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ADVANCED REVIEW

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Reduction-responsive polymers for drug delivery in cancer therapy—Is there anything new to discover?

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Abstract

Among various types of stimuli-responsive drug delivery systems, reduction-responsive polymers have attracted great interest. In general, these systems have high stability in systemic circulation, however, they can respond quickly to differences in the concentrations of reducing species in specific physiological sites associated with a pathology. This is a particularly relevant strategy to target diseases in which hypoxic regions are present, as polymers which are sensitive to in-situ expressed antioxidant species can, through a local response, release a therapeutic at high concentration in the targeted site, and thus, improve the selectivity and efficacy of the treatment. At the same time, such reduction-responsive materials can also decrease the toxicity and side effects of certain drugs. To date, polymers containing disulfide linkages are the most investigated of the class of reduction-responsive nanocarriers, however, other groups such as selenide and diselenide have also been used for the same purpose. In this review article, we discussed the rationale behind the development of reduction-responsive polymers as drug delivery systems and highlight examples of recent progress. We include the most popular design methods to generate reduction-responsive polymeric carriers and their applications in cancer therapy, and question what areas may still need to be explored in a field with already a very large number of research articles. Finally, we consider the main challenges associated with the clinical translation of these nanocarriers and the future perspectives in this area.

This article is categorized under:

Therapeutic Approaches and Drug Discovery > Nanomedicine for Oncologic Disease

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KEYWORDS

cancer nanotechnology, disulfide, drug delivery system, glutathione, micelles, nanomedicine, redox-responsive nanoparticles, reduction-responsive polymers, stimuli-responsive, tumor

1 | INTRODUCTION

Polymeric delivery systems have emerged as important tools to enhance the therapeutic efficacy of many drug compounds. This is because a number of candidate molecules are sub-optimal for patients due to undesirable pharmacokinetics and poor distribution, which lead to rapid drug clearance or extravasation into healthy tissue, or dose-limiting side effects and toxicity (Senapati, Mahanta, Kumar, & Maiti, 2018). The knock-on effects of this are issues regarding the therapeutic effect of the drug, as a consequence of the administration of suboptimal doses. This scenario is a common problem, especially in cancer treatment with widely-used current cytotoxic drugs (Allen & Cullis, 2004). Various delivery systems have been developed over many years to overcome these shortcomings (Jain & Stylianopoulos, 2010; Nie, Xing, Kim, & Simons, 2007). Nanoscale drug delivery systems containing diverse surface properties, architectures, and sizes have been designed, and have enabled advances in site-specific targeting and controlled release of anti-cancer agents.

Conventional drug delivery systems, also referred to as first generation nanotherapeutics, offer numerous advantages, including improved bioavailability and half-life of hydrophobic drugs, decreased immunogenic response and reduced side effects (J. Shi, Votruba, Farokhzad, & Langer, 2010). Despite many laboratory successes and encouraging results in preclinical animal models from these first generation nanotherapeutics, the clinical translation of both passive and active nanocarriers have encountered problems, of which nonspecific biodistribution and lack of control of drug release from the targeting nanocarriers are such examples (Liu, Yang, Xiong, & Gu, 2016; Mura, Nicolas, & Couvreur, 2013). Stimuli-responsive polymeric drug delivery systems have emerged as a promising platform for on-demand or triggered release, based on various exogenous and endogenous changes which can be categorized as physical, chemical, or biological depending upon the origins of the stimuli. Physical phenomena which can alter the chain dynamics of polymers includes light, magnetic fields, ultrasound, temperature, and so on (Pelaz et al., 2017). Chemical stimuli include redox-potential, pH, and ionic strength, which can modify the molecular interactions between polymer chains or cleave polymer-drug links (Traitel, Goldbart, & Kost, 2008). In contrast, agents of biological change, such as enzymes and receptors, can trigger more specific (bio)chemical reactions, and ligand-recognition mediated signaling, in order to actuate drug release or induce selective carrier internalization (Hu, Katti, & Gu, 2014). Bioreducible delivery systems in particular have been applied successfully in anti-viral, vaccine, nucleic acid, and peptide delivery (Becker et al., 2011; Blakney et al., 2020; Bulmus et al., 2003; Riber, Smith, & Zelikin, 2015; Soliman et al., 2012). These systems include, but are not limited to, polymeric nanoparticles, micelles, liposomes, dendrimers, and a broad of inorganic nanoparticles, such as quantum dots, iron oxide, and gold and metal nanoparticles (Mura et al., 2013).

A major focus in the design of stimuli-responsive nanocarriers has been to target cancer through treatment and diagnostic approaches, due to the numerous issues related to conventional chemotherapy and thus, improve the performance of the administered drugs (Rao, Ko, Lee, & Park, 2018). Regarding the tumor microenvironment specifically, the low pH, elevated temperature, redox, and reactive oxygen species (ROS) levels, and existence of certain overexpressed enzymes have been investigated intensively (Locatelli-Champagne, Suau, Guerret, Pellet, & Cloitre, 2017; Pelaz et al., 2017) (Han et al., 2019; R. Li & Xie, 2017; Z. Wu et al., 2019; Yu, Zhang, Du, & Li, 2018; M. Zhang et al., 2016).

Polymeric drug delivery systems sensitive to redox processes are generally designed to respond to internal microenvironmental stimuli, by taking advantage of the high gradient levels of glutathione (GSH) present in some type of cancers (Qiao et al., 2019). Reduction-responsive polymeric nanocarriers have been widely explored and are generally designed from polymers containing disulfide linkages that undergo rapid cleavage in the presence of reducing agents, particularly in the intracellular components of tumor cells (Quinn, Whittaker, & Davis, 2017). In some approaches, disulfide bridges can be incorporated into the backbone or into side chains, or through the insertion of a crosslinker (Locatelli-Champagne et al., 2017; Pelaz et al., 2017).

In this review, we discuss reduction-based polymeric drug delivery systems with special reference to the tumor-target applications. A brief discussion on the rationale behind the development of these drug delivery systems is included along with diverse examples of progress made to the field, including recent reports and a discussion of the versatile ways to design reduction-responsive polymeric carriers. Toward the end of this article, examples regarding the preclinical efficacy of some reduction-responsive polymeric drug delivery systems and the main challenges associated with the clinical translation of the nanocarriers are also included along with the future perspectives in the area.

