



Recent advances in prodrug-based nanoparticle therapeutics

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ABSTRACT

Extensive research into prodrug modification of active pharmaceutical ingredients and nanoparticle drug delivery systems has led to unprecedented levels of control over the pharmacological properties of drugs and resulted in the approval of many prodrug or nanoparticle-based therapies. In recent years, the combination of these two strategies into prodrug-based nanoparticle drug delivery systems (PNDDS) has been explored as a way to further advance nanomedicine and identify novel therapies for difficult-to-treat indications. Many of the PNDDS currently in the clinical development pipeline are expected to enter the market in the coming years, making the rapidly evolving field of PNDDS highly relevant to pharmaceutical scientists.

This review paper is intended to introduce PNDDS to the novice reader while also updating those working in the field with a comprehensive summary of recent efforts. To that end, first, an overview of FDA-approved prodrugs is provided to familiarize the reader with their advantages over traditional small molecule drugs and to describe the chemistries that can be used to create them. Because this article is part of a themed issue on nanoparticles, only a brief introduction to nanoparticle-based drug delivery systems is provided summarizing their successful application and unfulfilled opportunities. Finally, the review's centerpiece is a detailed discussion of rationally designed PNDDS formulations in development that successfully leverage the strengths of prodrug and nanoparticle approaches to yield highly effective therapeutic options for the treatment of many diseases.



Abbreviations: AA, adjuvant arthritis; Ada, adamantane; ADD, adjudin; AKP, acetone-based ketal-linked prodrug; API, active pharmaceutical ingredient; APTES, 3-aminopropyltriethoxysilane; AUC, under the curve; MAC, *Mycobacterium avium* complex; BBB, blood brain barrier; BTZ, bortezomib; CD, cyclodextrin; chol-but, cholestryl butyrate; CrEL, Cremophor EL®, a polyoxyethylated castor oil; DACH-Pt, 1,2-diaminocyclohexane-platinum(II); 2-DA-FITC-PTX, 2-glucosamine-glutamic acid fluorescein isothiocyanate paclitaxel; dex, dexamethasone; DFEE, diclofenac ethyl ester; DLin-MC3-DMA, heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate); DMF, dimethylformamide; DOPA, catecholic amino acid 3,4-dihydroxy-L-phenylalanine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOX, doxorubicin; DPPC, dipalmitoylphosphatidylcholine; DSPE-PEG, 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol); ECM, extracellular matrix; EMA, European Medicine Agency; EPR, enhanced permeability and retention; ER, estrogen receptors; ESA, ethyl salicylate; ETP, etoposide; FDA, Food and Drug Administration; FITC, fluorescein isothiocyanate; FTC, emtricitabine; GBT, guideline-based therapy; GO, graphene oxide; GOD, glucose oxidase; GSH, glutathione; GUS, glucuronidase; HA, hyaluronic acid; HAPE, hyper-branched aliphatic polyester; HDAC, histone deacetylase; HCPT, 10-hydroxycamptothecin; HCPT-Gu, guanidine-modified HCPT prodrug; HER2, human epidermal growth factor receptor 2; H₂O₂, Hydrogen peroxide; HPMA, hydroxypropylmethacrylamide; HSA, human serum albumin; IMDQ, imidazoquinoline; IV, intravenous; L-DOPA, levodopa; LRA, latency-reversing agent; MDR, multidrug resistance; Meo-PEG-b-PDPA, methoxyl-poly(ethylene glycol)-b-poly(2-(diisopropylamino)ethyl methacrylate); miRNA, microRNAs; MMP, matrix metalloproteinase; NAC, neoadjuvant chemotherapy; NDDP, *cis*-bis-neodecanoato-*trans*-R,R-1,2-diaminocyclohexane platinum II; NO, nitric oxide; NONOate, N-diazenuimidolate; NP, nanoparticle; OA, oleate; PAH, pulmonary arterial hypertension; PANO, panobinostat; Pd, palladium; PDLLA, poly(D,L-lactide); pDOX, DOX prodrug; PEG, polyethylene glycol; PEG2000-C-DMG, (R)-2,3-bis(octadecyloxy)propyl-1-(methoxy polyethylene glycol 2000) carbamate); PEO-*b*-PCL, poly(ethylene oxide)-*b*-poly(*ε*-caprolactone); PgP-HA, poly(L-glutamic acid)-g-methoxy polyethylene glycol conjugated- hyper-branched aliphatic polyester; PK, pharmacokinetic; PD, pharmacodynamic; P(LA-co-TMCC)-g-PEG, poly(D,L-lactide-co-2-methyl-2-carboxy-trimethylenecarbonate)-g-poly(ethylene glycol); PLGA, poly(lactic-co-glycolic acid); PLG-g-mPEG, poly(L-glutamic acid)-g-methoxy polyethylene glycol; PNDDS, prodrug-based nanoparticle drug delivery systems; POEG-co-PVD, poly(oligo(ethylene glycol))-co-poly(vinylbenzyl chloride); POEGMA-b-PVBA, poly(oligo(ethylene glycol) methacrylate-b-poly(vinyl benzaldehyde); Ppa, pyropheophorbide-a; PT, progesterone receptors; Pt, platinum; PTX, paclitaxel; PVA, polyvinyl alcohol; RGDS, Arg-Gly-Asp-Ser; RNAi, RNA interference; ROS, reactive oxygen species; SAHA, vorinostat; siRNA, small interfering RNAs; TAF, tenofovir alafenamide; TAM, tamoxifen; TLR, toll-like receptor; TM, telmisartan; TNBC, triple negative breast cancer; Tolf, tolferamic acid; WHO, World Health Organization.

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1. Introduction

In this review, two drug design and formulation concepts that can be used independently or in combination to help overcome some of the inherent challenges of drug development are introduced. The first is the utilization of a prodrug strategy emphasizing how chemical modification of an active pharmaceutical ingredient (API) can be a versatile approach to optimize the fundamental properties of a drug for pharmaceutical and pharmacological advantages. This section is supported by clinically relevant examples that highlight how prodrugs can be used to optimize pharmacokinetic (PK), pharmacodynamic (PD), and formulation properties, as well as the toxicity profile of an API. Some of the challenges encountered during prodrug development are also described.

Next, the key benefits of nanoparticle-based drug delivery systems are highlighted, which include increased drug loading, improved stability and bioavailability, controlled drug release and biodistribution, enhanced therapeutic efficacy, and reduced toxicity. This summary sets the stage for the use of nanoparticles as vehicles to modulate the delivery and enhance the therapeutic application of prodrugs in PNDSS.

While the field of PNDSS is nascent and emerging, examples of PNDSS products in development support the rationale of leveraging the advantages of both the prodrug and NP strategies in a combined product. However, the added complexity of a PNDSS will only be acceptable if it provides significant benefits. These may include improvements in the efficacy and safety of the prodrug through increased loading capacity, improved stability, bioavailability, or systemic exposure, or enhanced targeting and release to diseased tissue. While most examples apply to oncology indications, there is a trend towards using a combination prodrug-nanoparticle strategy in other disease areas such as infectious, inflammatory, cardiovascular, neurological, and pulmonary diseases. This section emphasizes that while the emerging field of PNDSS shows a lot of potential and promise, continued efforts are required to translate these new technologies from bench-to-bedside and validate PNDSS as a commercially viable drug development strategy.

2. Brief introduction to prodrugs

Prodrugs are pharmacologically inactive chemical constructs that typically consist of a parent drug and a pro-moiety that are connected by a labile chemical bond [1–3]. The prodrug becomes active when the pro-moiety is cleaved to release the API parent drug. Historically, prodrugs were used primarily as a salvage strategy to mask problematic functional groups on drugs that performed poorly in clinical trials due to toxicity, poor bioavailability, inappropriate PK or pharmacodynamic profiles, or lack of efficacy [4–8]. More recently, there is increasing recognition that modification of an API to form a prodrug allows control over a wide variety of fundamental drug properties yielding favorable clinical outcomes. As a result, serious consideration is being given to prodrug strategies from the onset of development efforts. This trend is reflected in the growing market share of prodrugs over the past two decades. Approximately 10% of all marketed drugs are prodrugs and over the past several years 20% of all small molecule drug approvals have been prodrugs [4]. In the following section, an overview of the benefits and chemistries of prodrugs is provided to describe how chemical properties of drug molecules can be fine-tuned to achieve specific biological, clinical, or formulation objectives.

2.1. Why consider a prodrug strategy?

Adjusting the chemical properties of a compound can accomplish a variety of tasks ranging from aiding the formulation process, to optimizing bioavailability, or even developing new intellectual property. Prodrugs marketed today are typically intended to 1) improve formulation properties, 2) customize PK properties, 3) optimize PD effects, 4) reduce toxicity, or 5) streamline development [1,3,9]. Fig. 1 shows each of these features in more detail.

Modifying a drug's aqueous solubility is an example of how formulation properties can be tuned to support an alternative route of administration or control bioavailability. This was done in the case of the anti-inflammatory glucocorticoid methylprednisolone sodium succinate (Solu-Medrol®), where a sodium succinate pro-moiety was linked to the parent drug methylprednisolone [10]. The addition of this pro-

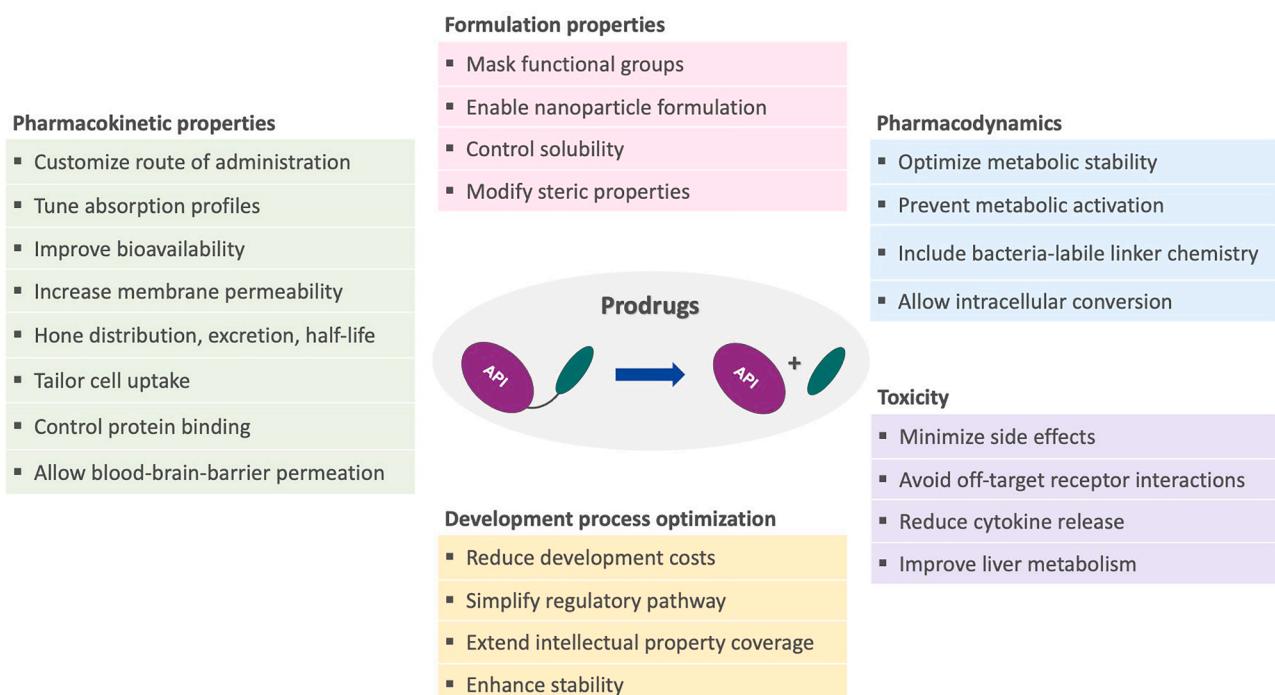


Fig. 1. Advantages of prodrug strategies.

moiety significantly increased the drug's aqueous solubility and enabled intravenous (IV) administration. In the case of tafluprost (Zioptan®), a prodrug strategy was used to optimize the PK properties of tafluprost acid, a selective agonist of the prostaglandin F receptor, for the treatment of intraocular hypertension in patients with open-angle glaucoma or ocular hypertension [11,12]. In this case, an isopropyl ester was introduced to mask a carboxylic acid, which resulted in increased lipophilicity and better ocular permeation. Other commercial examples of prodrugs aimed at increasing solubility include the N-phosphoryl prodrug fosaprepitant dimeglumine (Emend® for injection) [13,14], the N-phosphono prodrug ceftaroline fosamil (Teflaro®) [15,16], the phosphonooxymethyl ester fospropofol (Lusedra®) [17], and the methylene phosphate prodrug fostamatinib (Tavalisse®) [18]. An example showcasing the ability of a prodrug to reduce toxicity is tenofovir alafenamide, a second-generation prodrug of tenofovir that received U.S. Food and Drug Administration (FDA) approval in 2015 as part of the three-drug combination tablet called Genvoya® for the treatment of HIV [19]. In this example, the active component tenofovir is a nucleotide analog reverse-transcriptase inhibitor that has limited clinical use due to poor cellular uptake and poor bioavailability [20,21]. Initial development of a tenofovir prodrug was pursued by Gilead and yielded tenofovir disoproxil, a bis-diisopropoxy carbonyloxymethyl ester prodrug of tenofovir, featuring improved bioavailability [16,22]. However, tenofovir disoproxil is associated with nephrotoxicity [23]. To remedy these shortcomings, a second-generation tenofovir prodrug, tenofovir alafenamide, was developed using ProTide technology; a prodrug strategy designed specifically to optimize intracellular delivery of nucleotide analogs [24–26]. Tenofovir alafenamide features significantly enhanced cellular uptake allowing the drug to be delivered at lower doses, which is associated with fewer side effects [20,21].

2.2. Prodrug chemistries

Chemical modification of an API to form a prodrug can be a versatile process to optimize the fundamental properties of a drug for pharmaceutical advantage. As such, different prodrug chemistries can be used to alter the qualities of a compound in multiple directions including increasing or decreasing pKa, polarizability, or solubility. To this end, the nature of the pro-moiety linkage chemistry and pro-moiety structure

are the key determinants of the final chemical and biological characteristics. A large variety of linker configurations have been used for prodrug synthesis to date. The most frequently used chemistries are ester- and amine-based prodrugs [27–29]. Other linker configurations include carbamates [30], phosphates and phosphonates [5,8], hydrazines [31], azo [29], and N-Mannich Amines [28]. Table 1 shows the chemical structure for bonds commonly used to generate prodrugs together with their conversion products. Linker design also impacts how prodrugs are metabolized to the corresponding API. The transformation may be mediated by enzymatic conversions, as is the case for many ester-linked and phosphorylated prodrugs [27–29], changes in local conditions such as changes in pH [32,33], or a variety of non-enzymatic conversion mechanisms that do not require specific physiological conditions [9,34–37]. While linkage chemistry often dictates the conversion mechanism, the pro-moiety structure will impact both PK and PD [38,39]. Exactly how a prodrug component is chosen depends on many factors including available functional groups on the parent drug, the cleavage mechanism, and the desired *in vivo* stability profile. Importantly, the linkage chemistry and pro-moiety structure are intimately related and must be considered simultaneously along with the fate of the pro-moiety after cleavage during the selection process.

The most common prodrug approach is esterification. Ester linkages are suitable for parent drugs featuring either carboxylic acid or hydroxyl functionalities. Esters are susceptible to esterase enzymes that are ubiquitous in the body and found in every major organ [36]. Ester prodrug stability can be tuned based on the pro-moiety structure as the type and size of the pro-moiety determines whether the ester bond is accessible to the enzyme's active site.

