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# Microtubule-targeting agents for cancer treatment: Seven binding sites and three strategies

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## Abstract

Microtubules are pivotal in diverse cellular functions encompassing cell signaling, morphology, intracellular trafficking, and cell mitosis/division. They are validated targets for disease treatment, notably hematological cancers and solid tumors. Microtubule-targeting agents (MTAs) exert their effects by modulating microtubule dynamics, impeding cell proliferation, and promoting cell death. Recent advances in structural biology have unveiled novel perspectives for investigating multiple binding sites and mechanisms of action used by MTAs. In this review, we first provide an overview of the intricate structure and dynamics of microtubules. Then we explore the seven binding sites and the three primary strategies (stabilization, destabilization, and degradation) harnessed by MTAs. Furthermore, we introduce the emerging domain of microtubule-targeting degraders, exemplified by PROteolysis TARgeting Chimeras and small-molecule degraders, which enable precise degradation of specific microtubule-associated proteins implicated in cancer pathogenesis. Additionally, we discuss the promising realm of precision-targeted approaches, including antibody–drug conjugates and the utilization of photopharmacology in MTAs. Lastly, we provide a comprehensive overview of the clinical applications of microtubule-targeting therapies, assessing their efficacy and current challenges. We aim to provide a global picture of MTAs development as well as insights into the microtubule-targeting drug discovery for cancer treatment.

## KEYWORDS

cancer therapy, microtubule, microtubule-targeting agents, tubulin, tubulin-targeting sites

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## 1 | INTRODUCTION

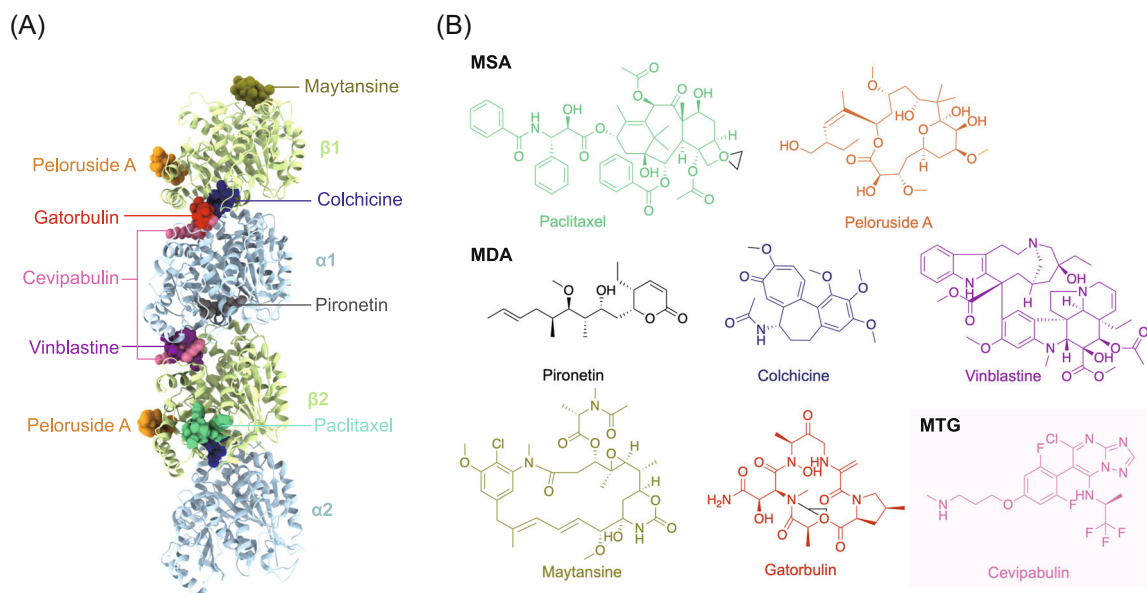
Cancer continues to be a global health challenge, necessitating the development of innovative therapeutic strategies. Among the diverse therapeutic approaches, microtubule-targeting agents (MTAs) have emerged as prominent candidates for cancer treatment.<sup>1–4</sup> Microtubules are integral components of the cell cytoskeleton. They are dynamic filaments that play a pivotal role in various cellular processes, including cell division, intracellular trafficking, and maintenance of cell shape and structure.<sup>5–7</sup> Their crucial involvement in these vital cellular functions has rendered microtubules an attractive target for anticancer interventions.

MTAs are regarded as highly promising drugs for treating diverse cancers, including lung, breast, ovarian, and prostate cancers.<sup>8</sup> By disrupting the normal function of microtubules, MTAs have demonstrated their effectiveness in halting cell cycle progression and inducing programmed cell death.<sup>9,10</sup> These agents can be classified into three primary categories based on their mechanisms of action: microtubule-stabilizing agents (MSAs), microtubule-destabilizing agents (MDAs), and microtubule-targeting degraders (MTGs). MSAs, exemplified by taxanes and laulimalide/peloruside-A, promote microtubule polymerization and stabilization. Conversely, MDAs, such as vinca

alkaloids and colchicine, inhibit microtubule polymerization and promote depolymerization. The third category, MTGs, represents a novel class of MTAs that induce microtubule denaturation and degradation. Despite different molecular mechanisms, all the three types of MTAs lead to microtubules abnormality and subsequent cell death.

Recent advances in structural biology have greatly contributed to our understanding of the mechanisms of MTAs at the atomic level. In particular, seven distinct binding sites for MTAs have been identified: those of taxanes, of peloruside-A/laulimalide, of colchicine, of vinca alkaloids, of maytansine, and of pironetin, and the recently discovered gatorbulin site (Figure 1).

In this review, we aim to provide a detailed and up-to-date overview of MTAs for cancer treatment. We summarize the existing knowledge regarding the dynamics and structure of microtubules and highlight the well-established binding sites for MTAs, offering a comprehensive overview of the current status and challenges associated with the clinical use of these agents. Furthermore, we will delve into the challenges associated with traditional MTA development and introduce emerging precision-targeted approaches that hold promise for enhancing therapeutic efficacy and minimizing adverse effects. This review aims to contribute to the advancement of microtubule-targeted



**FIGURE 1** Seven binding sites of microtubule-targeting agents (MTAs). (A) Seven MTA binding sites of tubulin. Two tubulin heterodimers are shown in cartoon ( $\alpha$ -subunits, sky blue;  $\beta$ -subunits, light green). The representative agents bound to the seven sites are shown in sphere representation: vinca site (vinblastine, purple, PDB ID 5J2T); maytansine site (olive, PDB ID 4TV8); peloruside A/laulimalide site (orange, PDB ID 4O4J); colchicine site (dark blue, PDB ID 4O2B); the seventh site/gatorbulin site (red, PDB ID 7ALR); pironetin site (gray, PDB ID 5LA6); and taxane site (paclitaxel, green, PDB ID 6WVR). Cevipabulin (pink, PDB ID 7CLD) binds to the vinca-site as a microtubule stabilizer and to the seventh site as a tubulin degrader, respectively. (B) The chemical structures of representative agents, colored according to Panel A. MDA, microtubule-destabilizing agent; MSA microtubule-stabilizing agent; MTG microtubule-targeting degrader.

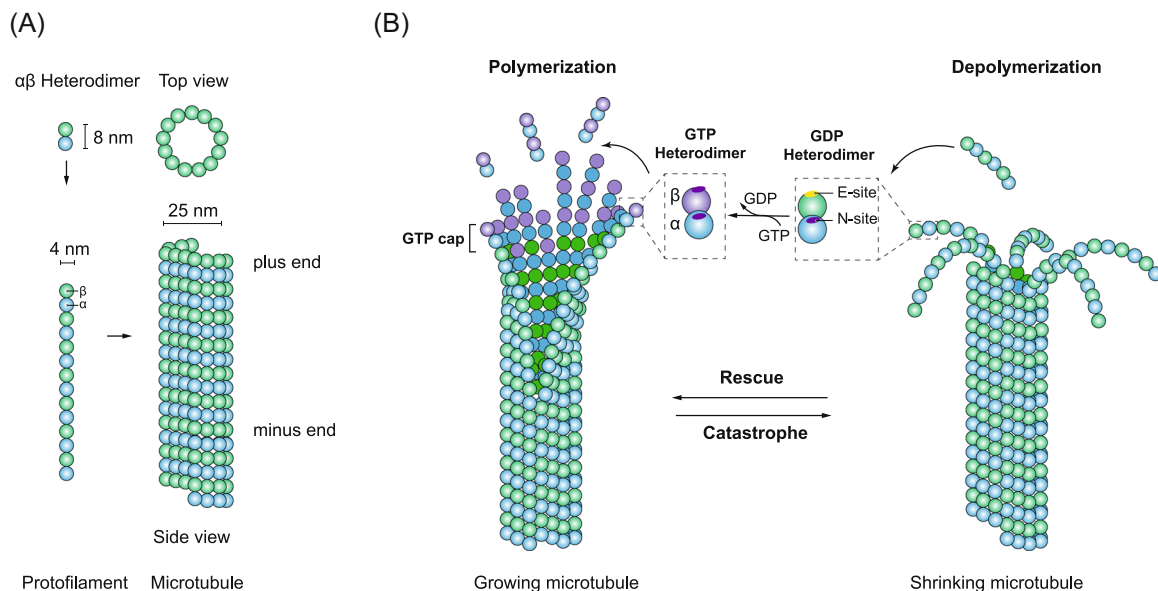
therapies, hoping to provide valuable insights that can be used to discover more effective treatments for cancer.