2 | RATIONALE OF DIFFERENT STIMULI-RESPONSIVE POLYMERIC NANOPLATFORMS

It has been long-established that GSH can be found at high concentrations (approximately 5 mM) in most cells and it is the most ubiquitous low molecular weight thiol-functional biomolecule. GSH is a tripeptide antioxidant composed of cysteine, glycine, and glutamic acid (Figure 1) and is responsible for a number of physiological functions, including maintenance of cellular redox homeostasis and metabolism/detoxification of xenobiotics and other molecules. GSH also modulates immune responses and lymphocyte functions, along with protection of cells from oxidative damage and has a role as a cell cycle regulator and a free-radical scavenger (Deneke & Fanburg, 1989; Forman, Zhang, & Rinna, 2009; Krezel & Bal, 2003; Kroemer & Reed, 2000; Meister, 1983). As shown in Figure 1 below, GSH exists in mammalian cells as a balance of two states: oxidized GSH (GSSG) and reduced GSH and the balance between them regulates the redox status of cells. Thus, a GSH/GSSG ratio higher than 100 can be found in healthy cells, whereas cells exposed to oxidative stress can have a much lower ratio of an average 1–10 (Pizzorno, 2014). The reactivity of the thiol group (SH) in the GSH molecule plays a major role on its physiological functions as it is responsible for the conjugation and reduction reactions (Krezel & Bal, 2003; Ulrich & Jakob, 2019).

Due to its importance in numerous physiological functions, changes in GSH levels and metabolism have been found to be associated with conditions such as cancers, Alzheimer's and Parkinson's diseases, as well as liver diseases, stroke, diabetes, and cystic fibrosis (Conway, Neptun, Garvey, & Popp, 1987; Estrela, Ortega, & Obrador, 2006; Townsend, Tew, & Tapiero, 2003; G. Wu, Fang, Yang, Lupton, & Turner, 2004). Increased GSH levels are observed in various tumors, such as, breast, bone marrow, colon, pancreas, larynx, and lung cancers. High GSH intracellular concentrations can enhance the antioxidant capacity and resistance to oxidative stress in cancer cells leading to tumors becoming more resistant to chemotherapy and also more prone to metastasis (Bansal & Simon, 2018; Cook et al., 1991; Perry, Mazetta, Levin, & Barranco, 1993). Hirono (1961) demonstrated that ascites cells with increased levels of nonprotein thiols were highly resistant to alkylating drugs in contrast to cells that were sensitive to these agents. Since then, many other articles have reported the critical role of GSH in protection of cells against ROS, electrophiles, and free radicals, and the relation of GSH to mechanisms of resistance in cancer cells (Calcutt & Connors, 1963; Connors, 1966; Harington, 1967; Hirono, 1961; Suzukake & DT, 1983). In this context, the correlation of altered GSH levels with cancers especially, have led to the development of many drug delivery systems aimed to target tumor cells selectively by exploiting the high intracellular GSH content. The main approaches used for the design of reduction-responsive polymeric drug delivery systems will be discussed in the next sections.

3 | DESIGN OF REDUCTION-RESPONSIVE POLYMERIC CARRIERS

Reduction-responsive polymeric carriers with disulfide bonds have been studied by many research groups. These systems have been designed mainly using two different strategies: insertion of a disulfide bond on the polymer backbone chains or the use of reduction-sensitive crosslink molecules which can be incorporated in the core or shell of the

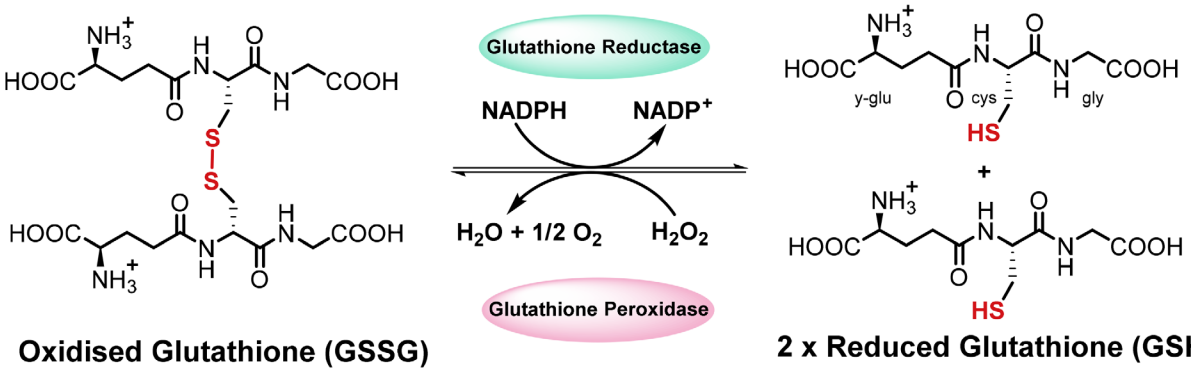

FIGURE 1 Structures of glutathione in its two states encountered in cells: oxidized glutathione (GSSG) reaction occurring through the glutathione peroxidase and reduced glutathione (GSH) through the reaction leaded by the enzyme glutathione reductase. Structures are presented in their fully protonated forms

appropriate nanocarriers (Conte et al., 2018; Z. Deng, Yuan, Xu, Liang, & Liu, 2018; Gulfam et al., 2017; Guo et al., 2018; X. Q. Li et al., 2011; Monteiro et al., 2020; C. Shi et al., 2014). In the presence of high levels of reduced GSH in tumor cells, disulfide bonds can, in principle, be easily broken down into sulfhydryl groups which results in the disassembly of carriers and consequently in the release of cargoes (Chakravarthi, Jessop, & Bulleid, 2006). However, it is important to note that GSH is a hydrophilic molecule which, in the absence of other reducing agents, must be able to react with the disulfide bonds in the polymer carrier to effect the desired thiol-disulfide exchange. This in turn leads to some specific design constraints for polymer carriers to ensure that GSH can access the disulfide bonds intracellularly. We describe below some selected examples of polymeric carriers with disulfide bonds as drug delivery systems and discuss subsequently how polymer architecture and disulfide bond placement can be critical in the reduction-response.

3.1 | Disulfide bonds in the polymer backbone

Disulfide bonds can be placed in the polymer backbone directly by using disulfide-containing monomers or by installing sulfhydryl (thiol) functionality followed by oxidation to generate the S–S-links. Carriers designed with these types of polymers/monomers generally break down at a more rapid rate than other reduction-responsive systems, which can be advantageous for release of entrapped drugs. However, the reactivity of the disulfide bond can also be detrimental for clinical application when compared to other polymeric drug delivery systems, as fast main-chain degradation may be problematic for storage stability in comparison with, for example, polyesters. In addition, further chemical modifications in these polymers can be challenging and can increase batch-to-batch variability, thus affecting the reproducibility of the entire process (Guo et al., 2018). Some of the most commonly used precursors for polymeric backbones containing disulfide bonds include cystine (J. Wu et al., 2015; X. Zhang, Kang, et al., 2019) and cystamine (L. Zhang, Zhou, Shi, Sang, & Ni, 2017). Other reactive thiol donor or acceptor molecules also used are N-succinnimidyl-3-(2-pyridyldithiol) propionate and disulfide-based dimethacrylate (Guo et al., 2018; Ling Zhang, Liu, Lin, Chen, & Stenzel, 2008).

