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REVIEW ARTICLE

Prodrugs as drug delivery system in oncology

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Abstract
The use of conventional chemotherapy in the treatment of cancer has been restricted by the lack of cell specificity, which causes toxicity regarding healthy cells resulting in limiting side effects responsible for low therapeutic efficiency. To overcome these drawbacks, the design of prodrugs has evolved and improved by covalently linking the drug through a degradable spacer. The use of these prodrugs as drug delivery systems, which are able to inactivate the drug during its biodistribution to specifically deliver the drug to its target, is an important breakthrough in cancer therapy. This strategy consisting in the covalent binding of a promoiety to daily used therapeutic compounds has been clinically proven in the design of targeted prodrugs leading enhanced therapeutic efficacy and increase of the therapeutic index. This review summarizes and compares several strategies that improve the therapeutic index of chemotherapy (i.e. conventional drugs) by their chemical transformation into prodrugs improving pharmacokinetic profiles and optimizing administration routes in comparison to the initial drug. This review provides an overview of the methods used to control the structure and function of prodrugs and, ultimately, their current and future potential in increasing the therapeutic index of daily used anticancer drugs. First, prodrugs' design and their activation within the tumor microenvironment or within the tumor cell will be exposed. Then, the different strategies used leading to these prodrugs will be presented.

Keywords Cancer therapy · Drug delivery systems · Microenvironment · Prodrugs · Targeting

Targeting for efficient care

Prodrug strategy

Chemotherapy (CT) has demonstrated important curative rates; nevertheless, the lack of specificity is still an issue as CT is responsible for side-effects especially towards fast dividing healthy cells. CT drugs act through an antiproliferative mechanism or by disruption of the cell cycle at a specific phase. Hence, these drugs, due to poor specificity, affect rapidly proliferating and dividing cells such as red blood cells, hair follicles, gut epithelial cells, bone marrow, and the lymphatic system, leading to numerous side-effects which limit their uses. High-dose CT is generally required to effectively inhibit tumor proliferation, especially in resistant solid tumors. The lack of specificity of CT could lead to lethal damages of the adjacent normal proliferating cells, resulting in a discontinuity of therapy. Therefore, improving the specificity of CT drugs used daily is a vital step to increase their therapeutic index. Many strategies have been developed for this purpose. Among them, a relevant strategy is based on the development of prodrugs. Initially, a prodrug is an inactive drug designed to be activated through a spontaneous process (e.g. hydrolytic degradation) or through a biocatalytic mechanism, near the pharmacological target allowing a specific release of the active entity at its target site [1].

Their design is commonly based on three components (Fig. 1): (1) the active moiety for anticancer activity (i.e. drug); (2) a cleavable labile chemical moiety (i.e. linker) which is conjugated to the active moiety through a functional

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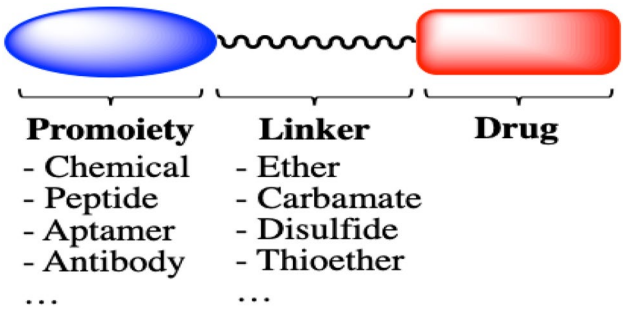


Fig. 1 Common structure of a prodrug

group (hydroxyl, carboxylic, amine, carbonyl, and phosphate groups, etc.); and (3) a targeting moiety for specific delivery to tumor cells such as polypeptides or monoclonal antibodies (mAb) (i.e. promoiety) [2].

The design of such compounds depends on two major principles: (1) the structural features of the active moiety with the ability of the targeted moiety to mask the pharmacodynamic activity of the anticancer drug. (2) The bioconversion mechanism of the drug release which can either gradually or immediately degrade the linker chosen for the conjugation [3]. The design of prodrugs provides a remarkable tool to improve the pharmacological properties of the active moiety. Conventional prodrug design aims mostly to: improve solubility in water or lipid membrane, improve chemical stability, increase oral or local absorption and brain permeability, mask unacceptable taste, reduce irritation or pain, modify pre-systemic metabolism, and reduce toxicity [4].

Paracelsus said some 500 years ago: "All things are poison and nothing is without poison, only the dose permits something not to be poisonous". In the sixteenth century, Paracelsus initiated the shift from conventional medicine to modern medicine based on chemical knowledge for a better development of pharmaceutics. For a long time, the galenic development of drugs has been limited to physicochemical stability concerns to be stored and administered. Better understandings on the fate and behavior of the drugs in the body (pharmacokinetics) have allowed designing "clever" drugs. In the beginning of the twentieth century, a German scientist named Paul Ehrlich thought that a compound could target selectively a disease-causing organism without harming the body itself and called the hypothetical agents "Zauberkugel", the "Magic Bullet" [5]. His idea has been extended to many drugs used in various diseases including malignant diseases. The dream of Paul Ehrlich is becoming true towards new vectorization concepts, with the development of new physicochemical concepts, new therapeutic targets, and news biomaterials. We are now faced with more than 110,000 publications (Pubmed) in the field of drug delivery systems. Today, controlling the duration and the localization of the drug release for optimized exposure is a reality (Fig. 2).

Temporally controlled drug delivery systems (TCDDS)

Controlling the chronology of drug release displays endpoints: (1) delay the drug release in order to reach the right tissue based on the pharmacokinetic and (2) increase the drug exposure by controlling its release (diffusion through a structure, erosion, etc.) and degradation/elimination process (decreasing the renal or hepatic clearance, protecting the drug from biologic events). Nowadays, drug release can be modulated through innovative structure, such as lipidic or polymeric scaffolds, sensible to biologic or external stimuli, and benefit from new knowledge of the specific microenvironment of the target.

Spatially controlled drug delivery systems (SCDDS)

In oncology, CT uses are limited because of their off-target toxicities; CT agents act by inhibiting the cellular division and, by consequence, have drastic effects on healthy cells, especially on cells with rapid cell divisions such as bone marrow cells, cells from the digestive tract and hair follicles. In addition, some cytotoxic agents have a specific toxicity profile such as platinum (Pt) with nephro-/ototoxicity, doxorubicin (DOX) with cardiotoxicity, leading to interruptions or cessation of treatment. DDS are now able to control the drug release to target a specific physiologic compartment

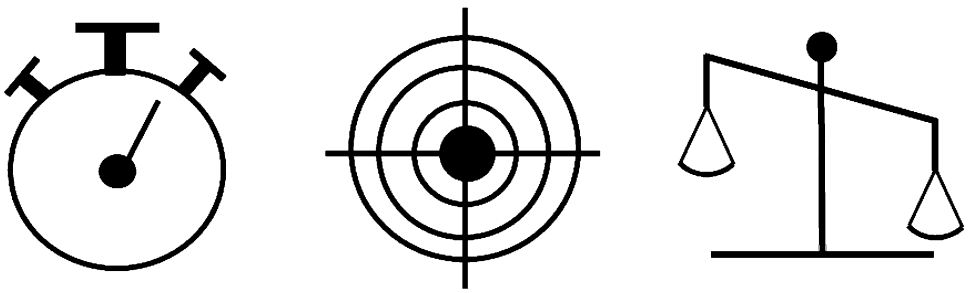


Fig. 2 Control of duration and localization for improved quantity release to the target

which could be a tissue involved in the disease. This strategy hugely improves the risk/benefit balance.

The strategy aiming to design new spatially controlled drug delivery systems (SCDDS) can be of interest in the targeting of specific organs or compartment and can be divided into: (1) the passive targeting with an indirect targeting focused on tumor microenvironment and (2) the active targeting by a direct targeting of the specific proteins or surface marker of tumor cells. It is necessary to keep in mind two characteristics of cancer cells related to targeting. First, the majority of cancer cells express surface molecular targets such as ligand, also present in healthy cells at lower levels. Secondly, the microenvironment of the cancer cells represents significant limitations for drug penetration especially in large solid tumors.

Quantity release-controlled drug delivery systems (QRCDDS)

Galenic developments play a role in the drug release at the right place and at the right time as well as the right delivered quantity. Therapeutic concentrations are a major concern especially in anticancer drugs. High cytotoxic exposure can cause collateral damages and poor exposure can lead to treatment failure. DDS provides the opportunity to control and optimize blood and/or tumor concentration and thus an increased therapeutic index (efficacy/toxicity balance). Besides, unfavorable physicochemical characteristics of some CT limited the effective doses, for example low water solubility for paclitaxel. DDS strategies allow developing structures to improve physicochemical properties.

