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Small Molecule-Drug Conjugates: Opportunities for the Development of Targeted Anticancer Drugs

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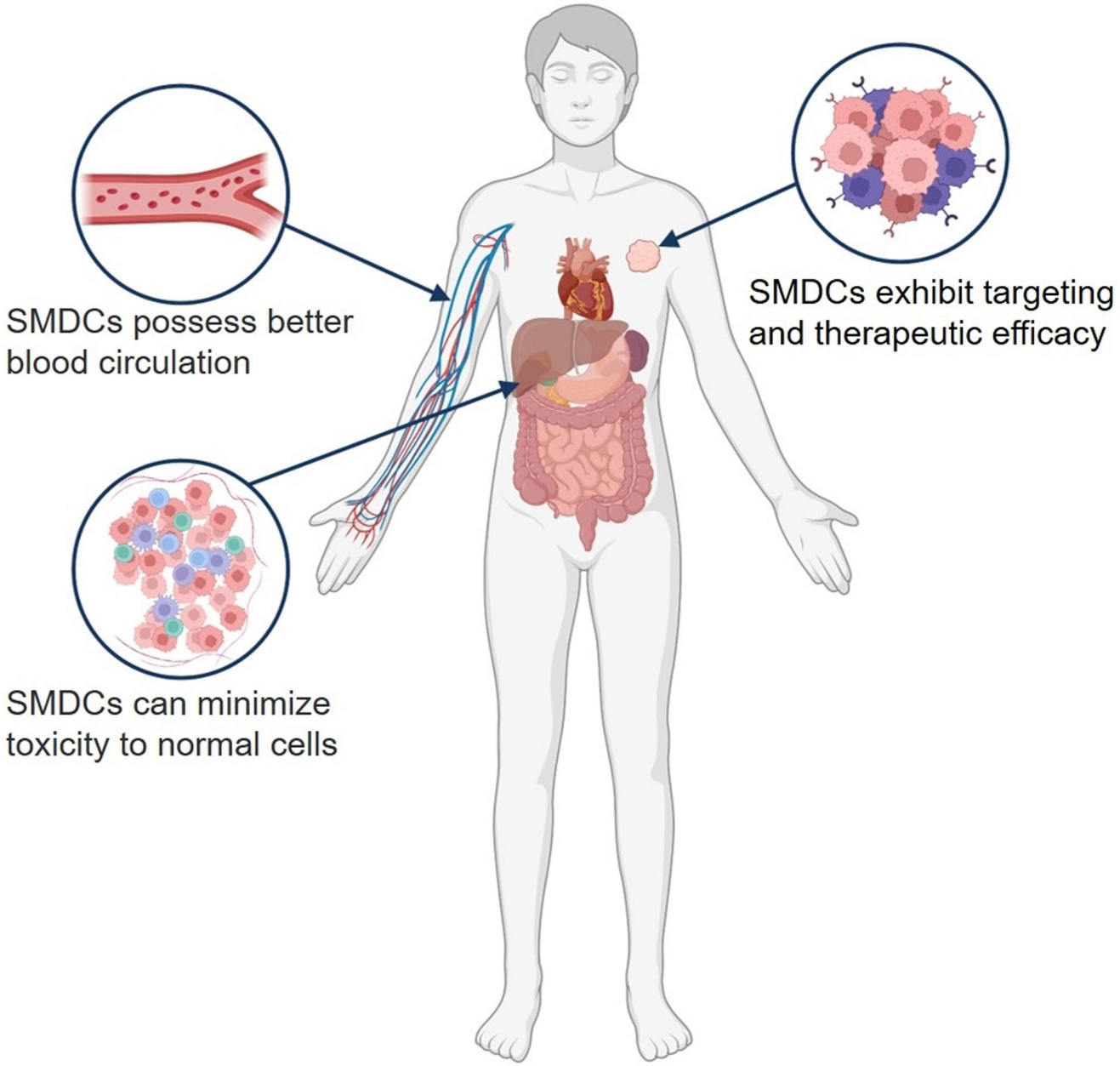

Conventional chemotherapy is insufficient for precise cancer treatment due to its lack of selectivity and inevitable side effects. Targeted drugs have emerged as a promising solution for precise cancer treatment. A common strategy is to conjugate therapeutic agents with ligands that can specifically bind to tumor cells, providing targeted therapy. Similar to the more successful antibody drug conjugates (ADCs), small molecule drug conjugates (SMDCs) are another promising class of targeted drugs, consisting of three parts: targeting ligand, cleavable linker and payload. Compared to ADCs, SMDCs have the advantages of smaller size, better permeability, simpler preparation process and non-immunogenicity, making them a promising alternative to ADCs. This review describes the characteristics of the targeting ligand, linker and payload of SMDCs and the criteria for selecting a suitable one. We also discuss recently reported SMDCs and list some successful SMDCs that have entered clinical trials.

Introduction

Currently, chemotherapy remains one of the most commonly employed methods for cancer therapy.[1] However, due to lack of selectivity, conventional cancer chemotherapies often exert non-specific effects on both malignant and healthy tissues, resulting different degree of side effects and limiting the dose to maintain therapeutic efficacy, thus restricting their clinical applications.[2] Consequently, over the past few decades, the focus of anticancer drug development has shifted from classical chemotherapy drugs to targeted therapy in order to enhance tumor specificity and minimize side effects.[3] The strategy that coupling cytotoxic molecules with tumor targeting ligands is an important research and development trend for the production of targeted therapeutic drugs.[4]

Antibody-drug conjugates (ADCs)[5] and small molecule-drug conjugates (SMDCs)[6] are two significant class of targeted agents. In recent years, ADCs have developed rapidly and made great progress by utilizing antibodies to selectively deliver drugs to tumor cells.[7] Despite the great promise, challenges persist in ADCs application such as inefficient delivery and instability due to site-specific chemical coupling of drugs to antibodies.[8,9] Similar to ADCs, SMDCs also consist of targeting ligand, linker, and cytotoxic molecule (Figure 1).[10] The primary distinction between SMDCs and ADCs lies in the targeting ligand used. While ADCs employ biological antibodies for drug localization targeting, SMDCs utilize small molecule instead.

Compared to ADCs, SMDCs possess several advantages including smaller size and lower molecular weight which facilitate better cell and tissue permeability,[11] better *in vivo* and *in vitro* stability,[12] leading to quicker and more even distribution throughout the tumor tissue. Furthermore, the ratio between payload (toxicity molecule) and antibody is usually difficult to determine during the preparation process of ADCs,[13] whereas both the small molecule targeting ligand as well as payload values can be accurately determined for SMDCs.[6] The lower cost-of-goods[14] and lack of immunogenicity[15] also make SMDCs better choice. Although still mainly being investigated at preclinical or clinical stage,[14,16] SMDCs are gaining increasing interest as a novel alternative for drug delivery and tumor targeting applications with promising development prospects. Radioisotope labeled small molecule conjugations are also a type of SMDCs, which have been discussed in a few reviews,[17,18] so we will not focus on this type of molecule in this review.

Targeting Ligand

The targeting ligands in SMDCs are equivalent to antibodies in ADCs, which can specifically bind to particular receptors that are overexpressed in cancer cells, are used to deliver drug molecules into cancer cells.[19] To select great small molecule ligands, factors such as target selectivity, binding affinity, and molecular size need to be taken into consideration.[6] Developing suitable small molecule ligands has been difficult, which often derived from derivatives of natural ligands.[20] However, the advancement of DNA-encoded chemical library technology is expected to promote the discovery process.[20,21] At present, the typical small molecular ligands include glutamic acid urea derivatives targeting prostate specific membrane antigen (PSMA),[22] folate derivatives targeting folate receptors,[23] somatostatin analogues targeting somatostatin receptors,[24] and some aromatic sulfonamides specially targeting carbonic anhydrase IX(CAIX) (Figure 2).[25]

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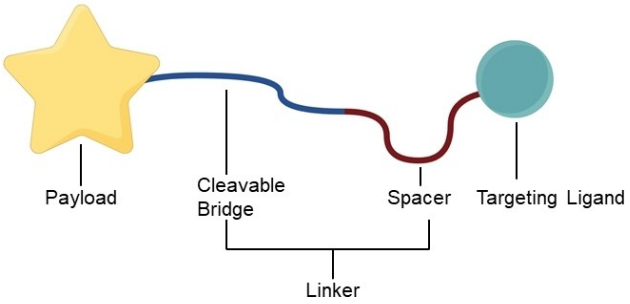


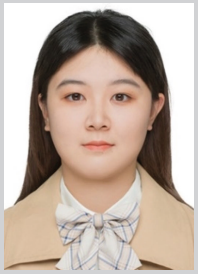
Figure 1. Schematic of a typical small molecule-drug conjugate.

