MMP, not only can be regarded as a biomarker for disease diagnosis and prognosis, but also represents a means for enzyme-triggered drug release in tumor.[123–126] Early studies on detailed degradation studies of oligopeptide sequences attached to polyHPMA-based PDCs identified the short peptide GFLG, specific for cathepsin B.[127] The polyHPMA PDCs with such linker have shown efficacy in various preclinical efficacy study and have entered the clinical trials.[128,129] Another widely used short peptide linker is citrulline-valine, which can be cleaved by specific lysosomal proteases but impart greater stability in plasma. Of note Brentuximab vedotin (Adcetris™ approved for the use in Hodgkin lymphoma) is an ADC containing such dipeptide linker to facilitate release of the drug, monomethylauristatin E.[130] Other short peptides linkers include PVGLIG (cleaved by MMP-2/MMP-9),[131] and SSKYQL (cleaved by prostate-specific antigen).[132] One can envision that the presence of certain enzymes as biomarkers potentially could be utilized to design a PDC for personalized medicine, once the concentration of enzymes at the tumor site should also be sufficient for the disruption of the PDC.

2.3.4 | Light sensitive linker

There has recently been growing interest in light-responsive NPs for triggered drug delivery. The use of an optical stimulus is appealing because it could provide a greater selectivity in terms of control over the moment and the location of drug release, and potentially transfer photonic energy to heat, acoustic wave, or induce reactive species such as singlet oxygen in photodynamic therapy.[133] In terms of light-

triggered chemical bond cleavage, several classes of light-sensitive linkers have been reported including the nitrobenzyl, coumarin-4-ylmethyl, *p*-hydroxyphenacyl, and 7-nitroindoline derivatives with ester, amide, carbonate, carbamate, and phosphate linkages for photolysis (Figure 4).[134] However, many photocaging groups required the irradiation by UV light or short wavelength visible light, which restricted primarily to superficial lesions unless fiberoptics or near-infrared (NIR) light can be used. Of note, NIR light, with wavelengths in the range of about 700–1000 nm, is more suitable for biomedical applications than UV or visible light; the irradiation is less detrimental to healthy cells, and the absorption and scattering by water and biological substances are reduced, which results in a greater tissue penetration depth (on the order of millimeters to centimeters).[135–137]

One way to use NIR light is to use two-photon excitation for many UV-light absorbing photosensitive linkers.[138,139] Two-photon excitation usually requires high-intensity pulsed laser ($MW{\cdot}cm^{-2}$ to $GW{\cdot}cm^{-2}$)[140]; however, many photocaging groups do not have large enough two-photon cross-sections to be efficiently activated by NIR light.[141] Alternatively, recent developed NIR-light sensitive linkers are showing promise. A near-IR (690 nm) light-initiated photolysis reaction was developed based on the C4'-dialkylamine-substituted heptamethine cyanine linker and has been used in a light-triggerable ADC; upon the irradiation, the photo-oxidation of the cyanine polyene could generate a secondary amine and promote the cleavage of the carbamate bond to release the drug (Figure 4).[142,143] The use of inorganic NPs such as gold NPs or upconversion NPs for triggered drug release offers another intriguing strategy and has been reviewed elsewhere.[144–146]

2.4 | In vivo drug delivery barriers for PDCs

2.4.1 | Circulation

To achieve therapeutic efficacy, NPs must first overcome systemic barriers with prolong circulation time, especially clearance by mononuclear phagocytic system (or so-called reticuloendothelial system), hepatobiliary system and urinary system.[147] In general, NPs with sizes below 100 nm are suitable for systemic (usually intravenous) distribution, as larger ones cause embolic phenomena[148]; while there seems no significant difference in circulation half-life for various sized NPs in the range of 30–100 nm.[149] To avoid rapid clearance by the kidneys, the NP's hydrodynamic size should be larger than 6 nm.[150] Notably, most HPMA PDCs in clinical trials have NPs size below 10 nm (Table 1) or moderate molecular weight (less than 40 kDa, Table 1), which may affect their in vivo circulation and accumulation profiles. The NP should keep its size in circulation and not be destabilized under flow or at physiological temperature.[151] In addition, the NP should not bind with proteins in blood that could lead to aggregation, or be uptaken by the macrophages in the mononuclear phagocytic system, all of which can lower the dose of NPs reaching tumors.[152] Coating of NPs with PEG that mimics a cell's glococalyx,[153–155] known as "PEGylation," can suppress protein absorption to NPs and delay the rate of NP uptake and clearance, greatly prolonging circulation time.[156] The NP's circulation half-life is impacted by the extent of PEGylation on NPs surface,[157,158] and may be reduced upon repetitive administration, which has been reviewed elsewhere.[159,160]

2.4.2 | Tumor penetration

When NPs reach tumor blood stream from circulation NPs extravasate from tumor vessels and penetrate up to hundreds of micrometers through the tumor stroma so that even cancer cells situated distal to the tumor vessel can be exposed to the anticancer agent at high enough concentrations. Thus, both NPs accumulation (total mass) and penetration depth from vessels over the time can determine the efficacy and have to be carefully examined in preclinical studies, which can be evaluated by both the drug's concentration in tumor and the area under the drug's intratumoral concentration–time curve. NP size is one crucial determinant of accumulation and penetration into tumor tissue. It is reported that polymeric micelles $\sim$ 30 nm showed enhanced tissue penetration and potent anti-tumor activity in pancreatic tumors, compared with larger NPs.[149] In another example, 50 nm NPs showed deeper tissue penetration and higher accumulation in breast tumors over time, compared with 20 nm or larger NPs.[161] One recent imaging study showed that the intercellular gaps and transcellular fenestrae in the tumor have dynamic changes that brief vigorous outward fluid flows into the tumor interstitial space, which allows for the 70 nm sized NPs extravasate into tumor tissues.[162] In general, current consensus is that sub-100 nm may be the optimal NP size range for passive tumor targeting, which may vary depending on individual NP's composition and formulation. Besides, the NP surface charge (see discussion in Section 3.5.2) and the aspect ratio of NPs can affect NP's, in vivo circulation time and tumor penetration capability[163] and NP's cell uptake.[164] Other strategies of improving NP's tumor penetration include co-injecting drugs to reduce tumor's extracellular matrix density,[165,166] and conjugating tumor-homing or tumor penetration ligands.[167,168]

2.4.3 | Tumor cell uptake

After reaching the tumor cells, NPs may need to cross the barrier of the cell membrane to deliver the loaded drugs into specific organelles to achieve efficacy. The surface modification of NPs with cell targeting ligands,[169] cell penetration peptides,[170] or lysosome-destabilizing agents[171] can greatly enhance intracellular uptake. Generally cancer cells may contain certain receptors or targets, such as transferrin, EGFR/HER-2, PSMA, VCAM, that can mediate the corresponding enhanced cellular uptake of targeted NPs.[172] Of note, the use of targeting ligands can enhance NPs' cellular uptake but not necessarily increase the tumor accumulation of NPs when compared with EPR-mediated accumulation.[173–175] Conversely, the introduction of targeting ligands onto NPs not only requests synthetic efforts but also sometimes compromises the prolonged circulation of PEGylated NPs.[176,177] The surface density of targeting ligands should be closely monitored to provide a desirable targeting effect without reducing NP's circulation or tumor penetration capability.

For NPs without targeting ligands, intracellular NPs are found mainly within endosomes or lysosomes. These organelles have acidic pH, and contain proteases for degradation. The rate of uptake and intracellular localization of NPs have been studied by many research groups.[178–180] Currently it is difficult to draw general conclusions about

optimal physicochemical properties of NPs for rapid cellular uptake, since the rate and mechanism of uptake are cell-type dependent and could vary between NPs with different size, charge, and other surface properties. However, some reports show that NPs of 20–50 nm are taken up more rapidly than smaller or larger NPs.[178,181]

For hydrophilic polymer based PDCs, it is found that some PDCs, such as polypeptides[182] or dextran,[183] cannot be naturally degraded into small fragments that can cross the lysosomal membrane; the accumulated polymers in the lysosome increase the osmotic pressure and adversely affect the biocompatibility.[184] Another study shows that most polyHPMA-based PDCs quickly and evenly diffuse throughout the cytoplasm and remain excluded from membrane-bound organelles; only strongly cationic HPMA copolymers can bound to microtubules; the nuclear entry kinetics were affected by the ratio of the HPMA to comonomer compositions.[185]

3 | NEW STRATEGIES IN PDC

The purpose of this section is to highlight some novel ways in which chemistry and nanotechnology are being applied to tackle challenges in PDC development.

3.1 | "Conjugation through" PDC

The "conjugation through" method in PDCs requires monomer-drug conjugates not interfere the polymerization.[89] The "conjugation through" method could address the drawbacks in the "conjugation to" strategy, such as inconsistent and uncontrolled site conjugation along the polymer backbone.[89] The drug loading can be controlled by adjusting the feed ratio of monomer-drug conjugates; and the drug release can be controlled by the judicious selection of the linker between the drug and the monomer, which could be stimulus-responsive (Figure 3b).[186] Such method thus allows for even higher drug-loadings than the "conjugation to" approach, by avoiding steric hindrance and accessibility limitation during the conjugation.

A few of monomer-drug conjugates have been synthesized to prepare PDCs and corresponding NPs. Ring-opening metathesis polymerization (ROMP) is often unitized in the "conjugation through" strategy. Examples include the norbornene-Doxo conjugate with the acid-sensitive carbamate linker,[187] or the similar norbornene-Doxo conjugate with the hydrazone linker.[188] Multiple drugs including Doxo, Cpt, and cisplatin were individually tethered to the norbornene monomer with different stimulus-responsive linkers; upon ROMP, precise ratio of drugs were controlled linked to the polymer, and resulted NPs could orthogonally triggered release individual drugs.[189,190] Similarly, the reversible addition fragmentation transfer (RAFT) polymerization is reported for "conjugation through" method; for example, Cpt-tethered acrylate with redox-sensitive disulfide linker was polymerized by RAFT to formulate redox-sensitive NPs.[191] However, PDCs synthesized by the ROMP or RAFT had non-biodegradable polymer backbones, which limits their potential clinical application.[186] Alternatively, the ring-opening polymerization (ROP) is applied in "conjugation through" method. For instance, a campothecin-tethered cyclic carbonate monomer was prepared with the disulfide linker between the drug and carbonate. The ROP of such drug-carbonate conjugates resulted in a biodegradable polycarbonate PDC which can be further formulated to redox-responsive NPs.[186]

3.2 | Polymerizable drug

The use of drug as the monomer could significantly increase the drug loading. However, not many drug molecules fit for such strategy. Often, drug molecules contain two functional groups that allow for polycondensation reaction. Another drawback lies in the polymerization chemistry especially in polycondensation reaction. Such polymerization cannot produce high molecular-weight polymer (e.g., over 10 kDa) and lacks polydispersity control. In addition, the introduction of stimulus-responsive group is not straightforward. Early work to prepare PDCs via such strategy often focus on the polyanhydrides which degrades through hydrolysis in vivo without burst release. Drugs such as ibuprofen, naproxen,[192] ferulic acid,[193] or morphine[194] have been used as monomer in the polymerization.