Prodrugs design

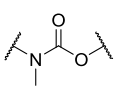
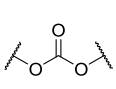
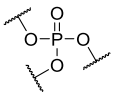
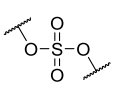
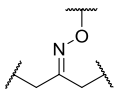
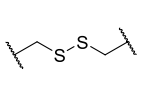
The term "prodrug" was first introduced by Albert in 1951; he defined a prodrug as "a molecule which does not have any intrinsic biological activity but which is capable, during the different phases of its metabolism, to generate a biologically active drug" [6]. Ideally, the prodrug should convert to the active drug as soon as the target is reached concomitantly with the release of the non-cytotoxic moiety followed by its subsequent elimination. This conversion can be obtained after an enzymatic or chemical degradation in vivo. According to the linked moiety, self-assembling properties can be observed giving access to a passive targeting. This new property leads to an increased specificity for the target site decreasing the exposure regarding normal cells and tissue which leads to an improved therapeutic index of the parent active drug.

Functional linker for an optimal release

The design of prodrug relies on the choice of the right functional group present between the drug and the promoiety. This functional group should be self-immolative or enzymatically/chemically cleavable so that the active drug can be liberated spontaneously or under triggerable condition. This process takes place either in the tumor microenvironment: such as in the presence of an overexpressed enzyme, a change in the pH, the presence of a characteristic stimulus, or at the surface of the tumor cell using a specific ligand–receptor combination. The promoiety linked to the active moiety (drug) plays a key role in the improvement of the drug-like properties and in overcoming physiological barriers or in the guidance of the drug to its target cells. The linker between the active moiety and the promoiety should be stable enough to remain intact in the blood circulation until it reaches the target cells. Optimization of the conjugation is highly suggested during the prodrug design to achieve the optimal stability and efficacy. Commonly, only a few functional groups are used for the design of prodrugs (Table 1).

Ester functional groups are among the most used as they are most easily cleaved by a large number of esterase in biological environments. They are usually introduced by the conjugation using charged groups such as hemi-esters, sulfates, phosphates or their salts, thus creating a variety of links. These functional groups can be readily hydrolyzed to release effective molecules by ubiquitous esterase, phosphatases, and sulfatases in the blood, liver, and other organs and tissues [7]. Depending on the differences in the structure of the prodrug or of the environmental conditions, the half-life of the ester bond can vary from several minutes to several hours according to accessibility of the bond [8].

Table 1 Commonly found linkers in prodrug conjugation strategy

Ester					Amide	Other linkers			
Carboxyl ester	Carbamate ester	Carbonate ester	Phosphate ester	Sulfate ester	Peptide bond	Oxime	Imine	Disulfide	Thioether
									

Amide group is usually designed to enhance oral absorption of drugs with amine and carboxylic functionalities. It has a relatively higher enzymatic stability than ester bond. Most of the amide bonds are stable for several hours or even several days in the plasma in the absence of specific enzymes [9]. However, the use of an amide linker in prodrugs is designed to be cleavable by a specific enzyme to increase the target-ability or reduce the toxicity.

In addition to ester and amide bonds, several other types of linkers including oximes/imines, disulfide bond or uncleavable thioether and ether bond, have also been used as functional groups [10].

Other functional groups have recently been proposed. Self-immolative linkers have significantly broadened the synthetic scope with regard to chemical functionalities amenable for prodrug design. These new types of linker are designed to rely on the cleavage of the promoiety and the active moiety with the activation of a precursor. This chemical activation generates a cascade of disassembling reaction leading to the spontaneous and fast release of the active moiety drug (Fig. 3) [11].

Self-assembling concept

Chemical modifications can also provide new physicochemical properties to the compound such as self-assembling property leading to prodrug-based nanoparticle drug delivery systems (PBN-DDS) [13]. These PBN-DDS could lead to a dual targeting property, by integrating prodrug strategy and nanomedicine strategy into one same drug delivering system. PBN-DDS have many advantages; on the one hand, prodrugs are inactive drugs and become active only after a specific metabolization or after a chemical reaction at the active site. On the other hand, the nanoparticle system plays the role of cargo resulting in the protection and guidance of the prodrug to the active site. As for today, PBN-DDS can be divided into two major groups according to the characteristic of the material used for its design which can be: (1) high weight polymeric structures; or (2) small molecular weight PBN-DDS. Each category corresponds to the conjugation of the drug to a polymeric block or a small molecular weight compound, respectively. The physicochemical property of the moiety enables the PBN-DDS to self-assemble into nano-object in aqueous media.

These PBN-DDS refer to amphiphilic conjugates in which the carrier material and the drug play an important role in the hydrophilic–lipophilic balance of the prodrug. These prodrugs are designed by the conjugation of the carrier material directly to the anticancer drug by a covalent bond. The resulting nano-object provides the protection of the prodrug from degradation and diminishes the premature burst release. In addition, the nanoparticular form provides an increased internalization of the drug leading to an increase of the intracellular drug concentration [14]. The first generation of PBN-DDS describes a drug delivery system that targets the lesion through passive mechanism using the so-called "Enhanced Permeability Retention" (EPR) effect proposed by Maeda [15]. The EPR effect is a phenomenon due to the anatomical and physiological characteristics of tumors. In contrast to normal tissues and organs, most tumors show a higher vascular density, such as in the case of angiogenesis which is one of the most important features of tumors to sustain their uncontrolled and rapid growth. Important differences in the structure between tumor vessels and normal blood vasculature are observed as normal cell vascularization is characterized by joined cells. On the contrary, tumor vessels are formed by non-binding cells leading to small gaps where PBN-DDS auto-agglutinate; this phenomenon characterizes the EPR effect [16]. Even if the EPR phenomenon is widely documented, controversies are discussed. The tumor physiology outlined three points: (1) heterogeneous blood vasculature which can be totally disorderly with loops and shunts leading to tight supplies, (2) elevated interstitial fluid pressure due to the plasmatic protein leakage as well as macromolecules which can block the drug uptake or promote its exclusion, and (3) distance into tumor interstitium for the macromolecules transport [17].

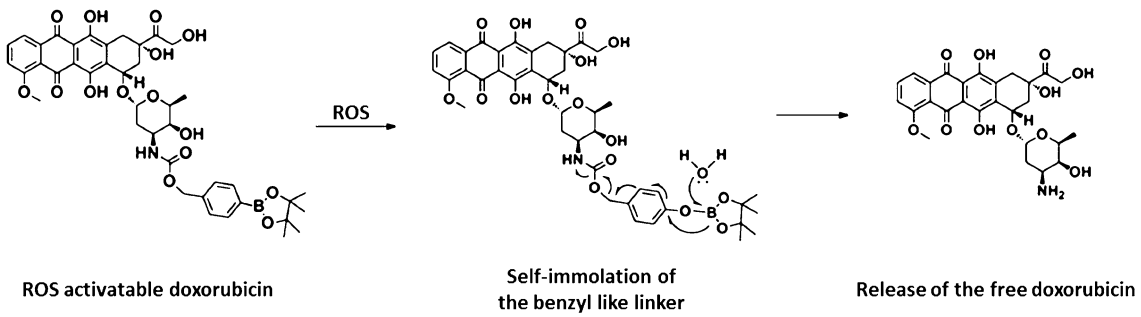


Fig. 3 Example of a ROS activatable self-immolative doxorubicin prodrug [12]

Surface modulation of this PBN-DDS is frequently observed by the use of poly(ethylene) glycol chains (PEG) which leads to "stealth" PBN-DDS with substantially increased circulation time and more likely an increased passive tumor activity (second generation) [18, 19]. The evolutions towards the third generation of PBN-DDS correspond to a dual PBN-DDS with the compilation of different functional moieties such as the drug and a ligand moiety specific for a tumor antigen/receptor (Fig. 4) [20].

Polymer PBN-DDS

This class corresponds to the covalent conjugation of drugs to polymer blocks. In the field of anticancer therapeutics, various polymer blocks have been used to design polymer PBN-DDS, such as poly-lactic-*co*-glycolic acid (PLGA), poly-lactic acid (PLA), poly-glutamic acid (PGA), polycaprolactone (PCL), *N*-hydroxypropyl-methacrylate copolymers (HPMA), and poly-amino acids. Among them, the mostly used are PLA, PLGA and PGA due to their favorable biocompatibility and biodegradability properties [21]. The use of these polymer blocks led to the development of various polymer PBN-DDS which afford different properties, such as water solubility optimization and self-assembling properties. Moreover, the choice of the optimal polymer is essential as it drives the final structure of the skeleton which plays an important role in the final physicochemical properties of the obtained nanosystem. Linear polymer blocks are usually used to synthesize self-assembling PBN-DDS polymer due to their low drug loading. Regarding the use of branched polymer blocks, they offer more functional groups and enable to increase the conjugation of numerous drugs on the same polymer but leads to an increase of the drug loading. However, the increase of the drug conjugation leads to an important modification of the skeleton which can result in the loss of the self-assembling property. In addition, branched polymers exhibit high steric hindrance which can be critical for cellular internalization and might often result in a delayed cellular entry [22]. The conjugation of paclitaxel to PLA (CT-2103) led to the development of a polymer PBN-DDS which has been studied in phase II trial for the treatment of ovarian, breast and lung cancer. Unfortunately, the study showed no increased overall survival in terms of efficacy compared to the control treatment [23]. More complex polymer can also be used such as polysaccharides which are interesting regarding their physicochemical and biological properties. Chitosan, for example, is an important polysaccharide which is biodegradable, biocompatible, showing antibacterial, antifungal and antioxidant properties. Nevertheless, the conjugation of drugs to chitosan is mainly fulfilled in the antimicrobacterial or antifungal research area and not in the cancer research [24, 25].