Wu et al. synthesized L-cysteine-based poly(disulfide amide) polymers for designing redox-triggered nanoparticles with high hydrophobic drug loading capacity and tuneable properties. As shown in Figure 2, reduction-responsive polymers were synthesized through polycondensation of L-cystine and fatty acids. Moreover, the authors altered the diacid

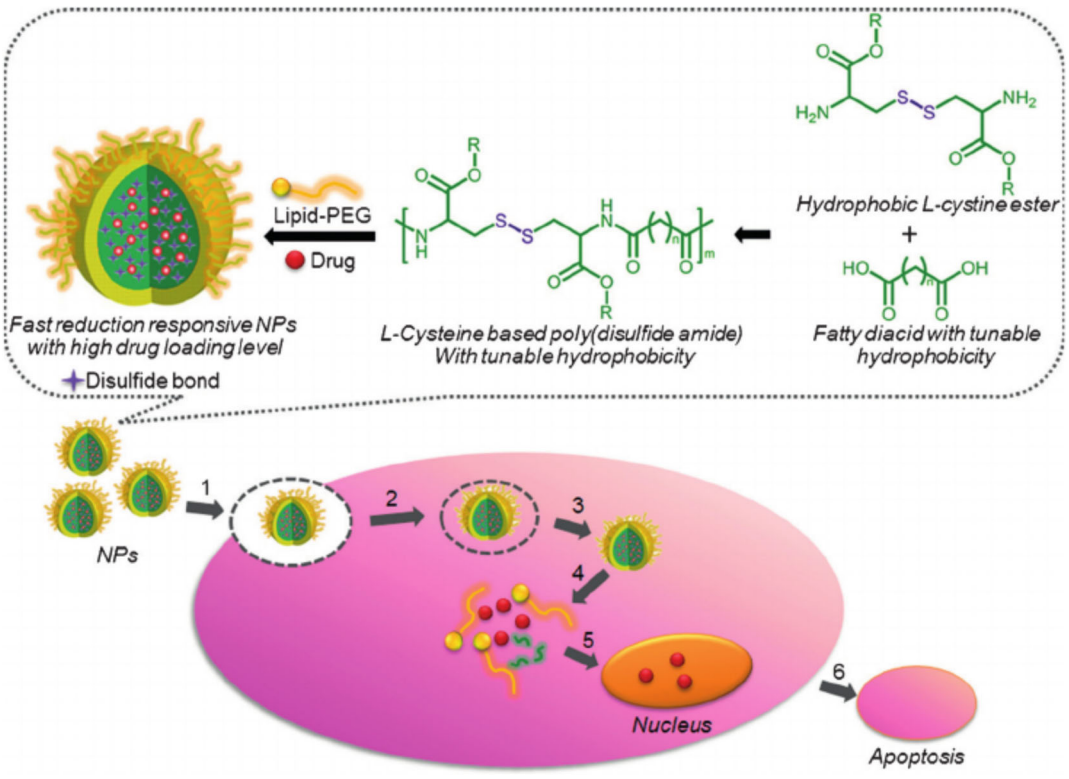

FIGURE 2 Illustration of L-cysteine-based poly (disulfide amide) (Cys-PSDA) and intracellular delivery of docetaxel-loaded redox-responsive Cys-PDSA nanoparticles (Reprinted with permission from J. Wu et al. (2015). Copyright 2015 John Wiley and Sons)

structure in order to tune the physicochemical properties of the polymers and nanoparticles in terms of size, hydrophobicity, responsiveness, degradation rate, and secondary self-assembly (after reductive dissociation of the nanoparticles). Docetaxel-loaded nanoparticles were evaluated in vivo in an A549 xenograft mouse model. The results demonstrated that free docetaxel and docetaxel-loaded PLGA nanoparticles (used for comparisons) showed only limited tumor inhibition, whereas the docetaxel-loaded L-cysteine-based nanoparticles suppressed tumor growth for longer. Thus, the in vitro and in vivo data obtained using the poly(disulfide amide) polymers showed that the designed nanoparticles were well-tolerated and gave significant antitumor performance (J. Wu et al., 2015).

The reactivity of the disulfide bond to GSH was effectively tuned by the different fatty diacid co-monomers, as not only was the overall assembly of the particles dependent on the fatty-acid chain length, but also the rate of dye release under reducing conditions varied with the fatty acid co-monomer. The placement of the disulfides in the main chain allowed very rapid disassembly of polymers built from C2 diacids, but much slower breakdown for analogous C8 and C10 diacid derived polymers.

A related approach was recently described by Jiang et al, who linked protamine-type peptides containing Vitamin E succinate units via cysteine termini into micelles for the delivery of miR-4638–5p and docetaxel (Jiang et al., 2019). Again, the ability to disassemble and release cargo was based on the reactivity of the disulfide bond, which was maintained accessible to the action of GSH via the positioning of the polypeptide micelles when complexed with RNA, and with docetaxel and the vitamin E components in the micellar interiors.

3.2 | Disulfide bonds in polymeric side chains and linking two moieties

An alternative method to introduce disulfide bonds into polymer-based carriers is to modify the polymeric chains with disulfide containing linkers, which themselves can be linked to drugs, promoting their delivery to the targeted sites. Specific ligands can also be attached in the polymer backbone according to the functional groups presented in the linkers improving, thus, the selectivity of the carriers. These methods provide more flexibility as the polymers are easier to modify in comparison to the systems containing disulfide bonds in the polymer backbone (Guo et al., 2018; Quinn et al., 2017; T. Yin et al., 2018).