## 2 | MICROTUBULE STRUCTURE AND DYNAMICS

Microtubules are essential components of the eukaryotic cell cytoskeleton and play a crucial role in various cellular processes, including cell division, intracellular transport, cell shape maintenance, and cell motility.<sup>6,11</sup> They are hollow cylinders of about 25 nm in diameter formed by 13 parallel protofilaments, themselves consisting of  $\alpha\beta$ -tubulin (50 kDa each in size) heterodimers assembled into in a head-to-tail fashion.<sup>12–14</sup> Thus, microtubules possess polarity. The  $\alpha$ -tubulin subunits are exposed at one end, known as the minus end, while the  $\beta$ -tubulin subunits are exposed at the other end, known as the plus end (Figure 2A). This polarity is essential for microtubule dynamics and their involvement in cellular processes such as cell division and intracellular transport.

The sophisticated dynamics of microtubules is exquisitely regulated both spatially and temporally in response to varied cellular requirements, primarily driven by guanosine triphosphate (GTP) hydrolysis

and guanosine diphosphate (GDP)–GTP exchange cycles.<sup>15,16</sup> Specifically, whereas GTP bound to the  $\alpha$ -tubulin subunit, at the N site, is nonhydrolyzable and nonexchangeable, the one bound to  $\beta$ -tubulin, at the E site, can be hydrolyzed and exchanged with free nucleotide.<sup>17</sup> The GTP-bound form promotes microtubule growth, while GDP-bound form does not polymerize. The continuous polymerization process requires the presence of a GTP-bound tubulin cap at the growing end of the microtubule.<sup>11</sup> The stochastic loss of this cap triggers a phenomenon known as catastrophe, characterized by a switch into a depolymerization phase.<sup>11,18</sup> The transition from the stable GTP-tubulin lattice to the metastable GDP-tubulin lattice lies at the core of the intricate dynamics observed in microtubule behavior.<sup>11,18</sup> Nonequilibrium dynamics of microtubules encompasses two distinct behaviors: dynamic instability and treadmilling. Dynamic instability, the most characteristic dynamic behavior, refers to individual microtubule ends switching between growing and shrinking periods, the transition between these two phases being called catastrophes and rescues (Figure 2B).<sup>19</sup> Whereas dynamic instability concerns both minus and plus ends in vitro, the plus end grows faster and undergoes more catastrophe and rescue events, that is, is more



**FIGURE 2** Microtubule structure and dynamics. (A) Microtubule structure. Tubulin heterodimers consisting of  $\alpha$ - (blue) and  $\beta$ -subunits (green) assemble into protofilaments in a head-to-tail fashion. Thirteen parallel protofilaments further polymerize to generate the hollow cylinder about 25 nm in diameter with the plus end exposing the  $\beta$ -tubulin and the minus end exposing the  $\alpha$ -tubulin. (B) Cartoon illustrating structural intermediates of microtubule dynamic instability. The polymerization–depolymerization cycle is powered by GTP hydrolysis and GDP/GTP exchange, during which the microtubule undergoes catastrophe and rescue events. The GTP bound to  $\alpha$ -tubulin subunit (called N site) is nonhydrolyzable and nonexchangeable, whereas the one bound to  $\beta$ -tubulin (called E site) can be hydrolyzed and exchanged with free GDP. Due to the delay of GTP hydrolysis, a GTP heterodimer-rich region ( $\beta$ -tubulin bound GTP showed in purple), named GTP cap, will form during tubulin polymerization. GDP, guanosine diphosphate; GTP, guanosine triphosphate.

dynamic, than the minus end.<sup>12</sup> By comparison, treadmilling is characterized by net growth at one end of the microtubule and balanced net shortening at the other end.<sup>12</sup> The exquisite dynamic behavior allows microtubules to rapidly remodel their structure and participate in cellular processes. Disruption of microtubule dynamics, such as through the use of antimetabolic drugs that suppress dynamic instability, can result in cell cycle arrest, abnormal formation of mitotic spindles, and apoptotic cell death.<sup>20</sup>

Microtubule dynamics is modulated by tubulin posttranslational modifications (e.g., acetylation, phosphorylation, and detyrosination),<sup>21–24</sup> and by the expression of diverse tubulin isotypes.<sup>25–27</sup> It is also tightly regulated through the coordination of microtubule-associated proteins (MAPs).<sup>28,29</sup> MAPs, encompassing structural MAPs and motor MAPs, are involved in the regulation of microtubule dynamics, organization, intracellular transport, and cell division.<sup>30</sup> Structural MAPs, exemplified by MAP4, MAP2, and Tau, contribute to microtubule polymerization, spacing regulation, and the formation of intricate microtubule architectures within specific cellular compartments.<sup>29</sup> Motor MAPs, including dyneins and kinesins, bind to microtubules and generate directed movements along them and facilitate the transportation of cargos along microtubules, enabling crucial cellular functions such as protein trafficking, neurotransmitter release, and organelle positioning.<sup>29</sup>

Increasing evidence has suggested that dysregulation of MAPs plays a role in cancer pathogenesis and is associated with tumor growth, metastasis, and resistance to cancer treatments.<sup>31,32</sup> For instance, the overexpression of MAPs, such as MAP4, can enhance microtubule stability, resulting in resistance to microtubule-targeting anticancer drugs and promoting tumor cell survival.<sup>33,34</sup> Dysregulation of motor MAPs involved in spindle formation, such as Eg5 (a kinesin-5 family member), can lead to chromosome missegregation and aneuploidy, commonly observed in cancer cells.<sup>35–37</sup> Moreover, MAPs have emerged as potential diagnostic and prognostic markers in diverse cancer types. Notably, in urothelial carcinoma, MAP1B shows significant upregulation associated with cancer progression.<sup>38</sup> Similarly, elevated expression of MAP4 is significantly correlated with enhanced metastatic potential of cancer cells and unfavorable long-term survival outcomes among patients diagnosed with lung adenocarcinoma.<sup>34</sup> In the context of non-small-cell lung cancer (NSCLC), the enriched expression of MAP2, MAP4, MAP6, MAP7, and MAP7D3 is associated with a favorable prognosis, improved patient survival outcomes, and a reduced likelihood of disease recurrence.<sup>39</sup>

The understanding of microtubule structure and dynamics establishes the groundwork for exploring

precise microtubule-targeting sites, while also yielding valuable insights into cancer biology and the identification of therapeutic targets.