Target Selectivity

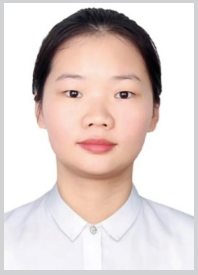
Selectivity is extremely important for targeting ligands as the original purpose of SMDCs is to specifically deliver drugs to tumor cells while minimizing harm to normal tissues.[16] To select appropriate targeting ligands, tumor specific receptors need to be focused. The ideal receptor would be overexpressed on tumor cells but with little or no expression on normal cells and should has sufficient quantities to effectively transport drugs into tumor cells.[16] The optimal targeting ligand should be able to recognize the corresponding receptor precisely while exhibit poor affinity towards other members within the receptor family. For example, in mammals, each of the integrin heterodimer contains an α-subunit and a β-subunit in a noncovalent complex, 18α and 8β-subunits give rise to a total of 24 functionally distinct heterodimeric transmembrane receptors.[26] Different integrin isoforms expressed in different tissues thus require precisely targeted ligands. For example, integrin $\alpha_v\beta_3$ specifically recognizes arginine-glycine-aspartic acid (RGD) moiety, thus RGD peptides have been widely used in the development of $\alpha_v\beta_3$-targeting drug delivery systems.[27]

Binding Affinity

Small molecule ligands must possess sufficient receptor affinity, as a high level of receptor affinity can effectively decrease the required drug concentration to achieve a specific therapeutic effect,[28] thereby minimizing potential adverse effects on normal tissues. The K_d value of a successful targeted ligand should be 10 nM or lower.[16] Suboptimal affinity can be improved by connecting multivalent ligands to the same carrier.[29] This strategy has been applied in the asialoglycoprotein receptor (ASGPR), which is highly expressed on the hepatocyte membrane and can specifically recognize galactose.[30] A targeted drug with β-elemene derivative W105



Jingjing Zhang is currently pursuing a master's degree at the School of Public Health, Xi'an Jiaotong University. Her research interests include nanomedicine, drug delivery, and the synthesis and application of small molecule drug conjugates.



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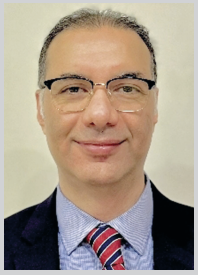

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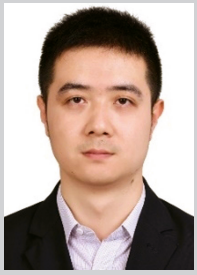

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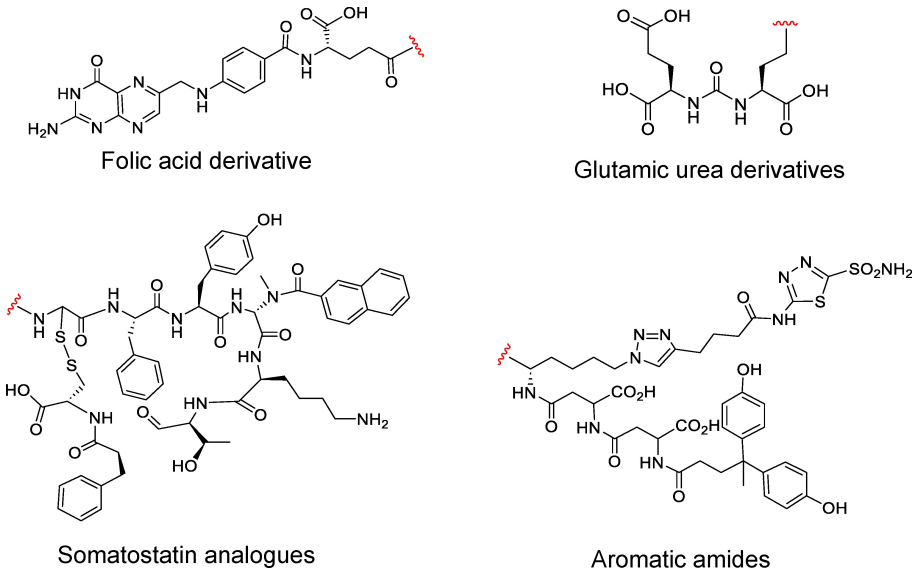


Figure 2. Typical small-molecular ligands.

as payload, galactose as hepatocyte targeting ligand and disulfide bond as linker was designed in a recent study.[31] To enhance targeting specificity, multiple galactose molecules were modified resulting in three prodrugs: monogalactose (W-1–5), digalactose (W-2–9), and trigalactose (W-3–8). Confocal fluorescence microscopy images demonstrated that all three prodrugs selectively entered HepG2 cells with their targeting ability ranked as follows: W-3–8 > W-2–9 > W-1–5. Fluorescence imaging of tissue slices also revealed a consistent pattern in the distribution of compounds within the liver: W-3–8 > W-2–9 > W-1–5, with comparatively lower distribution observed in other tissues. These findings suggest that the synthesized precursors possess remarkable hepatocyte-targeting properties, and increasing the number of ligands can effectively enhance the targeting efficacy.

Size of Ligand-Drug Conjugates

Compared to ADCs, one major advantage of SMDCs is the lower molecular weight. The size of molecular can affect drug penetration into solid tumors via diverse mechanisms, including permeability and retention (EPR) effects.[32] Although it has been reported that larger conjugates can enhance passive accumulation of the payload in tumor mass by enhanced permeability and retention effect,[28,33] however, further tumor tissue penetration of large-sized conjugates is often hindered by dense extracellular matrix and aberrant lymphatic system commonly found in most solid tumors.[11] This leads to difficulties for delivering drugs to cells deep within the tumor mass. In contrast, smaller conjugates are more easily released and can independently diffuse into the deep tissue of tumor. On the other hand, larger-sized conjugates have longer blood circulation time which may increase the chances of releasing drugs outside tumor cells, thereby reducing targeting specificity. Particles or molecules smaller than ~40 kDa are typically extracted from blood by glomeruli and rapidly excreted into urine.[16,34] This characteristic allows non-receptor-bound SMDCs to be quickly eliminated from the body, thus avoiding adverse effects caused by prolonged circulation of cytotoxic drugs. By adjusting the sizes of targeting ligands and linkers, the size of conjugates can be adjusted to achieve desired pharmacokinetics.

Typical Ligands

Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein located on cellular membrane, exhibiting remarkable tissue specificity.[35] It demonstrates minimal expression levels in lacrimal glands, duodenum and normal prostate tissues, but has 100–1000 times higher expression in prostate cancer (PCa) tissues compared to most normal tissues.[36] Notably, poorly differentiated, metastatic, and castration-resistant PCa tissues exhibit significantly elevated levels of PSMA expression.[37] Consist of 750 amino acids distributed across three domains, PSMA has multiple epitopes that can bind to ligands in both intracellular and extracellular domains.[38] These characteristics make it an ideal receptor for targeted drug delivery. Glutamic urea derivatives have high affinity and specificity for PSMA and can be rapidly internalized after binding to the receptor,[39] making them the most commonly used small-molecule targeting ligands for PSMA. The tumor-targeting efficacy of diverse PSMA ligands has been validated through experimentation on rodent models as well as clinical studies involving cancer patients.[20] In addition, PSMA-targeting ligands with different structures can be designed for multiple purposes. For example, in a recent study, a novel PSMA-targeting ligand suitable for bimodal coupling of diagnostic and therapeutic drugs was designed and synthesized,

and the efficacy of these compounds on PSMA-expressing PCa cells was significantly increased.[40]

Folate receptor α (FRα) is a folate-binding protein located on cell membrane that exhibits overexpression in several kinds of solid tumors including ovarian cancer, colon cancer, lung cancer, and breast cancer,[41] while in normal tissues or cells, FRα expression is minimal or absent.[42] Consequently, this receptor presents an appealing target for anticancer therapeutics. Other receptors such as FOLR2 and FOLR3 can also facilitate folate transport into cells, but their affinity for folate is comparatively lower than that of FRα.[43] Hence, the strategy of targeting FRα has proven successful in selectively delivering payloads to tumors. Drugs designed to target intracellular folate metabolism like Methotrexate and Pemetrexed have been effectively developed using this approach,[42] and some FRα-targeting drugs are currently undergoing phase II/III clinical trials.[44,45]

Carbonic anhydrase IX (CAIX, CA9) is a transmembrane protein that exhibits specific overexpression on the surface of hypoxic tumor cells.[46] It plays a critical physiological role in facilitating the transport of carbon dioxide and bicarbonate ions,[47] thereby regulating intracellular and extracellular pH balance and contributing to the establishment of a hypoxic and acidic tumor microenvironment (TME).[48] Extensive research has demonstrated a strong association between CAIX expression and both tumor metastasis and chemotherapy resistance.[49] Consequently, targeting CAIX for the development of anti-tumor drugs not only enhances drug specificity but also effectively inhibits its physiological function, leading to reduced tumor metastasis rates and improved drug resistance profiles, with significant implications for improving treat efficacy. Therefore, CAIX is a meaningful target for tumor therapy with widespread applications. A new SMDC targeting human renal cell carcinoma surface CAIX with acetazolamide derivatives was designed and synthesized.[50] Biodistribution studies of the conjugate labeled with a technetium-99 m chelating complex or red fluorophore in tumor-bearing mice have shown that the compound preferentially accumulates in tumors. Subsequently, the mentioned acetazolamide derivative was conjugated to potent cytotoxic drugs monomethyl auristatin E (MMAE) and PNU-159682 via a dipeptide linker, exhibiting remarkable anti-tumor activity in nude mice. In contrast, compounds lacking the acetazolamide moiety failed to demonstrate any discernible anticancer effects at equivalent doses.[50]