Yin et al. designed redox and pH co-responsive prodrug micelles by attaching doxorubicin (DOX) into a hyaluronic acid (HA) backbone through a linker containing disulfide and hydrazone bonds, as shown in Figure 3. The prodrug micelles showed high doxorubicin release at highly reducing and acid conditions, and the efficacy of the formulation

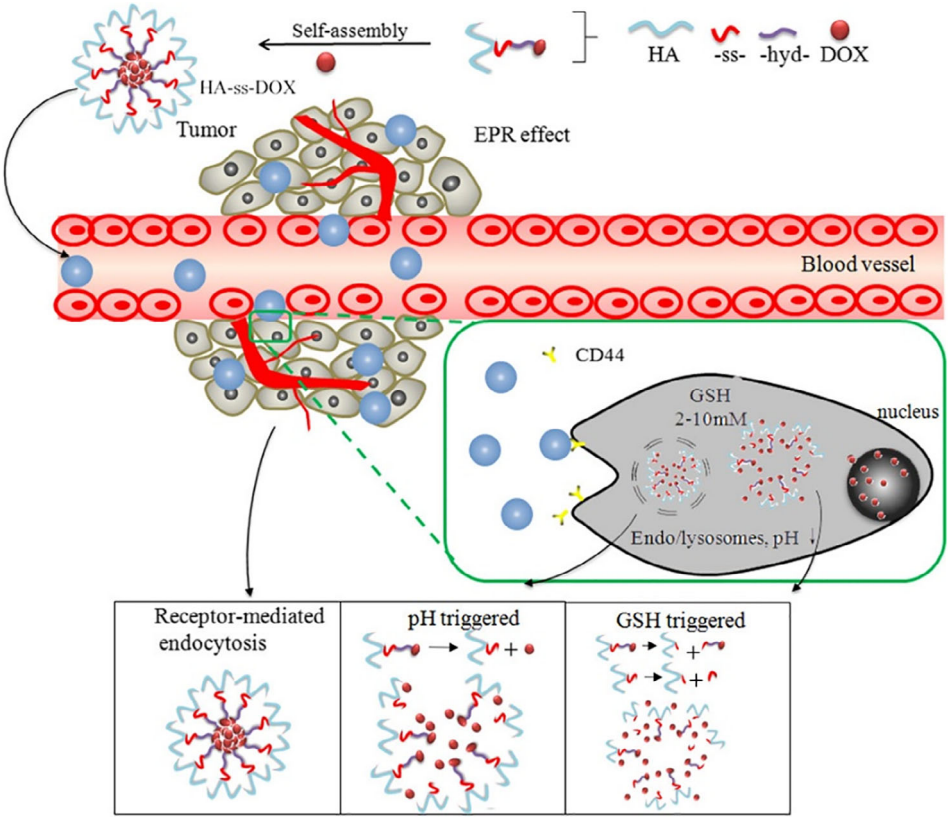

FIGURE 3 Tumor accumulation, self-assembly, intracellular trafficking of DOX/HA-S-S-DOX micelles (Reprinted with permission from T. Yin et al. (2018). Copyright 2018 American Chemical Society)

was also assessed in vitro on A549 human lung cancer cells and in vivo using a xenograft model. The authors also formulated doxorubicin-loaded redox-insensitive micelles which, as expected, showed a pH-dependent drug release, however, when compared to the reduction and pH co-responsive prodrug micelles, these carriers had the faster intracellular doxorubicin release profile due to their sensitivity to both reducing and acidic environments (T. Yin et al., 2018).

Recently, Chai et al developed a redox-responsive hyaluronic acid-ibuprofen micellar prodrug for the treatment of metastatic breast cancer. For designing the prodrug micelles, ibuprofen was bound to the hyaluronic acid backbone through a disulfide bond which allowed the polymer to self-assemble as micelles and the carrier was then loaded with doxorubicin. In these constructs, ibuprofen was used to target cyclooxygenase-2 (COX-2), which is believed to be upregulated in various tumors promoting proliferation and invasion of cancer cells. The authors hypothesized that the micelles had been internalized via CD44 receptors and both ibuprofen and doxorubicin were delivered inside the cancer cells. In addition, a nonreduction responsive control was prepared, and it was shown that the redox-responsive hyaluronic acid-ibuprofen micellar prodrug showed superior efficacy in suppressing the growth and proliferation of cancer cells in vitro and in vivo (Chai et al., 2020).

Moreover, disulfide linkers have also been used to promote the connection between two moieties, such as hydrophobic and hydrophilic blocks aiding the formation of amphiphilic polymers. Using this strategy, it is possible to design a micellar-type of carrier that can carry a payload and, when reaching a site of enhanced reduction potential, undergo a change in polymer structure in which the hydrophilic and the hydrophobic blocks separate (Conte et al., 2018; Sauraj et al., 2020; Wang et al., 2017). In turn, this breakdown of an amphiphilic material or particle can be used to release a drug directly or alter the cellular internalization kinetics of the nanoparticle. For example, Conte et al prepared poly(lactic-co-glycolic acid)-polyethylene glycol based nanoparticles with a disulfide bond attaching the hydrophilic and hydrophobic blocks of the polymer. The authors also prepared nonresponsive nanoparticles as control and the results indicated that the redox responsive nanoparticles were internalized more rapidly, released drugs more effectively in 2D, and penetrated 3D lung cancer spheroids to a greater extent when compared to the control nanoparticles (Conte et al., 2018).

3.3 | Disulfide bonds in cross-linked micellar-based carriers

Among the many examples of polymers for drug delivery, a large number have been based on amphiphilic copolymers which can self-assemble into micelles or micellar-like structures (Chiang, Yen, & Lo, 2015; Kim, Jeong, & Park, 2011; Xia et al., 2018). The formation or self-assembly into micelles occurs in aqueous solutions when the concentration of the hydrophobic block of the copolymers rises above a certain threshold, which is termed the critical micelle concentration (CMC). At the CMC, the hydrophobic constituents of block copolymers begin to associate to decrease the interaction with the aqueous environment, resulting in micellar-like or vesicular core-shell structures which have been shown to be very versatile as drug delivery systems (Lu, Zhang, Yang, & Cao, 2018). Polymeric amphiphiles exhibit significant advantages when compared with traditional drug delivery systems, including enhanced stability and controllable particle size compared to small molecule micelles and vesicles. In addition, polymer micelles and vesicle have thicker cores and membranes than their small molecule amphiphile counterparts, and can encapsulate hydrophilic and/or hydrophobic drugs dependent on their architecture. In turn this can help to reduce the toxicity of systemically-injected drugs and improve the transport of poorly soluble drugs (Biswas, Kumari, Lakhani, & Ghosh, 2016). However, for all micelle-based formulations, injection in vivo can lead to premature disassembly due to the dilution of the micelles below their CMC in the bloodstream, and subsequent interactions of micellar unimers with blood components and undesired release of encapsulated drugs. This can affect drastically the efficacy of the drug formulation, in addition to provoking unexpected side effects (Owen, Chan, & Shoichet, 2012; Talelli et al., 2015). To overcome this issue, core-crosslinking of micelles became a popular method for improving the in vivo stability of these formulations by ensuring full drug retention in the nanocarriers during systemic circulation. In this context, the use of responsive molecules, such as reduction-responsive crosslinkers to formulate core-crosslinked micelles can, in addition to enhancing the stability of the formulation kinetics, selectively and efficiently deliver drug in the pathological sites (Talelli et al., 2015).