For parent drugs containing a carboxylic acid functional group, the pro-moiety is often a simple alcohol though it may also be polymeric in nature [47]. Clinical examples of ester-based prodrugs include dimethyl fumarate (Tecfidera®) comprised of two methyl esters [48,49], telotristat ethyl (Xermelo®) containing a single methyl ester modification [50], and sacubitril (a component of Entresto®) which utilizes a single ethyl ester modification [51]. For parent drugs without a carboxylic acid functional group, ester prodrugs can be formed by using hydroxyl moieties as a chemical handle to prepare acetate-type esters with the pro-moiety contributing the carboxylic acid functionality. Examples of acetate-type esters include abiraterone acetate (Zytiga®) [52,53] and

Table 1

Common prodrug bonds and their transformation products.

Bond Type	Chemical Structure	Transformation Products	References
Ester			[27,40–43]
Carbamate			[30]
N-Acylsulfonamide			[44]
N-phosphoryl (R = OH) N-Phosphono (R = O')			[5,8]
Phosphonooxymethyl ester (X = O) N-methylene phosphate (X = NH)			[5,8]
Phenolic ether			[45,46]
Azo			[29]
Hydrazone			[28]

deflazacort (Emflaza®) [54] both of which contain a single acetate group, uridine triacetate (Xuriden®), which contains three acetate pro-moieties [55], and artesunate, which contains a succinic acid pro-moity [56,57].

Active therapeutic agents featuring an amine functional group can also be good targets for a prodrug strategy as they are amenable to a variety of chemical modifications including formation of carbamate, amide, alkyl, and sulfonamide bond types [28,29]. Examples of commercial carbamate prodrug derivatives include loratadine (Claritin®), gabapentin enacarbil (Horizant®), and dabigatran etexilate (Pradaxa®). Loratadine is an ethyl carbamate prodrug of desloratadine, an H₁-anti-histamine. Loratadine is activated by the hydroxylation-decarboxylation metabolic route; the products of this activation are desloratadine, ethanol and carbon dioxide [58,59]. As part of a life-cycle management strategy, the parent drug desloratadine was later developed as a stand-alone therapy branded as Clarinex® prior to expiration of loratadine patent protections in 2002 [60]. Gabapentin enacarbil is a N-[1-(isobutyryloxy)ethoxy]carbonyl prodrug of gabapentin that was developed to elicit extended release of gabapentin following oral administration. Gabapentin enacarbil is activated by non-specific esterases [61,62].

Interestingly, prodrugs based on amine chemistry do not always require the parent drug to contain an amine functionality. In the case of selexipag (Uptravi®), sofosbuvir (Sovaldi®) [63], and tenofovir alafenamide (Genvoya®; described above), an amine is included as a functional group on the pro-moity to impart the desired prodrug properties. In the case of selexipag, a non-prostanoid prostacyclin receptor agonist used for the treatment of pulmonary hypertension, a novel N-acylsulfonamide pro-moity is used to mask a carboxylic acid. The result is sustained release of the active metabolite ACT-333679 (previously known as MRE-269) [64,65]. Selexipag is metabolized by cytochrome P2C8 enzymes; however, the PK profile is sensitive to enzyme activity. As a result, selexipag is contraindicated for coadministration with cytochrome P2C8 inhibitors such as gemfibrozil and requires dose adjustment when co-administered with cytochrome P2C8 inducers such as rifampicin [66].

2.3. Codrugs

Several types of prodrugs do not exactly conform with the canonical API - pro-moity structure and function. These include a well-known sub-class of prodrugs that release more than one active compound during cleavage and are called codrugs, or double prodrugs [67,68]. Codrugs are typically designed to release two complementary or synergistic therapeutic agents or two identical APIs. In theory, codrugs may not require a pro-moity as they can be linked directly, though in practice one is often used to fine-tune their properties. A seminal example is sultamicillin (Unasyn®), a double prodrug that releases equimolar portions of sulbactam, a beta-lactamase inhibitor, and ampicillin, a beta-lactam antibiotic. This particular codrug strategy is effective because sulbactam is able to prevent hydrolysis of the beta-lactam ring in ampicillin, a bacterial resistance mechanism that would otherwise render ampicillin inactive [69]. Another codrug example is sulfasalazine (Azulfidine®). Sulfasalazine is unique because it combines both a codrug strategy and the utilization of a disease specific activation strategy as further described below [70]. Sulfasalazine relies on anaerobic bacteria in the lower bowel to reduce an azo bond and release the APIs mesalamine and sulfapyridine for the treatment of Crohn's disease, rheumatoid arthritis, and ulcerative colitis [71,72].

2.4. Bioprecursor prodrugs

Another class of non-canonical prodrugs are bioprecursor prodrugs, a family of therapeutics that require biotransformations other than enzymatic hydrolysis to yield an active compound. Examples of bioprecursor prodrugs that require cleavage of a pro-moity and subsequent biotransformation are valganciclovir (Valcyte®) and prasugrel

(Effient®). Activation of valganciclovir begins with the esterase-mediated hydrolysis of an L-valine pro-moity to produce the inactive metabolite ganciclovir, which is then metabolized by both viral and human kinases to the final active form ganciclovir triphosphate [73,74]. Metabolism of prasugrel begins with deacetylation to form an inactive thiolactone, which is then hydrolyzed to the active agent R-138727 [75,76]. Some bioprecursor prodrugs do not require cleavage of a pro-moity [77,78]. One such example is bempedoic acid (Nexletol®), a bioprecursor prodrug approved in 2020 for the treatment of hypercholesterolemia [75,76]. Bempedoic acid is activated in the liver by very-long-chain acyl-CoA synthetase-1; the activated metabolite inhibits adenosine triphosphate citrate lyase, an enzyme involved in cholesterol biosynthesis. Latanoprostene bunod (Vyzulta®) is a hybrid prodrug that combines bioprecursor and codrug strategies to deliver a prostaglandin analogue and a nitric oxide (NO) donating pro-moity that, like tafluprost, is used to treat patients with open-angle glaucoma and ocular hypertension. Initial codrug hydrolysis is catalyzed by corneal esterases to release an active prostaglandin, latanoprost acid, and a bioprecursor pro-moity, butanediol mononitrate, that is further metabolized to produce NO and 1,4-butanediol [79,80].

2.5. Prodrugs with disease-specific activation mechanisms

Some enzymatically activated prodrugs do not utilize patient-specific enzymes for conversion, but instead take advantage of unique enzyme activity arising from the disease they are intended to treat. Examples of this class of drugs are nitroimidazole prodrugs such as secnidazole (Solosec®) and benznidazole (Radanil®). Secnidazole, which is used for the treatment of bacterial vaginosis, contains a 5-nitroimidazole prodrug and is activated by the pyruvate-ferredoxin oxidoreductase complex [81]. Benznidazole, which is used to treat Chagas disease in children, contains a 2-nitroimidazole prodrug and is activated by parasite type I nitroreductase enzymes [82]. Both of these prodrugs are activated by bacterial reductase enzymes which are absent in eukaryotic cells ensuring a targeted effect.

Additional therapies employing a pathogen-mediated conversion approach are in pre-clinical development. These include the use of prokaryotic beta-lactamase enzymes to activate NO-donor prodrugs to help disperse bacterial biofilms [83–86], enterobactin conjugates that capitalize on siderophore uptake machinery for selective delivery to pathogenic *E. coli* [87,88], and reactive oxygen species (ROS) responsive prodrugs that release a wide variety of drugs upon exposure to high local levels of ROS caused by various pathological conditions [37].

In addition to prodrugs composed of small molecule pro-moieties that mask certain functional groups or that are intended to adjust the chemical properties of a drug, more complicated antibody-drug conjugate systems that selectively target the parent drug to a specific site within the body have been developed [89–91]. Though most antibody-drug conjugates retain activity as the whole construct and are not considered prodrugs, some do require release of a pro-moity to function and meet the prodrug definition. An example of that type is gemtuzumab ozogamicin (Mylotarg®), where a pH sensitive hydrazide-based linker is used to attach the active calicheamicin to an antibody; internalization of the antibody-drug conjugate results in hydrolytic release of the active drug [92–95].

Other more complex systems referred to as Directed Enzyme Prodrug Therapies involve a two-step treatment process involving targeted administration of an exogenous enzyme followed by administration of a complementary prodrug. The enzyme is targeted directly to a desired site in the body using antibodies, polymers, or genes [96–99], while the prodrug is designed specifically to release the active drug only after interacting with the exogenous enzyme. This type of system has been evaluated preclinically and several examples are in clinical trials, though no commercial examples exist.

2.6. Prodrugs with modified delivery route

An interesting case study highlighting the evolution of prodrug design strategy based around a single API in order to optimize the route of administration is the development of Sinemet® and subsequently Inbrija® for the treatment of Parkinson's disease. Parkinson's disease is characterized by progressive neurodegeneration that depletes dopamine from the central nervous system. Most treatments of Parkinson's disease rely on various strategies to replace dopamine. Dopamine replacement therapies are quite challenging because dopamine is highly hydrophilic and cannot permeate the blood brain barrier (BBB). To resolve this issue, zwitterionic levodopa was developed as a prodrug of dopamine that is activated by aromatic L-amino acid decarboxylase enzymes after crossing the BBB [100,101]. Levodopa can suffer from premature activation following oral administration and is often administered in conjunction with an enzyme inhibitor known as carbodopa (Sinemet®). Unfortunately, carbodopa only inhibits human enzymes and cannot inhibit bacterial decarboxylase enzymes that may be found in the intestines [102]. Differences in bacterial decarboxylase concentration, either patient-to-patient, day-to-day, or dose-to-dose, can complicate dosing and may introduce variability to levodopa efficacy. As a potential improvement to the orally administered tablet, levodopa inhalation powder (Inbrija) was developed as an inhaled dopamine replacement therapy that avoids gut metabolism and offers enhanced and more predictable PK relative to oral administration. Inbrija showcases how the combination of a prodrug strategy with a new route of administration can be effective to develop therapies for difficult-to-treat diseases like Parkinson's Disease.

2.7. Challenges and future directions

While there are many promising examples of prodrug technologies being utilized to improve existing drugs or design new drugs (both approved and in development/clinical trials), it is not possible to create successful prodrug versions of all APIs. For example, despite numerous attempts, prodrug development of the widely used chemotherapeutic agent cisplatin, and other approved platinum(II) agents have failed [103]. Ormaplatin (Tetraplatin, NSC 363812), a platinum(IV) complex that is converted *in vivo* to an active platinum(II) complex was studied in a phase I clinical trial but development was halted due to unpredictable neurotoxicity [104]. Iproplatin, a platinum(IV) complex structurally similar to ormaplatin, but less susceptible to reduction, advanced to phase III clinical trials where it failed to show activity [105,106]. Satraplatin, a platinum-based antineoplastic agent, also advanced to phase III clinical trials but did not achieve superiority to cisplatin, likely due to unfavorable PK and premature reduction to the active compound [107]. For examples like satraplatin, where a standard prodrug strategy based on oral delivery failed as a result of premature release, it is possible that a combined approach involving a prodrug to modulate PK and a nanoparticle formulation to facilitate delivery could be successful.

Another factor complicating prodrug development is enzymatic genetic polymorphism, as there are numerous reports in the literature where genetic variants influence drug metabolism and clinical outcomes [42,108]. Tamoxifen (Nolvadex®) is an estrogen modulator prodrug approved for the treatment of metastatic breast cancer. Tamoxifen is activated by the cytochrome P450 isozymes cytochrome P2D6 and cytochrome P3A4, to generate multiple active metabolites including endoxifen and afimoxifene [109,110]. In patients with genetic variants of the cytochrome P2D6 gene, metabolism of tamoxifen can be too slow, which results in reduced conversion rates and diminished activity of the drug [111,112]. A similar trend is observed in patients receiving sulindac (Clinoril®), a, where variations of flavin-containing monooxygenase 3 enzymes are associated with different drug metabolism rates and clinical outcomes [108,113,114].

Despite these challenges, given the many successful prodrugs on the market today, it is clear that prodrug strategies enable impressive levels

of fine-tuning and optimization of therapeutic agents that can be designed to achieve favorable clinical outcomes. In particular, the ability to attenuate challenging physical properties and toxicities of APIs while preserving their biological effect, make prodrug development a powerful tool to elicit unparalleled control over the pharmacology of a drug. As the cited examples show, prodrug strategies are not a one-size-fits-all approach and need to be considered on a case-by-case basis.

3. Nanoparticle-based drug delivery systems

Interest in nanoparticles as therapeutics has grown in recent decades due to their ability to carry large payloads of therapeutic drugs and preferentially deliver them to specific tissues or sites [115–118]. Fig. 2 summarizes various types of nanoparticle-based delivery systems [119] such as liposomes [120–124], solid lipid nanoparticles [125–127], micelles [128–132], cubosomes [133,134], polymeric nanoparticles [135–137], and inorganic nanocarriers [138–141]. Nanoparticle-based drug delivery systems have the potential to modify the PK characteristics of their APIs, which may include a longer half-life and greater distribution to the site of action; these features can lead to improved efficacy and a reduction in adverse effects [142]. For poorly soluble hydrophobic drugs, nanoparticle formulations can render them suitable for delivery by various routes of administration and improve their bioavailability [143,144]. Nanoparticles can also be designed to utilize targeting moieties to deliver drugs more precisely to the site of action [145] or to carry multiple drugs in a synergistic molar ratio to optimize clinical effect [146].

Other design elements are focused on the optimal release of the drug payload once the nanoparticle has been delivered to the target site [147]. Environmentally-responsive nanoparticle-based delivery systems allow controlled or sustained drug release from the matrix in response to changes in temperature, pH, or other physiological factors [148]. Additionally, some inorganic nanoparticles have stimuli-responsive functions such as remotely triggering the release of therapeutics by utilizing their surface plasmon resonance property [149] or by an external magnetic field [150]. Nanoparticle formulation is an inherently complex process that requires extensive understanding of underlying physical principals that contribute to particle formation, stability, and function [151]. Various means to engineer a nanoparticle-based drug delivery system that tailors to different purposes are schematically represented in Fig. 3 and have been discussed elsewhere [152–157].

While nanoparticle-based drug delivery systems have been used in the clinic since the early 1990 s, the field has continued to evolve in concert with innovations in technology to improve the delivery of therapeutics [158,159]. Progress and advances of clinically approved nanoparticle-based drug delivery systems that encapsulated unmodified drugs have been extensively reviewed [137–140,152,160–163]. In this section, we will focus on the challenges and future of these platforms.

3.1. Challenges and future directions

Despite the advantages that nanoparticle technologies can offer, the successful rate of clinical translation remains low. Some challenges that nanoparticle-based delivery systems still face include the need for even better control of the drug release kinetics and biodistribution as well as improvement of the formulation characteristics such as stability and encapsulation efficacy [164].