Somatostatin (SST) is a key regulatory polypeptide extensively distributed throughout the body, exerting inhibitory effects on the secretion of various hormones including growth hormone, cholecystokinin, glucagon, and insulin, as well as neuronal excitability modulation.[51] Moreover, SST has also been found to inhibit tumor growth.[52] Its physiological functions are mediated by five somatostatin receptors ($SSTR_{1-5}$), which belong to G-protein-coupled receptors (GPCRs) and exhibit widespread distribution in diverse tumors such as neuroendocrine tumors, gastric cancer, hepatocellular carcinoma, prostate cancer, and breast cancer.[51] The five receptors are structurally identical, but differ in cellular and subcellular localization as well as regulatory patterns.[24] Among them, SSTR2 and SSTR5 have emerged as major therapeutic targets for drug intervention in neuroendocrine tumors.[53] However, the clinical application of SST is significantly limited due to its short half-life.[24] Consequently, extensive research has focused on developing SST analogues like octreotide (OCT) possess improved metabolic stability along with high affinity for SSTR.[54]

In addition to the aforementioned four targeting ligands, numerous other targeting ligands have been employed in SMDC, such as RGD fragments that specifically target integrins. By covalently linking a peptide cyclo (Arg–Gly–Asp–D–Phe–Cys) ($cRGD_{fK}$), which targets αvβ3 integrin, with a polymethine fluorophore (IR-II-dye 5H5), a dual-mode probe exhibiting robust glioma-targeting ability was designed and synthesized. Enhanced tumor penetration and superior tumor-targeting contrast were observed in NIR-II PA/NIR-IIa fluorescence imaging.[55] Furthermore, an innovative approach by combining circulating iRGD with Proteolysis-targeting chimeras (PROTACs) was developed in another. The results demonstrated that this combination significantly improved the water solubility and tumor targeting capability of PROTACs, thereby facilitating their penetration into tumor tissues.[56]

Linker

The linker is a structure that connects the targeting ligand and the therapeutic payload. Typically, the linker consists of a spacer and a cleavable bridge.[6] Most of the linkers commonly used in SMDCs are similar to those used in ADCs.[19] When selecting a linker, it is important to ensure that the targeted drugs remain stable during circulation while can be efficiently released in the specific TME after internalization into target cells. Since modifying the structures of targeting ligand or the therapeutic payload can be challenging, optimization of targeted drugs is often achieved through linkers for improved pharmacokinetic and pharmacodynamic properties, and reduced impact of payload on the affinity of targeting ligands in space.[16]

Spacer

The spacer is typically located between the targeting ligand and the cleavable linker, which is critical for maintaining receptor binding in SMDCs. If the targeting ligand is too close to the drug payload, it may affect the affinity of the conjugate for target cells.[6] Spatial interference between ligands and cytotoxic molecules can be reduced by changing spacer length. However, an inappropriate spacer can also lead to a decrease in binding affinity.[57] For example, when designing platinum (IV) prodrugs specifically targeting cancer cells, it was found that using two monodisperse polyethylene glycol (PEG_{27}) polymers provided spatial separation between two targeting peptides, thereby enhancing receptor affinity, while shorter PEG_{10} chains lacked this function.[58] Another important function of the spacer is to improve the hydrophilicity of SMDCs. Targeting ligands and therapeutic payloads are usually hydrophobic in order to

diffuse through cell membranes and reach intracellular targets.[16] On the contrary, the whole SMDC structure must have sufficient hydrophilicity to avoid non-specific passive uptake by normal cells.[28] The use of water-soluble spacers such as polysaccharides, hydrophilic amino acids, PEGs and peptide glycans can impart improved hydrophilicity potential on SMDCs.[16] For example, biocompatible polyethylene glycol (PEG) chains used as linkers for coupling Ir(III) with Pt(IV) have enhanced solubility for conjugated products.[59]

Cleavable Linker

The cleavable linker is an important component of SMDCs, as it not only connects the target ligand and payload, but also has the ability to cleave within the tumor to release cytotoxic drugs.[60,61] These two functions require the linker to possess two characteristics: first, sufficient stability in the blood circulation system before reaching tumor cells. Second, after accumulating at the tumor or entering the target cell, it must be able to specifically cleave to release the payload (Figure 3).[62]

Suitable linkers can be selected based on the characteristics of the TME, which refers to the surrounding microenvironment where tumor cells exist, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, various signaling molecules, and extracellular matrix (ECM).[63] In the TME, there is an enhanced cell metabolism known as the Warburg effect which leads to a lower pH value (between 6.5 and 7.2) compared to normal tissues (around 7.4).[64] Additionally, intracellular biomarkers such as glutathione (GSH) are upregulated in cancer.[64] GSH concentration in cancer cells is ~20 mM compared to 5 mM in healthy cells.[65] Based on these characteristics, common cleavable linkers can be roughly divided into three categories: enzyme-cleavable linkers like ester bonds or amide bonds, acid-cleavable linkers like hydrazones or carbonate bonds, and reducible disulfides (Figure 4).[66]

Enzyme Cleavable Linker

Intracellular compartments within cancer cells such as nucleoli and lysosomes are rich in enzymes like esterases and amidases.[62] Based on this characteristic, ester or amide bonds that can be cleaved by enzymes have been used as linkers to selectively release cytotoxic drugs in TME or the cancer cell lysosomes. In ADCs, the use of linkers cleavable by Cathepsin B such as Valine-citrulline (Val-Cit) dipeptide linker has been well established,[28] and similar attempts in SMDCs are also underway. However, the instability in mouse serum has been a concern for its further applications.[67] Some studies showed that replacing Val-Cit with glutamic acid-valine-citrulline (EVCit) can improve the stability of ADCs in mouse serum,[67] but this effect has not been reported for SMDCs. Therefore, a study compared the effects of EVCit and VCit linkers on the stability and efficacy of SMDCs by connecting MMAE with PSMA-targeting ligands using these two linkers to synthesize two small molecule conjugates: EVCit-TFM and VCit-TFM.[68] Serum stability studies showed that EVCit-TFM exhibited significantly higher stability in mouse serum compared to VCit-TFM after 24 hours' incubation (71.3 ± 2.5% remained vs 20.9 ± 1.4% remained). The *in vivo* toxicity experiment in CD-1 mice indicated that mice treated with VCit-TFM experienced approximately 20% weight loss compared to control group, while those treated with EVCIt-TFM did not show significant decrease in body weight. The result indicated that the serum instability of VCIt-TFM induced significant toxicity towards mice. These results confirmed that replacing VCit with EVCit is a feasible strategy to improve the mouse serum stability of SMDC, as well

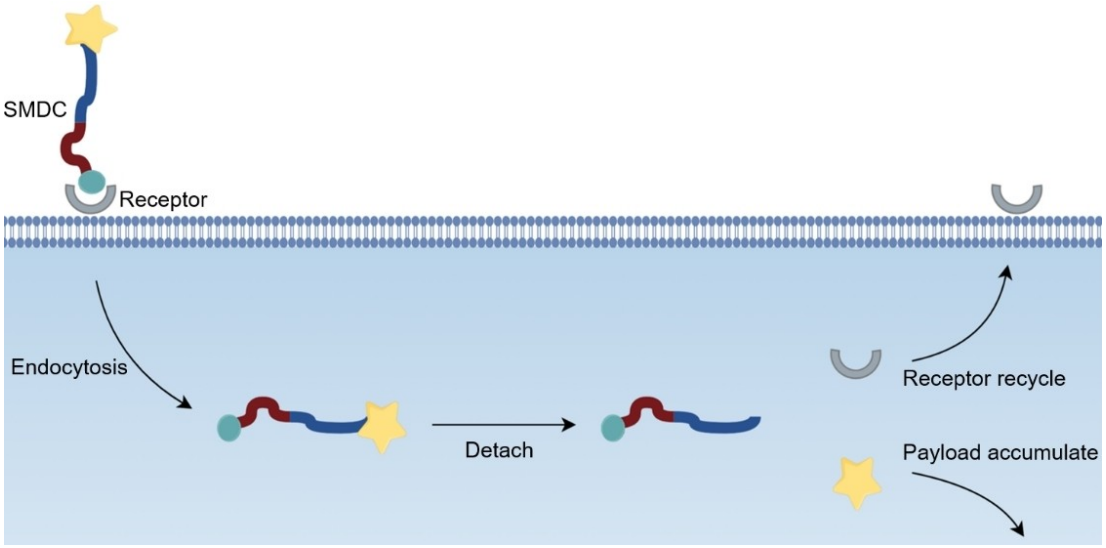


Figure 3. The mechanism of SMDCs entering the cell. Targeted ligands specifically recognize the corresponding receptor, enter the cell through endocytosis. Linker breaks under specific conditions (such as low pH, high GSH concentration), and releases the payload. Some of the internalized receptors can recycle to the cell surface.