Wu et al developed reduction and pH dual-sensitive reversibly core-crosslinked polypeptide micelles based on lipoic acid and cis-1,2-cyclohexanedicarboxylic acid decorated poly(ethylene glycol)-b-poly(l-lysine) block copolymers. As shown in Figure 4, the micelles were crosslinked in the presence of a catalytic amount of dithiothreitol (DTT) and loaded with doxorubicin. As part of the in vitro studies, the authors measured doxorubicin released under a range of pH values, in association or not, with GSH. The best formulation performance, that is, the fastest drug release was

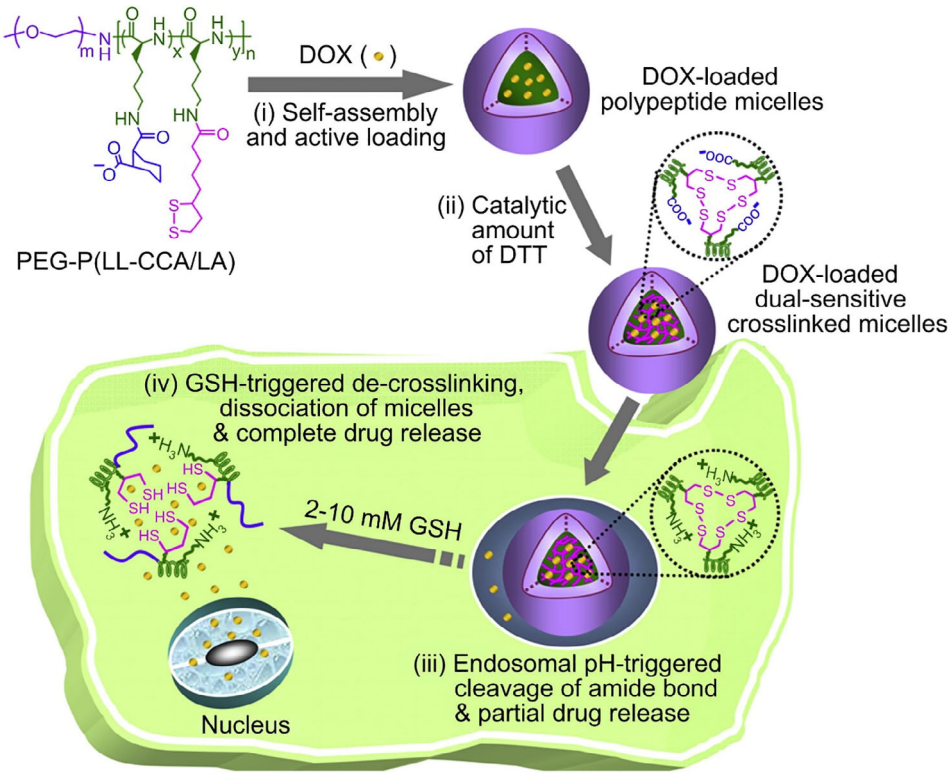

FIGURE 4 Illustration of reduction and pH dual-sensitive core-crosslinked lipoic acid and cis-1,2-cyclohexanedicarboxylic acid decorated poly(ethylene glycol)-b-poly(l-lysine) micelles for active loading and triggered intracellular release of DOX (Reprinted with permission from L. Wu et al. (2013). Copyright 2013 Elsevier Ltd)

achieved by the double responsive crosslinked micellar-based formulation using a combination of 10 mM of GSH and endosomal pH conditions. Through cell-based assays, the authors showed that the blank crosslinked polypeptide micelles did not show toxicity, whereas the doxorubicin-loaded micelles had high in vitro efficacy against HeLa and HepG2 tumor cells (L. Wu et al., 2013).

The above system offered the possibility of multiple S–S bonds and highly dense cross-links in the cores of the polymeric micelles. The consequence of this was high stability in the absence of reducing agents but the pH-responsive component may also have played a role in the response of these materials. This was because the change in the protonation state of the carboxycyclohexane-carboxamide side-chains following endosomolytic cleavage may have helped access of the intracellular fluid to the core, thus allowing greater penetration of GSH and faster micellar breakdown.

This aspect of core stability in micellar type particles was explored by Kataoka and co-workers. In experiments evaluating delivery of siRNA by polymeric micelles bearing cationic charges, disulfide-only cross-linking in the cores led to nanoparticles which were significantly less stable to reducing conditions than analogous micelles in which the siRNA was conjugated to cholesterol. The resultant mixed micellar systems were stabilized by hydrophobic interactions due to cholesterol components packing into the cores of the polyelectrolyte nanoparticles. The detailed mechanisms behind the enhanced stability were not fully explored, but it is likely that aqueous solvent ingress and reactivity to GSH were factors inherently related to the hydrophobicity of the cores and the packing of cholesterol units.

Another route to altered GSH access was described recently by Monteiro and co-workers, who prepared core-crosslinked micellar-like nanoparticles as potential treatments for triple negative breast cancer. The authors synthesized terpolymers containing polyethylene glycol (PEG) with both polylactide and a functionalised polycaprolactone regions, or polymers with functionalised polycaprolactone and PEG but no polylactide regions, to investigate the accessibility to reductive agents in polymers of different architectures. The aim was to achieve stability in transit, but fast breakdown of the micelles along with drug release, when the polymers entered breast cancer cells with increased levels of reducing agents. The in vitro efficacy data, in 2D and 3D mono and co-cultures of triple negative models presenting high levels of GSH, showed that the docetaxel loaded reduction-responsive crosslinked nanoparticles were of greater efficacy against triple negative breast cancer models and demonstrated deep penetration in 3D spheroids compared to controls. Furthermore, the key finding in this article was that placement of the disulfide cross-links in poly(caprolactone)-co-poly(lactide) regions of the "cores," compared to in poly(caprolactone) only regions, enhanced the reactivity toward GSH. This demonstrated that access of GSH to the disulfide links varied, dependent on the nature of the polymer backbone carrying the disulfides, and suggests that it might be possible to tune the reduction response of delivery systems via specific placement of disulfides in bespoke regions of a polymer (Monteiro et al., 2020).