Recently a new facile strategy has been reported to use the stimulus responsive group to induce further depolymerization in the PDCs using drugs as monomers. 10-hydroxycamptothecin, a diol drug, was polymerized with *o*-nitrobenzyl, a photosensitive group, caged 2,6-bis (hydroxymethyl)aniline via condensation polymerization. The resulted polycarbonate PDCs could be responsive to the UV-light triggering: the *o*-nitrobenzyl group was detached from the polymer and unfolded the aniline groups, which could successively trigger the depolymerization via the 1,4-elimination reaction (Figure 5) and released the drug.[195] Similar 10-hydroxycamptothecin-loaded polycarbonate caged with redox disulfide linker was also reported.[196]

3.3 | Light-responsive PDC

3.3.1 | Photosensitizer conjugate

Photodynamic therapy is a photochemistry-based approach for treating tumors or other diseases such as macular degeneration. It involves the administration of nontoxic dyes known as photosensitizers systemically or topically, followed by illumination of the lesion with visible or NIR light,[197] and then photosensitizers generate cytotoxic oxygen species (either singlet oxygen or oxygen radicals).[198] Most photosensitizers bind to normal cells as well as to cancer cells, leading to unwanted off-target activation from environmental (ambient) light.[199–202] The conjugation of photosensitizer to polymeric delivery vehicles is designed to improve photosensitizer's performance by increasing specificity and/or uptake in tumors, or decreasing phototoxicity to normal tissue.[203,204] Early photosensitizer-drug conjugates include polyHPMA, PEG and antibody conjugates.[205–208] Factors such as the charge and hydrodynamic size of the conjugates affect the cellular uptake rate and tumor accumulation of hydrophilic polymer-photosensitizer conjugates.[209,210] In many cases the covalent linkage between photosensitizer and polymer significantly reduced the quantum yield.[211] The enzymatic-cleavable linker, or environmental-sensitive linker, was introduced to enhance both the selectivity of photosensitizer and the quantum yield;

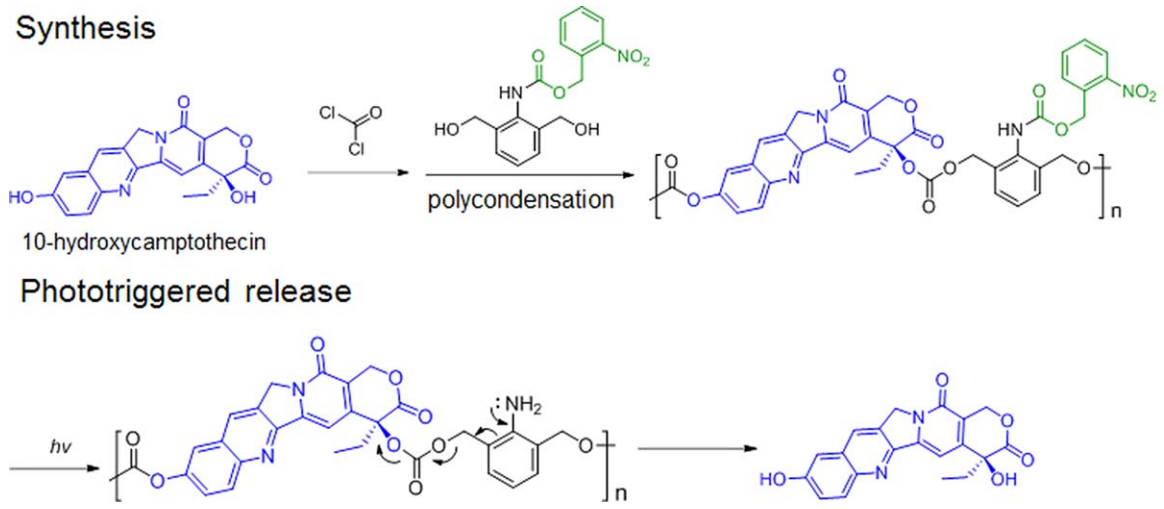

FIGURE 5 The synthesis and drug release of a light-triggerable polymer-10-hydroxylcamptothecin conjugate. The drugs are highlighted in blue, the linkers in green

the conjugates were quenched and non-toxic in the native state, but became fluorescent and produced singlet oxygen upon the cleavage of linkers by proteases in tumor.[211–214]

The use of lipid-photosensitizer conjugates to formulate light-sensitive liposomes that combine photothermal therapy with chemotherapy has recently garnered interest. Photothermal therapy may potentially improve the chemotherapy efficacy of polymeric NPs containing drugs.[215] For example, nanoliposomes composed of lipid conjugates of pyropheophorbide (a chlorin analogue) can efficiently absorb and transfer light energy into heat for photothermal therapy, as well as release the loaded drugs inside liposome.[216] Of note, the use of another porphyrin-lipid conjugates could also induce the transient increased permeability of the nanoliposome upon NIR light triggering; its mechanism remains unknown but not due to the photothermal effect.[217]

3.3.2 | Conjugated polymers

Conjugated polymers, or conductive polymers, containing light-absorbing units in their backbones with delocalized electrons (overlapping p-orbitals), have attracted interests in applications ranging from light-emitting diodes, photovoltaics to sensors.[218,219] Some conjugated polymers can generate ROS upon the irradiation of light, and become a new class of materials for photodynamic therapy.[220,221] A light-sensitive PDC can be formulated using redox-sensitive or ROS-cleavable thioacetal linker, between the drug and the conjugated polymer; upon light illumination, the generated ROS causes drug release through the cleavage of the thioacetal linker (Figure 6).[222] Such nanoparticulate PDCs can be triggered by visible or NIR light, providing new opportunities for both photodynamic therapy and chemotherapy delivery, as most light-responsive polymeric systems are still activated by UV light.[133]

3.4 | Thermal-responsive PDCs

One of the most promising thermal-responsive polymers used in PDCs is the elastin-like polypeptide (ELP). ELPs are biopolymers with the pentapeptide repeating unit Val–Pro–Gly–Xaa–Gly, where Xaa can be any of the natural amino acids except Pro.[223] Aqueous ELP solution undergoes an inverse temperature phase transition; the soluble solution becomes hydrophobically aggregation when heated up above its transition temperature, which can be adjusted ~ 40–42°C for hyperthermia application.[224] In such context, ELP-drug conjugates have prolonged circulation with the half-life over 8 hrs,[225] and could

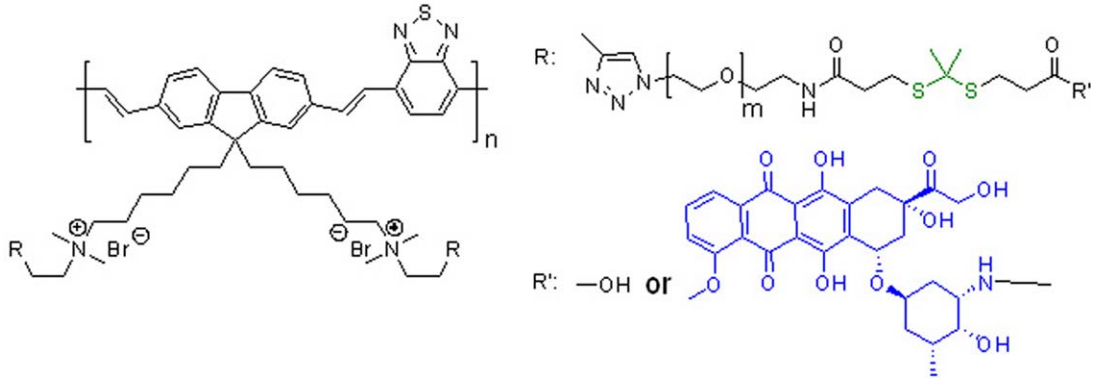

FIGURE 6 The chemical structure of a conjugated polymer-doxorubicin (blue) conjugate with redox-sensitive thioacetal linker (green)

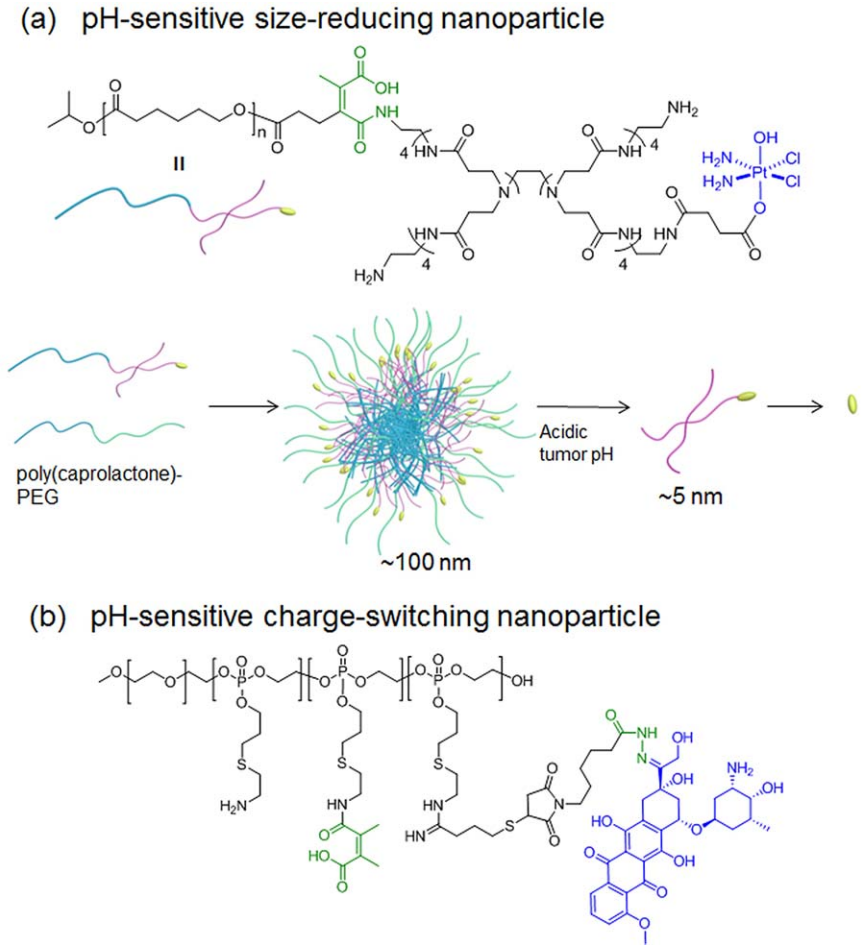

FIGURE 7 Two examples of polymer-drug conjugates that can change the nanoparticle's (a) size or (b) charge to improve their tumor penetration. The drugs are highlighted in blue, the pH-sensitive linkers in green

accumulate in the locally heated tumor region, which was confirmed by intravital fluorescence microscopy.[226] The use of acid- or redox-sensitive linkers in ELP-drug conjugates allowed for the intratumoral drug release.[227,228] It is showed that the most effective strategy to enrich the ELP NPs' tumor accumulation was to thermally cycle the tumors between 37 and 42°C, where NPs aggregated in the vasculature of tumors heated to 42°C and the aggregation reverted and extravasated into tumor tissues as the temperature decreased to 37°C.[226,229]