In one example, SPI-77, a long-circulating liposome encapsulating cisplatin currently in clinical development, achieved an extended circulation time compared to free cisplatin [165]. However, the slow and inefficient release of the encapsulated drug resulted in the lack of anti-tumor activity of SPI-77 in clinical trials [165,166]. In a second example, L-NDPP (Aroplatin™), a cisplatin analog (*cis*-bis-neodecanoato-*trans*-R, R-1,2-diaminocyclohexane platinum II) encapsulated within non-PEGylated multilamellar liposomes showed unfavorable accumulation in the liver limiting its clinical value [167]. Additionally,

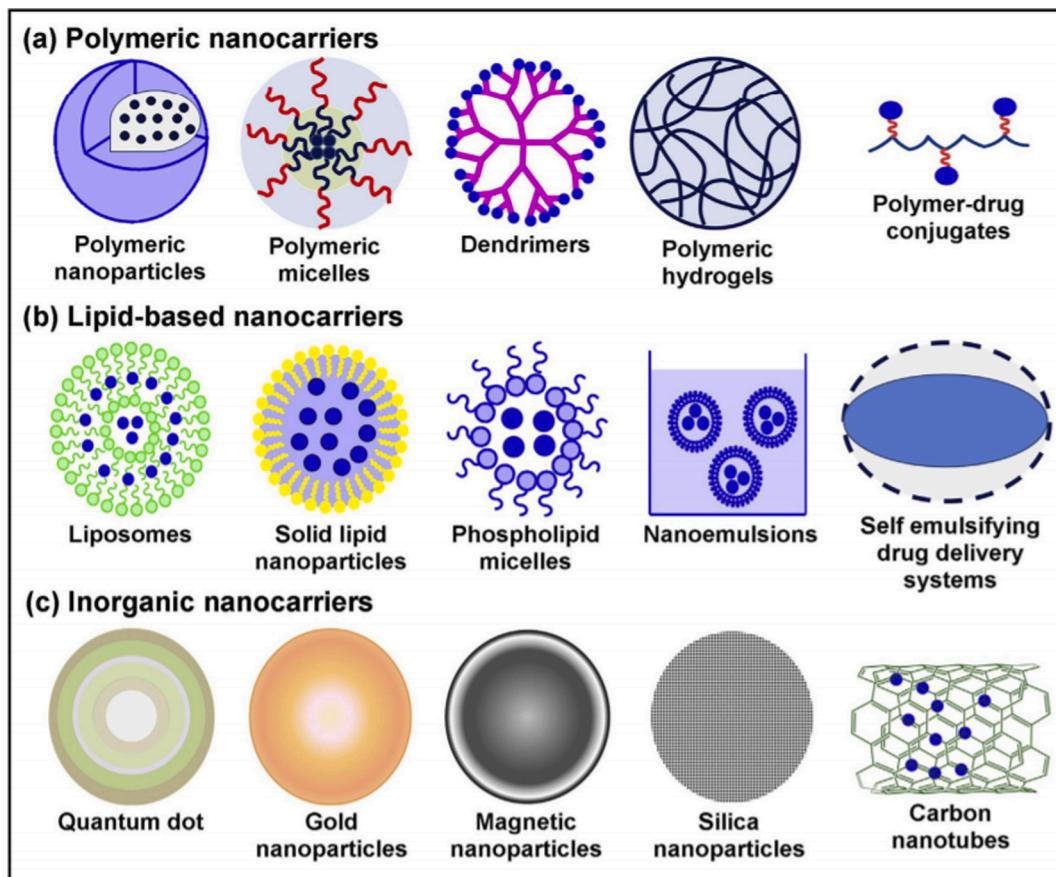


Fig. 2. Summary of various types of nanoparticle-based drug delivery systems. Reprinted from Trends in Food Science & Technology, 54, Divya Arora, Sundeep - Jaglan, Nanocarriers based delivery of nutraceuticals for cancer prevention and treatment: A review of recent research developments, 114–126, Copyright 2016, with permission from Elsevier.

high degradation levels of the complex were observed after reconstitution, resulting in the inactivation of the drug [120]. A further example is LiPlaCis®, a novel liposomal formulation of cisplatin with a triggered release property designed to be degraded by secretory phospholipase A₂ in tumor sites. However, renal toxicity and acute infusion reactions were observed in many patients [168]. The poor safety profile suggests that further reformulation and optimization are required.

Going forward, there is a need to better understand how biology affects nanocarrier PK to ensure optimized pharmacology and toxicity profiles. The adsorption of plasma proteins onto nanoparticles *in vivo* can alter their surface properties, affecting the interactions of nanoparticles with biomolecules and thus their pharmacological properties, therapeutic efficacy, and toxicity that contribute in part to the lack of correlation between *in vitro* and *in vivo* results [169]. This phenomenon is referred to as protein corona (or biocorona) formation and is perhaps the most significant factor affecting nanoparticle behavior and fate when administered intravenously. The structural and physicochemical complexity of the formulations can also limit their clinical translation potential, as it can be quite problematic to pharmaceutically manufacture complex nanoparticle systems at large-scale. Specific challenges include overcoming inadequate quality control and product purity, low product yield, insufficient batch-to-batch reproducibility and stability, and high cost [170]. Yet, the future of nanoparticle drug delivery systems as therapeutics is promising and their potential to have a major impact on human health will become even more compelling as these current limitations are addressed.

4. Progress with formulation of prodrugs within nanoparticles

In the preceding sections of this review, two key strategies were outlined to improve the PK, safety profile, and therapeutic efficacy of drugs. One is the direct chemical modification of an API to form a prodrug; the other is the encapsulation of therapeutics within nanocarriers. Despite the advantages mentioned in previous sections, both strategies have challenges to overcome. The unprotected nature of prodrugs can lead to their rapid clearance and premature degradation, while nanoparticle-based delivery systems face unfavorable drug loading and leakage. Combining these two strategies allows formulation scientists to exert additional control over the chemical and biological properties of therapeutics and has the potential to further enhance their clinical translation [171]. For example, with the protection of nanocarriers, the degradation of prodrugs can be minimized. By incorporating prodrugs within nanocarriers, additional administration routes can be explored. By modifying the surface chemistry of the nanocarriers, improved efficacy can be achieved. In this section, a selection of articles on PNDDs published in 2018 or later is reviewed, highlighting their potential advantages over prodrug-only or nanoparticle-only strategies. These examples were selected to provide a diverse perspective for this rapidly evolving field, featuring the most advanced and - in our view - most scientifically interesting studies categorized by indication. A list of each individual system described in this section is also summarized in Table 2. Additionally, Fig. 4 shows two generic PNDDs assembly strategies; i.e., prodrug encapsulation utilizing various types of nanocarriers and carrier-free self-assembled nanoparticles from prodrugs with amphiphilic characteristics.

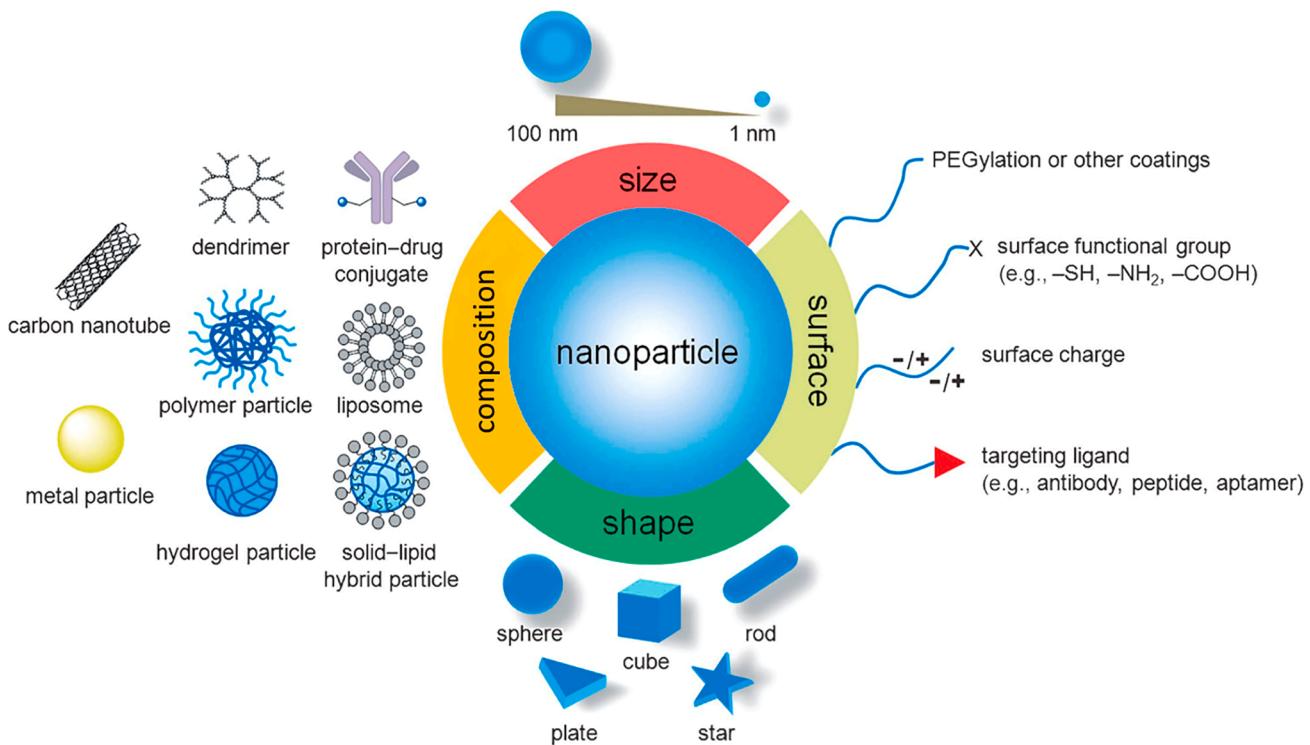


Fig. 3. Various means to engineer nanoparticle-based drug delivery systems. Reprinted with slight modification from Surface Science Reports, 72, Hendrik Heinz, Chandrani Pramanik, Ozge Heinz, Yifu Ding, Ratan K. Mishra, Delphine Marchon, Robert J. Flatt, Irina Estrela-Llopis, Jordi Llop, Sergio Moya, Ronald F. Ziolo. Nanoparticle decoration with surfactants: Molecular interactions, assembly, and applications, 1–58, Copyright 2017, with permission from Elsevier.

4.1. Oncology

While only one PNDD is currently approved, many candidates are in clinical development. Without question, the field of oncology has yielded the greatest number of prodrug-based nanoparticle formulations moving into the clinic. This is likely due to the great need for more tolerable cancer therapeutics. Numerous publications describe these efforts and many of them have been reviewed elsewhere [172]. Here, an overview of the most recent PNDDs for the treatment of cancer is catalogued by API.

4.1.1. Platinum-based drugs

Platinum-based drugs are among the most effective chemotherapeutics available today, despite several significant shortcomings. Chief among them are dose-limiting side effects as well as drug resistance due to inefficient cellular ingestion, deactivation by glutathione (GSH) and metallothionein detoxification [173]. In an effort to address these limitations, there have been many attempts to develop novel platinum-based therapeutics that minimize toxicity and improve therapeutic efficacy [174,175]. One example is a library of redox-responsive platinum-based prodrugs with varying hydrophobic carboxylate ligands (denoted as Pt(IV) n) [176]. When the Pt(IV) n-prodrugs are mixed together with amphiphilic lipid-PEG, the hydrophobic prodrugs self-assemble to form a Pt(IV) n prodrug-based nanoparticle delivery system (Pn NP) for cancer therapy [176]. As shown in Fig. 5, upon endocytosis and exposure to intracellular GSH, the resulting lead formulation (P6 NP) disintegrates, creating a GSH-exhausting effect, which reduces the probability of thiol-mediated platinum detoxification. The PEG functionalization on the nanoparticle resulted in longer blood circulation and higher tumor accumulation *in vivo*. Altogether, this PEGylated P6 NP delivery platform achieved better therapeutic outcomes in an *in vivo* tumor xenograft mouse model compared to free cisplatin and the Pt (IV) 6 prodrug itself, including overcoming drug resistance while minimizing off-target side-effects [176].

Several carrier-free therapies have been investigated as well. NC-6004 or Nanoplatin® is a micellar nanoparticle formulation prepared by exchanging cisplatin's chloride ligands with carboxylates from PEG poly(glutamic acid) block copolymers [177,178]. Coordination bonds cross-link the platinum metal center to block copolymers, creating a labile bond that allows this type of Pt drug to be classified as a prodrug, resulting in self-assembly into micelles. Nanoplatin was better tolerated and offered improved antitumor activity relative to cisplatin in a variety of animal models. In a phase Ia/II clinical trial in patients with advanced solid tumors, Nanoplatin, in combination with gemcitabine, achieved greater cisplatin equivalent doses without clinically significant toxicity issues, suggesting that a larger therapeutic window can be achieved [177].

Following a similar design strategy, DACH-Pt, 1,2-diaminocyclohexane-platinum(II), a platinum-based prodrug, was evaluated as a potential treatment for recurrent ovarian cancer using a variety of formulations [179,180]. These formulations are typically composed of a copolymer that serves to increase plasma half-life and a chelator that binds the inactive platinum species at physiological pH to prevent off-target effects. After exposure to the low pH environment found in tumors, the active platinum species is released to achieve targeted delivery. Chelation of the platinum species is required for nanoparticle formation. The most advanced formulation, termed ProLinDac or AP5346, uses a hydroxypropylmethacrylamide (HPMA) copolymer and an amidomalonato chelator and was both safe and efficacious in a phase II clinical trial [181]. More recently, the DACH-Pt prodrug was reformulated as part of a self-assembled, biodegradable dendritic copolymer-based drug delivery system (NP-TPGS-Pt) for the treatment of multidrug-resistant lung cancer [182]. In this work, a PAMAM-NH₂-G3 dendrimer was conjugated to glutamic acid, which provided carboxylate moieties that chelated the Pt metal center and an α-tocopherol PEG 1000 polymer unit. The NP-TPGS-Pt formulation suppressed growth of drug-resistant cancer cells *in vivo* and represents a novel strategy for treating multidrug-resistant tumors.

Table 2
Summary of highlighted PNDDs.