3.4 | Other reduction-responsive bonds used in polymeric carriers

As an alternative to disulfide bonds, diselenide bonds have also been investigated for the design of reduction-responsive polymeric carriers (Guo et al., 2018; C. Shi et al., 2014). It has been reported that Se—Se and C—Se bonds, which have lower bond energy (Se—Se 172 kJ/mol; C—Se 244 kJ/mol) in comparison to S—S bonds (268 kJ/mol), can increase the sensitivity to reducing environments (Ji, Cao, Yu, & Xu, 2014; Longshuai Zhang, Liu, et al., 2019).

In this regard, Sun et al. investigated the impact of different chemical linkages on reduction-responsive nanoassemblies for drug delivery. Prodrugs that self-assemble into nanoparticles were designed by synthesizing six paclitaxel-citronellol conjugates, containing thioether, disulfide, selenoether, diselenide, carbon or carbon–carbon bonds as linkages, as shown in Figure 5. in vitro drug release under redox conditions was assessed using DTT and hydrogen peroxide, and the nanoassemblies containing disulfide bonds were the most sensitive to reduction conditions, followed by the nanoparticles with diselenide bonds. The selenoether bond was more sensitive to hydrogen peroxide than the thioether bond and, as expected, nanoassemblies containing carbon–carbon bond linkages showed no response to the reducing conditions. Interestingly, it was found that the efficiency of prodrug nanoassemblies was affected by sulfur, selenoether, and carbon bonds, whereas the bond angles (dihedral angles) impacted the self-assembly, stability, and pharmacokinetics of the prodrug nanoassembles. The three prodrug carriers that achieved the highest cytotoxicities against human oral epidermoid carcinoma KB cells, human pulmonary carcinoma A549 cells, and mouse breast carcinoma 4T1 cells were those containing diselenide, selenoether, and disulfide bonds. However, it was noted that

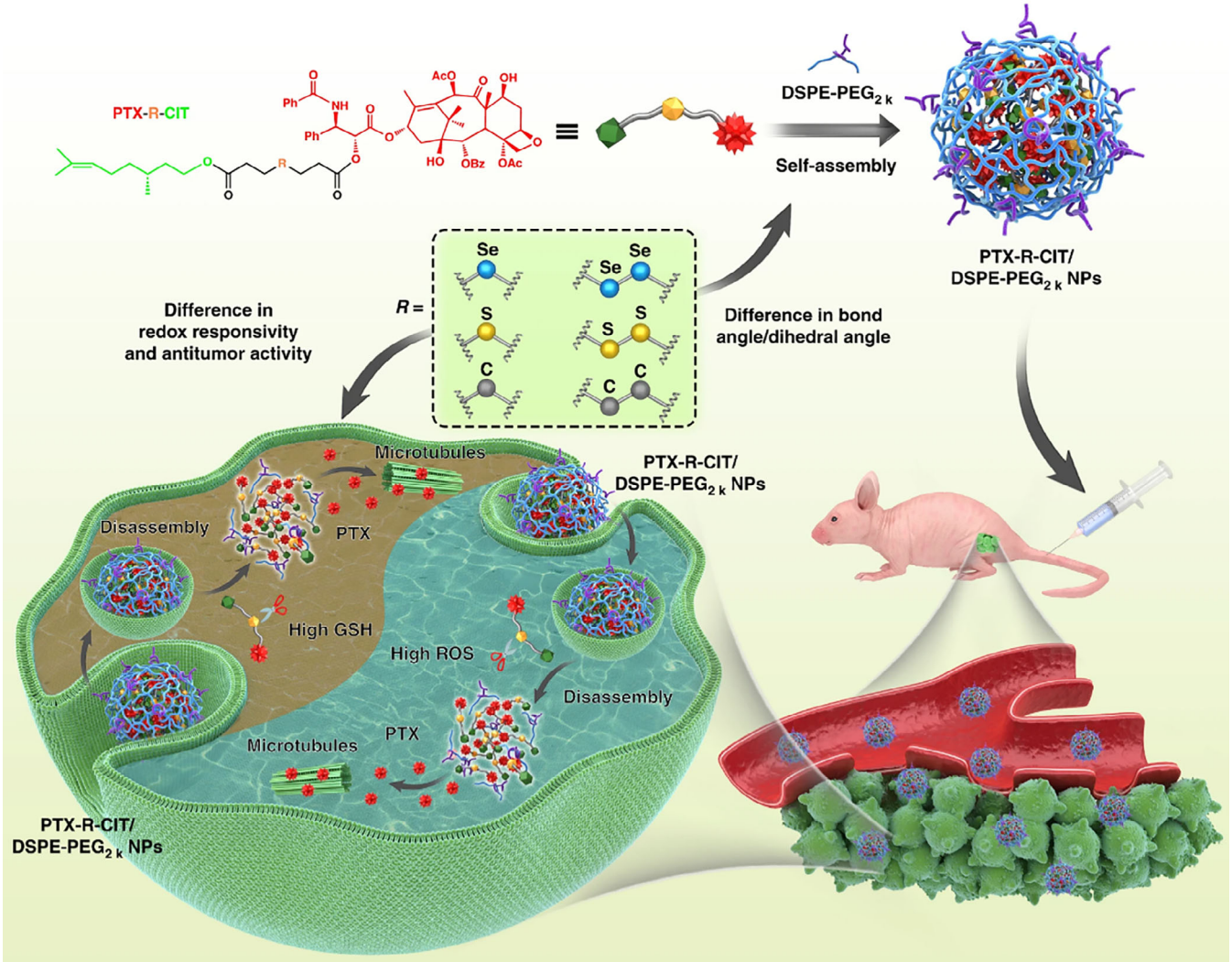

FIGURE 5 Illustration paclitaxel-citronellol conjugates containing thioether, disulfide, sulfur, selenoether, diselenide, carbon or carbon–carbon bonds as prodrug nanoassemblies for cancer therapy (Reprinted with permission from B. Sun et al. (2019). Spring Nature 2019)

selenoether and diselenide were also able to produce reactive oxygen species which improved the cytotoxicity of these polymers in comparison to those containing disulfide bonds (B. Sun et al., 2019).