### 3 | TUBULIN-BINDING SITES AND MECHANISMS OF ACTION OF MSAs

#### 3.1 | Taxane site

Paclitaxel (Taxol<sup>®</sup>), the first discovered MSA, was initially isolated from *Taxus brevifolia tree bark* in 1966.<sup>40</sup> It was found to possess antileukemic and antitumor properties. Due to its potent antiproliferative activity, paclitaxel was approved by the US Food and Drug Administration (FDA) in 1992 for treating metastatic ovarian cancer. Docetaxel (Taxotere<sup>®</sup>), a semisynthetic analog of paclitaxel, emerged as a second-generation broad-spectrum anticancer drug and is widely employed for solid tumor malignancies, including breast cancer, ovarian cancer, and NSCLC.<sup>41,42</sup>

Early investigations revealed that paclitaxel binds specifically and reversibly to microtubules, suggesting the existence of a distinct binding site.<sup>43</sup> The atomic-level structure of tubulin–paclitaxel was elucidated using electron crystallography in 1998.<sup>44</sup> Subsequent advances in X-ray crystallography provided high-resolution insights into taxane-site interaction. This is exemplified by the binding of taxane-site MSAs to tubulin, including epothilone, zampanolide, and taccalonolide.<sup>45–47</sup>

The taxane site, recognized as the initially identified microtubule-stabilizing site, plays a crucial role in the mechanism of action of MSAs.<sup>40,48</sup> These agents exhibit specific and reversible binding to microtubules, effectively inhibiting depolymerization and promoting stabilization.<sup>49</sup> The taxane-binding site agents, interact specifically with  $\beta$ -tubulin at the interface between neighboring subunits and form hydrogen bonds with specific residues on M-loop that a key element involved in establishing lateral contacts (between protofilaments) in microtubules.<sup>50,51</sup>

Binding of taxane-site agents induces conformational changes in the  $\beta$ -tubulin subunit, leading to structural alterations in the microtubule itself.<sup>52</sup> These conformational changes are crucial in preventing the curvature of protofilaments, a critical step in microtubule depolymerization.<sup>49</sup> By promoting the stability of protofilaments, taxane-binding site agents facilitate interactions between the M-loop of one  $\beta$ -monomer and a  $\beta$ -monomer of an adjacent protofilament.<sup>53,54</sup> Recently, Prota et al.<sup>55</sup> conducted a study that elucidated the preferential binding of taxanes to microtubules over tubulin, as well as the mechanism underlying the longitudinal expansion of microtubule lattices

driven by the accommodation of the taxane core. These findings provide new molecular insights into taxane-mediated microtubule stabilization.

### 3.2 | Laulimalide/peloruside-A site

Laulimalide and peloruside A, nontaxane MSAs derived from marine sponges,<sup>56,57</sup> have demonstrated potent antiproliferative activity against P-glycoprotein-mediated multidrug resistance (MDR) cells and paclitaxel-resistant cells.<sup>58–60</sup> These compounds offer improved aqueous solubility and enhanced tolerability compared to taxol. However, the clinical development of laulimalide and peloruside A have been hindered by their narrow therapeutic index, severe toxicity, and limited tumor inhibition.<sup>61</sup> Combination therapy involving nontaxane site and taxane-site agents shows promise in overcoming these challenges and has emerged as an important strategy in cancer treatment.<sup>61,62</sup>

The laulimalide/peloruside-A site, representing an alternative MSAs binding site, serves as a target for microtubule stabilization. Elucidation of the molecular model of tubulin-bound laulimalide and peloruside A has been achieved through structural biology, revealing that this site is located on the external surface of the microtubule.<sup>52,63</sup> Upon binding to the microtubule, laulimalide/peloruside-A deeply insert their side chain and macrocycle into a specific pocket formed by  $\beta$ -tubulin residues, inducing allosteric stabilization of the M-loop region without any regular secondary structure and establishing lateral contacts between adjacent tubulin subunits across protofilaments.<sup>63</sup> These interactions do not significantly alter the overall conformation of the  $\alpha\beta$ -tubulin dimer but effectively impede microtubule disassembly. Furthermore, an intriguing phenomenon of allosteric crosstalk between the laulimalide/peloruside A-binding site and the taxane pocket has been observed in both unassembled and assembled tubulin states. This discovery suggests a potential synergistic effect between laulimalide/peloruside and taxane-site ligands in modulating tubulin assembly and effectively inhibiting cancer cell growth.<sup>63</sup>

## 4 | TUBULIN-BINDING SITES AND MECHANISMS OF ACTION OF MDAs

### 4.1 | Colchicine site

Colchicine, initially extracted from the autumn crocus *Colchicum autumnale* in 1820,<sup>64</sup> has a long history for

the treatment of diseases such as gout and familial Mediterranean fever.<sup>65</sup> However, neither colchicine nor colchicine-binding site inhibitors (CBSIs) have been approved as antitumor drugs due to their high toxicity and narrow therapeutic index in disrupting microtubule function across different cell types.<sup>66,67</sup>

In 2004, Ravelli et al.<sup>68</sup> characterized the X-ray structure of tubulin in complex with colchicine, providing structural insights into its binding mechanism. In subsequent studies, the structure of many CBSIs bound to tubulin has been determined.<sup>69–71</sup> Zhang et al.<sup>70</sup> elucidated the crystal structures of tubulin complexed with a diverse set of CBSIs, including lexibulin, nocodazole, plinabulin, and tivantinib. The colchicine binding site is mainly contributed by the tubulin  $\beta$  subunit, near the interface with the  $\alpha$  subunit and adjacent to the GTP N-site of  $\alpha$ -tubulin. This binding pocket comprises two hydrophilic centers suitable for hydrogen bond formation and three hydrophobic centers: (i) the  $\alpha/\beta$  tubulin interface, (ii) the major hydrophobic core, and (iii) the “deep binding site” of some ligands.<sup>69</sup> Collectively, ligands targeting the colchicine site interact with unassembled tubulin and inhibit tubulin polymerization. This inhibition occurs by preventing curved (unassembled) tubulin from adopting a straight structure through steric clash between colchicine and  $\alpha$ -tubulin or by inhibiting the subdomain movements of tubulin during the curved-to-straight structural conversion.<sup>72</sup> Notably, a few compounds such as KXO-1, nocodazole, and tivantinib exhibit inhibitory effects on kinase signaling pathways as well as tubulin polymerization, suggesting a novel multitarget therapeutic approach.<sup>73–75</sup>

### 4.2 | Vinca alkaloids site

Vinca alkaloids, initially extracted from the Madagascar periwinkle *Catharanthus roseus* in the 1950s, were originally recognized for their antidiabetic and antimalarial properties.<sup>76–78</sup> Subsequent studies demonstrated the potent antileukemic effects of vinblastine, the prototype of vinca alkaloids, leading to its development as a promising chemotherapeutic agent against cancer. The vinca-site family of MDAs has since exhibited clinical success in the treatment of hematologic malignancies, lymphatic neoplasms, and solid tumors, including breast cancer, NSCLC, and small-cell lung cancer (SCLC).<sup>79–83</sup>

The binding site of vinca alkaloids was initially elucidated in 2005 when the structure of vinblastine bound to tubulin was determined.<sup>84</sup> Further structural studies revealed that various other drugs also bind to the same site, known as the vinca domain.<sup>84,85</sup> This domain is located at

the interdimer interface of a curved protofilament, proximal to the exchangeable GTP site. It consists of a core pocket that extends toward the GTP-binding site on  $\beta$ -tubulin at the plus end of the microtubule. The residues within the inner lumen of microtubules associated with tubulin stabilization through longitudinal protofilament contacts contribute to the vinca domain. Binding of vinca alkaloids at this site destabilizes microtubules by introducing a wedge at the interdimer interface between  $\alpha$ - and  $\beta$ -tubulin, thereby preventing the conformational transition of protofilaments from curved to straight which occurs during microtubule assembly.<sup>86</sup> Interestingly, the effects of both vinca alkaloids and colchicine on tubulin are concentration-dependent.<sup>87,88</sup> At high concentrations (e.g., 10–100 nmol/L in HeLa cells), tubulin depolymerization is promoted, mitotic progress is blocked, and mitotic spindles are disrupted.<sup>89</sup> Conversely, at low but clinically relevant concentrations (e.g., IC<sub>50</sub> 0.8 nmol/L in HeLa cells), they do not induce tubulin depolymerization but instead suppress microtubule dynamics, leading to apoptotic cell death.<sup>89</sup>

### 4.3 | Maytansine site

Maytansine, initially isolated from the bark of the African shrubs *Maytenus serrata* and *Maytenus ovatus* in 1972, has exhibited remarkable cytotoxicity against a diverse range of cancer cell lines.<sup>90</sup> However, its clinical application as a standalone therapeutic agent has encountered challenges due to dose-limiting toxicity and limited tumor specificity.<sup>91</sup> Nonetheless, the advent of antibody-drug conjugates (ADCs) has revived interest in maytansinoids for cancer chemotherapy. Notably, the FDA approval of mertansine (DM1) in 2013, which incorporates maytansine as a payload into trastuzumab emtansine (Kadcyla®), has proven successful in the treatment of advanced breast cancer.<sup>92</sup> Additionally, various analogs of maytansine, including DM4 (ravtansine/soravtansine), have shown promising potential as ADC payloads in clinical trials.<sup>93,94</sup>