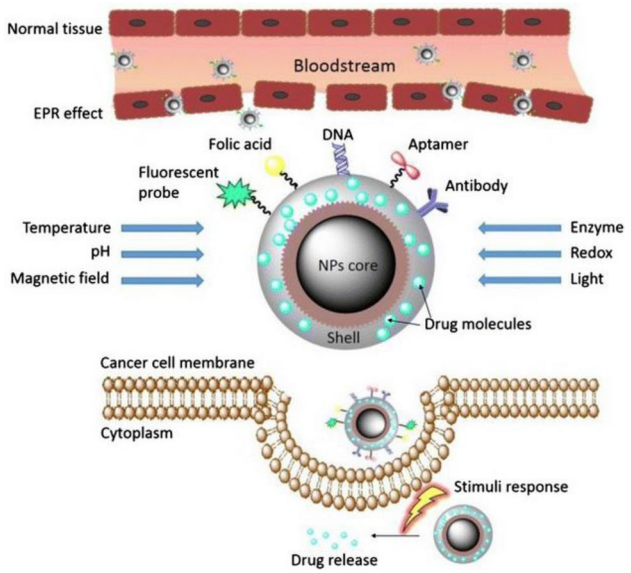


Fig. 4 Illustration underlining examples and targets of third-generation PBN-DDS taken from [20]

Small molecular weight PBN-DDS

Unlike polymer PBN-DDS, small molecular weight PBN-DDS use small molecules. Some of the most commonly used are polyethylene glycol, hyperbranched poly(ether–ester) (< 5 kD) or squalene [26]. The main advantage of these conjugates is their small size (lower than 250 nm) and their interesting higher drug loading efficiency. Among them, the ones using squalene or polyisoprenoid moieties have generated expanding attention in recent years [27]. Furthermore, to deceive the short circulation time in the blood stream, poor solubility and structural stability in time issues, the co-precipitation in the presence of amphiphilic long-chain PEG copolymers can be fulfilled to achieve an increased circulation time and a higher structural stability [28]. The liberation of the drug can be triggered by a variety of stimuli and many researchers have studied approaches to design stimuli-sensitive nanosystems by applying various specific and/or sensitive linkers. These stimuli can be endogenous redox, enzyme and pH or exogenous, such as light, ultrasound, magnetic fields or temperature [29].

The stimuli-responsive behavior can be achieved by grafting these moieties to the appropriate anticancer drug through cleavable bonds or using gatekeepers, which are ruptured in response to the specific stimuli. In this case, the nanosystem transports the drug to the target tissue and, once there, the presence of a particular stimulus will trigger the release of the trapped drug achieving better control over the administered dose and bringing specificity for the target [30].

To increase the specificity, different target can be defined. Among them, we have chosen to present the strategies used

to target either (1) the tumor microenvironment taking into account the general surrounding of the tumor (passive targeting), and, (2) the tumor cell more specifically by taking advantage of the ligand receptor interaction (active targeting) (Fig. 5).

Targeting strategies using prodrugs

Enzyme-specific release

One of the targeting strategies takes advantage in the elevated amounts of specific enzymes in the tumor tissues. The most common elevated enzymes are from the proteases family including lysosomal proteases, such as cathepsins, and proteases present in the extracellular matrix, such as matrix metalloproteases (MMP) (Table 2).

Matrix metalloproteinase

A methotrexate (MTX) prodrug has been developed by conjugation with a MMP-2 and MMP-9-cleavable peptide (PVGLIG). This prodrug was combined also with dextran in order to take advantage of the EPR effect. This strategy has demonstrated a better therapeutic index for the prodrug compared to MTX alone in fibrosarcoma and gliobastoma xenografted mouse models [31]. Other research teams have investigated this strategy, i.e. PVGLIG peptide with paclitaxel [32]. However, to date, no prodrug with this linker has reached clinical stage.

Prostate-specific antigen

Prostate-specific antigen (PSA) is a chymotrypsin-like protease overexpressed and secreted in the extracellular fluid of the prostate cancer microenvironment. Taking advantage of the high PSA level is one of the targeting strategies in prostate cancer research.

Denmeade et al. have synthesized a prodrug of DOX conjugated to a peptide (L-377202) specifically cleavable by PSA in prostate tumor tissue. The release of the active metabolites Leu-DOX and DOX in the prostate gland led to a localized antitumor action [33]. L-377202 has shown a good tolerance in patients at equivalent dose on a molar basis (NCT00987753) [34]. Nevertheless, the clinical phases II and III of the present clinical trial have never raised. Other cytotoxic drugs (paclitaxel, nitrogen mustard, vinblastine and methotrexate) have also been conjugated with PSA-activated peptide [35]. Among them, PSA-activated vinblastine prodrug has shown an excellent antitumor efficacy

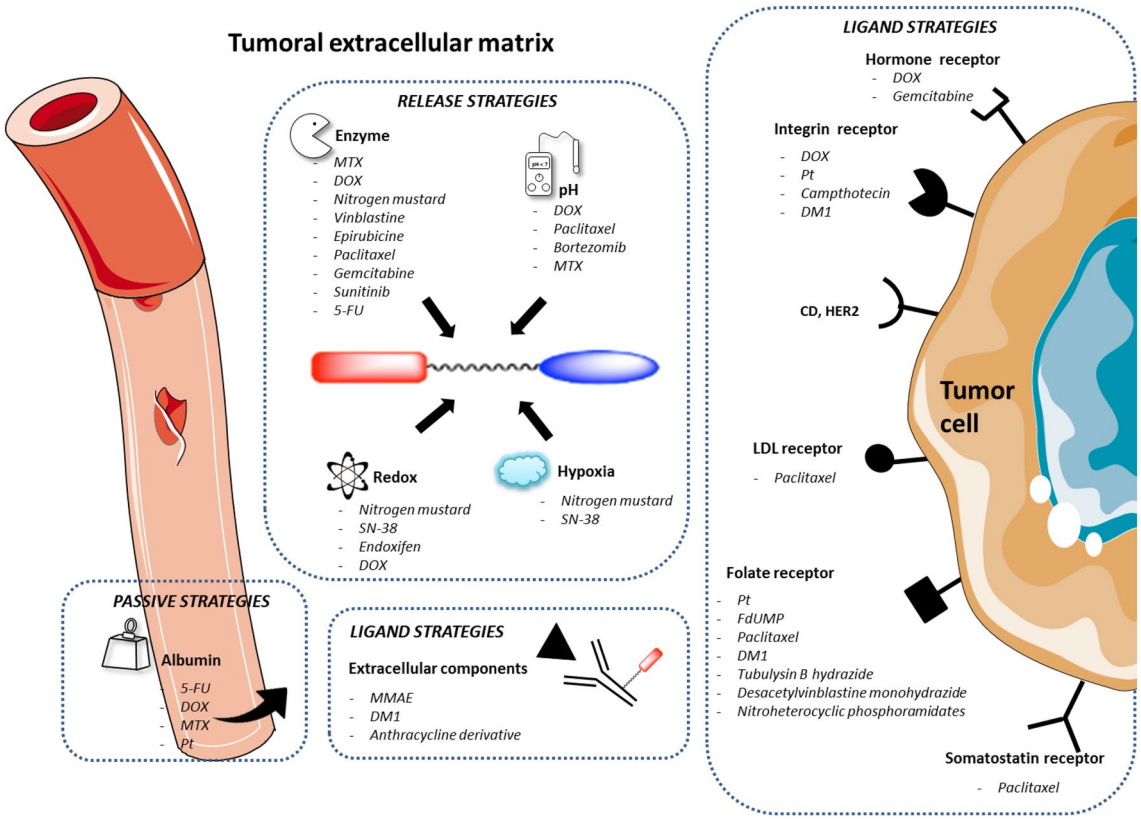


Fig. 5 Use of the tumor microenvironment or the tumor cell as a targeting strategy (Servier Medical Art)