4 | PRECLINICAL EFFICACY OF REDUCTION-RESPONSIVE POLYMERIC DRUG DELIVERY SYSTEMS

Although there have been many interesting and innovative reports presenting a range of different chemistries for the design of reduction-responsive polymeric drug delivery systems, only a relatively small number of these have included results of in vivo therapeutic efficacy of the developed systems. Furthermore, given the complexity of biological environments along with the high heterogeneity of tumors, it remains a challenge to achieve controllability of any redox-based molecular release mechanisms (Liu et al., 2016; Mura et al., 2013; Patra et al., 2018). In some cases, the in vivo evaluations have failed to demonstrate any real significant advantage of the reduction-responsive polymeric system compared to the conventional treatment, that is, free drug formulation, depending on the in vivo model adopted. Several factors can be associated with this issue, such as, the low stability of drug delivery systems in biological fluids which can lead to premature disassembly of the nanoparticles. In addition, the competition of nontarget organs and biological clearance systems, such as the mononuclear phagocytic system, with tumors lead to the fast blood elimination of nanoparticles. Moreover, the tumor microenvironment is another important factor that can alter accumulation of nanoparticles as it can be highly heterogeneous depending on the tumor type and the organ where the disease originated (Blanco, Shen, & Ferrari, 2015; Wilhelm et al., 2016). In this regard, it is important to consider whether the models chosen for evaluation are appropriate, and many investigators are now replacing conventional cell culture methods with three-dimensional models and cell co-culture systems along with relevant stem cells and primary cells that can better mimic the in vivo physiology to evaluate penetration, safety, and efficacy of nanocarriers. This can offer a chance to develop better and optimize drug delivery systems before attempting to perform in vivo assessments (Fang & Eglen, 2017).

In Table 1 below are some reported preclinical evaluations of reduction-responsive polymeric drug delivery systems.

In some of the examples listed in Table 1, the polymer core cross-linking or attachment of the drugs is via a self-immolative disulfide β-ester linker (Khan et al., 2014; Wohl, Smith, Jensen, & Zelikin, 2014). In such cases, generation of the thiol leads to rapid elimination of a thiolactone intermediate, and can afford accelerated drug release in contact with reducing agents. In the context of cytotoxic agents this is unlikely to be problematic as the potency of the drug is much greater than the biological effect of the eliminated thiolactone, however, for other therapies, such a strategy may incur additional regulatory hurdles.

5 | CHALLENGES FOR CLINICAL TRANSLATION OF REDUCTION-RESPONSIVE POLYMERIC DRUG DELIVERY SYSTEMS

Although the therapeutic potential of reduction-responsive polymeric drug delivery systems has been widely demonstrated, the clinical translation of these innovative systems is still challenging. This is first because even the best in vitro and preclinical models currently used to evaluate polymer drug delivery systems do not reflect the full complexity of tumor microenvironments in humans, and thus a promising reduction response in the lab does not lead to progress through early stage clinical trials. Cancer is a heterogeneous disease, with plenty of differences in terms of morphology, immunophenotype, and genotype, both between tumor types (inter-tumor heterogeneity) as well as within tumors (intra-tumor heterogeneity). Genetic and nongenetic factors, in fact, can affect gene expression, metabolism, motility, proliferation, and metastatic potential of tumor cells in different ways (Diaz-Cano, 2012; Tellez-Gabriel, Ory, Lamoureux, Heymann, & Heymann, 2016).

The tumor microenvironment plays a pivotal role in regulating the distribution and biological effects of polymeric drug delivery systems, including reduction-responsive nanocarriers. For this reason, endogenous stimulus-responsive nanosystems have been generally designed on the basis of the pathophysiological characteristics of the tumor microenvironment, with the aim to improve the efficacy of cancer treatments and to combat multi-drug resistant cancers. As widely previously reported, in fact, different cancers are characterized by increased levels of intracellular glutathione and an abnormal redox potential compared to healthy tissues. However, due to the high range of intracellular mechanisms and genes involved in the regulation of redox homeostasis, the redox responsiveness can be strongly

TABLE 1 Examples of reduction-responsive polymeric drug delivery systems and their in vivo evaluation

Drug loaded	Nanocarrier structure/composition	In vivo evaluation/clinical application	References
Paclitaxel (taxol), gemcitabine (GEM) and immunomodulating agent (NLG919)	Disulfide GEM-conjugated POEG-co-PVD-based micelles	Nanocarrier significantly inhibited the tumor growth (pancreatic ductal adenocarcinoma tumors) and was more effective than Taxol +GEM+NLG919 combination	(J. Sun et al., 2020)
Vorinostat (SAHA) and tamoxifen (TAM)	SAHA disulfide conjugated POEG-co-PVD prodrug nanoparticles	The co-delivery of SAHA and TAM showed synergy in inhibiting the proliferation of triple negative breast cancer cells in vitro and in vivo more efficiently than the free drugs combination	(Ma et al., 2020)
^{19}F and indocyanine green (ICG)	^{19}F-bearing groups attached to polyethylene glycol via disulfide linkers and formulated with ICG into NPs	The NPs showed stepwise two-stage activation/amplification cascade multiresponsive behavior in vitro and in vivo (HepG2 tumor-bearing mice)	(Tang et al., 2020)
Camptothecin (CPT)	Poly(ethylene glycol) CPT conjugated poly(methacrylate) redox and ROS responsive NPs (GR-BCP)	GR-BCP showed enhanced drug release and antitumor efficacy in vitro (HeLa) and in vivo (H22 mice tumor) compared to the free CPT and single responsive NPs (G-BCP and R-BCP)	(W. Yin et al., 2020)
Hydroxycamptothecin (HCPT)	Disulfide-doped organosilica-micellar hybrid nanoparticles modified on the surface with poly(ethylene glycol) and polyethylenimine (DOSN-PEI-SS-PEG)	HCPT loaded DOSN-PEI-SS-PEG NPs showed enhanced antitumor activity and reduced adverse effect in vivo (SMMC-7721 tumor)	(Jia et al., 2019)
Camptothecin (CPT)	Disulfide conjugated CPT to polymethacrylate (PMAA) prodrug nanogel	PMAA nanogel showed quick dual responsive CPT release (pH/redox), enhanced efficacy in vivo (Hep G2) and reduced toxicity compared to free CPT	(Qu et al., 2019)
Brominated BODIPY (BDP)	Disulfide linked BDP to poly(ethylene glycol) (PSSBDP) NPs	PSSBDP NPs showed the strongest tumor growth suppression compared to the unresponsive NPs and the free BDP in vivo (EMT6)	(Ruan et al., 2019)
Doxorubicin (DOX)	Poly(N-isopropylacrylamide)-based nanogels with pH-regulated charge reversal and GSH-responsive DOX release	The nanogel exhibited enhanced in vivo (H22) tumor inhibition and CSCs killing efficiency and reduced side effects compared to free DOX	(Yang et al., 2018)
Ovalbumin (OVA)	Polyethyleneimine-redox-responsive hyperbranched poly(amido amine) NPs (PAA-PEI_{600})/OVA	PAA-PEI_{600}/OVA enhanced OVA-specific cellular immune responses, inhibited tumor growth (E.G7-OVA tumor) and extended mice survival compared to OVA alone	(Lv et al., 2017)
Camptothecin (CPT) and doxorubicin (DOX)	Diselenide-PEG based polymeric crosslinked micelles (CPT/DOX-CCM)	CPT/DOX-CCM showed in vitro and in vivo tumor (EMT6) suppression with low dosage drugs and without any detectable side effects	(Zhai, Hu, Hu, Wu, & Xing, 2017)