Example	Prodrug	Prodrug Activation Mechanism	Delivery System	Ref
Oncology				
P6 NPs	Platinum(IV) prodrug with carboxylate ligands	Redox	Lipid-PEG nanoparticles	[176]
Nanoplatin	Platinum-based Polymer-metal complex	Platinum ligand exchange	Polymeric micelles	[178]
AP5346	Diaminocyclohexane-platinum	Platinum ligand exchange	Self-assembled nanoparticles from prodrug	[180]
NP-TPGS-Pt	Diaminocyclohexane-platinum	Platinum ligand exchange	Self-assembled nanoparticles from prodrug	[182]
Pt(IV)-NP	Platinum(IV) prodrug with diaxial decanoic acid ligands	Platinum ligand exchange	Self-assembled nanoparticles cloaked with pluronic block copolymers	[183]
PEG-Por-CD: oxaliPt(IV)-ada nanoparticles	Adamantane modified oxaliplatin prodrug	Redox	Self-assembled supramolecular nanocarriers	[184]
Onivyde	Irinotecan	Ester hydrolysis; Esterases	Liposomes	[187]
Irinotecan-C12	Irinotecan ester	Ester hydrolysis; Esterases	Self-assembled nanoparticles from prodrug	[189]
NK012	Macromolecular SN-38 ester	Ester hydrolysis; Esterases	Self-assembled nanoparticles from prodrug	[191]
P(CL/CL-g-SN38)-b-PMPC	SN-38 ester	Ester hydrolysis; Esterases	Polymer prodrug micelles	[192]
SN38-etcSS-OA	SN-38 ester	Ester hydrolysis; Esterases	Rod-shaped nanoaggregates	[193]
Cremophor EL/NPs	SN-38 ester	Ester hydrolysis; Esterases	Cremophor EL nanoparticles	[194]
PgP-HA/HCPT-Gu nanoparticles	Guanidine-modified 10-hydroxyacamptothecin	Redox	Poly(L-glutamic acid)-g-methoxy polyethylene glycol nanoparticles	[199]
2DA-FITC-PTX nanoparticles	2-Glucosamine-glutamic acid-paclitaxel	Ester hydrolysis; Esterases	Self-assembled nanoparticles from prodrug	[207]
PTX-S-OA/PPa-PEG2k nanoparticles	Paclitaxel oleate	Redox	Pyropheophorbide a PEG2000 nanoshell	[210]
PTX-S-OA/TPGS2k nanoparticles	Paclitaxel oleate	Redox	Tocopherol PEG2000 succinate nanoshell	[211]
TB@PMP	Polymer-paclitaxel conjugates	Redox	Self-assembled micelles from prodrug	[212]
G(TM)PPSP	Deselenide paclitaxel	Redox	Gelatin and platinum nanocarrier	[214]
HSA(S-Cy)	Paclitaxel dimeric prodrug	Redox	Albumin-bound nanoparticles	[216]
2' monoester of paclitaxel	Taxol-2'- methylpyridinium acetate	Ester hydrolysis; Esterases	Self-assembled hydrogel	[219]
Squalenoyl-Paclitaxel Nanoassemblies	Squalenoyl ester of paclitaxel	Ester hydrolysis; Esterases	Squalenoyl-paclitaxel nanoassemblies	[220]
PAM-co-PPEGMEA-Linker-PTX	Paclitaxel esters	Ester hydrolysis; Esterases	Self-assembled paclitaxel-copolymer micelles	[221,222]
Hyaluronic Acid - Paclitaxel Conjugate Micelles	Hyaluronic acid -paclitaxel ester	Ester hydrolysis; Esterases	Self-assembled Hyaluronic acid -paclitaxel conjugate micelles	[217,218]
Oxidation-responsive self-assembled PEGylated paclitaxel ester	PEGylated paclitaxel ester	Ester hydrolysis; Esterases	Self-assembled nanoparticles from prodrug	[223]
PRMO@DOX nanoparticles	Cyclodextrin polyrotaxanes polymer-doxorubicin conjugates	Acidic pH	Self-assembled supramolecular nanoparticles	[224]
GSP-pDOX-CD	Acylated doxorubicin	Palladium catalysis	Mesoporous silica nanoparticles	[231]
BTZ-PEP-RGD nanoparticle	Peptide-bortezomib conjugates	Acidic pH	Self-assembled nanoparticles from prodrug	[232]
Ketal glycoside prodrug NP	Glucose-etoposide conjugates	Acidic pH; Glucuronidases	Self-assembled nanoparticles from prodrug	[235]
GKNP	Polyketal gemcitabine	Acidic pH	mPEG-PDLLA nanoparticles	[238]
PEG5k-GL2-IMDQ	Pegylated imidazoquinoline	Esterases; Glucuronidases	Self-assembled nanoparticles from prodrug	[240]
PROMITIL®	Lipophilic mitomycin C	Redox	Pegylated liposomes	[246,247]
PTPPSN	Vitamin E-triptolide conjugates	Redox	PEG-linoleic acid nanoparticles	[253]
TAM-loaded POEG-co-PVDSAHA micelles	Vorinostat-polymer conjugates	Redox	POEG-co-PVDSAHA micelles	[263]
C4-Pt-ADD@PEG NPs	Co-drug of cisplatin and adjuvin	Redox	DSPE-PEG nanoparticles	[264]
LPTP NPs	Co-drug of cisplatin and tolafenamic acid	Acidic pH	PLGA nanoparticles	[270]
pGO-Pt/DOX	Pegylated graphene oxide-cisplatin conjugates	Acidic pH	Self-assembled nanoparticles from prodrug	[271]
VDNP	Palmityl doxorubicin	Acidic pH	P(LA-co-TMCC)-g-PEG nanoparticles	[274]
siRac1/DDP NP	Cisplatin prodrug with diaxial decanoic acid ligands	Acidic pH	Meo-PEG- <i>b</i> -PDPA nanoparticles	[275]
CNPPTCP/si(c-fos)	Platinum(IV)-polymer conjugates	Blue-light irradiation	Self-assembled nanoparticles from prodrug	[277]
Infectious (Viral)				
SOF-MSN-APTES nanoparticles	Sofosbuvir	Cellular enzymes and kinases	Mesoporous silica nanoparticles	[287]
TAF + FTC nanoparticles	Tenofovir alafenamide	Cellular enzymes and kinases	PLGA nanoparticles	[289]
LRA-loaded LCNP	PLGA-LRA conjugates (Ing3A, prostratin, panobinostat)	Hydrolysis; Esterases; Amidases	Hybrid lipid-coated PLGA nanocarriers	[295]
Infectious (Bacterial)				

(continued on next page)

Table 2 (continued)

Example	Prodrug	Prodrug Activation Mechanism	Delivery System	Ref
POEGMA-b-PVBA-GEN-NONOate nanoparticles	Polymer-gentamicin conjugates	Hydrolysis	POEGMA-b-PVBA nanoparticles	[298]
Cardiovascular				
T-APP	Aspirin polyconjugate	Redox	Co-polymer particles	[303]
ACL	Lovastatin	Acidic pH	Alginate/chitosan nanoparticle	[304]
Inflammatory (Rheumatoid Arthritis)				
AKP-dex-loaded NPs	Alcohol-dexamethasone conjugates	Acidic pH	Lipid-PEG nanoparticles	[301]
DFEE-TM	Diclofenac ethyl ester	Ester hydrolysis; Esterases	PEO-b-PCL micelles	[302]
Neurological				
L-DOPA-AuNPs	Levodopa	Ester hydrolysis; Esterases	Gold nanoparticles	[306]
Pulmonary Hypertension				
INS1009	Treprostinil palmitil	Ester hydrolysis; Esterases	Solid lipid nanoparticles	[317–320]

Ada, adamantane; ADD, adjudin; AKP, acetone-based ketal-linked prodrug; BTZ, bortezomib; CD, cyclodextrin; 2-DA-FITC-PTX, 2-glucosamine-glutamic acid fluorescein isothiocyanate paclitaxel; dex, dexamethasone; DFEE, diclofenac ethyl ester; DOX, doxorubicin; DSPE-PEG, 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol); FITC, fluorescein isothiocyanate; FTC, emtricitabine; GO, graphene oxide; HA, hyaluronic acid; HCPT, 10-hydroxycamptothecin; HCPT-Gu, guanidine-modified HCPT prodrug; HSA, human serum albumin; IMDQ, imidazoquinoline; L-DOPA, levodopa; Meo-PEG-b-PDPA, methoxyl-poly(ethylene glycol)-b-poly(2-(diisopropylamino)ethyl methacrylate; NONOate, N-diaziniumdiolate; NP, nanoparticle; OA, oleate; PDLLA, poly(D,L-lactide); pDOX, DOX prodrug; PEG, polyethylene glycol; PEO-b-PCL, poly(ethylene oxide)-b-poly(ϵ -caprolactone); PgP-HA, poly(L-glutamic acid)-g-methoxy polyethylene glycol conjugated-hyperbranched aliphatic polyester; P(LA-co-TMCC)-g-PEG, poly(D,L-lactide-co-2-methyl-2-carboxy-trimethylene carbonate)-g-poly(ethylene glycol); PLGA, poly(lactic-co-glycolic acid); POEG-co-PVD, poly(oligo(ethylene glycol))-co-poly(vinylbenzyl chloride); POEGMA-b-PVBA, poly(oligo(ethylene glycol) methacrylate-b-poly(vinyl benzaldehyde); Ppa, pyropheophorbide-a; Pt, platinum; PTX, paclitaxel; SAHA, vorinostat; TAF, tenofovir alafenamide; TM, telmisartan.

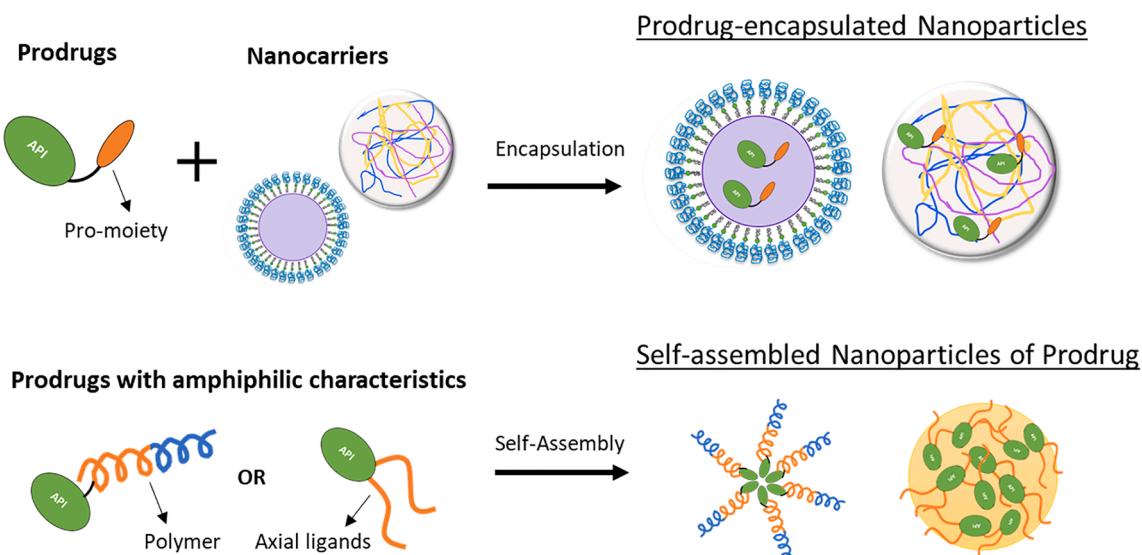


Fig. 4. Assembly strategies of PNDDs. Assembly of PNDDs can be achieved by either prodrug encapsulation using various types of nanocarriers (top) or carrier-free nanoparticles that can be generated via self-assembly of prodrugs with amphiphilic characteristics (bottom).

Based on a slightly different strategy, a Pt(IV) prodrug system was described that uses hydrophobic axial ligands to reduce toxicity and overcome drug resistance [183]. The axial ligands including linoleic acid, lauric acid, and hexanoic acid on a Pt(IV) Cl_2NH_2 core induce self-assembly in aqueous solution to form stable colloidal nanoparticles. For increased cellular internalization via endocytosis, the self-assembled nanoparticles were surface-coated with Pluronic block copolymers to generate a PEGylated nanoparticles formulation (Pt(IV)-NP) suitable for IV administration. *In vitro* and *in vivo* studies indicate that Pt(IV)-NP was more potent and less cytotoxic than cisplatin alone.

To further increase the therapeutic efficacy of chemotherapy, photosensitizers have been incorporated into PNDDs. For example, a supramolecular self-assembled oxaliplatin prodrug-based nanoparticle system was designed to facilitate photodynamic therapy [184]. Here, adamantane-modified oxaliplatin prodrugs (oxaliPt(IV)-ada) and β -cyclodextrin-modified porphyrin photosensitizers (PEG-Pro-CD) self-

assembled into nanoparticles that displayed good colloidal stability and disassembled in a reducing environment. Time-dependent generation and cellular accumulation of cytotoxic ROS were observed from the photosensitizer under light irradiation. The improved anticancer effect observed with these nanoparticles combined with light irradiation against 4 T1 cells *in vitro* compared to monotherapy, was attributed to the dual killing mechanism of ROS and platinum where the generation of ROS causes oxidative cellular apoptosis and the release of oxaliplatin causes DNA damage [185]. *In vivo*, these nanoparticles showed better tumor growth inhibition compared to monotherapy in 4 T1 tumor-bearing mice. Additionally, haematoxylin and eosin stained tumor tissues from the treatment group receiving these nanoparticles plus light irradiation indicated more pronounced tumor apoptosis without noticeable damage to other major organs. With the integration of photodynamic therapy and chemotherapy, this work demonstrates the potential to further improve the therapeutic efficacy of

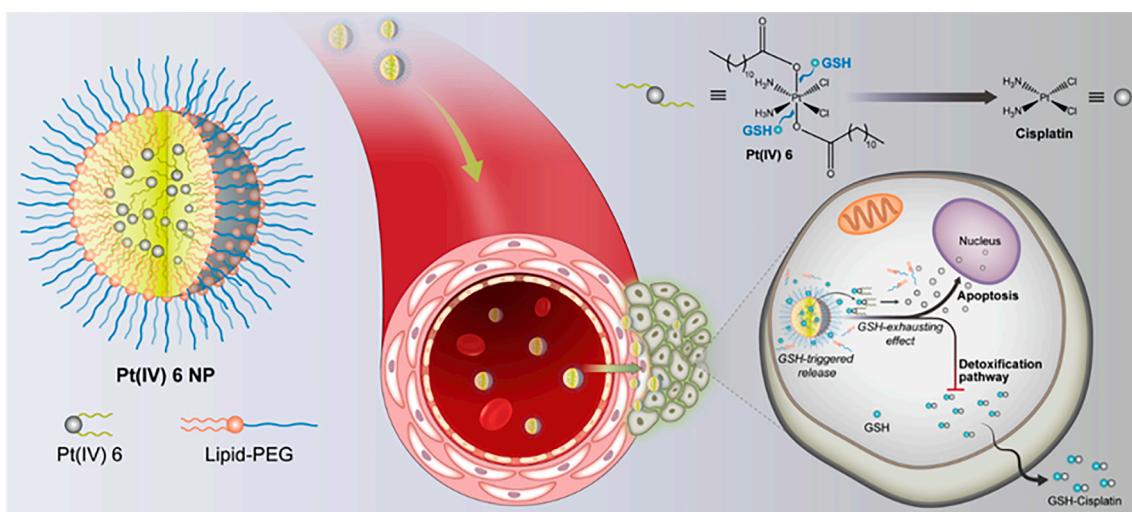


Fig. 5. Schematic representation of redox-responsive self-assembled Pt(IV) 6 NP for the treatment of cisplatin-resistance tumors. After endocytosis, Pt(IV) 6 NPs are disintegrated by intracellular GSH that reduces thiol-mediated detoxification of platinum-based drugs. Reprinted with permission from ACS Nano 2019, 13, 357–370. Copyright 2019 American Chemical Society. GSH, glutathione; NP, nanoparticle; Pt, platinum.

chemotherapeutics through nanomedicines.

4.1.2. Camptothecin

Another common chemotherapy drug is camptothecin. In order to improve its water solubility and antitumor activity, a lot of research has focused on synthesizing novel camptothecin analogs since its discovery. Two analogues of camptothecin, irinotecan and topotecan, have been approved for cancer treatment as a result [186]. Of these, irinotecan, a prodrug that converts to its active metabolite SN-38 via nonspecific carboxylesterases, is the most widely investigated. Unfortunately, irinotecan has several clinical disadvantages including rapid drug elimination and major dose-limiting toxicities such as diarrhea and neutropenia [187].

A rationally designed drug delivery system may offer the opportunity to overcome these limitations by protecting irinotecan from premature metabolism. An example of such a system is a liposomal formulation of irinotecan, called Onivyde®, that has been developed for the treatment of metastatic pancreatic cancer. It is indicated for patients who have previously been treated with gemcitabine and is given in combination with 5-fluorouracil and leucovorin [188] and was approved by the FDA in 2015. In the phase III NAPOLI-1 trial, the Onivyde/5-fluorouracil/leucovorin combination regimen maintained its overall survival benefit in the 12-month follow-up along with a adequate safety and tolerability profile compared to 5-fluorouracil/leucovorin treatment alone. Because Onivyde monotherapy did not demonstrate superior efficacy compared to the 5-fluorouracil/leucovorin treatment and was associated with more adverse events compared to combination therapy, the drug is only approved for use in conjunction with 5-fluorouracil/leucovorin [188]. There is also an ongoing phase I/II, open-label trial (NCT02551991) designed to assess the dose-limiting toxicities, safety, and tolerability of Onivyde when used in combination with 5-fluorouracil, leucovorin, and oxaliplatin, as a first-line treatment for patients with metastatic pancreatic cancer. This additional Onivyde combination therapy represents a promising strategy to improve the clinical outcome for the treatment of a variety of tumor types by ameliorating the limitations of single drug anticancer medicines. While this example utilized irinotecan as the prodrug to deliver the active metabolite SN-38 as part of a liposomal formulation, as discussed previously in the prodrug section of this review article, alternative prodrug design strategies can be imagined for almost any active compound through variation of the labile bond type and the pro-moiety structure. Indeed, researchers have developed alternative prodrug/nanoparticle approaches to deliver SN-38 [189–191]. Recent examples include development of SN-38 grafted

amphiphilic phosphorylcholine polymers [192], a redox-responsive lipophilic SN-38 prodrug [193], and hydrophobic SN-38 redox hypersensitive nanorods [194].