3.5 | Switching NP physicochemical properties for enhanced tumor penetration

3.5.1 | Size

The diffusion of NPs in solid tumor tissue is hindered by many factors including intratumoral dense extracellular matrix such as collagen and hyaluronic acid.[230,231] Small-sized NPs could penetrate deeper in tumor tissue, and not cleared from the tumor as rapidly as much smaller molecular drugs.[232] However, smaller polymeric NPs are often difficult to formulate and may not have the capacity to have high drug loadings. An alternative delivery approach was proposed to use relatively larger NPs with initial size (still sub-100 nm NPs), but once docking at tumor sites, NPs were switchable to small particles to facilitate tumor penetration.[233] The stimuli-responsive NPs that are able to shrink their sizes by responding to enzymes or light exhibited the enhanced tumor penetration of NPs and improved efficacy.[234,235] Recently, a new pH sensitive NP was prepared by poly(caprolactone)-*co*-poly(amidoamine)—platinum prodrug conjugate with a pH sensitive *cis*-aconityl linker (degrade ~pH 6.8) between poly(amidoamine) and poly(caprolactone) (Figure 7a). The initial 100 nm sized NPs degrade in acidic tumor interstitial spaces to 5-nm poly(amidoamine)—drug conjugates with enhanced diffusion capability.

3.5.2 | Surface charge

Positively charged NPs often have short circulation half-life compared with PEGylated or anionic NPs.[236–239] However cationic NPs may penetrate tumors deeper than neutral or anionic NPs due to the attractive electrostatic forces between cationic NPs and anionic endothelial glycocalyx.[240] Positively charged NPs also generally have better cellular uptake than negatively ones.[241,242] A pH-sensitive PDC-based NP was designed to achieve multi-stage charge changing to improve delivery efficiency: NP's surface charge maintained slightly anionic at pH 7.4; in tumor tissues with pH ~ 6.8, the pH-sensitive *cis*-aconityl group was cleaved from the surface and expose the positive amine groups, which

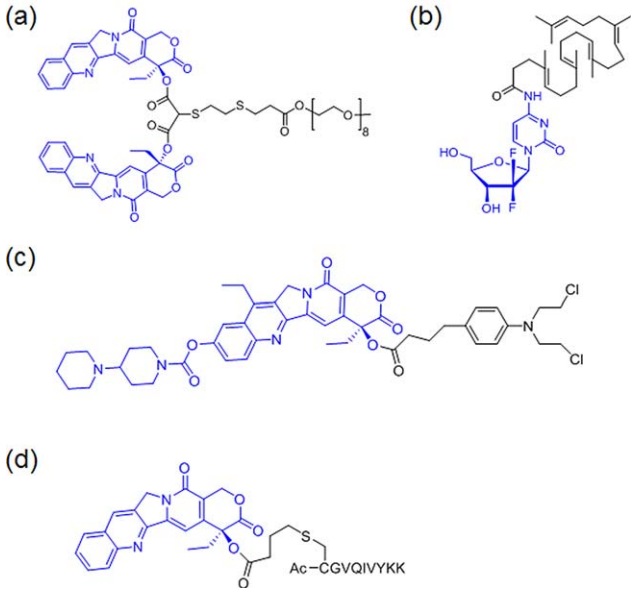

FIGURE 8 Four representative supramolecular drug conjugates that can assemble into nanoparticle for drug delivery

enhanced the tumor penetration and facilitate cellular uptake; the intracellular low pH in endosome and lysosome (~5.0) could further promote the intracellular Doxo released from the PDCs by the breakage of hydrozone linkers (Figure 7b).[243]

3.6 | Supramolecular prodrug conjugates

Prodrugs are pharmacologically inactive or less active drug derivatives, aiming to improve the solubility or pharmacokinetics of drugs. There are some lipid-drug conjugates in Phase I/II clinical trials, such as a docosahexaenoic acid conjugate of paclitaxel (Taxoprexin),[244] an elaidic acid conjugate of cytarabine (Elacytarabine),[245] and a cardiolipin conjugate of gemcitabine.[246] None of them are designed to assemble into nanostructures. Recently, there have been increased efforts to use well-designed prodrugs, such as lipid-drug conjugates, or peptide-drug conjugates to create NP objects. An obvious advantage of these prodrug conjugates is that they have well-defined chemical structures, similar to those of small-molecule drugs; while PDCs often have molecular-weight distributions and/or multiple components in their nanostructures. Therefore, the in vivo studies of the degradation, metabolism, and excretion of these prodrugs are foreseen more straightforward than those of PDCs.

It is known that amphiphlic or lipid-like molecules could potentially self-assemble into supramolecular nanostructures.[247] Taking advantages of the self-assembly properties of these small molecules, an amphiphilic prodrug conjugate was synthesized by conjugating two hydrophobic Cpt molecules to a short oligo(ethylene glycol) as the hydrophilic segment via a biodegradable β-thioester bond. Such amphiphilic prodrug conjugates have high drug loading and form stable 100 nm nanoliposome (Figure 8a).[248] Similar approach was applied to synthesize amphiphilic PEG-block-dendritic polylysine–CPT conjugate that could assemble to nanorod.[249] Another reported strategy is to conjugate hydrophobic squalene to hydrophilic drugs or prodrugs to construct NPs.[250] Doxo, gemcitabine and other drugs were "squalenoylated" and formulated into ~100–150 nm sized NPs (Figure 8b).[251] An extreme strategy is recently reported to synthesize an amphiphilic drug-drug conjugate by directly connecting the hydrophilic anticancer drug irinotecan to the hydrophobic anticancer drug chlorambucil via a hydrolyzable ester linker, which can be assembled to NPs with ~80 nm size (Figure 8c).[252] Similar conjugate was synthesized between the hydrophilic drug floxuridine with the hydrophobic drug bendamustine.[253]

Besides amphiphilic molecules or lipids, another interesting molecule to prepare prodrug conjugates is the small peptide. It is known that small peptides can assemble into filamentous supramolecular structures.[254] Such peptide-drugs conjugates have been intensively studied to formulate hydrogel system, and are reviewed elsewhere.[250,255–258] A recent study demonstrate that a rationally designed peptide-Cpt conjugate be formulated to nanostructures for drug delivery. A β-sheet-forming peptide sequence derived from the tau protein was conjugated to Cpt via a redox-sensitive disulfylbutyrate linker, and the resulting nanostructures could vary from long filaments to short filaments and then to nanotubes with high drug loadings (Figure 8d).[259–261] Studies also showed the choice of the degradable linker between the peptide and Cpt affect the nanostructure. The carbonate linker is more preferred than ester since it minimizes the potential aggregation in cell culture, which could compromise Cpt's potency.[262]

4 | OUTLOOK

The routine clinical use of PEGylated proteins since 1990s and the recent large investments in ADCs overshadow the development of PDCs. Although so many interesting designs and impressive data presented in this review, there seems a long and arduous journey to bring more PDCs or NPs into clinical practice.[129,263] Several recent publications try to provide their solutions for the whole nanomedicine field.[264–266] Progress in the field will depend on a fundamental understanding of chemistry, materials science, biology, and clinical practice to allow rational design of optimized NPs of PDCs, tools for delivering them and measure outcomes. In terms of chemistry design, one has to bear in mind the clinical application and whole-organism pharmacokinetics. One example is the in vivo studies in ADCs revealed an in vitro stable linker may have unexpected instability in vivo and cause reduced efficacy.[267,268] Many of the first generation PDCs were developed before the concept of nanomedicine and the study of the relationship between NPs' sizes and their in vivo circulation and intratumoral accumulation; thus such PDCs have moderate molecular weight and small particle sizes (Table 1), which may result in some of the failure in clinical trials. In addition, there lack standard or optimized preclinical or clinical protocols to evaluate PDCs' stability, tumor penetration, metabolism, and toxicity.[269] The development of labeling/imaging technique and nano-device system may help monitor the in vivo use of PDCs. Of all note that many data obtained in animal models cannot be

easily translated into humans; in the frequent-used subcutaneous tumor xenografts the access of the blood to the tumor interstitium is greater than that in solid tumors in patients.[270,271] Furthermore, the advances in cancer biology can change the landscape of the field rapidly, as seen in the recent promising therapeutics in cancer immunology.[272,273] Last but not least, nontrivial optimization and engineering has a bearing on the eventual translation of NPs from preclinical experimental models to daily clinical practice.[274] The PDC and NP preparation should not require complex multistep processes; the scalability of NPs should not represent a problem in industry; the NP product should be sufficiently stable under storage and easy to use in clinics, that is, no complex administration protocols or regimens.[172,275] All of these prudent considerations will ensure that the field of PDC-based NPs reaches its full potential for clinical impact in cancer therapy.

CONFLICT OF INTERESTS

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

[1] Duncan R. Polymer conjugates as anticancer nanomedicines. *Nat Rev Cancer*. 2006;6:688–701.

[2] Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. *FASEB J*. 2005;19:311–330.

[3] Langer R. Drug delivery and targeting. *Nature*. 1998;392:5–10.

[4] Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. *Nat Rev Clin Oncol*. 2010;7:653–664.