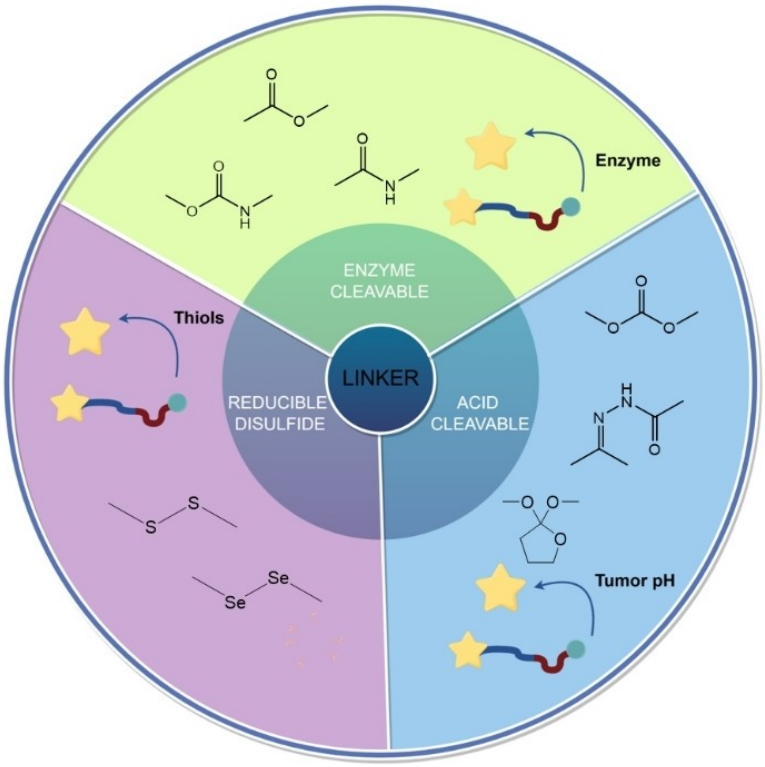


Figure 4. Classification of linkers. Common cleavable linkers can be roughly divided into three categories: enzyme-cleavable linkers like ester bonds or amide bonds, acid-cleavable linkers like hydrazones or carbonate bonds, and reducible disulfides.

as the importance of linker stability for reducing systemic toxicity caused by premature release of cytotoxic drugs.

Acid Cleavable Linker

As mentioned earlier, compared to the neutral pH condition in normal tissue, the pH of TME is relatively acidic. This allows for the design of linkers that are sensitive to acidic condition. Commonly used linkers include hydrazone and less studied ketal, carbonate ester bonds.[66] Acid-labile bonds can maintain stability during blood circulation (pH 7.4) but can be cleaved to release unmodified drugs in acidic TME (pH 6.5–6.9) and acidic cellular compartments such as endosome and lysosomes (pH 6.5–6.2).[62]

In a recent study, succinic acid (SA) was used as an acid cleavable linker to conjugate hydrophobic drug paclitaxel (PTX) with cell-penetrating peptide (CPP) resulting a pH-cleavable connecting conjugate.[69] *In vitro* stability experiments were conducted in 1x PBS (pH 7.4), freshly isolated mouse serum proteins, and complete cell culture medium (IMDM + 10% FBS), respectively. The results showed that the conjugate exhibited greater stability in all three media (> 90%, R_t = 6.541 min) compared to free PTX. This indicated excellent biocompatibility and biological stability of the conjugate. *In vitro* drug release experiments demonstrated that only minimal PTX was released from the conjugate (~ 10% within 24 h) at pH 7. The hydrolysis rate increased significantly as the pH decreased, and reached > 90% within 24 h at pH 5. PTX started release from the conjugate within 1 h (~22%) and gradually increased to 48.89%, 60.98%, 77.14%, 87.94%, and 91.45% after incubation for 3, 6, 12, 18, and 24 h, respectively. These results confirmed that the conjugate remained stable under normal physiological condition but can be rapidly hydrolyzed under lower pH condition. Cytoviability experiments on glioblastoma cell line showed that the conjugate (EC_{50} ~8 nM) exhibited significantly higher cytotoxicity than its parent molecule PTX (EC_{50} ~12 nM) and CPP (EC_{50} ~25 nM). Consistent with *in vitro* studies, the mice treated with the conjugate exhibited significant tumor growth inhibition, while both CPP and free PTX showed limited inhibition effect on tumor growth.

Reducible Disulfide Linker

Disulfide is the most commonly used cleavable linkers in prodrug design, which are stable at physiological condition but can be easily reduced by thiols through nucleophilic attack, releasing the payload.[62] The intracellular concentration of reduced glutathione (2–10 mM) is typically higher than that in the extracellular environment (2–10 μM),[70] resulting in a greater intracellular reducing potential.[71] Oxidative stress and hypoxia in the TME can further elevate the concentration of glutathione inside cancer cells (~20 mM),[72] thereby facilitating rapid release of thiol-based drugs within cancer cells. Consequently, disulfides exhibit enhanced stability during blood

circulation compared to inside cells. Many SMDCs have been designed with disulfide bonds, such as PTX-SS-DUPA which utilizes a reduction-sensitive disulfide bond as a linker to conjugate DUPA, a glutamate urea ligand that has high affinity for PSMA, with PTX to facilitate rapid release of PTX within tumor cells.[73] Another conjugate named PTX-DUPA was also synthesized as a control group, in which a carbon-carbon bond replaced the disulfide bond. *In vitro* drug release studies were conducted using DTT (a popular glutathione mimic) as the releasing medium for both PTX-DUPA and PTX-SS-DUPA. The results showed that less than 20% of PTX was released from PTX-SS-DUPA within 24 hours in blank PBS (pH 7.4). While ~80% of PTX was released within 4 hours under treatment with 1 mM or 10 mM DTT. In contrast, negligible PTX release (< 2.1%) was observed for PTX-DUPA in the presence or absence of 10 mM DTT. Consistent results were observed from cellular experiments by incubating two conjugates with 22RV1 cells, followed by ultrasonic lysis and UPLC-MS/MS analysis to determine intracellular concentrations of PTX; significantly higher levels of PTX were detected in cells treated with PTX-SS-DUPA compared to those treated with PTX-DUPA. *In vivo* antitumor experiments demonstrated moderate antitumor activity for PTX-DUPA, while mice treated with PTX-SS-DUPA showed significant reduction in tumor volume without significant difference compared to PTX itself. These findings suggest that drug release from non-cleavable PTX-DUPA is very slow, while PTX-SS-DUPA remains stable during blood circulation but allows for fast and significant redox-responsive drug release upon internalization into tumor cells. This fast and differential release may enhance antitumor efficacy as well as minimize systemic toxicity.

Payload

The drug payload is the component that exerting therapeutic effects in SMDCs, which makes the selection of appropriate drug payload critical in SMDC design. The optimal payload selection depends on aspects such as therapeutic efficacy against the target disease and the chemical composition. The selected drug must have a high potency and specific therapeutic effect against the target disease, be chemically capable of conjugating to the linker or the targeted ligand, and have adequate intracellular stability for maximum activity and minimum toxicity.[16]

First, when the conjugate can saturate the receptor to a significant extent, it is imperative for the selected drug to possess high potency to achieve the desired therapeutic effect. In a study with fixed ligand-receptor affinity, it was suggested that the drug molecule with an $IC_{50} < 10$ nM is sufficient when the number of receptors on each cancer cell exceeds 10^6. When the number surpassed 10^8, an IC50 of 1 μM proved to be adequate.[74] Conversely, if there are insufficient receptors present on the cancer cell membrane, higher drug potency becomes necessary. Similar to targeted ligands, increasing the payload quantity on each specific ligand can enhance the therapeutic efficacy efficiently.[28] However, it is generally easier to increase the potency of a drug molecule itself by ten-fold than connect ten identical payloads onto one ligand.[16] Second, it is critical for exceptional payloads to possess modifiable sites in their chemical structure,[6] such as hydroxyl (−OH), amine ($-NH_2$), carboxyl (−COOH) and thiol (−SH). This enables direct or functional group-mediated connection with a linker or targeting ligand. Here, the common payloads were classified into three categories according to their action mechanisms: (i) drugs that exert their effects on microtubule proteins, such as maytansine, auristatins and paclitaxel; (ii) drugs that act on DNA, including topoisomerase inhibitors like camptothecin; (iii) drugs that specifically target RNA, exemplified by α-amanitin.

Paclitaxel

Paclitaxel is a widely used anticancer drug in clinic and is considered one of the most extensively utilized natural agents against cancer.[75] Its action mechanism involves promoting the assembly of microtubule proteins into microtubules while preventing their disassembly, consequently hindering cell cycle progression, inhibiting mitosis, and effectively suppressing cancer cell growth.[76] However, paclitaxel exhibits certain limitations including poor water solubility, inadequate selectivity, and high toxicity.[51] Therefore, efforts have been made towards optimizing paclitaxel through the implementation of ligand-Targeted-Drug strategies to address these challenges.

A conjugate of bradykinin-potentiating peptide-paclitaxel (BPP-PTX) was designed and synthesized to target angiotensin-converting enzyme (ACE),[77] which is highly expressed in Triple-negative breast cancer (TNBC). The synthesis of the conjugate involved coupling BPP with PTX using a succinic linker. In ACE-positive cell lines, BPP-PTX (9.5 nM) demonstrated comparable cytotoxicity to PTX (3.1 nM). Overexpression experiments, knockout experiments, and competition assays provided evidence that the cytotoxicity of BPP-PTX relied on ACE while PTX lacked this characteristic, thus indicating excellent targeting ability of the synthesized conjugate. In the *in vivo* experiment, BPP-PTX demonstrated a higher maximum tolerated dose (BPP-PTX, 100 mg/kg; PTX, 20 mg/kg) and a lower release rate into circulation compared to PTX. *In vivo* antitumor experiments demonstrated that the mice treated with BPP-PTX exhibited an average reduction in tumor volume of ~15% compared to those treated with PTX and a remarkable reduction of 54% compared to those in the control group (1x PBS). Furthermore, there was a significantly lower decrease in body weight in the BPP-PTC group (2.8%) than that in the PTX group (6.2%). Moreover, white blood cell (WBC) count decreased much less for mice treated with BPP-PXT (17%) compared to those treated with PTX (52%). These findings strongly suggested that the compound BPP-PTX not only exhibited enhanced tumor enrichment but also significantly reduced toxicity compared to regular PTX. Further preclinical studies may demonstrate BPP-PTX to be a potential prodrug for targeted treatment of TNBC.