Table 2 Enzyme-specific prodrugs

Target	Parent drug	Linker	Activity	References
Matrix-metalloproteinase	MTX	Matrix-metalloproteinase specific peptide: Pro-Val-Gly-Leu-Ile-Gly (PVGLIG) Gly-Ile-Val-Gly-Pro-Leu (GIVGPL)	In vivo efficacy on Glioblastoma, fibrosarcoma and bladder carcinoma xenografted tumor	[31]
Prostate specific antigen	DOX	PSA-specific peptide: His-Ser-Ser-Lys-Leu-Gln-Leu	Enzymatic activation validation, in vitro activity on a panel of prostate cancer cell line	[33]
	Nitrogen mustards	PSA-specific peptide: Glu-Hyp-Ala-Ser-Chg-Gln	Validation of in vitro proof of concept	[35]
	Vinblastine	PSA-specific peptide: Hyp-Ser-Ser-Chg-Gln-Ser-Ser-Pro	In vivo proof of concept on tumor bearing mice	[36]
Cancer associated fibroblasts	Vinblastine	Fibroblast protein specific peptide: Gly-Pro	In vivo efficacy on a panel of xenografted tumor (lung, liver and breast cancer)	[38]
	DOX	Fibroblast protein specific peptide: Gly-Pro	Evaluation of the toxicological profile and the safety pharmacological property of FAPα-targeting prodrug of doxorubicin in vitro and in vivo	[39]
	Epirubicin	Fibroblast protein specific peptide: Gly-Pro	Similar in vivo efficacy and no visible cardiotoxicity side effects compared to free EPI	[40]
Cathepsin B	DOX	Ac-Phe-Lys: dipeptide specific for cathepsin B	In vivo efficacy and toxicity study	[42]
		Cathepsin B specific peptide: Gly-Phe-Leu-Gly	Phase II study in the treatment of breast, non-small cell lung and colorectal cancer	[44]
	Paclitaxel	Polyglutamate residue specifically digested by Cathepsin B	Phase III trial in non-small cell lung and ovarian cancer	[45]
	Gemcitabine	Cathepsin B-sensitive maleimide linker	In vivo efficacy superiority compared to gemcitabine	[67]
	Sunitinib	Cathepsin B specific peptide: Phe-Lys	In vitro and ex vivo Validation cathepsin B cleavage	[48]
	Vedotin	cathepsin B specific peptide: Val-Cit	Approved in refractory or recurrent Hodgkin's lymphoma and large cell anaplastic lymphoma, primary cutaneous large cell anaplastic lymphoma, Hodgkin's lymphoma stage III or IV	[47]
Thymidine phosphorylase	5-FU	Fluoropyrimidine carbamate moiety	Increased safety and efficacy compared to free 5-FU in more several tumor grafted models	[56]
		Conjugation of albumin	Improvement of the circulation in blood stream and better PK/PD properties	[59]
Endopeptidase	DOX	Endopeptidase specific peptide: Glu-Hyp-Ala-Ser-Chg-Gln-Ser-Leu	Phase I trial led to showing a good tolerable and safe prodrug with partial response in patient with soft-tissue sarcoma A phase II is recommended	[61]
Legumain	DOX	Legumain specific peptide: Ala-Ala-Asp-Leu peptide	In vivo efficacy and improved therapeutic index were observed	[68]

with slight side-effects on prostate human tumor xenograft and dogs [36].

Fibroblast activation protein α

Targeting tumor cancer-associated fibroblasts (CAFs) is also a strategy in order to release the cytotoxic drug into the tumor environment. Fibroblast activation protein α (FAPα) is an enzyme overexpressed by CAFs as well as in pericytes in human epithelial cancers [37]. Recently, Chen et al. have developed a FAPα-activated vinblastine prodrug by conjugation of the peptide Gly-Pro (Z-Gly-Pro), in order to overcome resistance to vascular disrupting agents through pericytes targeting, which has shown efficacy in tumor xenografts of carcinomas [38]. DOX was also tested in combination with the FAPα-cleaved peptide Z-Gly-Pro; the solubility of DOX was enhanced and the first clinical results in mouse and dog models have shown a reduced toxicity compared to DOX with a comparable antitumor efficacy [39]. The prodrug Z-Gly-Pro epirubicin also has shown a better therapeutic index in mouse model [40]. However, no clinical trials are ongoing for this kind of prodrugs.

Cathepsin B

Cathepsin B is a cysteine protease present in the lysosome as well as in the extracellular matrix. Its overexpression is correlated with invasive and metastatic cancers [41]. Regarding cathepsin B-cleavable tumor-targeting prodrug, Shao et al. have designed a cathepsin B-activated DOX prodrug, Ac-Phe-Lys-PABC-ADM [42], enhancing efficacy and reduced side-effects in gastric cancer peritoneal carcinomatosis mouse model. Other cathepsin B-activated prodrugs of DOX (PDOX) have been developed with a different linker, Gly-Phe-Leu-Gly. PDOX has shown a similar antitumor activity but reduced toxicities in carcinoma-bearing rabbits [43]. Another DOX prodrug made of [*N*-(2-hydroxypropyl) methacrylamide] copolymer and DOX linked by a tetrapeptide spacer cleavable by lysosomal cathepsins (FCE28068 or PK1) has been evaluated in patients with non-small cell lung, breast and colorectal cancer. PK1 takes advantage of the EPR effect and specific cleavage. Moreover, PK1 has shown a good tolerance and efficacy in phase II clinical trial [44]. Paclitaxel prodrug was also developed with the dual strategy "EPR and cathepsin B-cleavable linker" with combination of paclitaxel between degradable polymers made of L-glutamic acid residues (PPX for Paclitaxel poliglumex). PPX has shown in phase III in patients with non-small cell lung cancer a similar efficacy compared docetaxel but an increased neurotoxicity and lower neutropenia and alopecia [45]. Under the trade name Opaxio®, PPX has been withdrawn of the application for marketing authorization by the European Medicines Agency (EMA) in 2009 because of the neurotoxicity.

More recently, a multifunctional tumor-targeting cathepsin B-sensitive gemcitabine prodrug has been developed to take advantage of the specific cleavage by cathepsin B in the tumor cell as well as the in vivo covalently bound with the circulating albumin. This dual strategy has shown a better antitumor efficacy in mouse breast tumor xenografted model [46]. Finally, a new ADC obtained by the conjugation of Brentuximab, a mAb targeting specifically CD30 surface receptors on the surface of activated B and T cells, to the antimitotic agent vedotin was designed. The conjugation is fulfilled thru a valine–citrulline linker specifically cleaved inside the lysosome by lysosomal cysteine proteases such as cathepsin B. This ADC is approved in refractory or recurrent Hodgkin's lymphoma and large cell anaplastic lymphoma, primary cutaneous large cell anaplastic lymphoma, Hodgkin's lymphoma stage III or IV and is commercialized under the trade name Adcetris® [47].

In addition to the work on CT, developments are also being made on tyrosine kinase inhibitors, such as sunitinib, the VEGFR inhibitor. Sunitib prodrug was prepared by conjugation of sunitinib to a cathepsin B cleavable dipeptide. Preliminary in vitro data have shown similar efficacy compared to sunitinib alone; in vivo investigations are needed to appreciate the potential increased therapeutic index [48].

This strategy seems to be promising but no prodrugs have gone through the phase III of clinical trial.

Thymidine phosphorylase and others

The enzyme thymidine phosphorylase is necessary to convert fluoropyrimidines-based prodrug such as 5-fluorouracil (5-FU) into the antimetabolite agent. This enzyme is overexpressed in tumor cells, which leads to an increase of the fluoropyrimidine-based prodrug concentration into the tumor tissue [49]. 5-FU is a cytotoxic agent that has been widely investigated in order to improve its therapeutic index. Briefly, 5-FU is administered by intravenous injection (IV) due to an unpredictable gastrointestinal absorption and a fast degradation. To overcome the drawbacks of IV injection, such as infection risks, oral prodrugs of 5-FU have been developed. The first oral 5-FU prodrug, tegafur, was developed in 1967 [50] leading to the release of 5-FU after hepatic biotransformation (mainly by CYP2A6), but has shown neurotoxicities and is used in association with uracil to overcome these side-effects [51]. Tegafur has also been integrated in a therapeutic cocktail (S-1 cocktail) with an inhibitor of dihydropyrimidine dehydrogenase (DPD) to increase the 5-FU half-life, and, an inhibitor of 5-FU phosphorylation in the gastrointestinal tract to decrease the gastrointestinal toxicities. S-1 cocktail has shown a high efficacy but also high incidence of side-effects during the

phase II trial [52]; nevertheless it has been approved for the treatment of advanced gastric cancer by the EMA in 2011 (Teysuno®) but not by the Food and Drug Administration (FDA). Another oral prodrug has been developed taking advantage of metabolic pathway enabling the liberation of 5-FU to decrease the dose limiting due to hematotoxicity, the doxifluridine. The pyrimidine phosphorylase required for the transformation of doxifluridine into 5-FU is practically absent in the bone marrow [53]. A Japanese study did not show any significant advantages of doxifluridine over 5-FU in rectal cancer in clinic [54]. Besides, doxifluridine has been also studied with intravenous injections. Nevertheless, the phase II clinical trial of doxifluridine in patients with advanced ovarian cancer has shown toxic deaths and severe neurotoxicities; doxifluridine could pass through the blood–brain barrier more easily than 5-FU [55]. Capecitabin (Xeloda®), a derivative of doxifluridine, is not metabolized by intestinal cells with a nearly complete absorption and concentrates mostly into tumor cell to give 5-FU by metabolization through a cascade reaction of three enzymes with the last step by thymidine phosphorylase [56]. Nowadays, capecitabine is widely used in antitumor treatments. However, a recent meta-analysis has concluded no difference in overall survival and progression-free survival between 5-FU, capecitabine and S-1 in advanced gastric cancer, while lower toxicities with S-1 were observed [57]. Fukushima et al. have developed DFP-11207, which is a combination with precursor form of 5-FU (1-ethoxymethyl-5-FU), an inhibitor of 5-FU degradation (5-chloro-2,4-dihydroxypyridine) and an inhibitor of 5-FU phosphorylation (citrazinic acid). Pharmacokinetic study on rats has shown, after an oral administration, a lower C_{max} and longer half-life compared to the other 5-FU prodrugs, and a similar efficacy of 5-FU but lower toxicities [58]. A phase I is ongoing in solid tumors (NCT02171221).