[5] Xie J, Lee S, Chen XY. Nanoparticle-based theranostic agents. *Adv Drug Deliv Rev*. 2010;62:1064–1079.

[6] Cai W, Chen X. Nanoplatforms for targeted molecular imaging in living subjects. *Small*. 2007;3:1840–1854.

[7] Wagner V, Dullaart A, Bock AK, Zweck A. The emerging nanomedicine landscape. *Nat Biotechnol*. 2006;24:1211–1217.

[8] Weldon C, Tian B, Kohane DS. Nanotechnology for surgeons. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2011;3:223–228.

[9] Chow EKH, Ho D. Cancer nanomedicine: from drug delivery to imaging. *Sci Transl Med*. 2013;5:216rv4–216rv4.

[10] Matsumura Y, Maeda HA. New concept for macromolecular therapeutics in cancer-chemotherapy - mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS. *Cancer Res*. 1986;46:6387–6392.

[11] Hobbs SK, Monsky WL, Yuan F, et al. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. *Proc Natl Acad Sci USA*. 1998;95:4607–4612.

[12] Fang J, Nakamura H, Maeda H. The EPR effect: unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. *Adv Drug Deli Rev*. 2011;63:136–151.

[13] Gabizon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal doxorubicin - review of animal and human studies. *Clin Pharmacokinet*. 2003;42:419–436.

[14] Hawkins MJ, Soon-Shiong P, Desai N. Protein nanoparticles as drug carriers in clinical medicine. *Adv Drug Deliv Rev*. 2008;60:876–885.

[15] Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med*. 2013;369:1691–1703.

[16] Wang-Gillam A, Li C-P, Bodoky G, et al. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. *The Lancet*. 2016;387:545–557.

[17] Tong R, Cheng JJ. Anticancer polymeric nanomedicines. *Polymer Rev*. 2007;47:345–381.

[18] Haag R, Kratz F. Polymer therapeutics: concepts and applications. *Angew Chem Int Ed Engl*. 2006;45:1198–1215.

[19] Duncan R. The dawning era of polymer therapeutics. *Nat Rev Drug Discov*. 2003;2:347–360.

[20] Jatzkewitz H. An Ein Kolloidales Blutplasma-Ersatzmittel (Polyvinylpyrrolidon) Gebundenes Peptamin (Glycyl-L-Leucyl-Mezcalin) Als Neuartige Depotform Fur Biologisch Aktive Primare Amine (Mezcalin). *Zeitschrift Fur Naturforschung Part B-Chemie Biochemie Biophysik Biologie Und Verwandten Gebiete*. 1955;10:27–31.

[21] Kopecek J. Soluble biomedical polymers. *Polim Med*. 1977;7:191–221.

[22] Mathe G, Loc TB, Bernard J. Effet Sur La Leucemie 1210 De La Souris Dune Combinaison Par Diazotation Da-Methopterine Et De Gamma-Globulines De Hamsters Porteurs De Cette Leucemie Par Heterogreffe. *Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences*. 1958;246:1626–1628.

[23] Ringsdorf H. Sturcture and properties of pharmacologically active polymers. *J Polym Sci C Polym Symp*. 1975;135–153.

[24] Duncan R, Ringsdorf H, Satchi-Fainaro R. In: Satchi-Fainaro R, Duncan R, eds. *Polymer Therapeutics I*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2006:1-8.

[25] Abuchowski A, McCoy JR, Palczuk NC, Vanes T, Davis FF. Effect of covalent attachment of polyethylene-glycol on immunogenicity and circulating life of bovine liver catalase. *J Biol Chem*. 1977;252:3582–3586.

[26] Veronese FM. Peptide and protein PEGylation: a review of problems and solutions. *Biomaterials*. 2001;22:405–417.

[27] Alconcel SNS, Baas AS, Maynard HD. FDA-approved poly(ethylene glycol)-protein conjugate drugs. *Polym Chem*. 2011;2:1442–1448.

[28] Ulbrich K, Kopecek J. Radical polymerization of N-substitute methacrylamides. *Eur Polym J*. 1976;12:183–187.

[29] Kopeček J, Rejmanová P, Chytrý V. Polymers containing enzymatically degradable bonds, 1. Chymotrypsin catalyzed hydrolysis of p-nitroanilides of phenylalanine and tyrosine attached to side-chains of copolymers of N-(2-hydroxypropyl)methacrylamide. *Makromol Chem*. 1981;182:799–809.

[30] Duncan R, Kopeček J. Soluble synthetic polymers as potential drug carriers. *Adv Polym Sci*. 1984;57:51–101.

[31] Seymour LW, Ferry DR, Anderson D, et al. Hepatic drug targeting: Phase I evaluation of polymer-bound doxorubicin. *J Clin Oncol*. 2002;20:1668–1676.

[32] Vasey PA, Kaye SB, Morrison R, et al. Phase I clinical and pharmacokinetic study of PK1 N-(2-hydroxypropyl)methacrylamide copolymer doxorubicin: first member of a new class of chemotherapeutic agents - Drug-polymer conjugates. *Clin Cancer Res*. 1999;5:83–94.

[33] Seymour LW, Ferry DR, Kerr DJ, et al. Phase II studies of polymer-doxorubicin (PK1, FCE28068) in the treatment of breast, lung and colorectal cancer. *Int J Oncol*. 2009;34:1629–1636.

[34] Li C, Yu D-F, Newman RA, et al. Complete regression of well-established tumors using a novel water-soluble poly(L-glutamic acid)-paclitaxel conjugate. *Cancer Res.* 1998;58:2404–2409.

[35] Singer JW. Paclitaxel poliglumex (XYOTAX™, CT-2103): a macromolecular taxane. *J Control Release.* 2005;109:120–126.

[36] Homsi J, Simon GR, Garrett CR, et al. Phase I trial of poly-l-glutamate camptothecin (CT-2106) administered weekly in patients with advanced solid malignancies. *Clin Cancer Res.* 2007;13:5855–5861.

[37] Maeda H, Ueda M, Morinaga T, Matsumoto T. Conjugation of poly (styrene-co-maleic acid) derivatives to the antitumor protein neocarzinostatin: pronounced improvements in pharmacological properties. *J Med Chem.* 1985;28:455–461.

[38] Maeda H. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. *Adv Drug Deliv Rev.* 2001;46:169–185.

[39] Davis ME. Design and development of IT-101, a cyclodextrin-containing polymer conjugate of camptothecin. *Adv Drug Deliv Rev.* 2009;61:1189–1192.

[40] Pham E, Birrer MJ, Eliasof S, et al. Translational impact of nanoparticle–drug conjugate CRLX101 with or without bevacizumab in advanced ovarian cancer. *Clin Cancer Res.* 2015;21:808–818.

[41] Nakanishi T, Fukushima S, Okamoto K, et al. Development of the polymer micelle carrier system for doxorubicin. *J Control Release.* 2001;74:295–302.

[42] Cabral H, Kataoka K. Progress of drug-loaded polymeric micelles into clinical studies. *J Control Release.* 2014;190:465–476.

[43] Koizumi F, Kitagawa M, Negishi T, et al. Novel SN-38–incorporating polymeric micelles, NK012, eradicate vascular endothelial growth factor–secreting bulky tumors. *Cancer Res.* 2006;66:10048–10056.

[44] Nishiyama N, Yokoyama M, Aoyagi T, Okano T, Sakurai Y, Kataoka K. Preparation and characterization of self-assembled polymer−metal complex micelle from cis-dichlorodiammineplatinum(II) and poly(ethylene glycol)−poly(α,β-aspartic acid) block copolymer in an aqueous medium. *Langmuir.* 1999;15:377–383.

[45] Uchino H, Matsumura Y, Negishi T, et al. Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats. *Br J Cancer.* 2005;93:678–687.

[46] Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol.* 1995;146:1029–1039.

[47] Padera TP, Kadambi A, di Tomaso E, et al. Lymphatic metastasis in the absence of functional intratumor lymphatics. *Science.* 2002;296:1883–1886.

[48] Nichols JW, Bae YH. EPR: evidence and fallacy. *J Control Release.* 2014;190:451–464.

[49] Prabhakar U, Maeda H, Jain RK, et al. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. *Cancer Res.* 2013;73:2412–2417.

[50] Chauhan VP, Jain RK. Strategies for advancing cancer nanomedicine. *Nat Mater.* 2013;12:958–962.

[51] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144:646–674.

[52] Tong R, Langer R. Nanomedicines targeting the tumor microenvironment. *Cancer J.* 2015;21:314–321.

[53] Tong R, Cheng JJ. Ring-opening polymerization-mediated controlled formulation of polylactide-drug nanoparticles. *J Am Chem Soc.* 2009;131:4744–4754.

[54] Tong R, Yala LD, Fan TM, Cheng JJ. The formulation of aptamer-coated paclitaxel-polylactide nanoconjugates and their targeting to cancer cells. *Biomaterials.* 2010;31:3043–3053.

[55] Dawidczyk CM, Kim C, Park JH, et al. State-of-the-art in design rules for drug delivery platforms: lessons learned from FDA-approved nanomedicines. *J Control Release.* 2014;187:133–144.

[56] Yang J, Kopeček J. Design of smart HPMA copolymer-based nanomedicines. *J Control Release.* 2015;240:9–23.

[57] Posey JA, Saif MW, Carlisle R, et al. Phase 1 study of weekly polyethylene glycol-camptothecin in patients with advanced solid tumors and lymphomas. *Clin Cancer Res.* 2005;11:7866–7871.

[58] Sood P, Thurmond KB, Jacob JE, et al. Synthesis and characterization of AP5346, a novel polymer-linked diaminocyclohexyl platinum chemotherapeutic agent. *Bioconjugate Chem.* 2006;17:1270–1279.

[59] Nowotnik DP, Cvitkovic E. ProLindac™ (AP5346): a review of the development of an HPMA DACH platinum polymer therapeutic. *Adv Drug Deliv Rev.* 2009;61:1214–1219.

[60] Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. *Adv Drug Deliv Rev.* 2001;47:113–131.

[61] Meng FH, Zhong ZY, Feijen J. Stimuli-responsive polymersomes for programmed drug delivery. *Biomacromolecules.* 2009;10:197–209.

[62] Discher DE, Ortiz V, Srinivas G, et al. Emerging applications of polymersomes in delivery: from molecular dynamics to shrinkage of tumors. *Prog Polym Sci.* 2007;32:838–857.