In 2014, Prota et al.<sup>95</sup> determined the X-ray crystallography structure of maytansine bound to tubulin. Their findings unveiled a distinct binding site for maytansine, adjacent to the vinca alkaloids binding site.<sup>96,97</sup> This unique site is located at an exposed pocket of the  $\beta$ -tubulin subunit, at the longitudinal interdimer interface corresponding to the plus end of microtubules. By sterically hindering the normal formation of  $\alpha\beta$ -tubulin protofilaments, maytansine disrupts microtubule assembly.<sup>95</sup> The agents targeting the maytansine binding site exert their influence on microtubules through two distinct mechanisms. At high concentrations, they sequester tubulin subunits, forming complexes that are

incompetent for assembly. At lower concentrations, they impede the addition of further tubulin dimers to the plus ends of growing microtubules.<sup>95</sup>

### 4.4 | Pironetin site

Pironetin, originally isolated from the fermentation broth of *Streptomyces* species in the 1990s,<sup>98</sup> represents a unique natural compound that exclusively targets the  $\alpha$ -tubulin subunit. Initially characterized as a plant growth regulator,<sup>99</sup> pironetin and its derivatives were subsequently recognized as potent antiproliferative cytotoxic agents.<sup>100,101</sup> Notably, pironetin demonstrated remarkable activity against cell lines both sensitive and resistant to first-generation MTAs, including taxol, thereby highlighting its significant potential for the development of a new generation of anticancer drugs.<sup>102</sup>

Initial investigations into the binding mode of pironetin suggested its interaction with Lys-352 of  $\alpha$ -tubulin through systematic alanine scanning.<sup>103</sup> However, two recent X-ray crystallographic studies have provided valuable insights, unveiling a distinct binding mechanism wherein pironetin forms a covalent interaction with Cys-316 of  $\alpha$ -tubulin via a Michael-type addition reaction.<sup>104,105</sup> The crystal structure of the tubulin–pironetin complex has further revealed that the binding site is fully buried within the  $\alpha$ -tubulin subunit, positioned in close proximity to the interdimer interface crucial for the regulation of tubulin assembly. By perturbing the longitudinal tubulin contacts within microtubules, pironetin exerts its disruptive effect on tubulin assembly, employing a mechanism akin to that of vinca-site agents. Specifically, pironetin introduces a wedge at the interdimer interface between  $\alpha$ - and  $\beta$ -tubulin, effectively impeding the conformational transition from curved to straight.

The absence of GTPase activity and limited conformational changes in the  $\alpha$  subunit make agents targeting the pironetin-binding site on  $\alpha$ -tubulin potentially unaffected by mutations in  $\beta$ -tubulin and the overexpression of  $\beta$ III-tubulin in drug-resistant cells. The development of drugs that selectively target  $\alpha$ -tubulin holds significant promise in overcoming chemotherapeutic drug resistance and represents an area of substantial potential advancement.

### 4.5 | Gatorbulin-1 site or the seventh site

In addition to the six distinct MTA binding sites previously identified, a noteworthy discovery of a seventh binding site was made by Matthew et al.<sup>106</sup> using X-ray crystallography. This newly discovered site demonstrated specific affinity for a cyclic depsipeptide known as

gatorbulin-1 (GB1), which was derived from the marine cyanobacterium *Lyngbya cf. confervoides*.<sup>106</sup>

The structure of GB1 bound to tubulin clearly demonstrates the proximity and distinction between this seventh binding site and the colchicine binding site.<sup>106</sup> Similar to the colchicine site, the GB1 binding pocket is situated at the intra-dimer interface between  $\alpha$ - and  $\beta$ -tubulin, but with  $\alpha$ -tubulin contributing a greater buried area. This similarity implies that the seventh site and the colchicine site share a same mechanism of action. Specifically, GB1 prevents the critical curve-to-straight structural conversion of tubulin during assembly, effectively creating a wedge between the  $\alpha$  and  $\beta$  subunits within the tubulin heterodimer, hence inhibiting tubulin polymerization.<sup>106</sup>

The recently discovered seventh binding site of tubulin has demonstrated notable variability in the effects of compound binding. In a study conducted by Yang et al.,<sup>107</sup> it was demonstrated that cevipabulin (also known as TTI-237) exhibits binding affinity to this specific site. However, in contrast to GB1, the binding of cevipabulin to the seventh site elicits distinct consequences. It disrupts the critical hydrogen-bonding interaction between the nonexchangeable GTP and the T5 loop of  $\alpha$ -tubulin, leading to the conversion of the nonexchangeable GTP into an exchangeable state. This conversion event ultimately triggers the denaturation and degradation of tubulin.<sup>106,107</sup> The binding of cevipabulin to the seventh site demonstrates its potential as a tubulin degradation agent, providing a foundation for the development of future agents that specifically target tubulin degradation.

## 5 | MICROTUBULE-TARGETING DEGRADERS

Traditional MTAs primarily act by stabilizing or destabilizing microtubules, leading to mitotic arrest and subsequent cell death. Despite their initial efficacy, the emergence of resistance to these agents and the occurrence of associated side effects have underscored the need for alternative strategies. One such strategy involves targeted protein degradation by MTGs, which offers a promising avenue for overcoming these limitations.

### 5.1 | PROteolysis Targeting Chimeras

Targeted protein degradation, specifically PROteolysis Targeting Chimeras (PROTACs), has emerged as a promising strategy in cancer therapy.<sup>108,109</sup> This approach works by promoting the degradation of target proteins instead of solely inhibiting their activity holds

potential for overcoming resistance and improving therapeutic outcomes.<sup>110–112</sup> PROTACs are designed as heterobifunctional molecules comprising three essential components: a ligand that specifically binds to the protein of interest (POI), a ligand that recruits an E3 ubiquitin ligase, and a carefully designed linker that connects these components. By bridging the POI and the E3 ubiquitin ligase, PROTACs facilitate target protein degradation through the ubiquitin-proteasome system, a highly regulated cellular degradation pathway.<sup>113,114</sup> This unique mechanism of action addresses the limitations commonly associated with traditional small-molecule inhibitors, such as the emergence of drug resistance, which is particularly relevant in the context of MTAs.<sup>115</sup> Despite the promising outcomes observed in preclinical and clinical trials, the expanding application of PROTACs is confronted with several challenges. A pivotal concern lies in the assessment of drug resistance that may arise from the prolonged administration of PROTAC drugs in clinical settings.<sup>116</sup> Furthermore, the underlying mechanism of action employed by PROTACs involves intricate cellular entry, which limits its application to cell membrane proteins and extracellular proteins.<sup>116</sup>

Recently, Gasic et al.,<sup>117</sup> in a study conducted, explored the development of the first tubulin-targeting PROTACs that incorporate an E3 ubiquitin ligase cereblon (CRBN) moiety. Several compounds previously reported to induce tubulin degradation were incorporated into the design.<sup>118,119</sup> However, the study surprisingly revealed that none of the designed PROTACs were capable of inducing tubulin degradation.<sup>117</sup> These findings raise concerns regarding the potential limitations of CRBN-recruiting PROTAC degraders in facilitating tubulin degradation. Consequently, despite the considerable promise of PROTAC technology in overcoming drug resistance associated with MTAs, it is evident that there are remaining challenges and complexities that necessitate further investigation and refinement. Ongoing research efforts should primarily focus on optimizing PROTAC design by exploring alternative ligands, linkers, and E3 ubiquitin ligases to enhance the specificity and efficacy of tubulin-targeting PROTACs.<sup>120–122</sup> Additionally, comprehensive investigations into factors influencing tubulin degradation efficiency, including the cellular microenvironment and signaling pathways, are imperative for advancing the field.