Another camptothecin analog is 10-Hydroxycamptothecin (HCPT), which is more potent and less toxic than camptothecin; however, its poor water solubility and chemical instability - owing to the opening of its labile lactone ring at physiological pH - prevent HCPT from widespread clinical use [195]. Various nanomedicines have been designed to help HCPT achieve greater efficacy with lower toxicity [196–198]. Most recently, a ROS-responsive phenylboronic acid pinacol ester nanocarrier loaded with a guanidine-modified HCPT prodrug (HCPT-Gu) was developed [199]. The nanocarrier was synthesized by grafting hyper-branched aliphatic polyester (HAPE) to a pre-formed poly(L-glutamic acid)-g-methoxy PEG (PLG-g-mPEG) nanoparticle via Steglich esterification, resulting in PLG-g-mPEG-HAPE (PgP-HA). The PgP-HA nanocarriers showed very good stability and high drug loading capacity for HCPT-Gu, which is attributed to the superposition of hydrogen bonds from both the guanidine and carboxyl groups, hydrophobic interactions, and $\pi-\pi$ stacking interactions of the phenylboronic acid pinacol ester group of PgP-HA and the HCPT prodrug. *In vitro* uptake studies suggested that HCPT-Gu improved cellular uptake compared to HCPT alone as a result of guanidine's suspected ability to penetrate cells [200]. *In vivo* PK results demonstrated PgP-HA/HCPT-Gu nanoparticles had a longer circulation time than HCPT-Gu alone; hence, it may have greater potential to reach and sustain high levels of drug at the target tumor sites. Consistent with the superior PK profile, *in vivo* antitumor efficacy data showed that PgP-HA/HCPT-Gu nanoparticles displayed a growth inhibition rate of 80.6% with negligible toxicity. In contrast, both HCPT and HCPT-Gu only reduced tumor growth by 27.4% and 34.5%, respectively in addition to exhibiting moderate systemic toxicity. Altogether, this study demonstrates the potential of PNDDs fabrication for the advancement of cancer therapy.

4.1.3. Paclitaxel-based drugs

Paclitaxel (PTX) is another widely used chemotherapy agent and has been used as a cancer treatment since 1993 [201]. As discussed in the nanoparticle-based delivery system section, much research has been devoted to developing better drug delivery systems for PTX [202,203], many of which have been reviewed elsewhere [204–206]. A recent study combined a modified PTX prodrug with a targeting strategy [207]. Here, an amphiphilic and fluorescent prodrug of PTX, 2-glucosamine-glutamic acid fluorescein isothiocyanate PTX (2-DA-FITC-PTX), was synthesized and self-assembled into nanoparticles by nanoprecipitation. 2-DA,

which has a good tumor targeting effect, was selected as the targeting motif [208,209] to enhance tumor accumulation of the resulting formulation (2-DA-FITC-PTX NPs). Selective targeting and cytotoxicity were observed for the targeted formulation in an *in vitro* co-culture system of cancer and normal cell lines. In an MDA-MB-231 breast cancer model in mice, the nanoparticle assembly showed superior tumor accumulation and reduced tumor size two-fold compared to treatment with free PTX while extending survival 1.5-fold.

Another recent set of studies showed how a redox-sensitive prodrug of PTX, PTX-S-oleate (PTX-S-OA), took advantage of overproduction of GSH in tumor cells to achieve target-specific release of PTX. To further enhance site-specific release, PTX-S-OA was packaged into either an inert or a light-activated polymer shell [210,211]. The inert nanoparticle system utilized tocopheryl PEG 2000 succinate to form a PEG shell around the PTX-S-OA core with up to 66% drug-loading efficiency. PEGylation promoted stability and extended circulation time while reducing cytotoxicity. Treatment of KB-3 xenograft tumors in mice with this nanoparticle resulted in four times lower tumor volume compared to treatment with free drug [211]. The light-activatable nanoparticle system utilizes pyropheophorbide-a PEG 2000 (PPa-PEG2k) to encapsulate PTX-S-OA. Upon light-exposure at 660 nm, PPa-PEG2k generates ROS designed to work synergistically with endogenous ROS inside tumor cells to trigger the release of PTX. Hydrogen peroxide (H_2O_2)-dependent release of PTX from the nanoparticles was confirmed *in vitro* and the nanoparticle system had an improved circulation time *in vivo* compared to free drug. Treatment with these nanoparticles prevented growth of KB xenograft tumors in contrast to treatment with free non-ROS-sensitive PTX and PPa-PEG2k-encapsulated PTX which resulted in a three-fold and four-fold increase in tumor volume, respectively. Treatment benefit using free PTX-S-OA as a comparator was not measured [210].

An interesting example of a photodynamic PTX therapy is a combination therapy of photosensitizing self-assembled aggregation-induced emission fluorogens (AIEgenes) loaded into reduction-sensitive PTX prodrug polymer micelles termed TB@PMP [212]. First, PTX prodrugs were synthesized by conjugating PTX to an amphiphilic and redox-sensitive polymer backbone via disulfide bonds. Then the amphiphilic polymeric prodrugs self-assembled together with hydrophobic AIEgen photosensitizers in aqueous solution. Cellular uptake studies indicated that TB@PMP showed improved ROS generation compared to traditional photosensitizer treatments. In addition, *in vitro* studies in HeLa cells suggested that TB@PMP under light irradiation exhibited synergistic cytotoxic effects and enhanced cell apoptotic activity compared to PTX or photodynamic therapy alone. TB@PMP also accumulated selectively in tumor tissues after tail vein injection of HeLa tumor xenograft mice due in part to the EPR effect. Tumor growth was significantly inhibited as a result of TB@PMP treatment compared to micelles prepared without PTX or TB@PMP administered without light irradiation. These results demonstrate that TB@PMP has promising therapeutic potential for controlling malignant diseases due to its increased chemical stability, controlled drug release, high drug loading, preferential tumor accumulation, and delivery of synergistic drug combinations. Notably, TB@PMP was able to overcome aggregation-induced quenching that can result in reduced oxidant production and lower photodynamic therapy performance, which is a frequent problem for conventional photosensitizers [213].

The desire to combine photothermal therapy and traditional chemotherapy led to the development of a dual-drug combination platinum gelatin photothermal nanoparticle-based system loaded with telmisartan and PTX prodrug. The primary goal of this system was to overcome the treatment barrier created by excess extracellular matrix (ECM) that is produced by tumor associated fibroblasts in the tumor microenvironment, which is one of the main obstacles for nanoparticle penetration into tumor sites due to their dense collagenous matrix and high interstitial fluid pressure [214]. In this study, the nanoparticle formulation (G(TM)PPSP) was prepared by conjugating two different

nano particles together via an amide bond: Telmisartan (TM)-loaded gelatin nanoparticle (GNPs(TM)) and diselenide-PTX prodrug-loaded PEGylated platinum nanoparticles (PPSP). The GNPs(TM) component was designed for TM to be released after degradation of the gelatin nanoparticles by over-expressed matrix metalloproteinase (MMP)-2 in the tumor extracellular environment. This may prevent the build-up of ECM by attenuating transforming growth factor- β signaling and possibly immune cell recruitment into tumor sites. In contrast, the PPSP component was designed for ROS- and GSH-triggered PTX release via redox responsive diselenide bonds under near-infrared light. The PPSPs were prepared by attaching the diselenide-PTX prodrugs to platinum nanoparticles with PEG via a thio-platinum interaction. In a tumor spheroid penetration assay testing the fully assembled dual nanoparticle system, G(TM)PPSP penetrated deeper into tumor areas when MMP-2 was present compared to platinum nanoparticles alone or PPSP-linked gelatin nanoparticles without TM (GPPSP). The penetration depth was found to be dependent upon the size of the nanoparticle. In a co-culture system of 4 T1 and 3 T3 cells, G(TM)PPSP effectively disrupted tumor-associated fibroblasts through transforming growth factor- β down-regulation compared to PTX + TM or GPPSP. In a 4 T1 tumor bearing mouse model, interstitial fluid pressure was decreased when treating with G(TM)PPSP compared to PTX + TM or GPPSP. G(TM)PPSP also exhibited superior antitumor and tumor microenvironment remodeling effects over free PTX, PPSP, and GNPs(TM), especially when near-infrared light was used. These results suggest a potential use of telmisartan and PTX prodrug loaded platinum gelatin nanoparticle as a chemo-photothermal combination therapy for effective cancer treatment.

As described in the nanoparticle-based delivery system section, Abraxane is an injectable suspension of albumin-bound PTX nanoparticles approved by the FDA in 2005. While representing a significant advance over free PTX treatment, considerable drawbacks remain, which include the rapid dissolving of the nanoparticle formulation into soluble, albumin-bound PTX complexes after IV infusion [215] and overall low drug loading due to the high crystallinity of PTX. Built upon the concept of Abraxane, a theranostic “Abraxane-like” prodrug-based formulation (HSA(S-Cy) NP) was developed composed of human serum albumin (HSA) as the stealth shell and PTX dimeric prodrugs with thioether linkages (PTX₂-S) as well as photosensitizer IR780 iodides (Cy) as the core [216]. The dimeric prodrug design increased intermolecular hydrophobic interactions, thus preventing high crystallinity-induced self-aggregation of PTX. The flexible linker between PTX molecules additionally decreased its crystallinity and improved compatibility with its nanocarriers. Co-loading of the dimeric prodrug and IR780 iodide, a typical photothermal therapy agent that translates near-infrared light into heat inside the nanoparticle, resulted in redox-responsive drug release and light-activated spatio-temporal hyperthermia. *In vitro* stability testing in 100% fetal bovine serum substantiated 48-hour serum stability and *in vivo* biodistribution studies demonstrated enhanced tumor accumulation with prolonged blood circulation and optimized formulation stability in circulation. To evaluate *in vivo* efficacy, the nanoparticles were injected into mice with subcutaneous mouse breast tumor xenografts. In response to tumor irradiation with an 808 nm laser, the temperature of the tumors increased within 10 min and reached a plateau at 47 °C, demonstrating the formulation’s *in vivo* photothermal activity. As a result, high levels of tumor ablation were observed after treatment with the HSA(S-Cy) NPs under irradiation without systemic toxicity, indicating the synergistic antitumor efficacy of photothermal therapy and chemotherapy.

The introduction of amphiphilic groups into a drug molecule to promote the formation of carrier-free self-assembled nanoparticles has previously been described for other chemotherapeutic agents and has also been applied toward the preclinical development of PTX-based prodrug therapies as well [217,218]. An early example described the preparation of a PTX prodrug that used an ether linkage to connect a methylpyridinium-acetate salt pro-moiety [219]. In aqueous media this

prodrug self-assembled to form a transparent hydrogel with a diameter of approximately 10 nm. This represents one of the first attempts to develop a carrier-free self-assembling PTX-prodrug-based nanoparticle delivery system. Since this initial report, numerous similar strategies including squalenoylation to produce micelle-forming PEG-squalene-PTX [220], PEGylated copolymer systems [221,222], and PTX-hyaluronic acid conjugates that self-assemble into micelles [217,218] have been evaluated. Most recently, the development of an oxidation-responsive self-assembled PEG-PTX prodrug strategy for therapeutic applications was described [223], where *p*-(boronic ester)benzyl-based linkage chemistry was used to connect PTX to a PEG (5000 Da) pro-moiety. This construct self-assembles to form stable ~50 nm micelles that are activated upon exposure to elevated ROS present in tumors.

4.1.4. Doxorubicin

Doxorubicin (DOX) is a well-known anticancer drug used to treat many malignancies despite its several side-effects and limitations. To achieve targeted release and ameliorate systemic toxicities, DOX was covalently attached to a supramolecular macrocyclic cyclodextrin polyrotaxane polymer scaffold via a pH-sensitive hydrazone bond to generate an acid-labile self-assembling prodrug nanoparticle system (PRMO@DOX). PRMO@DOX nanostructures are less than 100 nm in diameter and display a pH-sensitive release profile with about 50% DOX released at pH 5, but less than 10% DOX released at pHs 6.8 and 7.4 after 48 h. In tumor-bearing nude mice, PRMO@DOX was as effective as free DOX in preventing tumor growth without any decline in body weight, in contrast to the free DOX treatment group with a 25% decline in body weight [224].

Another example of a DOX prodrug nanoparticle delivery system is one utilizing a palladium (Pd)-based nanocarrier to mediate in situ site-specific catalytic activation [225] of the prodrug instead of an enzyme-triggered transformation [226], which lacks exact tissue specificity and often leads to off-target toxicity [59,227]. In this work, a DOX prodrug (pDOX) was synthesized by acylation and combined with β -cyclodextrin (β -CD) to form pDOX-CD complexes, which were then adsorbed onto the surface or trapped within the pores of Pd-based mesoporous silica nanoparticles (SP) to yield the prodrug-loaded nanoparticle formulation (SP-pDOX-CD). In addition, glucose oxidase (GOD) was conjugated onto the surface of the SP-pDOX-CD particles to catalyze the oxidation of β -D-

glucose into gluconic acid and H_2O_2 in the glucose-rich tumor environment [228], resulting in the final formulation (GSP-pDOX-CD). At the tumor site, these Pd-based mesoporous silica nanoparticles (GSP-pDOX-CD) began to disassemble and GOD-mediated glucose oxidation further decreased the pH to fully disassemble the pDOX-CD complex. This triggers the activation of the pDOX prodrug by a Pd^0 -catalyzed bond cleavage mechanism. The GOD-mediated glucose oxidation process removes the excess β -D-glucose around the tumor, which facilitates the glucose oxidase-mediated cancer starvation [229,230] while the production of H_2O_2 further contributes to the tumor-killing activity (Fig. 6). *In vivo* data showed that this prodrug nanoparticle-based system could synergistically kill cancer cells and suppress tumor growth with high specificity compared to free DOX, DOX prodrug (pDOX), and the formulation without GOD (SP-pDOX-CD). This study demonstrates the potential of a multi-synergistic platform for cancer treatment [231].

4.1.5. Bortezomib

Bortezomib (BTZ) is a boronic acid-containing anticancer drug, whose unique chemistry was utilized to generate a biocompatible prodrug nanoparticle-based drug delivery system by conjugating cancer-targeting, mussel-derived peptides to bortezomib via pH-sensitive boronic acid ester bonds [232]. Specifically, a biomimetic peptide, PEP-RGD (FITC-(DOPA)₄-G5-RGDS), composed of the cancer-targeting sequence Arg-Gly-Asp-Ser (RGDS) at the C-terminus, a non-bioactive quintuple glycine spacer (G5), a tetrapeptide (DOPA)₄ with catechol groups, and a fluorescent molecule FITC for cell imaging at the N-terminal end, was synthesized. The catechol groups in the DOPA tetrapeptide were then reacted with bortezomib to obtain covalent pH-sensitive catechol/boronic acid ester bonds [233]. The resulting peptide-BTZ prodrug (BTZ-PEP-RGD) rapidly dissociated and released free BTZ in the weakly acidic endo/lysosome environment (pH 5.0–6.0) [234]. *In vivo* PK studies showed that the self-assembled BTZ-PEP-RGD nanoparticles had a prolonged blood circulation, which could facilitate BTZ accumulation at the tumor sites. After a 20-day treatment in a tumor xenograft mouse model, the BTZ-PEP-RGD nanoparticle group had the best tumor inhibition compared to the free BTZ, PEP-RGD peptide, and non-targeted BTZ-PEP-RGE nanoparticle groups, indicating the importance of specific uptake and drug release at the tumor site.