Other developments aim to increase the half-life and the biodistribution of 5-FU, such as an albumin-bound 5-FU prodrug, which forms a drug–albumin conjugate nanoparticle after administration [59].

Endopeptidase

More recently, a first-in-man phase I study investigated the safety and pharmacokinetic parameters of DOX prodrug DTS-201 (ALAL-DOX). This prodrug is cleaved into tumor tissue by extracellular tumor-specific endopeptidases, neprilysin and thimetoligopeptidase. Preclinical evaluations have shown clearly difference in pharmacokinetic and tissue distribution between DOX and DTS-201 after equimolar administration: with DTS-201, normal tissue were much less exposed to DOX (i.e. area under the curve (AUC) of DOX in the heart normal tissue is decreased of 93% after DTS-201 administration), whereas the exposure of the xenografted tumors was almost doubled [60].

It has shown a better cardiac safety profile at doses of DOX higher than those usually used (threefold increase) [61] and a phase II trial is ongoing. A second generation of tetrapeptidic enzyme-activated DOX prodrug, ALGP-DOX, has demonstrated a tolerated dose 30- to 40-fold higher compared to DOX in patient-derived soft tissue sarcoma xenograft model. The modification of the tetrapeptidic sequence could offer a better prodrug stability and tumor specificity [62].

Legumain

Legumain is a lysosomal protease elevated in several solid tumors such as breast, ovarian, colon and prostate cancer as well as in tumor-associated macrophages and intra tumoral blood vessels [63]. Wu et al. have developed a legumain-activated DOX prodrug (LEG-3) which has shown a better therapeutic index (lower toxicity and better efficacy) in CT26 mouse model [64]. More recently, DOX was conjugated to legumain-cleavable carboxyl-terminated poly(ethylene glycol) via the Ala-Ala-Asp-Leu peptide (4-arm PEG-AANL-DOX) which is known to be a good legumain substrate. Besides, this prodrug could self-assemble into nanoparticles and by consequence takes advantage of the so-called EPR effect. The 4-arm PEG-AANL-DOX has shown in vivo efficacy in human melanoma model comparable to DOX without toxicities [65]. The etoposide agent was also conjugated to legumain-cleavable peptide, Ala-Ala-Asp-ethylenediamine and this prodrug has shown a better cytotoxicity activity in vitro on tumor cell models [66]. Recent studies established on neuroblastoma mostly in children using high-dose of therapeutic treatment were fulfilled, but their use are so far limited. The authors synthesized 6-maleimidocaproyl-AANL-DOX (EMA-AANL-DOX) which has shown lower toxicities in vivo neuroblastoma human cell than DOX. The advantage of EMA-AANL-DOX over previous legumain-cleavable prodrugs is the presence of 6-maleimidocaproyl which is protective, as well as it can bind to serum albumin (SA) and by consequence benefit from the EPR effect [46]. The therapeutic strategy with legumain-cleavable based prodrug is recent and no clinical trial is yet on going.

pH-specific release

Abnormal structure of tumor vasculature along with abnormal blood flow lead to a heterogeneity of oxygen concentration which is lower in the microenvironment of the tumor. By consequence, the low level of oxygen triggers an anaerobic metabolism for the tumor cell and the production of lactic acid and CO_2. This phenomenon promotes an acidic microenvironment in tumor between pH 6.0 and 6.8 and

in endosome/lysosome (pH < 5.5) which is different from the physiological pH observed in healthy cells (pH ≈ 7.4). Among the design of prodrug strategies, pH-cleavable prodrugs are investigated to specifically target the acidity of tumors (Table 3). Different linkers have been developed using polymers responsive to tumoral low pH [polymers having imidazole groups or poly(β-amino ester)]. Paclitaxel has been linked to an imidazole derivative and has shown in vivo increased efficacy on carcinoma mouse model compared to paclitaxel alone [69]. On the other hand, linkers have been synthesized using polymers responsive to intracellular pH (mostly prodrugs with hydrazone bond) [70] with a main focus on the use of DOX as active entity [71–73]. Besides, one of the most interesting challenges of vectorized treatment is to target the bone tissue where metastases are often found. Recently, researchers have studied a bortezomib (BTZ) pH-based prodrug made of co-encapsulated BTZ with aryl boronate group as acidity-labile linker, and alendronate as bone-targeting ligand. This strategy takes advantage of the EPR effect, pH-cleavable linker and active targeting. This prodrug has shown a good efficacy in vivo in bone metastasis human breast adenocarcinoma model; decreased bone destruction was also underlined [74]. MTX was also studied as pH-sensible prodrug; it was coupled to 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[aldehyde-(polyethyleneglycol)-2000] (DSPE-PEG-Imine-MTX) and the obtained complex showed the ability to self-assemble into micellar nanoparticles. Combining the EPR effect and the imine-pH cleavable linker, DSPE-PEG-Imine-MTX has shown promising efficacy in vivo in human cervical carcinoma model [75].

Nevertheless, none of these pH-responsive prodrugs is involved on clinical trial for now.

Hypoxic-sensitive release

The formation of abnormal physiopathology of tumor tissue leads to hypoxia. Hypoxia is widely described as a biomarker of malignancy and an obstacle in drug therapy. Taking advantage of the hypoxic environment, several DDS have been developed with a specific hypoxic sensitivity. Among them, hypoxia-activated prodrugs (HAPs), which are specifically cleaved by reductive enzymes with a bioreductive group, have been designed (Table 4). Their developments involve the conjugation of the 2-nitroimidazole moiety to different anticancer drugs. One of the most clinically advanced hypoxia-activated prodrugs, evofosfamide (TH-302), was obtained by conjugation with the DNA cross-linking toxin bromo-isophosphoramide mustard (Br-IPM). However, TH-302 did not show effective results in phase III trials in locally advanced, unresectable or metastatic soft-tissue sarcoma [76]. Nevertheless, TH-302 is widely studied in others malignancies tumors; such as in i) relapse/refractory multiple myeloma with a positive phase I/II trial [77], ii) patients with aggressive human papillomavirus-negative head and neck squamous cell carcinoma [78], iii) combination with sunitinib in neuroblastoma [79], iv) melanoma xenograft models [80], v) nasopharyngeal carcinoma

Table 3 Acidic pH specific prodrugs

Target	Parent drug	Linker	Activity	References
Acidic pH	Paclitaxel	pH sensitive imidazole moiety	Similar cell toxicity and improved efficacy in vivo compared to free paclitaxel	[69]
	DOX	pH sensitive hydrazone moiety	Similar cell toxicity and improved efficacy in vivo compared to free DOX	[71]
	Bortezomib	pH sensitive aryl boronate moiety	Reduced systemic toxicity and improved therapeutic effects compared to free bortezomib	[74]
	MTX	pH sensitive imine moiety	Increased of the in vitro and in vivo anticancer activity	[75]

Table 4 Hypoxic environment specific prodrugs

Target	Parent drug	Linker	Activity	References
Hypoxic environment	Nitrogen mustard	Hypoxia sensitive nitroimidazole moiety	Promising phase II results in multiple myeloma patients	[77]
			Failed in phase III trial in soft tissue sarcoma	[76]
	Nitrogen mustard	Hypoxia sensitive nitroaromatic moiety	Validated a phase I clinical trial but increased hematotoxicity	[86]
			Phase I/II this prodrug is unlikely to be successful for long-term leukemia control because of on-target toxicity	[88]
	SN-38	Hypoxia sensitive nitroimidazole moiety	The proof of concept was validated in vitro	[85]

xenograft models [81], vi) recurrent bevacizumab-refractory glioblastoma with positive phase I trial [82], vii) osteosarcoma xenograft model [83], and viii) combination with radiotherapy in human orthotopic pancreatic tumor model [84]. This strategy was then applied to design a new prodrug by conjugation of the nitro-imidazole moiety to SN-38, the principal metabolite of irinotecan, showing the capacity of being a promising hypoxia-selective antitumor agent [85]. Besides, PR-104 is a phosphate ester that is hydrolyzed thought hypoxia-activated pathway releasing nitrogen mustard. Nevertheless, a clinical trial phase I in advanced solid tumors has shown severe side-effects such as myelotoxicity [86]. Indeed, Guise et al. have demonstrated a second mechanism for PR104 activation through hypoxia-independent aldo–keto reductase 1C3 enzyme [87], which is highly expressed in acute myeloid leukemia (AML) blast [78]. For this reason, PR-104 has been investigated in a phase I/II trial (NCT 01037556) in relapsed or refractory AML and has shown a threefold-higher AUCs due to overexpression of this enzyme into AML blasts compared to other solid tumors. Nevertheless, severe gastro-intestinal toxicities and myelosuppression have been reported and PR-104 did not provide efficacy evidence [88].