variable in different cancer types, individuals with the same cancer type, and between different cancer stages (Benfeitas, Uhlen, Nielsen, & Mardinoglu, 2017; Manda et al., 2015; Trachootham, Lu, Ogasawara, Nilsa, & Huang, 2008). Therefore, an extensive understanding of the mechanisms behind redox signaling dysregulation is

required to design personalized and efficient redox-sensitive polymer delivery agents. Following on from this is the difficulty in establishing proper preclinical models and in choosing the correct experimental endpoints in order to predict biological outcomes in patients (Day, Merlino, & Van Dyke, 2015). As noted above, current animal models cannot accurately reproduce the different tumor microenvironments in humans, and significant cancer heterogeneities and the corresponding variations in redox processes make the concept of a single "reduction-sensitive polymer" seem perhaps simplistic. Thus, different disease states would require polymers which respond to different levels of reducing agent proportionally, in order to target release to the desired location rather than in all regions of altered reduction potential.

The question then arises, is there anything new to discover for reduction-responsive polymers? A very large number of such polymers have been described, and the topic has been extensively reviewed (B. Deng, Ma, & Xie, 2015; Quinn et al., 2017). However, as noted above, there are large heterogeneities in cancers, and the varying redox states may mean that a polymer which is reducibly activated in one cell type may not be activated in another. In addition, for non-cancer applications, for example in anti-viral or in vaccines, the aberrant biology in an infected cell, or the need to deliver the reducibly activated polymer to an antigen-presenting cell, may require the polymer to be designed rather differently than has been the case for cancer therapies. There are also needs for combination therapeutics, where modulation of the site and rate of release of each individual drug may need to be varied on a single polymer chain, nanoparticle or implant. Accordingly, by altering the site at which a reducibly-activated/ cleaved bond is placed, some level of addition control may be afforded for time and location of drug release. In the case of disulfides, there have been many variations as to the placement of the link, the nature of adjacent functional groups, and modulation of the local environment via introduction of charge, hydrophilicity, hydrophobicity, and hydrogen-bonding. It is clear therefore, that while the concepts underlying reduction-responsive (and indeed any other stimuli-responsive) release have been very long established, there is still considerable "chemical and formulation space" to explore for these materials to tailor them specifically for individual therapeutic, and also diagnostic applications.

The final issue for clinical translation of polymeric nanoparticles in general, and in particular for the case of redox responsive nanocarriers is the need for materials which are easy to manufacture and demonstrably safe in application (Gaspar & Duncan, 2009). Moreover, in the case of more sophisticated smart materials, such as redox sensitive polymers, complex chemical synthesis is often required. This goes against the need for fast and easy production processes, and makes scale-up and validation harder, as batch-to-batch reproducibility becomes more difficult the more demanding a synthetic route becomes. In turn, these issues pose serious risks in gaining regulatory approval. Thus, the scale up of synthesis, the reproducibility of properties for both materials and formulations, and a good regulatory profile are key points to address in the future design of reduction-responsive polymer delivery systems. Only if these are addressed in full will reduction-responsive polymers have a good chance of reaching the clinic with the desired combination of safety, sustainability, and affordability with improved efficacy.

6 | CONCLUSIONS

As a result of progress in materials chemistry and the development of polymeric drug delivery systems, many advances have been made in the design of stimuli-responsive polymers, including those with reduction-responsive functionalities. These systems have been designed to overcome drawbacks of conventional drug delivery systems such as, but not limited to, biodistribution performance, higher stability and on-demand drug release. The major focus of reductive-responsive drug delivery systems to date has been to target cancer cells and consequently improve the therapeutic efficacy of chemotherapeutics. However, despite the great progress in the design, and reports of promising and relevant applications of reductive-responsive drug delivery systems in the laboratory, to the best of our knowledge, none has yet entered large-scale human clinical trials. Among the various factors related to this, the difficulty of obtaining systems simple in structure but with enough functionalities to ensure the treatment efficacy is certainly one of the challenges encountered. This affects directly the reproducibility and the ability to scale-up the production of drug delivery systems. Thus, it is important to consider the balance between carrier functionality and structural simplicity. Moreover, another important point is the choice of models during preclinical tests, as it is also crucial to consider models that are more clinically relevant due to, as previously mentioned, the high heterogeneity of tumor microenvironments, that is, the importance to set the right treatment for the right tumor type. In this context, more "human-like" models, such as patient-derived explants (PDEs), can add more relevance and provide more realistic results for the preclinical assessments, which in turn facilitate more rapid clinical translation. Therefore, the answer to the question "reduction-responsive polymers for drug delivery in cancer therapy—is there anything new to discover?" is not, unfortunately, a

simple "yes" or "no." On one side, with the constant improvement of structure–function relations for reductive-responsive drug delivery systems, along with the use of more relevant in vitro and in vivo methods to assess the performance of these materials, there are many new ways we might use these materials for patients as we now understand them much better than in the past. There is also still scope for the development of a more clinically safe redox-responsive polymer, which is easy to scale-up and which is also storage-stable. The routes to this may involve fine-tuning or minor variations on the well-established concepts of reduction-response, for example, by controlling access of reducing agents by functional group placement, or use of multiple stimuli to modulate core-shell structure and thus, disulfide or related linker reactivity. However, as a counter argument, the chemistry and formulation space remains wide for reduction-responsive materials, but the regulatory and clinical targets remain demanding, and these issues are not new to discover. It is likely that the best route to developing these interesting materials further, is to use the knowledge we already have on the regulatory and clinical issues to help us design in the desired reduction response into the simplest possible carrier structure. In this regard, there are still many things new to discover for reduction-responsive polymers.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Patricia Monteiro: Writing-original draft; writing-review and editing. **Alessandra Travanut:** Writing-original draft; writing-review and editing. **Claudia Conte:** Writing-original draft; writing-review and editing. **Cameron Alexander:** Supervision; writing-review and editing.

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