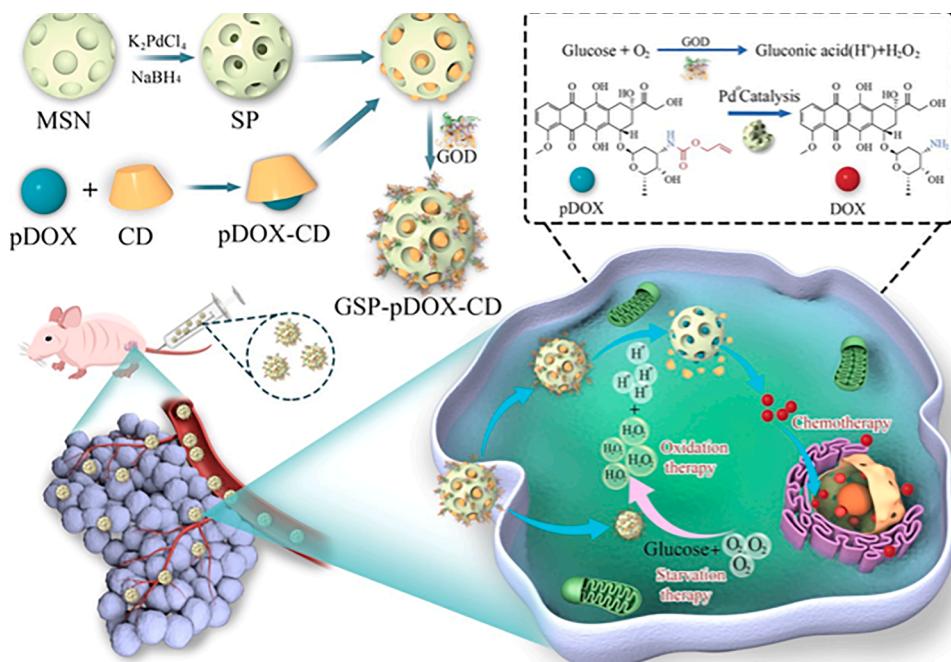


Fig. 6. Schematic illustration of a mesoporous silica nanoparticle delivery system (MSN) co-loaded with a metal palladium catalyst and a doxorubicin-prodrug (pDOX) solubilized using cyclodextrin (CD). Utilizing endogenous glucose oxidase (GOD), the prodrug is activated in situ by metal palladium resulting in multi-synergistic tumor-killing activity in the tumor tissue. Reprinted with permission from ACS Appl. Mater. Interfaces 2020, 12, 31, 34667–34677. Copyright 2020 American Chemical Society.

4.1.6. Etoposide

Glycoside prodrugs such as etoposide have been developed as a targeted chemotherapy against many types of tumors. However, the complexity of prodrug synthesis together with insufficient transformation to the API *in vivo*, have limited their usage. In order to address these issues, a ketal glycoside prodrug was recently designed to release its active metabolite following exposure to both glucosidase enzymes and low pH environments [235]. The ketal glycoside etoposide (ETP) prodrug was synthesized by conjugating hydroxyl group-containing monosaccharides with hydroxyl group-containing ETP using pH-sensitive acetone-based ketal linkages. The resulting amphiphilic ketal glycoside prodrugs were then self-assembled into glucose-decorated nanoparticles by nanoprecipitation. Hydrolysis studies showed that the ketal glycoside prodrug only released native ETP and glucose, indicating the prodrug fabricated from this method exhibited β -glucosidase- and acid-triggered self-immolative hydrolysis. These prodrug nanoparticles presented high cellular accumulation in A549 cells due to the glucose transporters expressed in this lung cancer cell, suggesting this strategy may be effective for tumors in which glucose transporters are overexpressed. The prodrug nanoparticles were also shown to accumulate preferentially in tumors in an A549 xenograft mouse model via a combination of the EPR effect and glucose transporter binding-mediated uptake. Greater hydrolysis was achieved in tumor tissue compared to other organs due to the β -glucosidase-rich acidic environment in the tumor cells. The tumor growth inhibitory effect of the prodrug nanoparticle was also higher than free ETP without any significant toxicity. These results demonstrate the potential of ketal glycoside prodrug glucose-decorated nanoparticles to be used as a glycoside prodrug monotherapy or a basic formulation for the development of stimulus-responsive self-immolative prodrugs for targeted chemotherapy.

4.1.7. Gemcitabine

Gemcitabine is a nucleoside analog used to treat many types of cancer. Yet, its clinical use has been hampered by premature metabolism, unfavorable PK, and off-target activity. In order to address these issues, several prodrug strategies have been explored by conjugating gemcitabine with long fatty acid chains or squalenic acid [236,237]. Recently, pH-sensitive gemcitabine polyketal prodrug nanoparticles were prepared with the goal to improve antitumor activity not only by increasing the systemic circulation time, but also by achieving efficient gemcitabine accumulation at the tumor site [238]. In this study, the prodrug was synthesized by conjugating gemcitabine to a polyketal backbone via pH-sensitive ketal linkages followed by nanoparticle encapsulation by nanoprecipitation. The prodrug nanoparticles demonstrated sustained release of the active metabolite in a pH-dependent manner during *in vitro* drug release studies. Improved antitumor effects were shown in the A2780 ovarian cancer cell line as well as a xenograft mouse model, yielding a better survival rate and improved tolerability compared to free gemcitabine. Together, this study demonstrates that pH-sensitive polyketal prodrugs have the potential to improve antitumor and antiviral activity of diol nucleoside analogues.

4.1.8. Imidazoquinoline

Immunotherapy has emerged as a promising strategy for the treatment of cancer [239]. Among several immune therapies, Toll-like receptor (TLR) 7/8 agonists like imidazoquinoline (IMDQ) have attracted attention due to their ability to activate antigen presenting cells and induce the presentation of antigen to T cells in secondary lymphoid organs. However, TLR agonists' clinical usage has been limited by severe side effects such as systemic inflammation and a short half-life after parenteral injection. As a strategy to increase tumor-specific immunity and thereby improve the response rate to IMDQ treatment, a novel amphiphilic polymer-prodrug conjugate of IMDQ loaded into a nanoparticle was prepared [240]. To this end, IMDQ was conjugated to PEG5k via the β -glucuronidase (β -GUS)-sensitive and self-immolative

linker GL2, followed by self-assembly into nanoparticles (PEG5k-GL2-IMDQ) in aqueous medium. Upon endocytosis by antigen presenting cells, esterase- and β -GUS-mediated cleavage revealed a labile intermediate that initiated the spontaneous and irreversible disassembly of the nanoparticle to release the active drug [241]. Interestingly, due to endosomal localization of esterases and glucuronidases, this release mechanism causes the highest rates of drug release to occur where the TLR7/8 receptors are located. *In vitro* release studies demonstrated efficient release of IMDQ from PEG5k-GL2-IMDQ nanoparticles in the presence of endosomal enzymes. *In vitro*, RAW Blue reporter cell assays further showed that TLR agonistic activity of PEG5k-GL2-IMDQ and GL2-IMDQ was superior after 72 h of incubation with PEG5k-GL2-IMDQ compared to free IMDQ, PEG5k-IMDQ, and GL2-IMDQ. In addition, subcutaneously injected PEG5k-GL2-IMDQ nanoparticles induced potent and prolonged innate immune activation in draining lymphoid tissues of *in vivo* mouse models compared to free IMDQ and PEG5k-IMDQ without systemic inflammation or toxicity. PEG5k-GL2-IMDQ also promoted the recruitment and maturation of dendritic cells *in vivo*. These data suggest that PEG5k-GL2-IMDQ may be a promising cancer immunotherapeutic utilizing an amphiphilic polymer-prodrug conjugation strategy.

4.1.9. Mitomycin c

Mitomycin-C (MMC) is a highly potent chemotherapeutic agent for various types of cancers; however, the accumulative toxicity limits its potential to use widely [242]. A PEGylated liposome delivery system platform utilizing a lipid-based prodrug of MMC known as MLP was designed to have an extended blood circulation time and favorable toxicity profile. This PEGylated prodrug-based liposomal formulation called Promitil® was composed of hydrogenated soybean phosphatidylcholine, methoxy-PEG-distearyl-phosphatidylethanolamine, and MLP at a molar ratio of 90:5:5 [243]. The lipid composition provides stability to the liposome and together with the PEGylation on the liposome surface resulted in good stability and prolonged PK *in vivo*. Due to the EPR effect, Promitil preferentially accumulates in tumors where the prodrug activation step occurs via the cleavage of a thiobenzyl bridge between the lipophilic pro-moiety and MMC. This allows for efficient and selective delivery of MMC to tumor tissue while minimizing systemic toxicity. Preclinical data showed a better therapeutic index and safety profile for Promitil compared to free MMC against various tumor models [244,245]. In a phase Ia/b clinical study, Promitil demonstrated an improved stabilization rate in metastatic colorectal carcinoma and helped prolong survival rate in patients with stable disease [246,247]. In addition, Promitil is currently being investigated for chemoradiotherapy [248] and targeted therapy [249] applications.

4.1.10. Triptolide

Triptolide, a diterpene lactone epoxide, exhibits potent therapeutic effects in various hard-to-treat types of cancer including prostate and pancreatic cancers by inhibiting cell proliferation and promoting apoptotic cell death [250]. However, its clinical application has been limited due to its narrow therapeutic window and poor aqueous solubility. There have been several efforts to overcome these limitations by synthesizing efficient and less toxic prodrugs [251,252]. In trying to expand upon the existing prodrug strategies and extend triptolide's clinical utilization, redox-responsive triptolide prodrug nanoparticles were prepared in order to increase drug solubility as well as improve tumor targeting [253]. To synthesize the prodrug, triptolide was conjugated to vitamin E using dithiodiglycolic acid, which was then co-dissolved with PEG2000-linoleic acid in ethanol and then self-assembled into nanoparticles by nanoprecipitation. This PEGylated triptolide prodrug self-assembly nanoparticles (PTPPSN) displayed a high drug loading capacity and improved stability. Their redox-responsive properties were achieved by rapid cleavage of the incorporated disulfide bonds in response to the GSH-rich tumor microenvironment, which resulted in the release of thioglycolic-triptolide ester. The

final conversion to triptolide was then mediated by ester hydrolysis to remove the thioglycolic acid pro-moiety. Consistent with this mechanism, *in vitro* drug release was shown to be GSH concentration dependent. Tumor volume was significantly decreased in H₂₂ tumor xenograft mice when TPPSN treatment was compared to prodrug alone and non-PEGylated prodrug nanoparticles (TPPSN) while no weight loss was observed. This study demonstrates the potential of triptolide prodrug-loaded PEGylated nanoparticles to improve tumor targeting using a redox-triggered release mechanism.

4.1.11. Two-drug combination therapy

The two-drug cocktail strategy has been widely used in the area of cancer therapeutics to overcome drug resistance, increase overall therapeutic effect, and reduce side effects brought on by the need for higher doses when giving the same medicines individually. However, in clinical applications, the co-delivery of drugs is difficult in part because the differing PK of the individual drugs can lead to reduced efficacy. Recently, the two-in-one co-delivery strategy was evolved to deliver two drugs within one delivery vehicle, which offers several advantages over free drug cocktail-based delivery therapies and has attracted more attention as a result [254]. Following are some PNDDs that combine multiple APIs, either as covalently-linked codrugs, or with at least one being a prodrug, to provide a more promising approach to chemotherapy.

Triple negative breast cancer (TNBC) is known to attribute to about 15% of all breast cancer cases and is defined by tumors that do not express estrogen receptors (ER) and progesterone receptors (PR) on their cell surface and also don't overexpress human epidermal growth factor receptor 2 (HER2) protein [255]. These triple negative tumors are more aggressive and result in worse outcomes because they respond poorly to targeted hormone therapies such as tamoxifen (TAM) or HER2-targeted therapy [256]. Interestingly, some studies have shown that silencing of ER gene expression is partially due to histone deacetylation and as a result, the utilization of histone deacetylase (HDAC) inhibitors can reactivate the expression of functional ER [257].

Translating this approach into clinical practice, Vorinostat (suberoylanilide hydroxamic acid, SAHA) was the first pan-HDAC inhibitor approved by the FDA in different types of cancers [258–260]. When dosed in combination with TAM, SAHA enhanced TAM efficacy and studies have shown that SAHA re-sensitized TAM-resistant cells to hormone therapy [261]. However, some major challenges to TAM/SAHA combination therapy are the limited oral bioavailability and poor stability of SAHA and the insufficient co-delivery of the two agents to the tumor sites [262]. To address these limitations, researchers have developed a SAHA prodrug-based nanoparticle delivery system to co-deliver SAHA and TAM for more effective combination therapy [263]. The SAHA-containing polymeric prodrug, POEG-co-PVDSAHA was formed via reversible addition-fragmentation transfer polymerization to incorporate SAHA into the polymer through redox-responsive disulfide linkages. In this novel construct, SAHA serves as part of the hydrophobic domain of the amphiphilic polymer, which facilitates the formation of stable micelles as well as TAM encapsulation. The resulting TAM-loaded POEG-co-PVDSAHA micelles exhibited enhanced and synergistic cytotoxicity against TNBC cell lines compared to free SAHA, free TAM, a combination of the two free drugs, POEG-co-PVDSAHA-micelle, and TAM-loaded micelles without SAHA. Moreover, compared with these controls, co-delivery of TAM and SAHA via the POEG-co-PVDSAHA micelle strategy led to significantly improved antitumor efficacy in a 4 T1.2 tumor model. However, more studies are needed to better understand the underlying synergistic mechanism of this combination therapy.

Another strategy to improve TNBC's sensitivity to cisplatin is the utilization of a structure-transformable co-drug-based nanoparticle system [264]. A series of self-assembled co-drugs were synthesized based on cisplatin and adjuvin (ADD) termed Pt(IV)-ADD. Due to these drugs' distinct mechanisms of action – mitochondria dependent

apoptosis [265,266] and DNA damage [267] – the Pt(IV)-ADD co-drug can act synergistically to induce cell death. In terms of nanoparticle formation, the amphiphilic nature of Pt(IV)-ADD and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-PEG (DSPE-PEG) resulted in small and homogenous nano-formulations (Pt(IV)-ADD@PEG NPs) via self-assembly with high drug loading. Interestingly, upon IV injection, one of the resulting NP formulations, C₄-Pt-ADD@PEG NP, was able to transform into a wire structure due to a dynamic equilibrium between ADD and the conjugated carbon chains, representing its thermodynamically stable morphology. *In vitro*, C₄-Pt-ADD@PEG NPs possessed enhanced therapeutic efficacy in both cisplatin-sensitive and cisplatin-resistant cell lines compared to free cisplatin and the non-transformable formulations. In cisplatin-resistant MDA-MB-231 tumor bearing mice, higher drug retention at the tumor site along with greater tumor inhibition were observed, which may be attributed to the synergistic effect of ADD and cisplatin as well as the secondary structure rearrangement, which may improve drug retention [268,269]. Taken together, this structure-transformable nanoparticle offers additional potential to combination therapy in fighting TNBCs.

In another example of a co-drug nanoparticle-based combination therapy, a two-in-one co-delivery design was implemented using a lipid-PLGA nanoparticle [270]. Here, the co-drug (Tolfplatin) was synthesized via a coordination reaction between cisplatin hydrate and tolframic acid (Tolf), a highly selective COX-2 inhibitor, which reduced the polarity of cisplatin via linkage to the highly hydrophobic Tolf and thereby enabled highly efficient encapsulation into lipid-PLGA nanoparticles, resulting in lipid-PLGA@Tolfplatin nanoparticles (LPTP NPs). The two active components rely on different mechanisms of action to induce apoptosis; cisplatin hydrate damages DNA while Tolf up-regulates p53 expression. In a breast tumor-bearing mouse model, the LPTP NPs passively targeted to and accumulated at the tumor site via the EPR effect, where the release of cisplatin hydrate and Tolf after intracellular endocytosis resulted in a synergistic antitumor effect (Fig. 7). Compared to free cisplatin, free Tolf, and the combination of the two free drugs, this co-drug-based nanoparticle delivery system demonstrated superior tumor accumulation and improved therapeutic efficacy without damaging off-target tissues compared to free cisplatin [270].