Docetaxel (DTX) is a derivative of PTX and has broad application prospects in clinic. In a recent study, a heptapeptide (p7) was conjugated with DTX to form the targeted drug

DTX–P7, in which p7 was used as the target ligand to specifically bind to cell surface heat shock protein 90 (Hsp90).[78] *In vivo* anti-tumor experiments showed that DTX–P7 (reduced tumor growth by 93.2% compared with control) had a more powerful therapeutic effect compared with DTX (reduced tumor growth by 35.9% compared with control). Further biodistribution analysis showed that DTX–P7 was preferentially distributed in tumor tissues with efficient targeting.

Camptothecin

Camptothecin (CPT) is a potent quinoline alkaloid that can effectively inhibit topoisomerase I, thereby disrupting DNA replication and leading to apoptosis.[79] Although extensive CPT derivatives have been synthesized over the past decades, only two analogs (Irinotecan and Topotecan) have gained approval for clinical cancer treatment.[80] Irinotecan functions as a prodrug of 7-ethyl-10-hydroxycamptothecin (SN38) and is recommended for the first-line treatment of metastatic colorectal cancer (CRC) in combination with fluorouracil (5-FU) and folinic acid protein.[81] However, its limited selectivity and low response rate impede its widespread applications. The clinical efficacy of irinotecan may be influenced by multiple resistance mechanisms, a recently reported mechanism suggests that pyruvate, a glucose metabolite, may diminishes Irinotecan-induced necrosis thus enhancing drug insensitivity.[82] On the other hand, ATP also diminishes irinotecan-induced apoptosis according to conventional perspectives.[82] Therefore, recent research synthesized a series of SMDCs that conjugating glucose transporter inhibitors (phlorizin or phloretin) with SN38 by utilizing two distinct linkers: disulfide bonds sensitive to GSH and valine-citrulline-para aminobenzyl alcohol (Val–Cit–PAB–OH) cleavable by cathepsin B.[83] *In vitro* stability assessments demonstrated that Val–Cit–PAB–OH as linker in the conjugates exhibited excellent stability (almost no SN38 release) in plasma compared to those using disulfide bonds as linkers (~ 40% release of SN38). The Val–Cit–PAB–OH linker conjugates showed enhanced antitumor efficacy in orthotopic CRC mouse model by reducing tumor growth of 40–75%. Notably, these conjugates did not exhibit significant adverse reactions such as weight loss. Biodistribution analysis revealed that the conjugates predominantly accumulated at gastrointestinal tract and colorectal tumor sites, while irinotecan itself distributed widespread across various organs. Moreover, the conjugates resulted in higher concentration of free SN38 in tumor tissue than irinotecan at the same administrated dose, indicating that the conjugates had better target property and efficient release of active drugs, thus improved the therapeutic effect. It also suggested glucose transport inhibitors to be useful in combatting glucose-related resistance to irinotecan.

Monomethyl Auristatin E (MMAE)

Monomethyl auristatin E (MMAE) is an auristatin derivatives that has potent anti-tumor activity by inhibiting tubulin polymerization and then blocking mitosis.[84] Despite its effectiveness, MMAE's high toxicity levels often hinder its application. Among the auristatin derivatives used in ADCs, MMAE is the most employed one.[85] Several ADC drugs loaded with MMAE have been successfully on the market, including Adcetris, Polivy, Padcev, Vedotin, and Tivdak.[86] As a promising payload option, MMAE can also be effectively utilized in SMDCs. In a recent comparison study, an ADC (7NP2–Gly–Pro–MMAE) and an SMDC (OncoFAP–Gly–Pro–MMAE) were synthesized by using fibroblast activation protein (FAP) as the same target and employing MMAE as the same payload.[87] Their targeting specificity and therapeutic efficacy were assessed through comprehensive *in vitro* and *in vivo* experiments. Both Enzyme linked immunosorbent assay (ELISA) and Fluorescence activated Cell Sorting (FACS) results demonstrated comparable high affinity for FAP. Fluorescence quantification of biodistribution revealed preferential accumulation of released MMAEs in tumor with minimal distribution in other tissues. Moreover, both approaches exhibited similar therapeutic efficacy and low toxicity in *in vivo* anticancer experiments. These findings suggest that SMDCs hold significant promise as potential alternatives to ADCs. Given their ease and cost-effectiveness in production, it is worth considering further exploration of SMDCs for the development of novel anticancer drugs. PSMA-1-VcMMAE is a new PSMA-targeting prodrug which exhibits exceptional targeting ability and therapeutic efficacy in both *in vivo* and *in vitro* studies. Notably, the maximum tolerated dose of PSMA-1-VCMMAE is increased by more than 10-fold compared to anti-PSMA antibody-MMAE conjugate (PSMA-ADC) and MMAE itself. This significant enhancement contributed to an improved therapeutic index, thereby enhanced its potential for clinical applications.[88] BT8009 is a Nectin-4 targeted bicycle toxin conjugate with high-affinity and selectivity for tumor targeting and has been undergoing clinical evaluation at present.[89]

Tubulysin

Tubulysins are cytotoxic tetrapeptides that can effectively hinder microtubule protein polymerization and impede mitosis, resulting in potent cytotoxicity against various tumor cell lines with an EC_{50} value in the picomolar range.[90] At present, folate conjugates including EC305, EC145, and EC510 are widely employed as small molecule ligands for precise tubulysin targeting. Moreover, the second-generation tubulysin conjugate, EC1456, has progressed into phase I clinical trials for solid tumors.[6] Tubulysin B conjugate was also reported to specifically target CAIX, in which the water solubility of the conjugate was improved by incorporating hydrophilic amino acids.[46] *In vitro* experiments demonstrated remarkable cytotoxicity against CAIX-transfected cells for this conjugate ($IC_{50} = 1.05 \pm 0.01$ nM). Notably, *in vivo* anticancer investigations revealed that the conjugate selectively delivered tubulysin B hydrazide to multiple solid tumors overexpressing CAIX, resulting in swift tumor regression. Another significant finding from this study was that although the CAL ligand (targeting CAIX) did not exhibit

internalization, it still displayed tumor-specific cytotoxicity, suggesting that non-internalized receptors can also be exploited for potent tumor-specific cytotoxic effects. Studies were also conducted on the conjugate that tubeolysin B was coupled with a ligand specifically binding to the cholecystokinin 2 receptor (CCK2R).[91] The conjugate, CRL—L1-TUBBH, was generated by linking cholecystokinin receptor ligand (CRL) to tubulysin B using a water-soluble ligand (L1), which enhances the water solubility of the conjugate. Remarkably, CRL—L1-TubBH exhibited selective inhibition towards CCK2R-positive tumors while displayed minimal toxicity to healthy tissues.

SMDCs in Clinical Trial

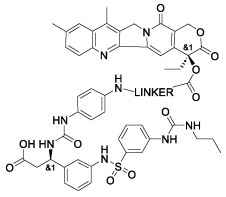
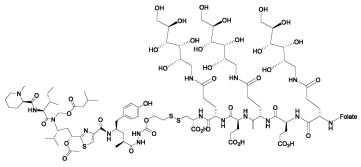
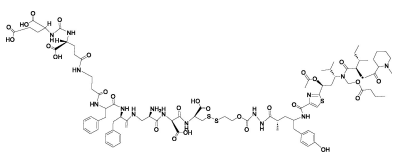
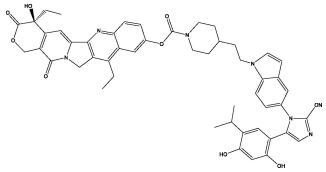
At present, there are no SMDCs that have been successfully approved for clinical use and most of them are still in the preclinical or clinical research stage. A few representative SMDCs that have entered clinical trials are briefly introduced as below (Table 1).

CBP-1008 and CBP-1018

CBP-1008 is a bi-specific ligand drug conjugate developed by Coherent Biopharma (CBP) company. It carries MMAE as payload and can target both folate receptor a (FRα) and vanilloid subfamily member 6 of transient receptor potential channels (TRPV6) which are overexpressed in various solid tumors including ovarian cancer.[92] CBP-1008 has already completed phase I clinical studies (NCT04740398) and entered Phase II clinical studies. Phase I study was conducted on 178 patients with different types of tumors, and the main purpose was to evaluate the safety and preliminary efficacy. Among 82 platinum-resistant ovarian cancer (PROC) patients that evaluable for efficacy assessment, the objective response rate (ORR) and the disease control rate (DCR) were 25.6% and 62.2%, respectively. The median progression-free survival (mPFS) was 3.7 months (95% CI: 2.7–5.1). The result showed that CBP-1008 has acceptable security, and antitumor activity was observed in PROC patients at dose of 0.15 mg/kg or above[92]

CPB-1018 is also a bi-specific ligand drug conjugate targeting both FRα and PSMA, carrying MMAE as payload. The completed Phase I clinical trial (NCT04928612) demonstrated good tolerance at dose levels of 0.03–0.14 mg/kg. Multiple Prostate-specific antigen (PSA) decrease were observed at dose levels of 0.08–0.14 mg/kg, suggesting preliminary antitumor activity in mCRPC patients.[93]

Table 1. SMDCs in clinical trial.