### 5.2 | Small-molecule degraders

Small-molecule degraders are a promising class of MTAs that induce the degradation of tubulin through a distinct mechanism of action. Unlike other targeted protein



degradation strategies, such as PROTACs, these degraders have the advantage of not requiring a bulky bifunctional structure.<sup>109</sup> They directly bind to the target protein and autonomously recruit the E3 ubiquitin ligase or interact with other components of the ubiquitin-proteasome system to initiate degradation.<sup>107,119,123–126</sup> Small-molecule degraders possess favorable characteristics, including reduced molecular weight, advantageous physicochemical properties, and improved bioavailability, making them a compelling approach for targeted protein degradation. However, it is worth noting that small-molecule degraders were initially discovered as microtubule modulators but were fortuitously discovered as degraders later on. Hence, small-molecule degraders also inherit the drawbacks associated with conventional small-molecule inhibitors, including a lack of target specificity and an increased risk of off-target effects.

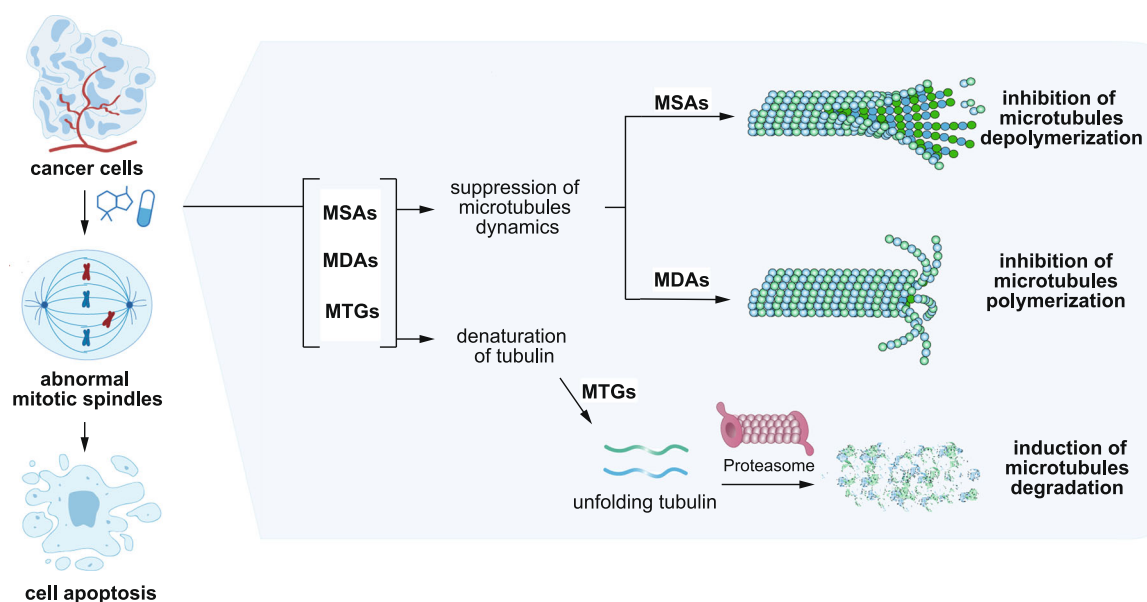
Recent studies have identified several MTAs, such as isothiocyanates,<sup>123</sup> thymoquinone,<sup>124</sup> withaferin A,<sup>125</sup> T0070907,<sup>119</sup> 3-(3-phenoxybenzyl)amino- $\beta$ -carboline (PAC),<sup>126</sup> and cevipabulin,<sup>107</sup> that exhibit protein-degrading properties. These findings highlight the potential application of these MTAs as small-molecule degraders in cancer therapy. Structural studies of ligand-tubulin complexes have provided insights into the mechanisms of tubulin degradation. For instance, T0070907 and T007-1 covalently bind to Cys-239 of  $\beta$ -tubulin, resulting in tubulin unfolding and

degradation.<sup>118</sup> Cevipabulin, on the other hand, interacts with a specific site near the nonexchangeable GTP, destabilizing tubulin and promoting its degradation.<sup>107</sup> PAC induces tubulin denaturation by forming a low-barrier hydrogen bond with  $\beta$ -tubulin, leading to tubulin aggregation and subsequent ubiquitin-mediated degradation.<sup>126</sup> These mechanisms disrupt tubulin stability and induce aggregation, ultimately causing cell cycle arrest and apoptosis through the ubiquitin-proteasome pathway. The understanding of these processes underscores the potential of small-molecule degraders as promising agents for targeted protein degradation in cancer treatment.

## 6 | AntiCANCER MECHANISMS OF MTAs

The diverse mechanisms of action employed by MTAs contribute to their potent anticancer effects, including disruption of cell division, induction of apoptosis, interference with signaling pathways, inhibition of angiogenesis, and modulation of tumor suppressor proteins.<sup>127,128</sup>

Primarily, MTAs perturb microtubule assembly and spindle formation, leading to impaired cell division and cell cycle arrest at the G2/M phase, resulting in abnormal chromosome segregation (Figure 3).<sup>129</sup> Moreover, MTAs induce apoptosis through intricate pathways involving



**FIGURE 3** Major mechanism of action of MTAs involved in cancer cell apoptosis. The interaction of chemotherapeutic agents that stabilize, destabilize, and denature microtubules leads to an increase in microtubule polymerization, depolymerization, and degradation. These actions subsequently trigger cellular apoptosis by the formation of an abnormal mitotic spindle or impaired microtubule-related functions. MDA, microtubule-destabilizing agent; MSA, microtubule-stabilizing agent; MTA, microtubule-targeting agent; MTG, microtubule-targeting degrader.

the phosphorylation of Bcl-2 and Bcl-xL, activation of E2F1, and subsequent release of cytochrome c.<sup>130,131</sup> Additionally, MTAs interfere with the protein kinase B/mammalian target of the rapamycin signaling pathway, inducing autophagy and impeding tumor cell proliferation.<sup>132</sup> Their antiangiogenic potential involves selective destruction of tumor blood vessels, potentially mediated by alterations in vascular endothelial growth factor expression.<sup>133</sup> Importantly, the damage inflicted to tumor vasculature by MTAs is reversible, making them suitable as vascular inhibitors.<sup>134</sup> Furthermore, some MTAs, such as paclitaxel, stimulate the production of the tumor suppressor protein p53, which is intricately linked to microtubule dynamics and the expression of diverse tubulin isotypes.<sup>135–137</sup> These multifaceted mechanisms collectively contribute to the therapeutic efficacy of MTAs for cancer therapy.

## 7 | PRECISION-TARGETED THERAPY APPROACHES

Conventional MTAs, such as taxanes (e.g., paclitaxel and docetaxel) and vinca alkaloids (e.g., vinblastine and vincristine), have demonstrated remarkable efficacy in cancer therapy. However, their clinical application is hindered by the emergence of off-target effects as they nonspecifically bind to tubulin in both normal and cancer cells, thereby compromising their therapeutic effectiveness. To overcome these limitations, researchers have developed and investigated various precision-targeted approaches. One successful example is the utilization of ADCs, which have shown promising results in clinical settings. Additionally, preclinical investigations have revealed the potential of light-triggered analogs of MTAs. Further exploration of these precision-targeted approaches may contribute to optimizing the clinical utility of MTAs and improving outcomes in cancer therapy.

### 7.1 | Antibody–drug conjugates

MTA-based ADCs represent a promising and rapidly advancing class of oncology therapeutics with therapeutic potential.<sup>138–140</sup> These innovative bioconjugates combine the potent cytotoxicity of MTAs with the specificity and selectivity of monoclonal antibodies (mAbs). This combination offers several advantages, including the reduction of off-target effects and the enhancement of cytotoxicity that specifically targets malignant cells. However, notable challenges of ADCs encompass the occurrence of off-target toxicity arising from the

premature release of cytotoxic small molecules into the blood circulation, and tumor-associated versus tumor-specific antigens and consequent unintended biodistribution and toxicity.<sup>141,142</sup> Addressing these challenges is critical for the successful application of ADCs in clinical settings.

In recent years, potent MTAs with well-established cytotoxic properties, have undergone extensive validation and exploration as payloads in ADCs.<sup>143</sup> These MTAs, including auristatin derivatives (MMAE, MMAF), maytansinoid derivatives (DM1, DM2, DM4), and tubulysins, disrupt microtubule assembly and affect mitosis, showing promising effects in preclinical and clinical studies.<sup>144–146</sup> Notably, ADCs based on MMAE, MMAF, DM4, and DM1 have achieved market approval, leading to huge commercial success.<sup>147,148</sup> MMAE and MMAF, known for their exceptional stability and potent antiproliferative activity by binding to vinca alkaloid sites on microtubules,<sup>149</sup> have demonstrated efficacy across various cancer types, including lymphoma, leukemia, and solid tumors (e.g., lung, gastric, and breast cancers).<sup>147,150,151</sup> Additionally, Maytansine-based ADCs, exemplified by trastuzumab emtansine (T-DM1), have exhibited promising results in treating HER2-positive metastatic breast cancer.<sup>152</sup> The diverse range of MTA-based payloads expands the opportunities for the development of ADCs and other targeted therapies in the realm of microtubule-targeting approaches.