[63] Lee CC, MacKay JA, Frechet JMJ, Szoka FC. Designing dendrimers for biological applications. *Nat Biotechnol.* 2005;23:1517–1526.

[64] Gao C, Yan D. Hyperbranched polymers: from synthesis to applications. *Prog Polym Sci.* 2004;29:183–275.

[65] Stiriba SE, Frey H, Haag R. Dendritic polymers in biomedical applications: from potential to clinical use in diagnostics and therapy. *Angew Chem Int Ed.* 2002;41:1329–1334.

[66] Kumar M. A review of chitin and chitosan applications. *React Funct Polym.* 2000;46:1–27.

[67] Gil ES, Hudson SM. Stimuli-reponsive polymers and their bioconjugates. *Prog Polym Sci.* 2004;29:1173–1222.

[68] Bellomo EG, Wyrsta MD, Pakstis L, Pochan DJ, Deming TJ. Stimuli-responsive polypeptide vesicles by conformation-specific assembly. *Nat Mater.* 2004;3:244–248.

[69] Liu ZH, Jiao YP, Wang YF, Zhou CR, Zhang ZY. Polysaccharides-based nanoparticles as drug delivery systems. *Adv Drug Deliv Rev.* 2008;60:1650–1662.

[70] Danhauser-Riedl S, Hausmann E, Schick H-D, et al. Phase I clinical and pharmacokinetic trial of dextran conjugated doxorubicin (AD-70, DOX-OXD). *Invest New Drugs.* 1993;11:187–195.

[71] Bernstein A, Hurwitz E, Maron R, Arnon R, Sela M, Wilchek M. Higher antitumor efficacy of daunomycin when linked to dextran: in vivo and in vitro studies. *J Natl Cancer Inst.* 1978;60:379–384.

[72] Shiah JG, Dvořák M, Kopečková P, Sun Y, Peterson CM, Kopeček J. Biodistribution and antitumour efficacy of long-circulating N-(2-hydroxypropyl)methacrylamide copolymer–doxorubicin conjugates in nude mice. *Eur J Cancer.* 2001;37:131–139.

[73] Kopeček J, Kopečková P, Minko T, Lu ZR, Peterson CM. Water soluble polymers in tumor targeted delivery. *J Control Release.* 2001;74:147–158.

[74] Seymour LW, Miyamoto Y, Maeda H, et al. Influence of molecular weight on passive tumor accumulation of a soluble macromolecular drug carrier. *Eur J Cancer.* 1995;31A:766–770.

[75] Paul A, Vicent MJ, Duncan R. Using small-angle neutron scattering to study the solution conformation of N-(2-hydroxypropyl)methacrylamide copolymer−doxorubicin conjugates. *Biomacromolecules*. 2007;8:1573–1579.

[76] Yamaoka T, Tabata Y, Ikada Y. Body distribution profile of polysaccharides after intravenous administration. *Drug Deliv*. 1993; 1:75–82.

[77] Dreher MR, Liu WG, Michelich CR, Dewhirst MW, Yuan F, Chilkoti A. Tumor vascular permeability, accumulation, and penetration of macromolecular drug carriers. *J Natl Cancer Inst*. 2006;98:335–344.

[78] Sadekar S, Ray A, Janàt-Amsbury M, Peterson CM, Ghandehari H. Comparative biodistribution of PAMAM dendrimers and HPMA copolymers in ovarian-tumor-bearing mice. *Biomacromolecules*. 2011;12:88–96.

[79] Ulbrich K, Šubr V. Structural and chemical aspects of HPMA copolymers as drug carriers. *Adv Drug Deliv Rev*. 2010;62: 150–166.

[80] Fox ME, Szoka FC, Fréchet JMJ. Soluble polymer carriers for the treatment of cancer: the importance of molecular architecture. *Acc Chem Res*. 2009;42:1141–1151.

[81] Bates FS, Fredrickson GH. Block copolymer thermodynamics - theory and experiment. *Annu Rev Phys Chem*. 1990;41:525–557.

[82] Thompson RB, Ginzburg VV, Matsen MW, Balazs AC. Predicting the mesophases of copolymer-nanoparticle composites. *Science*. 2001;292:2469–2472.

[83] Discher DE, Eisenberg A. Polymer vesicles. *Science*. 2002;297: 967–973.

[84] Zhang L, Eisenberg A. Multiple morphologies of "crew-cut" aggregates of polystyrene-b-poly(acrylic acid) block copolymers. *Science*. 1995;268:1728–1731.

[85] Letchford K, Burt H. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *Eur J Pharm Biopharm*. 2007;65:259–269.

[86] Cheng J, Teply BA, Sherifi I, et al. Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. *Biomaterials*. 2007;28:869–876.

[87] Al-Jamal WT, Al-Ahmady ZS, Kostarelos K. Pharmacokinetics & tissue distribution of temperature-sensitive liposomal doxorubicin in tumor-bearing mice triggered with mild hyperthermia. *Biomaterials*. 2012;33:4608–4617.

[88] Jiang SY, Cao ZQ. Ultralow-fouling, functionalizable, and hydrolyzable zwitterionic materials and their derivatives for biological applications. *Adv Mater*. 2010;22:920–932.

[89] Delplace V, Couvreur P, Nicolas J. Recent trends in the design of anticancer polymer prodrug nanocarriers. *Polym Chem*. 2014;5: 1529–1544.

[90] Nicolas J. Drug-initiated synthesis of polymer prodrugs: combining simplicity and efficacy in drug delivery. *Chem Mater*. 2016;28: 1591–1606.

[91] Doronina SO, Mendelsohn BA, Bovee TD, et al. Enhanced activity of monomethylauristatin F through monoclonal antibody delivery: effects of linker technology on efficacy and toxicity. *Bioconjugate Chem*. 2006;17:114–124.

[92] Sievers EL, Senter PD. Antibody-drug conjugates in cancer therapy. *Annu Rev Med*. 2013;64:15–29.

[93] Erickson HK, Park PU, Widdison WC, et al. Antibody-maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing. *Cancer Res*. 2006;66:4426–4433.

[94] Helmlinger G, Yuan F, Dellian M, Jain RK. Interstitial pH and pO2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. *Nat Med*. 1997;3:177–182.

[95] Höckel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst*. 2001;93: 266–276.

[96] Schumacker PT. Reactive oxygen species in cancer cells: live by the sword, die by the sword. *Cancer Cell*. 2006;10:175–176.

[97] Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell*. 141:52–67.

[98] Wang H, Tang L, Tu C, et al. Redox-responsive, core-cross-linked micelles capable of on-demand, concurrent drug release and structure disassembly. *Biomacromolecules*. 2013;14:3706–3712.

[99] Fleige E, Quadir MA, Haag R. Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: concepts and applications. *Adv Drug Deliv Rev*. 2012;64:866–884.

[100] Wang Y, Byrne JD, Napier ME, DeSimone JM. Engineering nanomedicines using stimuli-responsive biomaterials. *Adv Drug Deliv Rev*. 2012;64:1021–1030.

[101] Ge Z, Liu S. Functional block copolymer assemblies responsive to tumor and intracellular microenvironments for site-specific drug delivery and enhanced imaging performance. *Chem Soc Rev*. 2013; 42:7289–7325.

[102] Lee S, Saito K, Lee HR, et al. Hyperbranched double hydrophilic block copolymer micelles of poly(ethylene oxide) and polyglycerol for pH-responsive drug delivery. *Biomacromolecules*. 2012;13: 1190–1196.

[103] Roos A, Boron WF. Intracellular pH. *Physiol Rev*. 1981;61:296–434.

[104] Thomas JA, Buchsbaum RN, Zimniak A, Racker E. Intracellular pH measurements in ehrlich ascites tumor cells utilizing spectroscopic probes genetrated insitu. *Biochemistry*. 1979;18:2210–2218.

[105] Ulbrich K, Šubr V. Polymeric anticancer drugs with pH-controlled activation. *Adv Drug Deliv Rev*. 2004;56:1023–1050.

[106] Zou J, Zhang F, Zhang S, et al. Poly(ethylene oxide)-block-polyphosphoester-graft-paclitaxel conjugates with acid-labile linkages as a pH-sensitive and functional nanoscopic platform for paclitaxel delivery. *Adv Healthcare Mater*. 2014;3:441–448.

[107] Choy CJ, Geruntho JJ, Davis AL, Berkman CE. Tunable pH-sensitive linker for controlled release. *Bioconjugate Chem*. 2016;27: 824–830.

[108] Boghaert ER, Khandke KM, Sridharan L, et al. Determination of pharmacokinetic values of calicheamicin-antibody conjugates in mice by plasmon resonance analysis of small (5 μl) blood samples. *Cancer Chemother Pharmacol*. 2008;61:1027–1035.

[109] Lee CC, Cramer AT, Szoka FC, Fréchet JMJ. An intramolecular cyclization reaction is responsible for the in vivo inefficacy and apparent pH insensitive hydrolysis kinetics of hydrazone carboxylate derivatives of doxorubicin. *Bioconjugate Chem*. 2006;17:1364–1368.

[110] Hudecz F, Ross H, Price MR, Baldwin RW. Immunoconjugate design: a predictive approach for coupling of daunomycin to monoclonal antibodies. *Bioconjugate Chem*. 1990;1:197–204.

[111] Yoo HS, Lee EA, Park TG. Doxorubicin-conjugated biodegradable polymeric micelles having acid-cleavable linkages. *J Control Release*. 2002;82:17–27.

[112] Reményi J, Balázs B, Tóth S, Falus A, Tóth G, Hudecz F. Isomer-dependent daunomycin release and in vitro antitumour effect of cis-aconityl-daunomycin. *Biochem Biophys Res Commun*. 2003;303: 556–561.

[113] Kakinoki A, Kaneo Y, Ikeda Y, Tanaka T, Fujita K. Synthesis of poly (vinyl alcohol)-doxorubicin conjugates containing *cis*-aconityl acid-cleavable bond and its isomer dependent doxorubicin release. *Biol Pharm Bull*. 2008;31:103–110.

[114] Du C, Deng D, Shan L, et al. A pH-sensitive doxorubicin prodrug based on folate-conjugated BSA for tumor-targeted drug delivery. *Biomaterials*. 2013;34:3087–3097.

[115] Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med*. 2001;30:1191–1212.

[116] Saito G, Swanson JA, Lee KD. Drug delivery strategy utilizing conjugation via reversible disulfide linkages: role and site of cellular reducing activities. *Adv Drug Deliv Rev*. 2003;55:199–215.