In a third example of nanoparticle-based combination therapy incorporating a cisplatin prodrug, a PEGylated graphene oxide nanoparticle delivery system was used for the co-delivery of cisplatin and DOX [271]. Graphene oxide is a derivative of graphene that is modified with oxygen-containing functional groups such as epoxide, phenolic hydroxyl, carboxyl, and carbonyl on the surface, providing great water solubility and biocompatibility. Additionally, graphene oxide possesses a high surface area (2,600 m²/g) that confers high drug loading efficacy [272]. In this system, PEGylated graphene oxide nanosheets (pGO) were prepared by mixing nano-sized graphene oxide with PEG and subsequently attached to a cisplatin prodrug (Pt) through an amino-bonding reaction to formulate pGO-Pt nanoparticles. Pt was prepared by oxidizing cisplatin with H₂O₂, followed by reaction with succinic anhydride. The final dual-drug delivery system (pGO-Pt/DOX) was generated by loading DOX onto pGO-Pt nanoparticles via a non-covalent reaction. The high drug loading efficiency of pGO-Pt/DOX suggests that graphene oxide-based nanoparticles could load significantly higher amounts of Pt compared to other types of nanoparticles such as carbon nanotubes or gelatin hydrogels. The release of both cisplatin and DOX from pGO-Pt/DOX was pH-dependent and well controlled. *In vitro* cell viability studies showed that growth inhibition and apoptotic effect of pGO-Pt/DOX were significantly higher than for the single delivery system (pGO-Pt and pGO-DOX) or free drugs. *In vivo* results using a CAL-27 xenograft mice model also suggested that pGO-Pt/DOX increased drug accumulation in tumors without any systemic toxicity compared to the free drug mixture of Pt/DOX.

The development of multidrug resistance (MDR) where cancer cells become tolerant to therapeutic agents at clinical doses is one of the major obstacles in conventional chemotherapy. As a promising

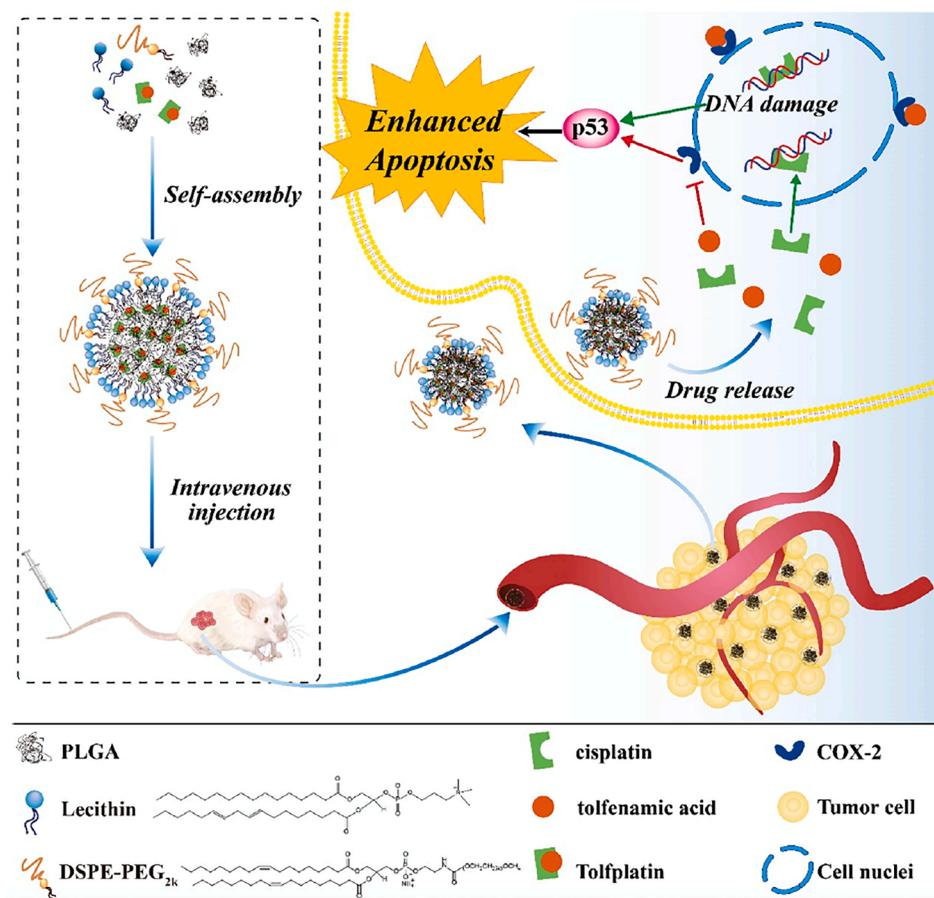


Fig. 7. Nanoparticles (LPTP NPs) containing PLGA, lecithin and DSPE-PEG self-assemble around the co-drug combination of cisplatin and tolvenamic acid. Upon intravenous injection, the LPTP NPs accumulate within the tumor site. Following intracellular endocytosis, both drugs are released, resulting in synergistic antitumor effect. Reprinted with permission from *Mol. Pharmaceutics* 2020, 17, 4, 1300–1309. Copyright 2020 American Chemical Society. DSPE-PEG, 1, 2-Distearoyl-sn-glycero-3-phosphoethanolamine-Poly(ethylene glycol); PLGA, poly(lactic-co-glycolic acid).

approach to overcome MDR, mitochondrial targeting has been widely studied. Mitochondria are the organelles that generate adenosine triphosphate required for the activity of efflux pumps that are overexpressed on MDR cancer cells which is widely considered to be the main mechanism of drug resistance. Another common feature of MDR cells are more polarized mitochondria compared with non-MDR cells [273]. A well-designed prodrug nanoparticle system targeting mitochondria may provide a potential treatment benefit compared to chemotherapy alone.

To generate a mitochondria-specific delivery system (VDNP), mitochondrial-targeting cationic lipid vitamin E succinate modified with octahistidine-octaarginine (VES-H₈R₈), which was previously shown to depolarize mitochondria and inhibit P-gp efflux pumps in MDR breast cancer cells, was combined with a palmitoyl-modified hydrophobic DOX prodrug (pDox) as well as the amphiphilic polymer P(LA-co-TMCC)-g-PEG [274]. pDox was synthesized using a hydrazone linkage to promote encapsulation at neutral pH while releasing DOX in the acidic endo/lysosomal environment of cancer cells. DOX release from VDNPs was shown to be pH-dependent *in vitro*. ROS generation and apoptosis *in vitro* were increased after VDNPs treatment compared to each singly loaded nanoparticle (DNPs or VNPs), or a mixture of DNPs and VNPs. Cellular uptake and retention of DOX was increased when treated with VDNPs possibly through VES-H₈R₈-mediated P-gp efflux inhibition. A synergistic antitumor effect was observed against MDR breast cancer cells when VES-H₈R₈ and pDox were co-delivered in a single VDNP nanoparticle, but not individually. These results demonstrate that a nanoparticle co-encapsulating a novel mitochondrial depolarizer and P-gp inhibitor and a chemotherapeutic has the potential to overcome MDR mechanisms.

Another approach to overcome chemoresistance to neoadjuvant chemotherapy (NAC) is to target the small GTPase Rac 1, which is

upregulated in chemo-resistant breast cancers [275]. To that end, an endosomal pH-responsive nanoparticle was developed to co-deliver Rac1-targeting siRNA and a cisplatin prodrug. Here, nanoparticles (siRac1/DDP NP) were prepared by mixing siRNA of Rac1 (siRac1), GO-C14 (cationic amphiphilic dendrimer) [276] and a cisplatin polymer prodrug with methoxyl-poly (ethylene glycol)-b-poly(2-(diisopropylamino) ethyl methacrylate) (Meo-PEG-b-PDPA) polymer dissolved in DMF, followed by dropwise addition to water. The Meo-PEG-b-PDPA polymer imparted endosomal pH-responsive properties to the nanoparticle through enabling effective endosomal escape via the “proton sponge” effect. The cisplatin prodrug (DDP), a cisplatin conjugated with two hydrophobic tails, was prepared to enhance its encapsulation into nanoparticles as well as release intact cisplatin upon exposure to GSH in the cytoplasm. After incubating the siRac1/DDP NPs with MDA-MB-231 breast cancer cells, siRac1 was shown to efficiently escape from the endosome into the cytoplasm and suppress Rac1 expression. No siRac1 escape and Rac1 expression were observed with non-pH responsive nanoparticles prepared by Meo-PEG-b-PLGA. siRac1 and DDP of the siRac1/DDP NPs also exerted a synergistic inhibitory effect in MDA-MB-231 cells. Systemic circulation and tumor accumulation of siRac1/DDP NPs were significantly increased compared to naked siRNA after IV injection into healthy mice and a patient-derived xenograft (PDX) mouse model, respectively. More importantly, tumor growth was significantly suppressed in PDX mice after siRac1/DDP NPs treatment compared to blank nanoparticles, DDP, or siRac1-only loaded nanoparticles, indicating that siRac1/DDP NPs could reverse the chemoresistance of breast cancers. Overall, the data suggests that Rac1-targeted siRNA and cisplatin prodrug delivering nanoparticles are an attractive strategy to overcome acquired drug resistance to chemotherapy in breast cancer.

Another platform showcasing the promise of combining

chemotherapy and gene therapy to tackle drug resistance at the genetic level utilized a photoactivatable Pt(IV) prodrug-backboned polymer to form a self-assembled nanoparticle system that was composed of the Pt(IV) prodrug and siRNA of c-fos (si(c-fos)) [277]. This gene product is often overexpressed in patients that have failed to respond to platinum-based chemotherapy [278] and the combination of chemotherapy and siRNA therapy is intended as a synergistic therapy to treat platinum-resistant ovarian cancer. High siRNA loading efficiency (93.9%) was achieved by condensing the si(c-fos) into the Pt(IV)-prodrug-backboned cationic polymer-assembled nanoparticles via electrostatic interactions. To further improve nanoparticle stability and offer tumor targeting capability to the formulation [279], a negatively charged CD44-receptor-targeting PEG-grafted hyaluronic acid was coated onto the nanoparticle surface, resulting in CD44-coated Pt(IV)-prodrug/siRNA nanoparticles (CNP_{PtCP/si(c-fos)}). As one of the major challenges for co-delivery of drug and gene delivery is degradation and deactivation of therapeutics, efficient endo/lysosomal escape is key. To that end, the Pt(IV) prodrug (Pt(IV)-azide complex) was designed to generate oxygen-independent azidyl radicals with mild oxidation energy in a visible-light-triggered manner to facilitate endo/lysosomal escape [280]. This is in contrast to conventional ROS-assisted photochemical internalization [281–283], which may damage or deactivate the drug molecules [284–286]. Along with the activation of the Pt(IV) prodrug, CNP_{PtCP/si(c-fos)} disassembled and released both active Pt(II) and si(c-fos). IC₅₀ values for CNP_{PtCP/si(c-fos)} with irradiation within the safe light-irradiation dosage against an A2780 ovarian cancer cell line and its platinum-resistant variant (A2780^{DDP}) were 5.7- and 7.1-fold lower, respectively, than those in the dark. Additionally, the CNP_{PtCP/si(c-fos)} formulation showed much greater cytotoxicity than the Pt(IV)-prodrug nanoparticle without siRNA and even higher cytotoxicity than free cisplatin. Furthermore, c-fos protein expression levels were down-regulated to 47.7% for A2780 cells and 37% for A2780^{DDP} cells after light irradiation. Due to the tumor-targeting ligands, CNP_{PtCP/si(c-fos)} accumulated more efficiently in tumors after IV injection than the non-targeted nanoparticle control or free cisplatin. In the A2780^{DDP} xenograft mouse model, tumor growth after treatment with free cisplatin, a control formulation without si(c-fos) (CNP_{PtCP/si(NC)}), and CNP_{PtCP/si(c-fos)} under dark conditions increased gradually after three days and reached 1060, 1700, and 1230 mm³, respectively by day 27 while the tumor size of the light-irradiated CNP_{PtCP/si(c-fos)} group was at least five times smaller (185 mm³). These results indicate that CNP_{PtCP/si(c-fos)} exhibited tumor-targeting specificity, drug release controllability, and superb synergistic effect of gene silencing and antitumor efficacy.

4.2. Other indications

Although PNDDS have been most widely investigated in the field of oncology, they have also been explored in other indications such as infectious, inflammatory, cardiovascular, neurological, and pulmonary diseases.

4.2.1. Infectious diseases

A handful of PNDDS have been used in the treatment of both viral and bacterial infectious diseases. For example, sofosbuvir, a ProTide-type prodrug approved for the treatment of hepatitis C that was described in the prodrug section, was adsorbed onto amino-decorated mesoporous silica nanoparticles [287]. A rat PK study showed that the total plasma exposure of sofosbuvir doubled and the time to C_{max} tripled when sofosbuvir was delivered using a nanoparticle vehicle compared to without. Furthermore, the drug release profile could be controlled by surface functionalization of the nanoparticle, either using polyvinyl alcohol (PVA) or 3-aminopropyltriethoxysilane (APTES). For example, APTES coating of mesoporous silica nanoparticles demonstrated an initial burst release of sofosbuvir of approximately 30% in the first hour followed by sustained release of the remaining sofosbuvir over the course of 16 h. In contrast, the PVA-coated particles exhibited an initial

release of approximately 10% in the first hour, followed by rapid release of up to 85% of sofosbuvir after four hours; note that for PVA coated particles 100% release was not achieved. These results demonstrate once again that modification of the nanoparticle structure can impact drug delivery and that fine-tuning of the entire formulation is often required to obtain the desired properties.

For tenofovir alafenamide, a prodrug optimized for enhanced cell uptake in anti-HIV combination therapy that was also discussed in the prodrug section of this review article, formulation as a drug delivery system may further improve outcomes for populations at high risk of HIV infections by increasing compliance with pre-exposure prophylaxis therapy. Compliance is often hindered by the frequent administration events or the on-demand nature of the prescribed dosing regimens. One example of a therapy with the potential to increase adherence was recently described by Mandal et al., who developed a long-acting release formulation encapsulating the anti-HIV prodrug combination therapy tenofovir alafenamide /emtricitabine (TAF/FTC) inside Pluronic F-127 and PLGA-based nanoparticles [288]. Compared to TAF/FTC drug solution, the nanoparticle formulation had an at least 6.5-times longer half-life at the site of infection in vaginal tissue and a three to five-times larger AUC. When tested in a humanized mouse model of HIV, 60% of the mice were protected from infection after treatment with the controlled release nanoparticle compared to 0% in the free drug control group when dosed 7 or 14 days prior to viral challenge. Together, these results suggest that a reduced dosing frequency of pre-prophylaxis therapy may be attainable with a nanoparticle-based approach, which could have a significant effect on compliance and treatment outcomes [289].

To overcome the resistance mechanism of virus within latent reservoirs, a “shock and kill” treatment approach has been extensively explored [290,291]. This strategy uses latency-reversing agents (LRAs) to elicit viral replication coupled with HIV-1 cell-specific cytotoxic agents or immune-mediated clearance to eradicate the infection. However, this approach is still controversial and has shown limited success in clinical settings, which can be attributed to insufficient potency of LRA monotherapy, off-target toxicity or nonspecific T cell activation, as well as low drug concentration at the target sites [292–294]. The use of nanoparticle-based delivery systems could provide an attractive strategy to address these challenges. Cao et al. developed lipid-coated PLGA hybrid nanoparticles (LCNPs) that allow the co-delivery of multiple LRAs with distinct mechanisms [295]. The LRAs (JQ1, DSF, Ing3A, cholestryl butyrate (chol-but), prostratin, and panobinostat (PANO)) were loaded to the LCNPs by either physical encapsulation of hydrophobic LRAs (JQ1/LCNP, DSF/LCNP, Ing3A/LCNP), insertion into the lipid bilayer (chol-but LCNP), or through chemical conjugation to PLGA with either an ester or amide bond (Ing3A-PLGA, Prs-PLGA, and PANO-PLGA) and self-assembly into LRA-loaded LCNPs (i.e., Ing3A-LCNP, Prs-LCNP, and PANO-LCNP). Higher drug loading was achieved with PLGA-conjugation compared to physical drug encapsulation. Additionally, slower drug release kinetics were observed for LRA-LCNPs compared to free LRAs or physically encapsulated LRAs, which minimized the undesired initial burst release. All LRA-LCNPs showed equal or lower cytotoxicity compared to the free drug in the J-Lat Tat-GFP cell line model, suggesting LCNPs could deliver higher doses for better efficacy while reducing toxicity. Balancing the trade-off between toxicity, potency, and synergy, the combination of Ing3A-LCNP and JQ1/LCNP was identified as the best option, displaying synergistic latency reversal with low cytotoxicity. Additionally, treatment with this combination resulted in synergistically increased HIV-1 mRNA expression levels in CD4⁺ T cells from infected individuals that were maintained on suppressive highly active antiretroviral therapy. Incorporation of CD4 antibodies on the surface of LCNPs as the active targeting motif, the Ing3A-LCNPs accumulated in lymph nodes after subcutaneous administration and selectively bound to and activated CD4⁺ T cells in mice. More studies are still needed to further evaluate the latency reactivation in a non-human primate simian immunodeficiency virus model [295], but the promising

findings from this research demonstrate the potential of this emerging field for developing a cure for HIV.