### 7.2 | Photopharmacology of MTAs

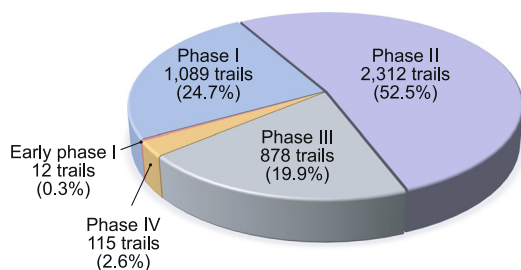
Photopharmacology, as a swiftly advancing research field, integrates the classical pharmacological approach with photochemical light control, enabling precise regulation of drug activity to mitigate systemic toxicity, minimize off-target effects, and enhance treatment precision.<sup>153,154</sup> Hence, extensive efforts have been dedicated to the development of light-triggered MTAs, aiming to achieve precise modulation of microtubule dynamics.<sup>155</sup> Among these efforts, analogs based on combretastatin A-4, paclitaxel, epothilone, and colchicinoid have garnered attention.<sup>156–160</sup> These photoswitchable MTAs have demonstrated potential for elucidating the intricate mechanisms underlying microtubule-related processes and have proven to be useful tools in the study of various fields such as embryology, neuroscience, and cytoskeleton research.<sup>161</sup> Importantly, these photopharmaceuticals also show potential for targeted therapeutic interventions by precisely modulating microtubule dynamics in cancer cells or disease-specific microtubule networks.<sup>155</sup> However, the field of photopharmacology is still in its nascent stage of development. There is still a

considerable distance to traverse before they progress towards clinical application. It is important to note that further research is still necessary to optimize their pharmacokinetic properties, enhance their selectivity towards specific microtubule networks, and refine their photoswitching capabilities to achieve a more precise and effective modulation of microtubule function in disease contexts.

## 8 | CLINIC APPLICATION

Microtubules, critical components involved in numerous cellular processes, have attracted significant attention in cancer therapy. A breakthrough in the development of microtubule-targeting drugs occurred in 1963 with the FDA approval of vincristine sulfate (Oncovin®) for the treatment of leukemia and lymphoma.<sup>162</sup> Subsequent extensive research and drug discovery endeavors have further highlighted the importance of MTAs as crucial components in the development of effective chemotherapeutic agents against cancer.

By 2023, extensive clinical investigation has been conducted on MTAs candidates (Figure 4), and the market provides a wide range of over 20 tubulin-targeting drugs for treating diverse diseases. Approved MTAs are broadly categorized into four classes: taxanes, vinca alkaloids, colchicine, and MT-ADCs (Table 1 and Figure 5). Taxanes, such as paclitaxel and docetaxel, are widely recognized as potent MSAs and have demonstrated significant clinical efficacy in the treatment of diverse cancers, including breast, ovarian, lung, and prostate cancers.<sup>163–165</sup> Vinca alkaloids, including vincristine and vinblastine, are commonly acknowledged as MDAs, and are extensively



**FIGURE 4** Tubulin modulators in clinical trial phases up to April 2023. Data are obtained from <https://clinicaltrials.gov>. Total of 4406 trials, either active or completed. Active: The study is ongoing, and participants are receiving an intervention or being examined, but potential participants are not currently being recruited or enrolled. Completed: The study has ended normally, and participants are no longer being examined or treated (i.e., the last participant's last visit has occurred).

employed in the treatment of hematological malignancies, such as lymphoma and leukemia, as well as solid tumors like SCLC.<sup>83,166</sup> Apart from their significant role in cancer treatment, MTAs have also demonstrated therapeutic potential in other disease areas. For instance, colchicine, traditionally employed for gouty arthritis and Familial Mediterranean fever, has expanded its therapeutic application to encompass conditions like osteoarthritis, pericarditis, and atherosclerosis.<sup>167–169</sup> The success of MTAs in treating different diseases underscores their significant contributions to the field of medicine.

However, the clinical application of conventional MTAs is hindered by a number of limitations, including MDR, high toxicity such as peripheral neuropathy and myelosuppression, and poor solubility.<sup>163,170,171</sup> To overcome these limitations, researchers are actively investigating more targeted therapies, including nanoparticle-based drug delivery systems,<sup>172,173</sup> ADCs,<sup>138,174,175</sup> and combination therapies with targeted agents.<sup>176–178</sup> For example, albumin-bound paclitaxel nanoparticles (Abraxane®) have gained clinical approval in 2005 for the treatment of metastatic breast cancer, due to their remarkable activity and tolerability compared to conventional paclitaxel formulations.<sup>179,180</sup> Moreover, researchers have developed nanotechnology-based delivery systems for a diverse array of MTAs, including colchicine, LY293, etoposide, and others.<sup>181–183</sup> These advancements have demonstrated effects such as increased drug release, enhanced cytotoxicity, and precise targeting of tumor cells in vitro.<sup>181–183</sup>

The combination of MTAs with other anticancer agents is widely recognized as the most applied strategy to enhance therapeutic effects, reduce toxicities, and overcome MDR. Now, combination therapies become standard clinical protocols. Several approaches have been explored to achieve synergistic effects and improve treatment outcomes. For instance, the combination of MTAs with receptor tyrosine kinase inhibitors (such as KX2-391),<sup>184</sup> histone deacetylase inhibitors,<sup>185</sup> DNA damage agents,<sup>186</sup> or topoisomerase inhibitors,<sup>187</sup> has been developed and has shown promising results in preclinical and clinical studies.<sup>188,189</sup>

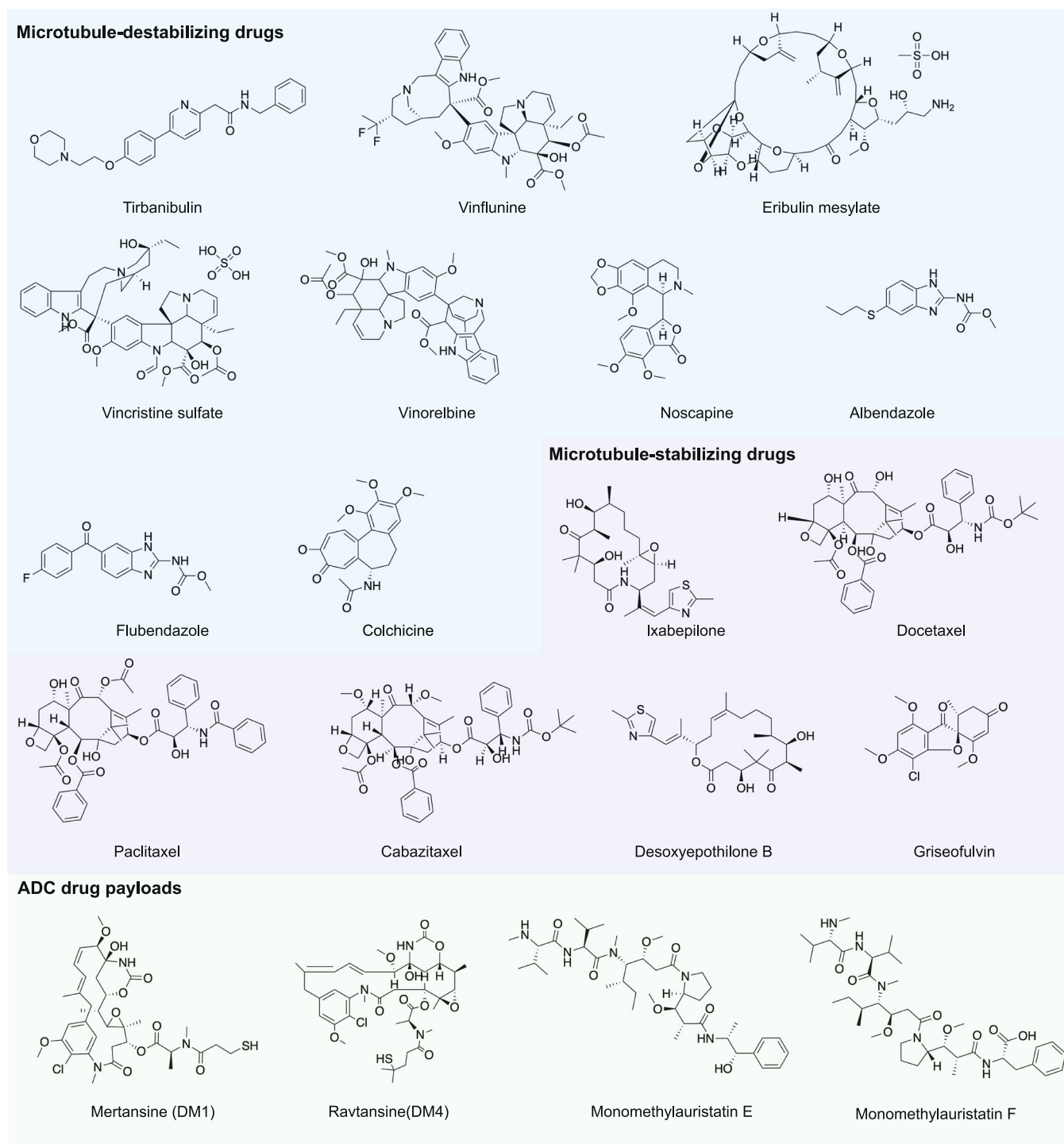
Notably, MT-ADCs have emerged as successful therapeutic options in clinical settings.<sup>174</sup> Out of the commercially introduced microtubule-targeting drugs, a specific subgroup of eight drugs stands out as antibody-conjugated formulations (Table 1). They have shown great efficacy in hematological tumors (e.g., Brentuximab vedotin [BV], Polatuzumab vedotin [PV], Belantamab mafodotin [Bm]) and solid tumors (e.g., Enfortumab vedotin, Tisotumab vedotin, Trastuzumab emtansine, Mirvetuximab soravtansine, Disitamab vedotin).