[117] Kuppusamy P, Li H, Ilangovan G, et al. Noninvasive imaging of tumor redox status and its modification by tissue glutathione levels. *Cancer Res*. 2002;62:307–312.

[118] Gamcsik MP, Kasibhatla MS, Teeter SD, Colvin OM. Glutathione levels in human tumors. *Biomarkers*. 2012;17:671–691.

[119] Bartsch H, Petruzzelli S, De Flora S, et al. Carcinogen metabolism in human lung tissues and the effect of tobacco smoking: results from a case-control multicenter study on lung cancer patients. *Environ Health Perspect*. 1992;98:119–124.

[120] Baur G, Wendel A. The activity of the peroxide metabolizing system in human-colon carcinoma. *J Cancer Res Clin Oncol*. 1980;97: 267–273.

[121] Meng F, Hennink WE, Zhong Z. Reduction-sensitive polymers and bioconjugates for biomedical applications. *Biomaterials*. 2009;30: 2180–2198.

[122] Cuchelkar V, Kopečková P, Kopeček J. Synthesis and biological evaluation of disulfide-linked HPMA copolymer-mesochlorin e6 conjugates. *Macromol Biosci*. 2008;8:375–383.

[123] Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer*. 2002;2:161–174.

[124] Coussens LM, Fingleton B, Matrisian LM. Cancer therapy - matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science*. 2002;295:2387–2392.

[125] Hu J, Zhang G, Liu S. Enzyme-responsive polymeric assemblies, nanoparticles and hydrogels. *Chem Soc Rev*. 2012;41:5933–5949.

[126] Law B, Tung CH. Proteolysis: a biological process adapted in drug delivery, therapy, and imaging. *Bioconjug Chem*. 2009;20:1683–1695.

[127] Rejmanová P, Kopeček J, Pohl J, Baudyš M, Kostka V. Polymers containing enzymatically degradable bonds, 8. Degradation of oligopeptide sequences in N-(2-hydroxypropyl)methacrylamide copolymers by bovine spleen cathepsin B. *Makromol Chem*. 1983; 184:2009–2020.

[128] Kopecek J, Kopeckova P, Minko T, Lu ZR. HPMA copolymer-anticancer drug conjugates: design, activity, and mechanism of action. *Eur J Pharm Biopharm*. 2000;50:61–81.

[129] Duncan R, Vicent MJ. Do HPMA copolymer conjugates have a future as clinically useful nanomedicines? A critical overview of current status and future opportunities. *Adv Drug Deliv Rev*. 2010; 62:272–282.

[130] Senter PD, Sievers EL. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. *Nat Biotechnol*. 2012;30: 631–637.

[131] Chau Y, Tan FE, Langer R. Synthesis and characterization of dextran− peptide−methotrexate conjugates for tumor targeting via mediation by matrix metalloproteinase II and matrix metalloproteinase IX. *Bioconjugate Chem*. 2004;15:931–941.

[132] Chandran SS, Nan A, Rosen DM, Ghandehari H, Denmeade SR. A prostate-specific antigen–activated N-(2-hydroxypropyl) methacrylamide copolymer prodrug as dual-targeted therapy for prostate cancer. *Mol Cancer Ther*. 2007;6:2928–2937.

[133] Tong R, Kohane DS. Shedding light on nanomedicine. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2012;4:638–662.

[134] Tong R, Tang L, Ma L, Tu CL, Baumgartner R, Cheng JJ. Smart chemistry in polymeric nanomedicine. *Chem Soc Rev*. 2014;43: 6982–7012.

[135] Weissleder R. A clearer vision for in vivo imaging. *Nat Biotechnol*. 2001;19:316–317.

[136] Srinivasan S, Pogue BW, Jiang S, et al. Interpreting hemoglobin and water concentration, oxygen saturation, and scattering measured in vivo by near-infrared breast tomography. *Proc Natl Acad Sci USA*. 2003;100:12349–12354.

[137] Ntziachristos V, Ripoll J, Wang LHV, Weissleder R. Looking and listening to light: the evolution of whole-body photonic imaging. *Nat Biotechnol*. 2005;23:313–320.

[138] Denk W, Strickler JH, Webb WW. Two-photon laser scanning fluorescence microscopy. *Science*. 1990;248:73–76.

[139] Furuta T, Wang SSH, Dantzker JL, et al. Brominated 7-hydroxycoumarin-4-ylmethyls: photolabile protecting groups with biologically useful cross-sections for two photon photolysis. *Proc Natl Acad Sci USA*. 1999;96:1193–1200.

[140] Starkey JR, Rebane AK, Drobizhev MA, et al. New two-photon activated photodynamic therapy sensitizers induce xenograft tumor regressions after near-IR laser treatment through the body of the host mouse. *Am Assoc Cancer Res*. 2008;14:6564–6573.

[141] Pawlicki M, Collins HA, Denning RG, Anderson HL. Two-photon absorption and the design of two-photon dyes. *Angew Chem Int Ed Engl*. 2009;48:3244–3266.

[142] Gorka AP, Nani RR, Zhu J, Mackem S, Schnermann MJ. A near-IR uncaging strategy based on cyanine photochemistry. *J Am Chem Soc*. 2014;136:14153–14159.

[143] Nani RR, Gorka AP, Nagaya T, Kobayashi H, Schnermann MJ. Near-IR light-mediated cleavage of antibody–drug conjugates using cyanine photocages. *Angew Chem Int Ed*. 2015;54:13635–13638.

[144] Timko BP, Dvir T, Kohane DS. Remotely triggerable drug delivery systems. *Adv Mater Weinheim*. 2010;22:4925–4943.

[145] Jain PK, Huang XH, El-Sayed IH, El-Sayed MA. Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. *Acc Chem Res*. 2008;41:1578–1586.

[146] Wang F, Banerjee D, Liu YS, Chen XY, Liu XG. Upconversion nanoparticles in biological labeling, imaging, and therapy. *Analyst*. 2010;135:1839–1854.

[147] Longmire M, Choyke PL, Kobayashi H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. *Nanomedicine (Lond)*. 2008;3:703–717.

[148] Kohane DS. Microparticles and nanoparticles for drug delivery. *Biotechnol Bioeng*. 2007;96:203–209.

[149] Cabral H, Matsumoto Y, Mizuno K, et al. Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. *Nat Nanotechnol*. 2011;6:815–823.

[150] Choi HS, Liu W, Misra P, et al. Renal clearance of quantum dots. *Nat Biotechnol*. 2007;25:1165–1170.

[151] Tan JF, Shah S, Thomas A, Ou-Yang HD, Liu YL. The influence of size, shape and vessel geometry on nanoparticle distribution. *Microfluid Nanofluid*. 2013;14:77–87.

[152] Shah NB, Vercellotti GM, White JG, Fegan A, Wagner CR, Bischof JC. Blood–nanoparticle interactions and in vivo biodistribution: impact of surface PEG and ligand properties. *Mol Pharm*. 2012;9:2146–2155.

[153] Allen TM, Hansen C, Martin F, Redemann C, Yauyoung A. Liposomes containing synthetic lipid derivatives of poly (ethylene glycol) show prolonged circulation half-lives in vivo. *Biochim Biophys Acta*. 1991;1066:29–36.

[154] Allen TM. The use of glycolipids and hydrophilic polymers in avoiding rapid uptake of liposomes by the mononuclear phagocyte system. *Adv Drug Deliv Rev*. 1994;13:285–309.

[155] Papahadjopoulos D, Allen TM, Gabizon A, et al. Sterically stabilized liposomes - improvements in pharmacokinetics and antitumor therapeutic efficacy. *Proc Natl Acad Sci USA*. 1991;88:11460–11464.

[156] Walkey CD, Olsen JB, Guo H, Emili A, Chan WCW. Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake. *J Am Chem Soc*. 2012;134:2139–2147.

[157] Perrault SD, Walkey C, Jennings T, Fischer HC, Chan WCW. Mediating tumor targeting efficiency of nanoparticles through design. *Nano Lett*. 2009;9:1909–1915.

[158] Albanese A, Tang PS, Chan WCW. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu Rev Biomed Eng*. 2012;14:1–16.

[159] Ishida T, Kiwada H. Accelerated blood clearance (ABC) phenomenon upon repeated injection of PEGylated liposomes. *Int J Pharm*. 2008;354:56–62.

[160] Yang Q, Lai SK. Anti-PEG immunity: emergence, characteristics, and unaddressed questions. *WIREs Nanomed Nanobiotechnol*. 2015;7:655–677.

[161] Tang L, Yang X, Yin Q, et al. Investigating the optimal size of anticancer nanomedicine. *Proc Natl Acad Sci USA*. 2014;111:15344–15349.

[162] Matsumoto Y, Nichols JW, Toh K, et al. Vascular bursts enhance permeability of tumour blood vessels and improve nanoparticle delivery. *Nat Nanotech*. 2016;11:533–538.

[163] Smith BR, Kempen P, Bouley D, et al. Shape matters: intravital microscopy reveals surprising geometrical dependence for nanoparticles in tumor models of extravasation. *Nano Lett*. 2012;12:3369–3377.

[164] Gratton SEA, Ropp PA, Pohlhaus PD, et al. The effect of particle design on cellular internalization pathways. *Proc Natl Acad Sci USA*. 2008;105:11613–11618.

[165] Diop-Frimpong B, Chauhan VP, Krane S, Boucher Y, Jain RK. Losartan inhibits collagen I synthesis and improves the distribution and efficacy of nanotherapeutics in tumors. *Proc Natl Acad Sci USA*. 2011;108:2909–2914.

[166] Stylianopoulos T, Martin JD, Chauhan VP, et al. Causes, consequences, and remedies for growth-induced solid stress in murine and human tumors. *Proc Natl Acad Sci USA*. 2012;109:15101–15108.

[167] Laakkonen P, Porkka K, Hoffman JA, Ruoslahti E. A tumor-homing peptide with a targeting specificity related to lymphatic vessels. *Nat Med*. 2002;8:751–755.

[168] Sugahara KN, Teesalu T, Karmali PP, et al. Coadministration of a tumor-penetrating peptide enhances the efficacy of cancer drugs. *Science*. 2010;328:1031–1035.

[169] Trapani G, Denora N, Trapani A, Laquintana V. Recent advances in ligand targeted therapy. *J Drug Target*. 2012;20:1–22.

[170] Oh E, Delehanty JB, Sapsford KE, et al. Cellular uptake and fate of PEGylated gold nanoparticles is dependent on both cell-penetration peptides and particle size. *ACS Nano*. 2011;5:6434–6448.