Reactive oxygen species-sensitive release

It has now been demonstrated that cancer cells exhibit increased intrinsic oxidative stress leading to an increased amount of reactive oxygen species (ROS) compared to normal healthy cells, such as superoxide anion, hydrogen peroxyde and the hydroxyl radicals. These high levels of ROS in cancer cells contribute to cancer-cell proliferation, DNA alterations, apoptosis, metastasis, angiogenesis and alteration in the cellular sensitivity to anticancer agents [89]. Consequently, ROS production has been used as a target to design new ROS-activable prodrug which would be activated in the presence of high amounts of ROS (Table 5). As described by Saravanakumar et al., numerous ROS-responsive linkers can be used to design these prodrugs [90]. However, boron-based linkers have been widely developed in the past decade by designing several prodrugs by conjugation of numerous anticancer compounds to boronate moieties. Among the anticancer drugs used with this strategy, we can find nitrogen mustards [91], SN-38 [92], endoxifen (non-steroidal selective estrogen receptor modulator) [93] and more recently DOX [12]. All these new prodrugs showed interesting results as the proof of concept of ROS activation was validated in vitro. Nevertheless, the type of linker, its sensitivity to be cleaved and its placement in the molecule structure govern the kinetic of the drug release as each linker has its own advantages and limitations. In regards to arylboronate linkers, there is the generation of harmful quinone methide upon degradation of the arylboronate, which remains a main concern for the clinical development [90].

High weight molecule-conjugate prodrug: serum albumin

The abnormality of the blood vasculature and lymphatic drainage lead to an accumulation of plasmatic protein such as SA into the tumor tissues. Moreover, SA has a biological half-life of 19 days, mostly due to its recognition by FcRn. Researchers have investigated the binding between SA and chemotherapy agents (Table 6) in order to take advantage of EPR effects and they have shown enhanced pharmacokinetic parameters of the active moiety. Since 1994, researchers have investigated albumin–drug conjugates; Gabor et al. have shown promising in vitro results of albumin–DOX conjugates against Ewing's sarcoma [94]. DOXO-EMCH (also called INNO-206 or aldoxorubicin) binds to the circulating albumin and accumulates into the tumor. Moreover, this DOX-derivative contains an acid-sensitive hydrazone linker that releases the cytotoxic drugs in acidic microenvironment [95]. The phase I and phase II in soft tissue sarcoma have shown a better antitumor activity compared to DOX with a well-tolerated cumulative dose (more than fivefold the median cumulative dose of DOX) [96]. Pharmacokinetic study has shown a narrow volume of distribution (0.3 L/kg versus 72 L/kg at DOX equivalent dose) which means that aldoxorubicin remains into the blood stream and does not accumulate in the body [95]. Then a small concentration of free-DOX and DOX metabolite has been found into the systemic compartment and urine that means aldoxorubicin has bounded to albumin and few DOX was released from

Table 5 ROS specific prodrugs

Target	Parent drug	Linker	Activity	References
Reactive oxygen species (ROS)	Nitrogen mustards	ROS sensitive arylboronate or boronic acid moieties	The proof of concept was validated in vitro	[91]
	SN-38	ROS sensitive arylboronate or boronic acid moieties	The proof of concept was validated in vitro and in vivo	[92]
	Endoxifen	ROS sensitive arylboronate or boronic acid moieties		[93]
	DOX	ROS sensitive arylboronate or boronic acid moieties		[12]

Table 6 Albumin targeting prodrugs

Target	Parent drug	Linker	Activity	References
Albumin	DOX	Acid-sensitive hydrazone linker	Clinical trials in sarcomas, small-cell lung cancer, glioblastoma	[95]
	DOX	Ile-Ala-Gly-Gln (MMP2-sensitive)	In vivo: melanoma model	[98]
	DOX	Ala-Leu-Ala-Leu (Cathepsin B-sensitive)	In vivo: colon model	[99]
	DOX	6-Maleimidocaproic acid-Arg-Arg-Ser-Ser-Tyr-Tyr—Ser-Gly (PSA-sensitive)	In vivo: prostate adenocarcinoma model	[100]
	MTX	No linker: simple Amide bond	Phase II in RCC: failed	[103]
	Oxaliplatin	Mono-maleimide moiety	In vivo in CT-26 model: tumor regression	[104]

RCC renal cell carcinoma

aldoxorubicin [97]. Phase III is being investigated in soft tissue sarcomas (NTC02049905), a phase II in recurrent small cell lung cancer (NTC02200757), a phase II in glioblastoma (NTC2014844) and a phase II in HIV-related Kaposi's sarcoma (NTC02029430). Albumin-binding prodrugs of DOX have been investigated with other enzyme-sensible linkers such as Ile-Ala-Gly-Gln for a cleavage by MMP-2 [98], Ala-Leu-Ala-Leu for a cleavage by cathepsin B [99], 6-maleimidocaproic acid-Arg-Arg-Ser-Ser-Tyr-Tyr—Ser-Gly for a cleavage by PSA [100].

Other cytotoxic agents have been investigated such as MTX, drug with a short half-life due to renal elimination leading also to nephrotoxicity. MTX has been bounded to SA in order to limit the renal elimination and promote the tumor uptake rate. The bioconjugate has shown therapeutic activity in various cancers without toxic side-effects [101]. A phase I in cancer patients confirmed a well-tolerated treatment [102]. Unfortunately, no objective responses have been demonstrated in phase II in renal cell carcinoma [103]. Platinum complexes (oxaliplatin and cisplatin) bounded to monomaleimide as promoiety have been studied; only oxaliplatin derivatives that have shown outstanding antitumor activity are presented, which will now be further developed towards clinical phase I trials [104]. The difference in antitumor activity between oxaliplatin and cisplatin derivatives might be explained by different extravasation potency or different affinity for glycoprotein 18 and 30, which are involved in the recognition of conformationally modified albumin molecules.

Tumor-specific ligand

The active targeting strategy takes advantage of the cellular difference between healthy cells and tumor cells. The targeting ligands can be classified into three groups according to the place where tumor-specific or overexpressed antigen is presented: (1) the tumor cell surface, (2) the endothelial cell surface or (3) the extracellular matrix cells surface. Proteins (monoclonal antibody), peptides (Arg-Gly-Asp (RGD) peptides, poly-Arg peptides) and small molecules have been investigated to target selectively cancer cells (Table 7).

Immuno-driven DDS

Antibody–drug conjugates The coupling of a cytotoxic agent to a monoclonal antibody (mAb) is called immunoconjugate or antibody–drug conjugate (ADC). ADC offers a strategy to improve the tolerance/efficacy balance for highly cytotoxic molecules. Indeed, mAb brings target specificity and, consequently, improves the pharmacokinetics (PK) and pharmacodynamics (PD) of these molecules and some of them have obtained their approval from either the FDA or the EMA (Table 8). There are two types of release of the agents: ADC can be cleaved externally via a cleavable linker (non-internalizing mAb) or inside the cell after endocytosis with a non-cleavable linker (internalizing mAb). The highest advantage of non-cleavable linkers compared to cleavable linkers is their plasma stability and by consequence, an optimized therapeutic window mostly due to reduced off-target toxicity. The PK of ADC is complex because it takes into account the PK characteristics of the cytotoxic molecule, the mAb, as well as the physicochemical properties of the linker. It is nevertheless greatly influenced by the PK of the mAb as the latter represents more than 90% of the molecular weight. Besides, sizes ranging of ADC are from 10 to 100 nm and can benefit of EPR effect as well. The first ADC to receive marketing authorization (MA) was Mylotarg®, conjugation of ozogamicin with gemtuzumab (mean drug:Ab ratio (DAR) 1.5) bounded by an acid-cleavable hydrazine [105]. Ozogamicin is a derivative of calicheamicin, a highly cytotoxic antibiotic, and gemtuzumab is an IgG4 anti-CD33, a surface marker expressed on cells of the myeloid line. Mylotarg® received the MA in 2000 from the FDA for the indication of relapsed AML but was withdrawn in 2010 and then reinstated in 2017 with a dosage regimen optimization including a lower dose [106]. Its withdrawal in 2010 was due to off-target reactions, particularly in hepatic tissue where Küpffer cells also express