Name	Structure	Payload	Target	Status	Indication
VIP236		VIP126	$\alpha_V\beta_3$ integrin	Phase 1 clinical trial	Advanced solid tumors
CBP-1008	Unpublished	Monomethyl auristatin E (MMAE)	FRα and TRPV6	Phase 2 clinical trial	Advanced solid tumors
CBP-1018	Unpublished	MMAE	FOLR1 and PSMA	Phase 1 clinical trial	Advanced solid tumors
EC1456		tubulysin	FOLR1	Phase 1 clinical trial	Advanced solid tumors
EC1169		tubulysin	PSMA	Phase 1 clinical trial	Recurrent metastatic, castration-resistant prostate cancer (MCRPC)
Pen-866		SN38	HSP90	Phase 2 clinical trial	Advanced solid tumors

EC1169 and EC1456

EC1169 is a PSMA-targeted tubulysin conjugate connected by disulfide bonds and a hydrophilic peptide-based spacer, exhibiting better hydrophilicity. The preclinical studies have demonstrated sufficient cytotoxicity of EC1169 towards PSMA positive cells, while being non-toxic to PSMA negative cells at a concentration up to 1 μM. EC1169 can significantly diminish PSMA-positive LNCaP tumors, but did not exert obvious effect on PSMA-negative KB tumors, indicating high specificity and potency of EC1169 against PMSA-expressing tumors. In addition, EC1169 showed more powerful antitumor activity compared to docetaxel, which is commonly used to treat end-stage prostate cancer. Unlike docetaxel, which causes significant weight loss, EC1169 did not exhibit such side effect.[94] EC1169 has completed phase I clinical trials (NCT02202447), and was well-tolerated under different dosing regimen.[95]

EC1456 is structurally similar to EC1169 but is a FR-targeted tubulysin conjugate, which also completed phase I clinical trials (NCT01999738) to demonstrate a good tolerance.[45]

VIP236

VIP236 is a SMDC that specifically targets the $\alpha_v\beta_3$ integrin receptor overexpressed on cancer cell membranes.[96] It consists of an $\alpha_v\beta_3$ integrin binder and VIP126, a modified CPT, connected by a linker that can be cleaved by Neutrophil elastase (NE). Pharmacokinetic studies have shown that VIP236 has remarkable stability in plasma, as a tumor-to-plasma ratio (AUC_{tumor}/AUC_{plasma}) of 6 compared to 0.6 when equimolar doses of VIP126 were directly administered. This ten-fold increase in the tumor-to-plasma ratio unequivocally validated the exceptional targeting efficacy of VIP236 and indicated its potential therapeutic advantages. *In vivo* antitumor experiment further demonstrated the efficacy of VIP236 in inducing sustained regression of several tumors, including non-small cell lung cancer, colon cancer, renal cell carcinoma, and triple-negative breast cancer. Notably, the Investigational New Drug application for VIP236 has been approved by the Food and Drug Administration (FDA) at present.

PEN-866

PEN-866 is a novel SMDC that targeting HSP90 and using SN38 as payload. Preclinical studies have shown the strong inhibitory effect on various xenograft tumors. At present, PEN-866 has completed phase I clinical trial (NCT03221400),[97] and entered Phase II clinical trial (NCT04890093) in November 2023. Phase I clinical trials were conducted in patients with progressive and advanced solid malignancies, revealed that PEN-866 was well tolerated and initially demonstrated antitumor activity. Preliminary determination of the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) was found to be 175 mg/m^2.

Conclusion and Perspective

The development of targeted drugs represents a significant breakthrough in the field of pharmaceutical research. Compared to conventional chemotherapy, well-designed targeted drugs possess the ability to precisely accumulate at the target sites, then effectively release the payloads under specific conditions to exert their effects. The smart mechanism can reduce their exposure to normal cells and minimize unnecessary toxicity.[16] SMDCs exhibit distinctive advantages such as the previously mentioned smaller volume, better blood circulation stability and non-immunogenicity. On the other hand, the variable three-part structure of SMDCs allows for great flexibility in drug optimization. Diverse molecules with varying targeting effects can be selectively chosen until a satisfying specificity. Similarly, there is also an option to conjugate various potent molecules until a satisfactory effect is achieved. Linkers can also be optimized to attain desired pharmacokinetics and pharmacodynamics. Compared to the currently available ADCs, SMDCs may exhibit considerable targeting and therapeutic efficacy.[25,87] In addition, SMDCs can not only be used for targeted treatment of cancer, but also has been applied to kidney disease and inflammation.[6] Moreover, SMDCs can be extensively utilized in the fields of imaging,[98] radiation therapy,[59] and immunotherapy.[99]

However, the application of SMDCs faces certain challenges. First, SMDCs are typically administered via injection and pose difficulties in oral formulation, thereby causing inconvenience in clinical practice. Second, the development of SMDCs requires more efficient targeted ligands, as only a limited number have been utilized to date. Therefore, future endeavors will focus on validating novel targeting ligands and identifying new targets. Furthermore, there is a strong desirement for more adaptable and stable linkers that can ensure precise payload delivery while minimizing toxicity arising from premature drug release during blood circulation. Although there is still no successful SMDC in clinic, emerging applications are being observed with an increasing number of SMDCs undergoing clinical trials, indicating a promising future for this field.

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Conflict of Interests

The authors declare no conflict of interest.