[171] Sasaki K, Kogure K, Chaki S, et al. An artificial virus-like nano carrier system: enhanced endosomal escape of nanoparticles via synergistic action of pH-sensitive fusogenic peptide derivatives. *Anal Bioanal Chem*. 2008;391:2717–2727.

[172] Torchilin VP. Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. *Nat Rev Drug Discov*. 2014;13:813–827.

[173] Hu-Lieskovan S, Heidel JD, Bartlett DW, Davis ME, Triche TJ. Sequence-specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma. *Cancer Res*. 2005;65:8984–8992.

[174] Bartlett DW, Su H, Hildebrandt IJ, Weber WA, Davis ME. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging. *Proc Natl Acad Sci USA*. 2007;104:15549–15554.

[175] Kirpotin DB, Drummond DC, Shao Y, et al. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. *Cancer Res*. 2006;66:6732–6740.

[176] Gu F, Zhang L, Teply BA, et al. Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. *Proc Natl Acad Sci USA*. 2008;105:2586–2591.

[177] Hak S, Helgesen E, Hektoen HH, et al. The effect of nanoparticle polyethylene glycol surface density on ligand-directed tumor targeting studied in vivo by dual modality imaging. *ACS Nano*. 2012;6:5648–5658.

[178] Jiang W, KimBetty YS, Rutka JT, ChanWarren CW. Nanoparticle-mediated cellular response is size-dependent. *Nat Nanotechnol*. 2008;3:145–150.

[179] Iversen TG, Skotland T, Sandvig K. Endocytosis and intracellular transport of nanoparticles: present knowledge and need for future studies. *Nano Today*. 2011;6:176–185.

[180] Sahay G, Alakhova DY, Kabanov AV. Endocytosis of nanomedicines. *J Control Release*. 2010;145:182–195.

[181] Chithrani BD, Chan WCW. Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Lett*. 2007;7:1542–1550.

[182] Chiu HC, Kopečková P, Deshmane SS, Kopeček J. Lysosomal degradability of poly(α-amino acids). *J Biomed Mater Res*. 1997;34:381–392.

[183] Chiu HC, Koňák C, Kopečková P, KopeČek J. Enzymatic degradation of poly(ethylene glycol) modified dextrans. *J Bioact Compat Polym*. 1994;9:388–410.

[184] Lloyd JB. Lysosome membrane permeability: implications for drug delivery. *Adv Drug Deliv Rev*. 2000;41:189–200.

[185] Callahan J, Kopečková P, Kopeček J. Intracellular trafficking and subcellular distribution of a large array of HPMA copolymers. *Biomacromolecules*. 2009;10:1704–1714.

[186] Liu J, Liu W, Weitzhandler I, et al. Ring-opening polymerization of prodrugs: a versatile approach to prepare well-defined drug-loaded nanoparticles. *Angew Chem Int Ed Engl*. 2015;54:1002–1006.

[187] Bertin PA, Smith D, Nguyen ST. High-density doxorubicin-conjugated polymeric nanoparticles via ring-opening metathesis polymerization. *Chem Commun*. 2005;3793–3795.

[188] Rao NV, Mane S, Kishore A, Das Sarma J, Shunmugam R. Norbornene derived doxorubicin copolymers as drug carriers with pH responsive hydrazone linker. *Biomacromolecules*. 2012;13:221–230.

[189] Liao L, Liu J, Dreaden EC, et al. A convergent synthetic platform for single-nanoparticle combination cancer therapy: ratiometric loading and controlled release of cisplatin, doxorubicin, and camptothecin. *J Am Chem Soc*. 2014;136:5896–5899.

[190] Gao AX, Liao L, Johnson JA. Synthesis of acid-labile PEG and PEG-doxorubicin-conjugate nanoparticles via brush-first ROMP. *ACS Macro Lett*. 2014;3:854–857.

[191] Hu X, Hu J, Tian J, et al. Polyprodrug amphiphiles: hierarchical assemblies for shape-regulated cellular internalization, trafficking, and drug delivery. *J Am Chem Soc*. 2013;135:17617–17629.

[192] Rosario-Meléndez R, Yu W, Uhrich KE. Biodegradable polyesters containing ibuprofen and naproxen as pendant groups. *Biomacromolecules*. 2013;14:3542–3548.

[193] Ouimet MA, Griffin J, Carbone-Howell AL, et al. Biodegradable ferulic acid-containing poly(anhydride-ester): degradation products with controlled release and sustained antioxidant activity. *Biomacromolecules*. 2013;14:854–861.

[194] Rosario-Meléndez R, Harris CL, Delgado-Rivera R, Yu L, Uhrich KE. PolyMorphine: an innovative biodegradable polymer drug for extended pain relief. *J Control Release*. 2012;162:538–544.

[195] Zhang Y, Yin Q, Yin L, Ma L, Tang L, Cheng J. Chain-shattering polymeric therapeutics with on-demand drug-release capability. *Angew Chem Int Ed Engl*. 2013;52:6435–6439.

[196] Cai K, Yen J, Yin Q, et al. Redox-responsive self-assembled chain-shattering polymeric therapeutics. *Biomater Sci*. 2015;3:1061–1065.

[197] Dougherty TJ, Gomer CJ, Henderson BW, et al. Photodynamic therapy. *J Natl Cancer Inst*. 1998;90:889–905.

[198] Ochsner M. Photophysical and photobiological processes in the photodynamic therapy of tumours. *J Photochem Photobiol B*. 1997;39:1–18.

[199] McCarthy JR, Perez JM, Brückner C, Weissleder R. Polymeric nanoparticle preparation that eradicates tumors. *Nano Lett*. 2005;5:2552–2556.

[200] Vrouenraets MB, Visser GWM, Snow GB, van Dongen G. Basic principles, applications in oncology and improved selectivity of photodynamic therapy. *Anticancer Res*. 2003;23:505–522.

[201] Carcenac M, Dorvillius M, Garambois V, et al. Internalisation enhances photo-induced cytotoxicity of monoclonal antibody-phthalocyanine conjugates. *Br J Cancer*. 2001;85:1787–1793.

[202] Vrouenraets MB, Visser GWM, Stewart FA, et al. Development of meta-tetrahydroxyphenylchlorin-monoclonal antibody conjugates for photoimmunotherapy. *Cancer Res*. 1999;59:1505–1513.

[203] Castano AP, Mroz P, Hamblin MR. Photodynamic therapy and anti-tumour immunity. *Nat Rev Cancer*. 2006;6:535–545.

[204] Giuntini F, Alonso CMA, Boyle RW. Synthetic approaches for the conjugation of porphyrins and related macrocycles to peptides and proteins. *Photochem Photobiol Sci*. 2011;10:759–791.

[205] Mew D, Wat CK, Towers GH, Levy JG. Photoimmunotherapy: treatment of animal tumors with tumor-specific monoclonal antibody-hematoporphyrin conjugates. *J Immunol*. 1983;130:1473–1477.

[206] Matthew Peterson C, et al. Combination chemotherapy and photodynamic therapy with N-(2-hydroxypropyl)methacrylamide copolymer-bound anticancer drugs inhibit human ovarian carcinoma heterotransplanted in nude mice. *Cancer Res*. 1996;56:3980–3985.

[207] Hamblin MR, Miller JL, Rizvi I, Ortel B, Maytin EV, Hasan T. Pegylation of a chlorin(e6) polymer conjugate increases tumor targeting of photosensitizer. *Cancer Res*. 2001;61:7155–7162.

[208] van Dongen GAMS, Visser GWM, Vrouenraets MB. Photosensitizer-antibody conjugates for detection and therapy of cancer. *Adv Drug Deliv Rev*. 2004;56:31–52.

[209] Soukos NS, Hamblin MR, Hasan T. The effect of charge on cellular uptake and phototoxicity of polylysine chlorin(e6) conjugates. *Photochem Photobiol*. 1997;65:723–729.

[210] Hamblin MR, Miller JL, Rizvi I, Loew HG, Hasan T. Pegylation of charged polymer-photosensitiser conjugates: effects on photodynamic efficacy. *Br J Cancer*. 2003;89:937–943.

[211] Campo MA, Gabriel D, Kucera P, Gurny R, Lange N. Polymeric photosensitizer prodrugs for photodynamic therapy. *Photochem Photobiol*. 2007;83:958–965.

[212] Chen J, Stefflova K, Niedre MJ, et al. Protease-triggered photosensitizing beacon based on singlet oxygen quenching and activation. *J Am Chem Soc*. 2004;126:11450–11451.

[213] Choi Y, Weissleder R, Tung CH. Selective antitumor effect of novel protease-mediated photodynamic agent. *Cancer Res*. 2006;66:7225–7229.

[214] Lovell JF, Liu TWB, Chen J, Zheng G. Activatable photosensitizers for imaging and therapy. *Chem Rev*. 2010;110:2839–2857.

[215] Kong G, Braun RD, Dewhirst MW. Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature. *Cancer Res*. 2001;61:3027–3032.

[216] Lovell JF, Liu TWB, Chen J, Zheng G. Porphysome nanovesicles generated by porphyrin bilayers for use as multimodal biophotonic contrast agents. *Nat Mater*. 2011;10:324–332.

[217] Carter KA, Shao S, Hoopes MI, et al. Porphyrin–phospholipid liposomes permeabilized by near-infrared light. *Nat Commun*. 2014;5:3546.

[218] Jiang H, Taranekar P, Reynolds JR, Schanze KS. Conjugated polyelectrolytes: synthesis, photophysics, and applications. *Angew Chem Int Ed*. 2009;48:4300–4316.

[219] Feng LH, Zhu CL, Yuan HX, Liu LB, Lv FT, Wang S. Conjugated polymer nanoparticles: preparation, properties, functionalization and biological applications. *Chem Soc Rev*. 2013;42:6620–6633.

[220] Lu LD, Rininsland FH, Wittenburg SK, Achyuthan KE, McBranch DW, Whitten DG. Biocidal activity of a light-absorbing fluorescent conjugated polyelectrolyte. *Langmuir*. 2005;21:10154–10159.

[221] Xing C, Xu Q, Tang H, Liu L, Wang S. Conjugated polymer/porphyrin complexes for efficient energy transfer and improving light-activated antibacterial activity. *J Am Chem Soc*. 2009;131:13117–13124.