Table 7 Immuno-driven prodrugs

Target	Parent drug	Ligand	Linker	Activity	References
Integrin receptor	DOX	RGD	Polyethylene glycol chain	Increased in vivo efficacy	[143]
	Pt	RGD	Amide bond	In vitro: greater the number of bounded RGD, greater is the internalization and efficiency	[120]
	Campthotecin	RGD	Dipeptide alanine–citrulline or phenylalanine–lysine + glycol chain	In vitro: shorter is the glycol chain, lower are the solubility and the affinity for receptor	[121]
	DM1	RGD	PEGP (TMC-*co*-Poly(pyridyldisulfide cyclic carbonate) [PDSC]) and Mal-PEG-P (TMC-*co*-PDSC) copolymers	In vivo: increase efficacy in human breast model	[123]
Hormone receptor	DOX	Small peptide agonist to LHRH receptor	Peptidic bond	Phase II ovarian cancer, prostate cancer), III (endometrial cancer)	[125–127]
	Gemcitabine	Gonadotrophin releasing hormone receptor ligand peptide	Succinate linker	Efficacy in human prostate model	[128]
LDL receptor	Paclitaxel	Angiopep-2	Cleavable ester linkage	Phase I in recurrent glioma, phase II for brain metastasis from breast or lung cancer	[130, 144]
Somato-stanin receptor	Paclitaxel	Octreotide	Polyethylene glycol chain	Efficacy in non-small cell lung cancer model	[132]
Folate receptor	Nitroheterocyclic phosphoramidates	Pteroic acid	Lysine	In vitro: no satisfactory cytotoxicity	[133]
	Pt	Folic acid	Polyethylene glycol	In vitro: no increased cytotoxicity compared PEG-Pt	[134]
	N 5-fluoro2′-deoxyuridine-5′-*O*-monophosphate (FdUMP)	Folic acid	Phosphodiester linkage	In vitro: increased cytotoxicity on 5-FU resistant cells	[135]
	Paclitaxel	Folic acid	Ester linker	In vivo: no increased efficacy	[136]
	DM1	Folic acid	Disulfide linker	In vitro: increased cytotoxicity	[137]
	Desacetylvinblastine monohydrazide	Folic acid	Endosome-cleavable disulfide bond	Phase III in Pt-resistant ovarian cancer: failure in improvement progression-free survival	[139]
	Tubulysin B hydrazide	Folic acid	Water soluble saccharo-peptide spacer	Phase I in advanced solid tumors	[145]
	Paclitaxel	Folic acid + evans blue (derivative for albumin bonding)	Ester linker	In vivo: Increased circulation half-life, more delivered paclitaxel into tumor and better efficacy	[141]
	DOX	*trans*-Activating factor	Ala-Ala-Asp	Breast cancer model	[142]

Table 8 Approved ADC by FDA and EMA

Target	Parent drug	Ligand	Linker	Activity	References
CD33	Ozogamicin	Gemtuzumab	Acid-cleavable hydrazone	Approved drug in relapsed AML	SmPC Mylotarg®
CD30	Vedotin	Brentuximab	Maleimide group-valine–citrulline-para-aminobenzyl carbamate	Approved in refractory or recurrent Hodgkin's lymphoma and large cell anaplastic lymphoma, primary cutaneous large cell anaplastic lymphoma, Hodgkin's lymphoma stage III or IV	SmPC Adcetris®
CD22	Ozogamicin	Inotuzumab	Acid-labile acetyl butyrate	Relapsed or refractory acute lymphoblastic leukemia with B precursor	SmPC Besponsa®
HER2	DM1	Trastuzumab	Amide bond	HER2 positive, metastatic or locally advanced, unresectable breast cancer	SmPC Trastuzumab®

SmPC summary of product characteristics

CD33 [107], leading to patient deaths (study SWOG S0106). The second ADC to receive MA by FDA (2011) and EMA (2012) was Adcetris® for refractory or recurrent Hodgkin's lymphoma and large cell anaplastic lymphoma. This ADC has also received MA in primary cutaneous large cell anaplastic lymphoma (FDA-2017 and EMA-2017) and more recently in first-line treatment against Hodgkin's lymphoma stage III or IV (FDA-2018) [47]. Adcetris® is composed of the antimitotic monomethylauristatin E (MMAE or vedotin) and the IgG1 brentuximab. MMAE which belongs to the antimitotic and antimicrotubule family is 50- to 200-fold more active than vinca-alkaloid. Brentuximab specifically targets CD30 surface receptors on the surface of activated B and T cells. The binding between the mAb and the cytotoxic agent (mean DAR 3.9) is made of a maleimide group, protease (cathepsin)-cleavable linker (valine–citrulline) and para-aminobenzyl carbamate spacers. This peptide-based linker is stable under physiological conditions, but, after internalization, is cleaved inside the lysosome by lysosomal cysteine proteases such as cathepsin B. Recently, the ADC, named Besponsa®, received MA in 2017 for in acute lymphoblastic leukemia (ALL) with B precursor, CD22 positive, relapsed or refractory. This ADC is made of ozogamicin and IgG4 inotuzumab (mean DAR 5.1) linked by an acid-labile acetyl butyrate linker, more stable in bloodstream than the linker used in Mylotarg® [108]. Inotuzumab specifically targets the CD22 surface protein present on B cells.

Regarding treatment of solid tumors, the PK biodisponibility is more complex because ADC has to diffuse to the targeting tissue. The parent drug maytansine—or DM1—is widely investigated. It is a vinca-alkaloid derivative, a potent inhibitor of microtubule assembly, 25–400 times more active than paclitaxel and 100–5000 times more active than DOX [109]. However, it failed as an anticancer agent in human clinical trials because of its lack of tumor specificity associated with an important systemic toxicity. DM1 has been linked to trastuzumab to afford T-DM1 with a mean DAR 3.4 via succinimidyl-*trans*-4(maleimidylmethyl] cyclohexane-1-carboxylate, a stable thioether linker that binds the mAb lysine residues [110]. Trastuzumab (Herceptin®) is a IgG1 mAb targeting extracellular domain of human epidermal growth factor receptor 2 (HER2 receptor, also known as neu, ErbB2, $p185^{HER2}$) and is widely used in the treatment of HER2 receptor positive breast cancer since 1998 in the United States and 2000 in Europe. This new ADC showed a similar drug release compared to Cantuzumab-DM1. Unlike Cantuzumab-DM1, the bond that connects the two entities is not cleavable by enzymes or reducing agents (e.g. glutathione) but the release of the cytotoxic agent is done by catabolism of the antibody after internalization in the targeted cancer cell. T-DM1 conjugate design led to favorable pharmacokinetic properties with an increased half-life from 2 to 44 h and an increased area under the curve by 60-fold [111]. T-DM1 (Kadcyla®) received MA in 2013 (FDA) and 2014 (EMA) in HER2 positive, metastatic or locally advanced, unresectable breast cancer previously treated with trastuzumab and a taxane. However, many DM1 or DM4-coupled mAbs failed as anticancer agents in clinical trials [112]. The reasons for discontinued ADCs are due to off-target effect with unacceptable toxicities (for example Bivatuzumab-DM1 has shown a fatal dermal toxicity in phase I leading to stop the development; Bivatuzumab targets CD44v6, receptor abundantly present in head and neck squamous cell carcinomas but also on normal keratinocytes [113]). It was discontinued also for the lack evidence of clinical activity [112].

The previously mentioned ADC target a receptor present on the surface of the tumor cell. A recent strategy aims to develop non-internalizing ADC targeting antigens overexpressed in the tumor extracellular matrix such as collagen IV, fibrin, fibronectin and tenascin-C [114]. Indeed Neri's team is investigating DM1 coupled with an antibody directed against fibronectin [115], MMAE and anthracycline derivative coupled with an antibody directed against tenascin-C through a valine–citrulline linker [114]. These ADCs have shown promising results in vivo in teratocarcinoma and human epidermoid carcinoma. The notable advantages are the accessibility of the extracellular matrix components and

the release of cytotoxic into the tumoral microenvironment may facilitate the diffusion and internalization in neighboring cells.

Approximately 222 clinical trials have been reported describing 82 different new ADCs that are listed on the clinicaltrials.gov website, 123 of them are currently under clinical evaluation.

Cancer-targeting ADC and more generally mAbs' uses are limited by the pharmacokinetic properties: Cellular up-take clearance due tumor tissue infiltration and reticulo-endothelial elimination via Fc fragments is the major parameter of the distribution of ADC.

Fragment-based antibody Over the past decades, the use of antibody-based therapeutics has grown exponentially leading to a revolution in biological drugs and has revolutionized targeted cancer therapies. However, the use of such therapeutics has limitation due to the large size of the scaffolds leading to the following: (1) poor penetration in the tumor, (2) important systemic accumulation and (3) slow clearance profiles. As the targeting capacity of Ab is achieved by a small variable loop, the selection of these small fragments has led to the design of small fragment-based prodrugs [116]. Aubrey et al. have recently developed two promising auristatin prodrugs targeting specifically HER2-positive breast cancer using this strategy. Their work was fulfilled by conjugation of auristatin to an engineered scFv fragment through maleimide linker. Their strategy was validated in vitro on HER2-positive breast cancer cells with activities in the subnanomolar range and no activity on HER2-negative breast cancer cells [117].