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[1] P. Gotwals, S. Cameron, D. Cipolletta, V. Cremasco, A. Crystal, B. Hewes, B. Mueller, S. Quaratino, C. Sabatos-Peyton, L. Petruzzelli, J. A. Engelman, G. Dranoff, *Nat. Rev. Cancer.* **2017**, *17*, 286–301.
[2] E. I. Vrettos, G. Mező, A. G. Tzakos, *Beilstein J. Org. Chem.* **2018**, *14*, 930–954.
[3] K.-H. Altmann, *Chimia.* **2018**, *72*, 154–155.
[4] S. Muro J, *Controlled Release.* **2012**, *164*, 125–137.
[5] N. Joubert, A. Beck, C. Dumontet, C. Denevault-Sabourin, *Pharmaceuticals.* **2020**, *13*, 245.
[6] C. Zhuang, X. Guan, H. Ma, H. Cong, W. Zhang, Z. Miao, *Eur. J. Med. Chem.* **2019**, *163*, 883–895.
[7] R. V. J. Chari, M. L. Miller, W. C. Widdison, *Angew. Chem. Int. Ed.* **2014**, *53*, 3796–3827.
[8] R. A. Davis, T. Ganguly, R. Harris, S. H. Hausner, L. Kovacs, J. L. Sutcliffe, *J. Med. Chem.* **2023**, *66*, 9842–9852.
[9] N. Diamantis, U. Banerji, *Br. J. Cancer.* **2016**, *114*, 362–367.
[10] T. K. Patel, N. Adhikari, S. A. Amin, S. Biswas, T. Jha, B. Ghosh New, *J. Chem.* **2021**, *45*, 5291–5321.
[11] R. K. Jain, T. Stylianopoulos, *Nat. Rev. Clin. Oncol.* **2010**, *7*, 653–664.
[12] Y. T. Adem, K. A. Schwarz, E. Duenas, T. W. Patapoff, W. J. Galush, O. Esue, *Bioconjugate Chem.* **2014**, *25*, 656–664.
[13] A. Martelet, V. Garrigue, Z. Zhang, B. Genet, A. Guttman, *J. Pharm. Biomed. Anal.* **2021**, *201*, 114094.
[14] S. Cazzamalli, B. Ziffels, F. Widmayer, P. Murer, G. Pellegrini, F. Pretto, S. Wulhfard, D. Neri, *Clin. Cancer Res.* **2018**, *24*, 3656–3667.
[15] X. Liu, J. Guo, S. Han, L. Yao, A. Chen, Q. Yang, H. Bo, P. Xu, J. Yin, Z. Zhang, *Vaccine.* **2012**, *30*, 6527–6533.
[16] M. Srinivasarao, C. V. Galliford, P. S. Low, *Nat. Rev. Drug Discovery.* **2015**, *14*, 203–219.
[17] M. Ora, N. Soni, A. H. Nazar, M. Dixit, R. Singh, S. Puri, M. M. Graham, S. Gambhir, *J. Nucl. Med.* **2023**, *64*, 1001–1008.
[18] B. M. Privé, M. A. Boussihmad, B. Timmermans, W. A. van Gemert, S. M. B. Peters, Y. H. W. Derks, S. A. M. van Lith, N. Mehra, J. Nagarajah, S. Heskamp, H. Westdorp, *Eur. J. Nucl. Med. Mol. Imaging.* **2023**, *50*, 1906–1918.
[19] A. Y. Deneka, Y. Boumber, T. Beck, E. A. Golemis, *Cancers.* **2019**, *11*, 1279.
[20] G. Casi, D. Neri, *J. Med. Chem.* **2015**, *58*, 8751–8761.
[21] N. Krall, J. Scheuermann, D. Neri, *Angew. Chem. Int. Ed.* **2013**, *52*, 1384–1402.
[22] S. M. Hillier, K. P. Maresca, F. J. Femia, J. C. Marquis, C. A. Foss, N. Nguyen, C. N. Zimmerman, J. A. Barrett, W. C. Eckelman, M. G. Pomper, J. L. Joyal, J. W. Babich, *Cancer Res.* **2009**, *69*, 6932–6940.
[23] P. S. Low, W. A. Henne, D. D. Doorneweerd, *Acc. Chem. Res.* **2008**, *41*, 120–129.
[24] T. Günther, G. Tulipano, P. Dournaud, C. Bousquet, Z. Csaba, H.-J. Kreienkamp, A. Lupp, M. Korbonits, J. P. Castaño, H.-J. Wester, M. Culler, S. Melmed, S. Schulz, E. H. Ohlstein, *Pharmacol. Rev.* **2018**, *70*, 763–835.
[25] S. Cazzamalli, A. Dal Corso, F. Widmayer, D. Neri, *J. Am. Chem. Soc.* **2018**, *140*, 1617–1621.
[26] X. Pang, X. He, Z. Qiu, H. Zhang, R. Xie, Z. Liu, Y. Gu, N. Zhao, Q. Xiang, Y. Cui, *Signal Transduct. Target Ther.* **2023**, *8*, 1.
[27] T. Chatzisideri, G. Leonidis, T. Karampelas, E. Skavatsou, A. Velentza-Almpani, F. Bianchini, C. Tamvakopoulos, V. Sarli, *J. Med. Chem.* **2021**, *65*, 271–284.
[28] M. Srinivasarao, P. S. Low, *Chem. Rev.* **2017**, *117*, 12133–12164.
[29] C. Chittasupho, *Ther. Delivery.* **2012**, *3*, 1171–1187.
[30] C. A. Sanhueza, M. M. Baksh, B. Thuma, M. D. Roy, S. Dutta, C. Préville, B. A. Chrunyk, K. Beaumont, R. Dullea, M. Ammirati, S. Liu, D. Gebhard, J. E. Finley, C. T. Salatto, A. King-Ahmad, I. Stock, K. Atkinson, B. Reidich, W. Lin, R. Kumar, M. Tu, E. Menhaji-Klotz, D. A. Price, S. Liras, M. G. Finn, V. Mascitti, *J. Am. Chem. Soc.* **2017**, *139*, 3528–3536.
[31] M. Wang, Z. Li, F. Liu, Q. Yi, C. Pu, Y. Li, T. Luo, J. Liang, J. Wang, *J. Med. Chem.* **2021**, *64*, 14793–14808.
[32] A. A. Manzoor, L. H. Lindner, C. D. Landon, J.-Y. Park, A. J. Simnick, M. R. Dreher, S. Das, G. Hanna, W. Park, A. Chilkoti, G. A. Koning, T. L. M. ten Hagen, D. Needham, M. W. Dewhirst, *Cancer Res.* **2012**, *72*, 5566–5575.
[33] Y. Li, J. Cui, C. Li, C. Deng, G. Deng, H. Zhang, F. An Chin, *Chem. Lett.* **2023**, *34*, 108180.
[34] J. Xin, S. Han, M. Zheng, C. Xu, Z. Huang, B. Wang, C. Yu, F. An, Y. Ren Chin, *Chem. Lett.* **2023**, 109165.
[35] J. Zhang, A. Rakhimbekova, X. Duan, Q. Yin, C. A. Foss, Y. Fan, Y. Xu, X. Li, X. Cai, Z. Kutil, P. Wang, Z. Yang, N. Zhang, M. G. Pomper, Y. Wang, C. Bařinka, X. Yang, *Nat. Commun.* **2021**, *12*, 5460.
[36] Y. He, W. Xu, Y.-T. Xiao, H. Huang, D. Gu, S. Ren, *Signal Transduct. Target Ther.* **2022**, *7*, 198.
[37] Y. Kinoshita, K. Kuratsukuri, S. Landas, K. Imaida, P. M. Rovito, C. Y. Wang, G. P. Haas, *World J. Surg.* **2006**, *30*, 628–636.
[38] F. Wang, Z. Li, X. Feng, D. Yang, M. Lin, *Prostate Cancer Prostatic Dis.* **2021**, *25*, 11–26.
[39] S. Pastorino, M. Riondato, L. Uccelli, G. Giovacchini, E. Giovannini, V. Duce, A. Ciarmiello, *Curr. Radiopharm.* **2020**, *13*, 63–79.
[40] N. Y. Zyk, A. S. Garanina, E. A. Plotnikova, A. P. Ber, E. A. Nimenko, N. S. Dashkova, A. A. Uspenskaia, R. R. Shafikov, D. A. Skvortsov, S. A. Petrov, A. A. Pankratov, N. V. Zyk, A. G. Majouga, E. K. Beloglazkina, A. E. Machulkin, *Int. J. Mol. Sci.* **2023**, *24*, 11327.
[41] J. Holm, S. I. Hansen Biochim Biophys Acta, *Biochim. Biophys. Acta, Proteins Proteomics* **2020**, *1868*, 140466.
[42] M. Scaranti, E. Cojocaru, S. Banerjee, U. Banerji, *Nat. Rev. Clin. Oncol.* **2020**, *17*, 349–359.
[43] J. Liu, H. Chen, Y. Liu, Y. Shen, F. Meng, H. Ü. Kaniskan, J. Jin, W. Wei, *J. Am. Chem. Soc.* **2021**, *143*, 7380–7387.
[44] U. Banerji, A. H. l. Garces, V. Michalarea, R. Ruddle, F. l. Raynaud, R. Riisnaes, D. N. Rodrigues, N. Tunariu, J. C. Porter, S. E. Ward, M. Parmar, A. J. Turner, S. Seeramreddi, E. Hall, E. J. Dean, B. Basu, A. George, S. B. Kaye, S. N. Banerjee, J. S. D. Bono, *J. Clin. Oncol.* **2017**, *35*, 2503–2503.
[45] M. Edelman, J. Sachdev, W. Harb, A. Armour, D. Wang, L. Garland, *J. Thorac. Oncol.* **2017**, *12*, S1288-S1289.
[46] l. S. Marks, S. S. Gardeen, S. J. Kurdziel, S. T. Nicolaou, J. E. Woods, S. A. Kularatne, P. S. Low, *Mol. Pharmaceutics.* **2018**, *15*, 2289–2296.
[47] S. Pastorekova, R. J. Gillies, *Cancer Metastasis Rev.* **2019**, *38*, 65–77.
[48] J. Chiche, K. Ilc, J. Laferrière, E. Trottier, F. d.r Dayan, N. M. Mazure, M. C. Brahimi-Horn, J. Pouysségur, *Cancer Res.* **2009**, *69*, 358–368.
[49] Y. Li, M. Dong, W. Sheng, L. Huang, *Pathol. Oncol. Res.* **2015**, *22*, 277–286.
[50] S. Cazzamalli, A. Dal Corso, D. Neri, *Mol. Cancer Ther.* **2016**, *15*, 2926–2935.
[51] M. Huo, Q. Zhu, Q. Wu, T. Yin, L. Wang, L. Yin, J. Zhou, *J. Pharm. Sci.* **2015**, *104*, 2018–2028.
[52] B. Mariniello, I. Finco, P. Sartorato, A. Patalano, M. Iacobone, V. Guzzardo, A. Fassina, F. Mantero, *J. Endocrinol. Invest.* **2011**, *34*, E131–E138.
[53] Y. Si, S. Kim, J. Ou, Y. Lu, P. Ernst, K. Chen, J. Whitt, A. M. Carter, J. M. Markert, J. A. Bibb, H. Chen, L. Zhou, R. Jaskula-Sztul, X. M. Liu, *Cancer Gene Ther.* **2020**, *28*, 799–812.
[54] A. Di Cianni, A. Carotenuto, D. Brancaccio, E. Novellino, J. C. Reubi, K. Beetschen, A. M. Papini, M. Ginanneschi, *J. Med. Chem.* **2010**, *53*, 6188–6197.
[55] S. Lyu, S. Lu, C. Gui, C. Guo, J. Han, Y. Xiao, R. Zhang, X. Hong, *J. Med. Chem.* **2024**, *67*, 1861–1871.
[56] S. He, Y. Fang, M. Wu, P. Zhang, F. Gao, H. Hu, C. Sheng, G. Dong, *J. Med. Chem.* **2023**, *66*, 16828–16842.
[57] C. P. Leamon, F. You, H. K. Santhapuram, M. Fan, I. R. Vlahov, *Pharm. Res.* **2009**, *26*, 1315–1323.
[58] A. C. Conibear, S. Hager, J. Mayr, M. H. M. Klose, B. K. Keppler, C. R. Kowol, P. Heffeter, C. F. W. Becker, *Bioconjugate Chem.* **2017**, *28*, 2429–2439.
[59] A. Gupta, A. K. Pandey, T. Mondal, J. Bhattacharya, P. K. Sasmal, *J. Med. Chem.* **2023**, *66*, 8687–8704.
[60] Y. Li, C. Zhang, G. Li, G. Deng, H. Zhang, Y. Sun, F. An, *Acta Pharm. Sin. B* **2021**, *11*, 2220–2242.
[61] Y. Li, T. Mei, S. Han, T. Han, Y. Sun, H. Zhang, F. An Chin, *Chem. Lett.* **2020**, *31*, 3027–3040.
[62] J. D. Bargh, A. Isidro-Llobet, J. S. Parker, D. R. Spring, *Chem. Soc. Rev.* **2019**, *48*, 4361–4374.
[63] M. Wang, J. Zhao, L. Zhang, F. Wei, Y. Lian, Y. Wu, Z. Gong, S. Zhang, J. Zhou, K. Cao, X. Li, W. Xiong, G. Li, Z. Zeng, C. Guo, *J. Cancer.* **2017**, *8*, 761–773.
[64] H.-H. Han, H.-M. Wang, P. Jangili, M. Li, L. Wu, Y. Zang, A. C. Sedgwick, J. Li, X.-P. He, T. D. James, J. S. Kim, *Chem. Soc. Rev.* **2023**, *52*, 879–920.
[65] B. Niu, K. Liao, Y. Zhou, T. Wen, G. Quan, X. Pan, C. Wu, *Biomaterials.* **2021**, *277*, 121110.
[66] M. Alas, A. Saghaeidehkordi, K. Kaur, *J. Med. Chem.* **2020**, *64*, 216–232.