[222] Yuan YY, Liu J, Liu B. Conjugated-polyelectrolyte-based polyprodrug: targeted and image-guided photodynamic and chemotherapy with on-demand drug release upon irradiation with a single light source. *Angew Chem Int Ed*. 2014;53:7163–7168.

[223] Chilkoti A, Dreher MR, Meyer DE, Raucher D. Targeted drug delivery by thermally responsive polymers. *Adv Drug Deliv Rev*. 2002;54:613–630.

[224] Meyer DE, Kong GA, Dewhirst MW, Zalutsky MR, Chilkoti A. Targeting a genetically engineered elastin-like polypeptide to solid tumors by local hyperthermia. *Cancer Res*. 2001;61:1548–1554.

[225] Liu W, Dreher MR, Furgeson DY, et al. Tumor accumulation, degradation and pharmacokinetics of elastin-like polypeptides in nude mice. *J Control Release*. 2006;116:170–178.

[226] Dreher MR, Liu WG, Michelich CR, Dewhirst MW, Chilkoti A. Thermal cycling enhances the accumulation of a temperature-sensitive biopolymer in solid tumors. *Cancer Res*. 2007;67:4418–4424.

[227] Furgeson DY, Dreher MR, Chilkoti A. Structural optimization of a "smart" doxorubicin-polypeptide conjugate for thermally targeted delivery to solid tumors. *J Control Release*. 2006;110:362–369.

[228] MacKay JA, Chen MN, McDaniel JR, Liu WG, Simnick AJ, Chilkoti A. Self-assembling chimeric polypeptide-doxorubicin conjugate nanoparticles that abolish tumours after a single injection. *Nat Mater*. 2009;8:993–999.

[229] McDaniel JR, MacEwan SR, Li X, et al. Rational design of "heat seeking" drug loaded polypeptide nanoparticles that thermally target solid tumors. *Nano Lett*. 2014;14:2890–2895.

[230] Brown E, McKee T, diTomaso E, et al. Dynamic imaging of collagen and its modulation in tumors in vivo using second-harmonic generation. *Nat Med*. 2003;9:796–800.

[231] Netti PA, Berk DA, Swartz MA, Grodzinsky AJ, Jain RK. Role of extracellular matrix assembly in interstitial transport in solid tumors. *Cancer Res*. 2000;60:2497–2503.

[232] Chauhan VP, Stylianopoulos T, Martin JD, et al. Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. *Nat Nanotechnol*. 2012;7:383–388.

[233] Wong C, Stylianopoulos T, Cui JA, et al. Multistage nanoparticle delivery system for deep penetration into tumor tissue. *Proc Natl Acad Sci USA*. 2011;108:2426–2431.

[234] Tong R, Hemmati HD, Langer R, Kohane DS. Photoswitchable nanoparticles for triggered tissue penetration and drug delivery. *J Am Chem Soc*. 2012;134:8848–8855.

[235] Tong R, Chiang HH, Kohane DS. Photoswitchable nanoparticles for in vivo cancer chemotherapy. *Proc Natl Acad Sci USA*. 2013; 110:19048–19053.

[236] Park YS, Maruyama K, Huang L. Some negatively charged phospholipid derivatives prolong the liposome circulation in vivo. *Biochim Biophys Acta*. 1992;1108:257–260.

[237] Torchilin VP, Omelyanenko VG, Papisov MI, et al. Poly(ethylene glycol) on the liposome surface: on the mechanism of polymer-coated liposome longevity. *Biochim Biophys Acta*. 1994;1195:11–20.

[238] Hirn S, Semmler-Behnke M, Schleh C, et al. Particle size-dependent and surface charge-dependent biodistribution of gold nanoparticles after intravenous administration. *Eur J Pharm Biopharm*. 2011;77:407–416.

[239] Xiao K, Li Y, Luo J, et al. The effect of surface charge on in vivo biodistribution of PEG-oligocholic acid based micellar nanoparticles. *Biomaterials*. 2011;32:3435–3446.

[240] Dellian M, Yuan F, Trubetskoy VS, Torchilin VP, Jain RK. Vascular permeability in a human tumour xenograft: molecular charge dependence. *Br J Cancer*. 2000;82:1513–1518.

[241] Harush-Frenkel O, Debotton N, Benita S, Altschuler Y. Targeting of nanoparticles to the clathrin-mediated endocytic pathway. *Biochem Biophys Res Commun*. 2007;353:26–32.

[242] Thorek DLJ, Tsourkas A. Size, charge and concentration dependent uptake of iron oxide particles by non-phagocytic cells. *Biomaterials*. 2008;29:3583–3590.

[243] Du JZ, Du XJ, Mao CQ, Wang J. Tailor-made dual pH-sensitive polymer–doxorubicin nanoparticles for efficient anticancer drug delivery. *J Am Chem Soc*. 2011;133:17560–17563.

[244] Bedikian AY, DeConti RC, Conry R, et al. Phase 3 study of docosahexaenoic acid–paclitaxel versus dacarbazine in patients with metastatic malignant melanoma. *Ann Oncol*. 2011;22:787–793.

[245] Roboz GJ, Rosenblat T, Arellano M, et al. International randomized phase III study of elacytarabine versus investigator choice in patients with relapsed/refractory acute myeloid leukemia. *J Clin Oncol*. 2014;32:1919–1926.

[246] Moysan E, Bastiat G, Benoit JP. Gemcitabine versus modified gemcitabine: a review of several promising chemical modifications. *Mol Pharm*. 2013;10:430–444.

[247] Stupp SI, LeBonheur V, Walker K, et al. Supramolecular materials: self-organized nanostructures. *Science*. 1997;276:384–389.

[248] Shen Y, Jin E, Zhang B, et al. Prodrugs forming high drug loading multifunctional nanocapsules for intracellular cancer drug delivery. *J Am Chem Soc*. 2010;132:4259–4265.

[249] Zhou Z, Ma X, Jin E, et al. Linear-dendritic drug conjugates forming long-circulating nanorods for cancer-drug delivery. *Biomaterials*. 2013;34:5722–5735.

[250] Vemula PK, Ma X, Jin E, et al. Prodrugs as self-assembled hydrogels: a new paradigm for biomaterials. *Curr Opin Biotechnol*. 2013;24: 1174–1182.

[251] Maksimenko A, Dosio F, Mougin J, et al. A unique squalenoylated and nonpegylated doxorubicin nanomedicine with systemic long-circulating properties and anticancer activity. *Proc Natl Acad Sci USA*. 2014;111:E217–E226.

[252] Huang P, Wang D, Su Y, et al. Combination of small molecule prodrug and nanodrug delivery: amphiphilic drug–drug conjugate for cancer therapy. *J Am Chem Soc*. 2014;136:11748–11756.

[253] Zhang T, Huang P, Shi L, et al. Self-assembled nanoparticles of amphiphilic twin drug from floxuridine and bendamustine for cancer therapy. *Mol Pharm*. 2015;12:2328–2336.

[254] Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphile nanofibers. *Science*. 2001;294:1684–1688.

[255] Estroff LA, Hamilton AD. Water gelation by small organic molecules. *Chem Rev*. 2004;104:1201–1218.

[256] Appel EA, del Barrio J, Loh XJ, Scherman OA. Supramolecular polymeric hydrogels. *Chem Soc Rev*. 2012;41:6195–6214.

[257] Du X, Zhou J, Xu B. Supramolecular hydrogels made of basic biological building blocks. *Chem Asian J*. 2014;9:1446–1472.

[258] Zhao F, Ma ML, Xu B. Molecular hydrogels of therapeutic agents. *Chem Soc Rev*. 2009;38:883–891.

[259] Cheetham AG, Zhang P, Lin Ya, Lock LL, Cui H. Supramolecular nanostructures formed by anticancer drug assembly. *J Am Chem Soc*. 2013;135:2907–2910.

[260] Chen Z, Zhang P, Cheetham AG, et al. Controlled release of free doxorubicin from peptide–drug conjugates by drug loading. *J Control Release*. 2014;191:123–130.

[261] Lock LL, Tang Z, Keith D, Reyes C, Cui H. Enzyme-specific doxorubicin drug beacon as drug-resistant theranostic molecular probes. *ACS Macro Lett*. 2015;4:552–555.

[262] Cheetham AG, Ou YC, Zhang P, Cui H. Linker-determined drug release mechanism of free camptothecin from self-assembling drug amphiphiles. *Chem Commun (Camb)*. 2014;50: 6039–6042.

[263] Min Y, Caster JM, Eblan MJ, Wang AZ. Clinical translation of nanomedicine. *Chem Rev*. 2015;115:11147–11190.

[264] Crommelin DJA, Florence AT. Towards more effective advanced drug delivery systems1. *Int J Pharm*. 2013;454:496–511.

[265] Venditto VJ, Szoka Jr F. C. Cancer nanomedicines: So many papers and so few drugs!. *Adv Drug Deliv Rev*. 2013;65:80–88.

[266] Wilhelm S, Tavares AJ, Dai Q, et al. Analysis of nanoparticle delivery to tumours. *Nat Rev Mater*. 2016;1:16014.

[267] Shen BQ, Xu K, Liu L, et al. Conjugation site modulates the in vivo stability and therapeutic activity of antibody-drug conjugates. *Nat Biotechnol*. 2012;30:184–189.

[268] Lyon RP, Setter JR, Bovee TD, et al. Self-hydrolyzing maleimides improve the stability and pharmacological properties of antibody-drug conjugates. *Nat Biotechnol*. 2014;32:1059–1062.

[269] Lammers T, Kiessling F, Hennink WE, Storm G. Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress. *J Control Release*. 2012;161:175–187.

[270] Killion JJ, Radinsky R, Fidler IJ. Orthotopic models are necessary to predict therapy of transplantable tumors in mice. *Cancer Metastasis Rev*. 1998;17:279–284.

[271] Richmond A, Su YJ. Mouse xenograft models vs GEM models for human cancer therapeutics. *Dis Model Mech*. 2008;1:78–82.

[272] Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature*. 2011;480:480–489.

[273] Irvine DJ, Hanson MC, Rakhra K, Tokatlian T. Synthetic nanoparticles for vaccines and immunotherapy. *Chem Rev*. 2015;115:11109–11146.

[274] Gaspar R, Duncan R. Polymeric carriers: preclinical safety and the regulatory implications for design and development of polymer therapeutics. *Adv Drug Deliv Rev*. 2009;61:1220–1231.

[275] Tong R, Kohane DS. New strategies in cancer nanomedicine. *Annu Rev Pharmacol Toxicol*. 2016;56:41–57.