BV (Adcetris®) and PV (Polivy®) are effective for the treatment of hematological tumors, especially

TABLE 1 Tubulin-targeting ADCs and small-molecule drugs approved by FDA, EMA, or NMPA up to April 2023.

Drug	Brand name	Type	Mechanism	Condition	Time	Payload/site
Brentuximab vedotin	Adcetris®	ADC	Microtubule destabilizers	Lymphoma, Hodgkin lymphoma	2011	Monomethylauristatin E (MMAE)
Trastuzumab emtansine	Kadcyla®	ADC	Microtubule destabilizers	Cancer, breast metastatic	2013	Maytansinoid Mertansine (DM1)
Enfortumab vedotin	Padcev®	ADC	Microtubule destabilizers	Cancer, urothelial carcinoma	2019	Monomethylauristatin E (MMAE)
Polatuzumab vedotin	Polivy®	ADC	Microtubule destabilizers	Lymphoma, diffuse large B-cell	2019	Monomethylauristatin E (MMAE)
Belantamab mafodotin	Blenrep®	ADC	Microtubule destabilizers	Multiple myeloma	2020	Monomethylauristatin F (MMAF)
Tisotumab vedotin	Tivdak®	ADC	Microtubule destabilizers	Cancer, cervix	2021	Monomethylauristatin E (MMAE)
Disitamab vedotin	Aidixi®	ADC	Microtubule destabilizers	Cancer, stomach; urothelial	2021	Monomethylauristatin E (MMAE)
Mirvetuximab soravtansine	Elahere	ADC	Microtubule destabilizers	Cancer, ovarian	2022	Maytansinoid Ravtansine (DM4)
Vinblastine sulfate	Velban®	Small molecule	Microtubule destabilizers	Cancer, breast	1961	Vinca alkaloids site
Vincristine sulfate	Oncovin®	Small molecule	Microtubule destabilizers	Acute lymphocytic leukemia	1963	Vinca alkaloids site
Vindesine	Eldisine®	Small molecule	Microtubule destabilizers	Acute lymphoblastic leukemia	1979	Vinca alkaloids site
Vinorelbine	Navelbine®	Small molecule	Microtubule destabilizers	Cancer, lung (NSCLC)	1989	Vinca alkaloids site
Eribulin mesylate	Halaven®	Small molecule	Microtubule destabilizers	Cancer, breast metastatic	2010	Vinca alkaloids site
Vinflunine	Javlor®	Small molecule	Microtubule destabilizers	Cancer, lung (NSCLC/SCLC), prostate	2010	Vinca alkaloids site
Noscipine	Narcotussin®	Small molecule	Microtubule destabilizers	Antitussives, lymphoma, multiple myeloma	1963	Colchicine site
Albendazole	Albenza®	Small molecule	Microtubule destabilizers	Infection, helminthic	1996	Colchicine site
Colchicine	Colcrys®	Small molecule	Microtubule destabilizers	Gout, Familial Mediterranean fever	2002	Colchicine site
Flubendazole	Fluvermal®	Small molecule	Microtubule destabilizers	Filariasis	2006	Colchicine site
Tirbanibulin	Klisyri®	Small molecule	Microtubule destabilizers	Keratosis, actinic	2021	Colchicine site
Griseofulvin	Fulvicin®	Small molecule	Microtubule stabilizers	Infection, fungal	1958	Taxane site
Paclitaxel	Taxol®	Small molecule	Microtubule stabilizers	Cancer, breast	1992	Taxane site
Docetaxel	Taxotere®	Small molecule	Microtubule stabilizers	Cancer, breast	1996	Taxane site
Ixabepilone	Ixempra®	Small molecule	Microtubule stabilizers	Cancer, breast metastatic	2007	Taxane site
Cabazitaxel	Jevtana®	Small molecule	Microtubule stabilizers	Cancer, prostate metastatic	2010	Taxane site
Uftelone	Youtidi®	Small molecule	Microtubule stabilizers	Cancer, breast metastatic	2021	Taxane site

Abbreviations: ADCs, antibody–drug conjugates; EMA European Medicine Agency; FDA, Food and Drug Administration; NMPA National Medical Products Administration; NSCLC, non-small-cell lung cancer; SCLC small-cell lung cancer.



**FIGURE 5** Chemical structures of a set of clinical small-molecule and ADC drugs targeting microtubules. ADC, antibody–drug conjugate.

lymphoma. They use MMAE as the payload, targeting CD30-positive lymphocytes and CD79b in malignant lymphoma, respectively.<sup>190,191</sup> Through disruption of mitosis, cell cycle arrest, and induction of apoptosis, BV and PV demonstrate direct cytotoxicity and exhibit antitumor effects through various mechanisms. BV received FDA approval in 2011 for Hodgkin lymphoma

and systemic anaplastic large cell lymphoma after treatment failure,<sup>192</sup> while PV gained approval in 2019 for refractory or relapsed diffuse large B-cell lymphoma in combination with bendamustine and rituximab.<sup>193</sup> Besides, BM employs MMAF as the payload to target BCMA in multiple myeloma.<sup>194</sup> Following binding, BM internalizes and releases MMAF, resulting in the

TABLE 2 Clinical trials of MTA-based antibody-drug conjugates.