[67] Y. Anami, C. M. Yamazaki, W. Xiong, X. Gui, N. Zhang, Z. An, K. Tsuchikama, *Nat. Commun.* **2018**, *9*.
[68] T. U. Amin, R. Emara, A. Pal, H. Aldawod, G. Jiang, D. Liang, M. T. Haque Tuhin, A. Balgoname, A. D. Patel, M. M. Alhamadsheh, *J. Med. Chem.* **2022**, *65*, 15473–15486.
[69] S. F. A. Rizvi, N. Abbas, H. Zhang, Q. Fang, *J. Med. Chem.* **2023**, *66*, 8324–8337.
[70] G. Yang, C. Chen, Y. Zhu, Z. Liu, Y. Xue, S. Zhong, C. Wang, Y. Gao, W. Zhang, *ACS Appl. Mater. Interfaces.* **2019**, *11*, 44961–44969.
[71] V. I. Lushchak, *J. Amino Acids.* **2012**, *2012*, 736837.
[72] C. Lin, H. He, Y. Zhang, M. Xu, F. Tian, L. Li, Y. Wang, *RSC Adv.* **2020**, *10*, 3084–3091.
[73] Q. Lv, J. Yang, R. Zhang, Z. Yang, Z. Yang, Y. Wang, Y. Xu, Z. He, *Mol. Pharmaceutics.* **2018**, *15*, 1842–1852.
[74] N. Parker, M. J. Turk, E. Westrick, J. D. Lewis, P. S. Low, C. P. Leamon, *Anal. Biochem.* **2005**, *338*, 284–293.
[75] Z. P. Zhang, L. Mei, S. S. Feng, *Expert Opin Drug Delivery* **2013**, *10*, 325–340.
[76] L. Zhu, L. Chen, *Cell. Mol. Biol. Lett.* **2019**, *24*, 40.
[77] X.-M. Guo, M. B. Yadav, M. Khan, C.-W. Hao, C.-Y. Lin, T. Huang, J. Wu, B.-M. Fan, Z.-X. Bian, *J. Med. Chem.* **2021**, *64*, 17051–17062.
[78] Y. Jiang, W. Huang, X. Sun, X. Yang, Y. Wu, J. Shi, J. Zheng, S. Fan, J. Liu, J. Wang, Z. Liang, N. Yang, Z. Liu, Y. Liu, *J. Hematol. Oncol.* **2022**, *15*, 73.
[79] D. Sriram, P. Yogeeswari, R. Thirumurugan, T. Ratan Bal, *Nat. Prod. Res.* **2005**, *19*, 393–412.
[80] F. Z. Li, T. Jiang, Q. Y. Li, X. Ling, *Am. J. Cancer Res.* **2017**, *7*, 2350–2394.
[81] J. Y. Douillard, D. Cunningham, A. D. Roth, M. Navarro, R. D. James, P. Karasek, P. Jandik, T. Iveson, J. Carmichael, M. Alakl, G. Gruia, L. Awad, P. Rougier, *The Lancet.* **2000**, *355*, 1041–1047.
[82] C.-Y. Huang, Y.-C. Pai, L. C.-H. Yu, *Oncology.* **2022**, *100*, 555–568.
[83] P.-F. Chiu, C.-K. Chang, P.-S. Huang, Y.-Y. Lin, C.-S. Lin, H.-Y. Yang, L.-C. Hsu, L. C.-H. Yu, P.-H. Liang, *J. Med. Chem.* **2023**, *66*, 9684–9696.
[84] L. Buckel, E. N. Savariar, J. L. Crisp, K. A. Jones, A. M. Hicks, D. J. Scanderbeg, Q. T. Nguyen, J. K. Sicklick, A. M. Lowy, R. Y. Tsien, S. J. Advani, *Cancer Res.* **2015**, *75*, 1376–1387.
[85] M. Akaiwa, J. Dugal-Tessier, B. A. Mendelsohn, *Chem. Pharm. Bull.* **2020**, *68*, 201–211.
[86] R. L. Best, N. E. LaPointe, O. Azarenko, H. Miller, C. Genualdi, S. Chih, B.-Q. Shen, M. A. Jordan, L. Wilson, S. C. Feinstein, N. J. Stagg, *Toxicol. Appl. Pharmacol.* **2021**, *421*, 115534.
[87] A. Zana, C. Puig-Moreno, M. Bocci, E. Gilardoni, C. Di Nitto, L. Principi, D. Ravazza, G. Rotta, E. Prodi, R. De Luca, D. Neri, S. Cazzamalli, *Bioconjugate Chem.* **2023**, *34*, 1205–1211.
[88] X. Wang, A. Shirke, E. Walker, R. Sun, G. Ramamurthy, J. Wang, L. Shan, J. Mangadlao, Z. Dong, J. Li, Z. Wang, M. Schluchter, D. Luo, Y. Wang, S. Stauffer, S. Brady-Kalnay, C. Hoimes, Z. Lee, J. P. Basilion, *Cancers.* **2021**, *13*, 417.
[89] G. E. Mudd, H. Scott, L. Chen, K. van Rietschoten, G. Ivanova-Berndt, K. Dzionek, A. Brown, S. Watcham, L. White, P. U. Park, P. Jeffrey, M. Rigby, P. Beswick, *J. Med. Chem.* **2022**, *65*, 14337–14347.
[90] J. Eirich, J. L. Burkhart, A. Ullrich, G. C. Rudolf, A. Vollmar, S. Zahler, U. Kazmaier, S. A. Sieber, *Mol. BioSyst.* **2012**, *8*, 2067–2075.
[91] C. Wayua, J. Roy, K. S. Putt, P. S. Low, *Mol. Pharm.* **2015**, *12*, 2477–2483.
[92] J. Gong, X. Hu, J. Zhang, Y. Du, R. Huang, Y. Teng, W. Tan, L. Shen, *J. Clin. Oncol.* **2021**, *39*, 3077–3077.
[93] K. Li, J. Wu, S. Ye, Y. liu, H. Huang, F. fan, Y. lai, S. Zhuang, L. Zhou, R. Huang, Y. Teng, X. Chai, J. Zhang, Y. Shi, H. Huang, *Ann. Oncol.* **2023**, *34*, S991.
[94] C. P. Leamon, J. A. Reddy, A. Bloomfield, R. Dorton, M. Nelson, M. Vetzel, P. Kleindl, S. Hahn, K. Wang, I. R. Vlahov, *Bioconjugate Chem.* **2019**, *30*, 1805–1813.
[95] S. Ejadi, N. J. Vogelzang, A. O. Sartor, A. Habbe, B. Nguyen, A. W. Tolcher, *J. Clin. Oncol.* **2015**, *33*, e13527–e13527.
[96] H.-G. Lerchen, B. Stelte-Ludwig, C. Kopitz, M. Heroult, D. Zubov, J. Willuda, T. Schlange, A. Kahnert, H. Wong, R. Izumi, A. Hamdy, *Cancers.* **2022**, *14*, 391.
[97] G. S. Falchook, J. C. Bendell, S. V. Ulahannan, S. Sen, R. Vilimas, K. Kriksciukaite, L. Mei, G. Jerkovic, N. Sarapa, M. Bilodeau, J. Bloss, A. Thomas, *J. Clin. Oncol.* **2020**, *38*, 3515–3515.
[98] J. Millul, G. Bassi, J. Mock, A. Elsayed, C. Pellegrino, A. Zana, S. Dakhel Plaza, L. Nadal, A. Gloger, E. Schmidt, I. Biancofiore, E. J. Donckele, F. Samain, D. Neri, S. Cazzamalli, *Proc. Nat. Acad. Sci.* **2021**, *118*, e2101852118.
[99] C.-F. Lo, T.-Y. Chiu, Y.-T. Liu, P.-Y. Pan, K.-L. Liu, C.-Y. Hsu, M.-Y. Fang, Y.-C. Huang, T.-K. Yeh, T.-A. Hsu, C.-T. Chen, L.-R. Huang, L. K. Tsou, *J. Med. Chem.* **2022**, *65*, 12802–12824.

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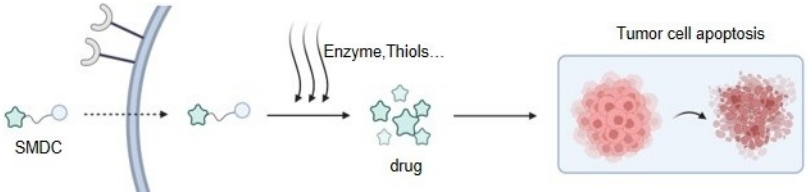

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Consisting of three parts: targeting ligand, cleavable linker and payload, small molecule drug conjugates (SMDCs) are a promising class of targeted drugs. Giving the advantages of smaller size, better permeability, simpler preparation process and non-immunogenicity, SMDCs might be a promising alternative to antibody drug conjugates (ADCs).