For the treatment of bacterial infections, nitric oxide (NO)-releasing polymers and polymer-encapsulated NO-prodrugs are being investigated. NO exerts bactericidal activity by inactivating metabolic enzymes, causing DNA damage, as well as reducing the integrity of the bacterial cell wall without the risk of emergence of antibiotic resistance. However, the non-specific nature of NO-induced damage poses challenges to the clinical utility of this approach [296]. To address this challenge, applications of NO treatments at sub-tissue-damage-inducing concentrations are of particular interest. In one study, NO induced the dispersal of bacterial biofilms at picomolar and nanomolar concentrations, generating planktonic bacteria that were re-sensitized to antibiotic treatment [297]. Making use of this effect, Ngyuen et al. generated combined NO and antibiotic micelles by directly conjugating an NO-donor (N-diazeniumdiolate (NONOate)) to a gentamicin-decorated amphiphilic block copolymer of poly((oligoethylene glycol) methyl ether methacrylate) and 3-vinylbenzylaldehyde (POEGMA-*b*-PVBA) to form 15 nm diameter particles. Treatment of biofilms with these micelles *in vitro* demonstrated an 83% reduction in biofilm mass compared to minimal reductions after treatment with free gentamicin alone, NO-donor alone or gentamycin-polymer micelles. Similar effects were observed when evaluating biofilm viability, suggesting that a dispersing agent can be instrumental in increasing the efficacy of classic antibiotic drugs against biofilm-associated infections [298].

4.2.2. Inflammatory diseases

PNDDS have also been explored for the treatment of inflammatory diseases such as arthritis. One of the limitations for non-prodrug nanoparticle-based delivery systems such as polymeric micelles and protein nanoparticles, in which drugs are physically encapsulated, is that they tend to exhibit burst release behavior, low drug loading, or both [299,300]. Thus, the implementation of innovative prodrug strategies to optimize the design of the delivery systems can help boost their clinical translation. Xu et al. designed modular pH-sensitive acetone-based ketal-linked prodrugs of dexamethasone (AKP-dexs) and formulated them into nanoparticles for the treatment of rheumatoid arthritis [301]. Eight AKP-dexs with different length carbon chains were synthesized and co-assembled into nanoparticles with an amphiphilic polymer, DSPE-mPEG200. The use of long-carbon-chain alcohols as pro-moieties resulted in better compatibility between dexamethasone and DSPE – mPEG2000 enabling the formation of stable nanoparticles with high encapsulation efficiency. In a collagen-induced arthritis rat model, the AKP-dex-loaded nanoparticles showed enhanced accumulation in arthritic joints as well as effective release of dexamethasone in the acidic microenvironment of the arthritic joints compared to the free water-soluble prodrug, dexamethasone sodium phosphate. This study showing improved therapeutic efficacy with low systemic side effects demonstrates that pH-sensitive prodrug nanoparticles could serve as an attractive platform for the treatment of inflammatory disorders.

Another example of a PNDDS that has potential for the treatment of inflammatory diseases is polymeric micelles incorporating diclofenac ethyl ester, a prodrug of diclofenac. Nonsteroidal anti-inflammatory drugs like diclofenac are some of the most widely prescribed medications for pain and inflammation. However, they are known to have various side effects such as enhanced cardiovascular risk, which is directly correlated to the extent of drug presence in the heart. Al-Lawati et al. showed that delivery with polymeric micelles reduced the cardiovascular risks of diclofenac by decreasing its distribution to the heart [302]. In this study, diclofenac ethyl ester was mixed with block copolymer PEO-*b*-PCL conjugated with the near-infrared probe cyanine-5.5 azide to prepare traceable polymeric micelles (DFEE-TM) using a cosolvent evaporation method. After a single IV dose, higher levels of fluorescence were observed in the inflamed joints of adjuvant arthritis (AA) rats compared to the joints in healthy rats in *ex vivo* near-infrared optical whole-body images, suggesting that the DFEE-TM were localized

in the inflamed areas due in part to the long circulating property of PEO. Furthermore, the concentration of diclofenac in the heart of AA rats was significantly reduced after seven daily doses of DFEE-TM compared to dosing with free diclofenac. Consistent with this finding, a key cardiotoxicity biomarker was reduced in the heart and plasma of AA rats. This study showed that prodrug-incorporating polymeric micelles can reduce the toxicity of conventional drugs by altering their bio-distribution and increasing their accumulation in the permeable vasculature of pathological lesions like inflamed joints.

4.2.3. Cardiovascular diseases

An example of a PNDDS with applications in cardiovascular therapy is a thrombus-targeting aspirin particle for the treatment of thrombotic disease. Here, the anti-coagulant and anti-inflammatory agent ethyl salicylate (ESA; aspirin) were first synthesized into a polymer-drug conjugate (APP) and then formulated into 33 kDa nanoparticles by combining APP with fibrin-binding peptide (Gly-Pro-Arg-Pro-Pro (GPRPP)- lipid conjugates, DSPE-PEG-GPRPP, via H₂O₂-scavenging peroxalate linkages to generate thrombus-targeting aspirin poly-conjugate particles (T-APP) with anti-inflammatory and anti-coagulant properties as shown in Fig. 8. Release of ESA from T-APP was H₂O₂-dependent and yielded a reduction in measured H₂O₂ and thus intracellular ROS when tested in H₂O₂-stimulated arterial endothelial cells. Evaluating T-APP in a mouse model of tail bleeding and arterial thrombosis revealed that treatment with T-APP resulted in about a two-fold increase in bleeding time compared to free aspirin treatment suggesting a stronger anti-thrombotic effect. Furthermore, in a mouse model of carotid arterial thrombosis, T-APP was highly bound to an artificially formed thrombus after IV injection. This effect was abolished when the animals were pre-treated with free fibrin-targeting GPRPP-peptide to block binding of T-APP. Together with T-APP's potent anti-inflammatory effects, these data suggest a promising new application for aspirin in the treatment of life-threatening blood clots in the body [303].

Nanoparticles comprised of chitosan and alginate have also been used to tune the release of FDA approved prodrugs like lovastatin to improve therapeutic outcomes [304]. Lovastatin is a cholesterol-lowering prodrug that is hydrolyzed *in vivo* to its active hydroxy acid form, which inhibits 3-hydroxy-3-methylglutaryl-coenzyme A reductase to regulate cholesterol biosynthesis [305]. However, due to its short half-life (~three hours), highly time-sensitive evening administration is required, which decreases patient compliance and limits its application. In this study, lovastatin-encapsulating alginate/chitosan nanoparticles were formulated by ionic gelation to control the release of lovastatin and create a favorable absorption and distribution profile. The polymer and lovastatin in the final formulation (ACL nanoparticles) interact via hydrogen bonding and dipolar-dipolar interactions, which dictate the structure and shape of the nanoparticles. The rate of lovastatin release from ACL nanoparticles increased with increasing solution pH. The release was rapid in the first 10 h (80–90%) followed by sustained release of the remaining 10–20% for up to 30 h. The ACL nanoparticles were also shown to be nontoxic in acute and sub-chronic toxicity studies after oral administration in healthy mice. These results demonstrate that ACL nanoparticles could be utilized to improve the pharmacological effect of lovastatin through its controllable release properties.

4.2.4. Neurological diseases

The combined prodrug-nanoparticle strategy has also been applied to the treatment of neurological disorders. As cited in the prodrug section, a prodrug of dopamine, the FDA-approved Parkinson's medication levodopa (L-DOPA), can penetrate the BBB efficiently. However, it is rapidly metabolized in the gastrointestinal tract and systemic circulation. While there are a few other dopamine prodrugs in development to address this issue, utilizing a nanocarrier to protect levodopa against serum decarboxylase activity while efficiently penetrating the BBB and specifically targeting the brain represents an intriguing strategy.

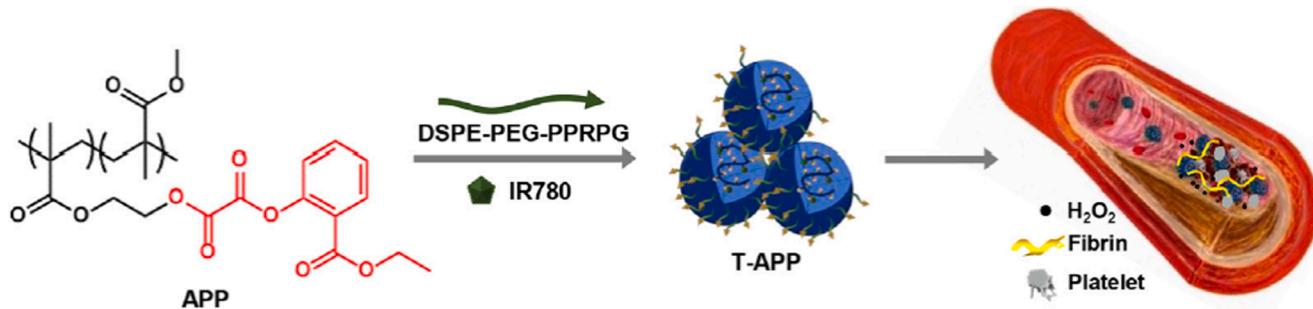


Fig. 8. Chemical structure of H_2O_2 -activatable aspirin polyconjugate and a schematic representation of fibrin-binding PPRPG peptide-conjugated aspirin polyconjugate particles (T-APP). Upon intravenous injection, T-APP binds to fibrin-rich thrombi, where they exert a localized anti-thrombotic effect. Figure reproduced with modifications with permission from the *Journal of Controlled Release*, 304, Jeonghun Lee, Lipjeong Jeong, Eunkyeong Jung, Changgon Ko, Semee Seon, Jounghyun Noh, Dongwon Lee, Thrombus targeting aspirin particles for near infrared imaging and on demand therapy of thrombotic vascular diseases, 164–172, 2019. Copyright 2019 Elsevier H_2O_2 , hydrogen peroxide.

Utilizing L-DOPA as a targeting ligand and therapeutic, Gonzalez-Carter et al. developed L-DOPA-ligand functionalized gold nanoparticles (L-DOPA-AuNFs) as a potential brain-penetrating delivery system [306]. They demonstrated more L-DOPA-AuNFs transported across the brain using an *in vitro* human BBB model (hCMEC/D3 cell line) compared to what has been reported in the literature [307–309] and their transportation rate across the BBB monolayer was comparable to that observed in monolayers composed of peripheral human umbilical vein endothelial cells. Additionally, L-DOPA-AuNFs were highly internalized by brain macrophages *in vitro* without inducing overt inflammation. While the pharmacological benefit of these prodrug-loaded gold nanoparticles has not been evaluated, these promising findings provide hope for advances in the treatment of brain disease.

4.2.5. Pulmonary diseases

PNDDS can also be an effective treatment option for pulmonary diseases. For example, pulmonary arterial hypertension (PAH) is a progressive disease associated with increased pulmonary arterial pressure caused by occluded and/or constricted pulmonary vasculature [310,311] and despite several approved therapies for the treatment of PAH, the 5-year survival rate remains low [312]. Furthermore, many of the existing PAH therapies are associated with adverse effects due to systemic exposure. Treprostinil, a vasodilator, is one of three prostacyclin analogues approved to treat PAH and is available as a continuous infusion (Remodulin®), a solution for inhalation (Tyvaso®), and as an oral tablet (Orenitram®) [313]. While the nebulized Tyvaso formulation provides local delivery of the API to the lung, it requires four times daily dosing and is associated with local adverse effects including throat irritation and cough [314,315]. To improve upon these shortcomings, a novel treprostinil-prodrug based nanoparticle formulation was designed and is currently in clinical development for the treatment of PAH [316]. The prodrug, treprostinil palmitil, uses palmitil alcohol as the pro-

moity and an ester bond to mask the carboxylic acid functional group of treprostinil acid resulting in a significantly altered solubility profile and decreased water solubility [317,318]. The chemical structures of the treprostinil palmitil prodrug and treprostinil acid API are shown in Fig. 9. The increased lipophilicity allows for the prodrug to be packaged into a lipid nanoparticle composed of squalane and DSPE-PEG2000. The squalane acts as a hydrophobic filler while DSPE-PEG2000 provides a “stealth” coating that promotes formulation stability and slows prodrug release. A cartoon schematic of the solid lipid nanoparticle, as well as TEM images, are shown in Fig. 9. Release of the active treprostinil species relies on a two-step mechanism that involves escape of the prodrug from the nanoparticle followed by subsequent esterase-mediated hydrolysis. Preclinical studies confirmed that this combined inhaled-prodrug-nanoparticle approach resulted in sustained-release of treprostinil and reduced cough sensitivity in rodents [318–320].

5. Conclusion and future perspective

This review summarizes recent advances in the development of PNDDS to provide a perspective on how combining prodrug synthesis and nanoparticle delivery systems can represent an avenue to the development of superior medicines, especially when the individual approaches fall short on their own. By harnessing the unique strengths of prodrug synthesis including optimization of PK and masking problematic functional groups together with the high drug loading capacity, bioavailability, and target selectivity of nanoparticle formulations, researchers can design elegant drug delivery systems that improve efficacy and reduce toxicity of conventional therapeutic agents. The successful implementations of this approach also demonstrate the importance of cross-functional collaborations for the development of innovative solutions to the most difficult clinical challenges. While many of the described PNDDS are still early in development and additional

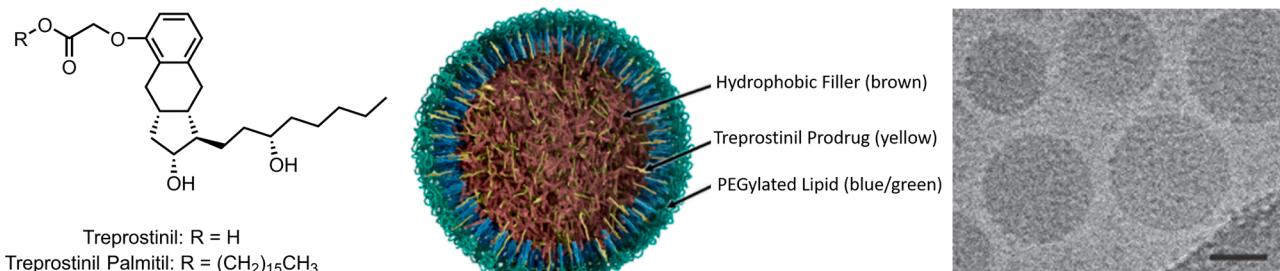


Fig. 9. (Left) Chemical structure of treprostinil palmitil, an ester prodrug of treprostinil acid. (Middle) A schematic representation of the solid lipid nanoparticle encapsulating treprostinil palmitil; the nanoparticle structure provides sustained release of treprostinil. (Right) A cryo-transmission electron microscopy image showing the treprostinil palmitil solid lipid nanoparticles juxtaposed with the 50 nm scale bar. Reprinted with permission from *Drug Research* 2018, 68(11): p. 605–614. Copyright 2018 Georg Thieme Verlag KG.

innovations are required to increase their clinical translation, the impressive advances achieved to date highlight the potential these systems have to revolutionize the treatment of many diseases.

6. Disclosures

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