ADCs	Status	Indication	Target	mAb	Payload	Reference
IMGN901	Phase 2	Hematologic-blood cancer, sarcoma, neuroblastoma, SCLC, MM.	CD56	huN901	DM1 <sup>a</sup>	NCT02420873
IMGN529	Phase 1	Lymphoma, NHL, CLL.	CD37	K7153A	DM1	NCT01534715
IMGN289	Phase 1	EGFR-positive solid tumors.	EGFR	J2898A	DM1	NCT01963715
AMG 172	Phase 1	RCC.	CD27L	Anti-CD27L	DM1	NCT01497821
AMG 595	Phase 1	Glioma, AA.	EGFRvIII	Anti-EGFRvIII	DM1	NCT01475006
BAY94-9343	Phase 2	NSCLC, mesothelioma, solid tumors.	Mesothelin	Anti-mesothelin	DM4 <sup>a</sup>	NCT03926143
SAR3419	Phase 2	LBCL.	CD19	huB4	DM4	NCT01472887
BT062	Phase 1/2	MM.	CD138	nBT062	DM4	NCT01001442
SAR428926	Phase 1	Solid tumors.	LAMP-1	853K3	DM4	NCT02575781
SAR566658	Phase 1	Solid tumors.	CA6	huDS6	DM4	NCT01156870
CDX-011	Phase 2	Breast cancer, melanoma, osteosarcoma, NSCLC.	GPNMB	CR-011	MMAE <sup>b</sup>	NCT01997333
DCDT2980S	Phase 2	NHL, DLBCL.	CD22	Anti-CD22	MMAE	NCT01691898
DCDS4501A	Phase 3	NHL, DLBCL, CLL.	CD79b	Anti-CD79b	MMAE	NCT03274492
DNIB0600A	Phase 2	Ovarian cancer, NSCLC.	NaPi2b	Anti-NaPi2b	MMAE	NCT01363947
PSMA ADC	Phase 2	Prostate cancer.	PSMA	Anti-PSMA	MMAE	NCT01695044
DMOT-4039A	Phase 1	Pancreatic cancer, ovarian cancer.	MSLN	MMOT-0530A	MMAE	NCT01469793
AGS-22M6E	Phase 1	Genitourinary cancer, solid tumors.	Nectin-4	Anti-Nectin-4	MMAE	NCT01409135
SGN-LIV1A	Phase 1	Breast cancer.	LIV1	Anti-LIV1	MMAE	NCT01969643
HuMax-TF-ADC	Phase 1/2	Solid tumors.	Tissue factor	TF-011	MMAE	NCT02001623
AGS-15E	Phase 1	Urothelial.	SLITRK6	AGS15	MMAE	NCT01963052
DLYE-5953A	Phase 1	breast cancer, NSCLC, solid tumors.	LY6E	Anti-Ly6E	MMAE	NCT02092792
DEDN-6526A	Phase 1	Melanoma.	EDNRB	Anti-EDNRB	MMAE	NCT01522664
AGS-67E	Phase 1	Hematologic-blood cancer, AML.	CD37	Anti-CD37	MMAE	NCT02175433
AGS-16M8F	Phase 1	RCC.	ENPP3	Anti-AGS-16	MMAF <sup>b</sup>	NCT01114230
ABT-414	Phase 3	Glioblastoma multiforme, NSCLC, brain cancer, solid tumors.	EGFRvIII	ABT-806	MMAF	NCT02573324
ARX-788	Phase 2	Breast cancer.	Her2	Anti-Her2	MMAF	NCT04829604
GSK-2857916	Phase 2	MM.	BCMA	J6M0	MMAF	NCT03525678
MEDI4276	Phase 1	Breast cancer, gastric cancer.	HER2	39S	AZ13599185 <sup>c</sup>	NCT02576548
M1231	Phase 1	Metastatic solid tumors, esophageal cancer, NSCLC.	EGFR	Anti-EGFR	Hemiasterlin <sup>d</sup>	NCT04695847

Note: Payload based on <sup>a</sup>maytansinoid; <sup>b</sup>dolastatin; <sup>c</sup>tubulysin; <sup>d</sup>hemiasterlin.

Abbreviations: AA, anaplastic astrocytomas; ADCs, antibody–drug conjugates; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B cell lymphoma; LBCL, large B-cell lymphoma; mAb, monoclonal antibody; MM, multiple myeloma; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin F; MTA, microtubule-targeting agent; NHL, non-Hodgkin's lymphoma; NSCLC, non-small-cell lung cancer; PSMA, prostate-specific membrane antigen; RCC, renal cell carcinoma; SCLC, small-cell lung cancer.

Source: <https://clinicaltrials.gov>.

disruption of the intracellular microtubule network, cell cycle arrest, and apoptosis. BM demonstrates potent antitumor activity through MMAF-induced apoptosis, antibody-dependent cytotoxicity, and antibody-dependent cellular phagocytosis.<sup>195</sup> In 2020, it received FDA approval for the treatment of relapsed or refractory multiple myeloma.<sup>194</sup>

Furthermore, MT-ADCs have demonstrated promising results in the treatment of various solid tumors, including urothelial cancer, cervical cancer, breast cancer, and epithelial ovarian cancer.<sup>138</sup> Enfortumab vedotin (Padcev<sup>®</sup>) is an FDA-approved ADC comprising a human mAb (AGS-22C3) that selectively targets nectin-4, a transmembrane protein highly expressed in urothelial carcinoma.<sup>196</sup> The antibody is conjugated to the cytotoxic agent MMAE through a protease-cleavable linker. It received accelerated approval in December 2019 for the treatment of locally advanced or metastatic urothelial cancer.<sup>197</sup> Tisotumab vedotin (Tivdak<sup>®</sup>) is a recently approved ADC containing a fully humanized antibody against tissue factor, which is overexpressed in various solid tumors. The antibody is linked to MMAE via a cleavable linker.<sup>147</sup> FDA approval was granted in September 2021 for recurrent or metastatic cervical cancer.<sup>198</sup> Ado-trastuzumab emtansine (Kadcyla<sup>®</sup>) is an ADC targeting HER2-positive breast cancer.<sup>199</sup> It consists of a humanized antibody linked to DM1, a potent cytotoxic agent, through a noncleavable linker. Kadcyla<sup>®</sup> was first approved in 2013 for HER2-positive metastatic breast cancer and subsequently gained expanded approval in May 2019 for adjuvant treatment of HER2-positive early breast cancer.<sup>92,199</sup> Mirvetuximab soravtansine-gynx (IMGN853) is an ADC designed to target folate receptor alpha (FR $\alpha$ ), which is overexpressed in various epithelial tumors. It incorporates a cleavable disulfide linker that connects the antibody (M9346A) to the genotoxic compound DM4.<sup>200</sup> In November 2022, IMGN853 received accelerated approval for the treatment of FR $\alpha$ -positive, platinum-resistant epithelial ovarian, fallopian tube, or primary peritoneal cancer based on positive outcomes from the phase 3 SORAYA trial (NCT04296890).<sup>200,201</sup> Moreover, a number of MT-ADC candidates are currently undergoing active clinical trials. Their corresponding status, therapeutic indications, targets, mAbs, payloads, and NCT numbers are summarized in Table 2. Data were generated from the ClinicalTrials.gov. The development of microtubule-targeting drugs and MT-ADCs provides more promising options for various types of cancers. However, further studies are needed to develop ADCs with tumor-restricted mAbs and effective linkers for successful clinical application.

## 9 | CONCLUSIONS AND PERSPECTIVES

Recent breakthroughs in the field of tubulin structural biology have led to compelling discoveries of novel tubulin-targeting sites. The exploration of the seven binding sites within microtubules, along with the understanding of the three primary strategies employed by MTAs (stabilization, destabilization, and degradation) have not only fostered drug discovery endeavors targeted at effective cancer treatment but also provided more promising options for therapeutic intervention in various disease contexts.

Notably, tubulin encompasses seven distinct binding sites, with five primarily localized on  $\beta$ -tubulin. However, the presence of the E-site within  $\beta$ -subunits, which possesses GTP hydrolysis ability, renders them vulnerable to drug resistance mediated by  $\beta$ -tubulin mutations and heightened  $\beta$ III-tubulin expression. Consequently, targeting  $\alpha$ -subunits MTAs offers a promising strategy to surmount drug resistance. Furthermore, it is important to mention that conventional tubulin inhibitors necessitate high systemic drug exposures, increasing the likelihood of undesirable off-target effects. The microtubule-targeting degradation strategy shows promise as a third novel class of tubulin inhibitors. As we look to the future, further investigations into microtubule sites and their functional roles will continue to inspire discoveries for cancer treatment.

### AUTHOR CONTRIBUTIONS

**Xingyu Wang:** Investigation (lead); visualization (lead); writing—original draft (lead). **Benoît Gigant:** Writing—review and editing (equal). **Xi Zheng:** Funding acquisition (supporting); project administration (supporting); validation (supporting). **Qiang Chen:** Conceptualization (lead); funding acquisition (lead); project administration (lead); writing—review and editing (lead). All authors have read and approved the final manuscript.

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**CONFLICT OF INTEREST STATEMENT**

The authors declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

Not applicable.

**ETHICS STATEMENT**

Not applicable.

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