Peptides and small molecule-driven DDS

Investigation of peptide as drug delivery system conjugate offers some advantages compared to mAbs. Indeed peptides are easier to use (no tertiary structure, control of the drug ratio, lower immunogenicity), easier and less expensive to produce in large quantities. Upon peptide-receptor binding, the complex can undergo receptor-mediated endocytosis.

Integrin receptor targeting $\alpha_v\beta_3$ integrin is a protein involved in cell adhesion playing a major role in angiogenesis and tumor metastasis. This protein is overexpressed on the surface of tumor cell in glioma, melanoma, breast cancer and ovarian cancer [118]. RGD peptide has been shown to exhibit excellent binding affinity and selectivity for $\alpha_v\beta_3$ integrin [119]. Synthesis of RGD-derivative DOX, Pt and camptothecin has been investigated in order to give specificity and decrease toxicity, particularly cardiotoxicity for DOX and nephrotoxicity for Pt. Nevertheless, only promising in vitro data are available and need more investigations [120, 121]. Moreover, many studies are investigating in the development of integrin-targeting DDS [122]. Recent study on cyclic RGD-functionalized micellar DM1 prodrug; compared to T-DM1, the cytotoxic loading is higher as well as the stability and the efficacy has been proved in vivo in human breast model [123].

Hormone receptor targeting Luteinizing hormone releasing hormone (LHRH) receptors is over-expressed in prostate and gynecological cancers [124]. A small peptide agonist to LHRH receptor has been conjugated to the DOX (AEZS-108). AEZS-108 has shown positive clinical results in ovarian cancer (phase II [125]), in castration- and taxane-resistant prostate cancer (phase I: [126]; phase II: [127]) and in endometrial cancer but the phase III did not improve efficacy or safety compared to DOX alone (NCT01767155). Besides, gemcitabine was coupled with gonadotrophin releasing hormone receptor ligand peptide with a succinate linker. The complex has shown efficacy in vivo in a castration resistant prostate cancer model [128].

LDL receptor targeting ANG1005, called GRN1005 or paclitaxel trevatide, is a prodrug designed from three molecules of paclitaxel coupled with angiopep-2. Angiopep-2 targets low-density lipoprotein receptors which are overexpressed on solid tumor and enhances the uptake thought the blood–brain-barrier [129]. Its half-life is shorter compared to nab-paclitaxel (3.6 h vs 21.6 h, respectively) and paclitaxel alone (20.5 h) [129]. Several trials have been investigated with this prodrug in recurrent malignant glioma [130] (NCT01967810), in non-small cell lung cancer patients with brain metastases (NCT01497665; NCT02048059) and in breast or lung cancer patients with brain metastases (NCT01679743-ended in 2018).

Somatostatin receptor targeting Somatostatin receptor are G-protein-coupled receptors overexpressed in various cancer, therefore targeting somatostatin receptor has been proposed for anticancer strategy. Octreotide, an octapeptide that mimics natural somatostatin pharmacologically, has been used to develop drug delivery system. Huo et al. have studied the somatostatin receptor as a target for the delivery of paclitaxel. Paclitaxel presents a poor water solubility (0.3 µg/mL); the currently formulation mixes Cremophor®EL and ethanol (Taxol®) in order to solubilize paclitaxel for intravenous injection. However, Cremophor®EL can lead to serious acute hypersensitivity reactions and other toxicities [131], therefore researchers have investigated new paclitaxel analog in order to increase specificity and especially solubility. The authors have developed a paclitaxel prodrug made of taxane and octreotide linked by polyethylene glycol chain (OCT-PEG-PTX). The paclitaxel prodrug solubility was hugely increased (30,000-fold) compared paclitaxel, and has shown a higher antitumor efficacy in somatostatin receptor

overexpressing non-small cell lung cancer cells [132] but no clinical data are currently available.

Folate receptor targeting The overexpression of folate receptor in many human cancers leads to developed folate-targeting prodrugs. DNA alkylating agents [133], platinum derivatives [134], 5-fluorofluracil [135], paclitaxel [136] conjugates have been investigated for this purpose. Nevertheless, all cited folate receptor-specific prodrugs have shown no increased cytotoxicity in vitro and in vivo (for paclitaxel prodrug) compared to the parent drug except for folic acid (FA)-derivated 5FU which has proven enhanced efficacy on 5FU-resistant cells. FA has been linked to FdUMP and this conjugate is 10- to 25-fold more cytotoxic than 5-FU on human colorectal tumor cell lines. Ladino et al. have investigated the synthesis of DM1 bound to FA which has demonstrated high cytotoxicity on folate receptor positive carcinoma (KB), ovarian cancer (SK-OV-3) and colon cancer (Lo Vo, SW620) cell lines [137]; 10 years later Reddy et al. has confirmed the high specificity of folate-conjugated DM1and the optimized therapeutic window. However, to our knowledge, this was only demonstrated in preclinical experiments [138].

EC145 (vintafolide), a conjugate of folic acid and a vinca-alkaloid compound (desacetyl vinblastine hydrazide) through an endosome-cleavable disulfide bond, has been evaluated in a phase I and II trial in advanced non-small lung cancer, ovarian and endometrial cancers [139]. The drug received orphan drug status in Europe in 2012 for the treatment of folate-positive Pt-resistant ovarian cancer in combination with pegylated liposomal DOX, but in 2014, Merck and Endocyte stopped its commercialization because of drug failure in improvement progression-free survival. Guertin et al. proposed the hypothesis that this failure is due to the implication of P-glycoprotein pump that trigger a folate-prodrug resistance [140]. A second-generation FA drug conjugate linked to tubulysin B hydrazide is under investigation in patients with advanced solid tumors (NCT01999738). EC1456) delivers higher doses than EC145,

Recently, Shan et al. have developed a dual-targeting prodrug of paclitaxel with bifunctional folate and albumin binding moieties for both passive and active targeted cancer therapy [141]. This prodrug has shown a prolonged blood circulation, enhanced drug accumulation in tumors and higher therapeutic index in vivo on human breast adenocarcinoma model.

Others Liu et al. have designed a DOX derivative conjugated to cell-penetrating peptides (TAF, *trans*-activating factor) with a legumain-sensible linker (alanine-alanine-asparagine), and loaded on liposomes (using PEG 2000) in order to combine different targeting ways [142]. TAF is known to promote the cell internalization capability of liposomal NPs. The addition of the legumain-sensible linker to TAT has shown, in vitro, a decrease of 72.65% for its transmembrane transport capacity. Therefore legumain-sensible targeting has been used in vivo in breast cancer model to effectively recover the internalization capacity of TAF in tumor cells and tumor-associated macrophages which both upregulate legumain expression.

This complex DDS has shown a better therapeutic window on breast cancer mouse model.

Most of these the studies fulfilled with peptide-associated prodrug were carried out in the last decades. Taking into account the relative recentness of these studies the proof of concept in clinics is hard to establish as for today. Thus, none of the peptide-associated prodrug has successfully reached the therapeutic market, yet.

Conclusion

The intratumoral chemotherapies, surgery and radiotherapy, are widely used but their success is guaranteed only if the tumor presents a well-defined localization. In the field of cancer chemotherapy, the main goal is to facilitate the accumulation of active drugs in tumor tissues or in its microenvironment compared to normal tissues. The therapeutic effect of daily used chemotherapeutic agents is far from being optimal due to their poor specificity and/or insufficient access to the tumor. Indeed, non-local administration of chemotherapy needs the delivery of anticancer drugs from the blood to the tumor cell through a complex transport process. The antitumor drug vectorization (i.e. targeted drug delivery systems), allows, on one hand, to target the malignant cells, and on the other hand to avoid toxicities on non-malignant cells. Prodrug design has become an established concept and a powerful tool in optimizing daily used chemotherapy agents, overcoming their physicochemical and pharmaceutical issues. These modifications aim to improve tumor targeting, as the active compound is released only after an activation process is triggered near the target. Targeted prodrug drug delivery systems should be designed to facilitate the targeted delivery of anticancer agents to the tumor. In the present review, we have reported that several PBN-DDS strategies achieve this goal by combining the advantages of both prodrugs and nanotechnology. Nowadays, numerous PBN-DDS are under clinical trials suggesting that these new targeted compounds/DDS could have great potential to be used in clinical practice in the future. These prodrug delivery strategies are novel. Most of the investigations are from academic laboratories and are currently in preclinical or early stages clinical studies. The way to FDA or EMA approval is long and numerous prodrugs fail to obtain this invaluable sesame, the market authorization. However, these failures pave the way for future pharmaceutical development as in oncology,

and in other therapeutic areas, medicine will be always more precision (i.e. specific) medicine.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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