Targeting Microtubules for Cancer Chemotherapy

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Abstract: Chemical compounds that interfere with microtubules such as the vinca alkaloids and taxanes are important chemotherapeutic agents for the treatment of cancer. As our knowledge of microtubule-targeting drugs increases, we realize that the mechanism underlying the anti-cancer activity of these agents may mainly lie in their inhibitory effects on spindle microtubule dynamics, rather than in their effects on microtubule polymer mass. There is increasing evidence showing that even minor alteration of microtubule dynamics can engage the spindle checkpoint, arresting cell cycle progression at mitosis and eventually leading to apoptotic cell death. The effectiveness of microtubule-targeting drugs for cancer therapy has been impaired by various side effects, notably neurological and hematological toxicities. Drug resistance is another notorious factor that thwarts the effectiveness of these agents, as with many other cancer chemotherapeutics. Several new microtubule-targeting agents have shown potent activity against the proliferation of various cancer cells, including cells that display resistance to the existing microtubule-targeting drugs. Continued investigation of the mechanisms of action of microtubule-targeting drugs, development and discovery of new drugs, and exploring new treatment strategies that reduce side effects and circumvent drug resistance may provide more effective therapeutic options for cancer patients.

Key Words: Cancer chemotherapy, microtubule, tubulin, microtubule-targeting drug, microtubule dynamics, angiogenesis, side effect, drug resistance.

INTRODUCTION

During eukaryotic cell division, in order for each daughter cell to inherit one and only one copy of each chromosome, the mother cell must replicate its chromosomes exactly once in the synthetic phase, and then must separate the replicated chromosomes evenly at the end of the mitotic phase to the two daughter cells. Defects in the coordination of chromosome replication and chromosome segregation can have severe consequences leading to genetic instability and aneuploidy, and eventually fostering tumor malignancy [1-3].

To ensure faithful transmission of chromosomes during cell division, eukaryotic cells have evolved cellular regulatory mechanisms termed cell cycle checkpoints [4]. The checkpoints prevent or delay cell cycle progression if certain cellular processes or proteins are disrupted, to gain time to repair the damage before cell division occurs. When the damage is irreparable, the cell undergoes apoptosis through the triggering of specific biochemical pathways [5]. However, cancer cells often harbor defective cell cycle checkpoints allowing for uncontrolled cell proliferation, even when cell division does not occur properly. Therefore, effective cancer treatment can be achieved by drugs that target certain processes or proteins impinging on the cell cycle machinery [6]. In particular, chemical compounds that target microtubules and inhibit the normal function of the mitotic spindle, have proven to be one of the best classes of cancer chemotherapeutic drugs available to date [7]. In this article, we will focus on the underlying mechanisms by which compounds that target microtubules exert their chemotherapeutic effects, and how the side effects and drug resistance hinder their clinical applicability. In addition, we will discuss the therapeutic potential of several recently discovered microtubule-targeting agents.

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MICROTUBULES: A VALIDATED TARGET FOR ANTI-CANCER DRUGS

Microtubules, together with actin filaments and intermediate filaments, are the major cytoskeletal components in all eukaryotic cells. Microtubules are crucial for the maintenance of cell shape and polarity, intracellular transport of vesicles and organelles, and beating of cilia and flagella. Moreover, during cell division, microtubules form the mitotic spindle, which is the key machinery driving the alignment of replicated chromosomes to the equatorial plane and mediating the subsequent segregation of chromosomes to the two daughter cells [8]. The critical role that microtubules play in cell division makes them a very suitable target for the development of chemotherapeutic drugs against the rapidly dividing cancer cells. The effectiveness of microtubule-targeting drugs has been validated by the successful use of several vinca alkaloids and taxanes for the treatment of a wide variety of human cancers. Their clinical success has prompted a worldwide search for compounds with similar mechanisms of action but improved characteristics. This search has resulted in the discovery of a number of novel microtubule-targeting drugs, the majority of which are natural products. Their natural sources and chemical structures are remarkably diverse, making

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microtubules the only target for which such a diverse group of anti-cancer agents has been identified.

MICROTUBULE STRUCTURE AND DYNAMICS

Microtubules are long, hollow, cylindrical protein polymers composed of α/β-tubulin heterodimers. The tubulin heterodimers assemble in a head-to-tail fashion to form the protofilaments. The protofilaments (13 in most cells) associate longitudinally to form a sheet, which then closes up to form a microtubule with a diameter of 25 nm [9]. α-Tubulin and β-tubulin are about 50% identical to each other at the amino acid level, and each has a molecular weight of about 50 kDa [10]. The tandem polymerization of α/β-tubulin heterodimers results in an inherent heterogeneity between the two ends of the microtubule; α-tubulin is exposed at the less dynamic end (called minus end), and β-tubulin is exposed at the more dynamic end (called plus end) [11-14]. Within a cell, microtubules are anchored by their minus ends at the microtubule-organizing center, disposing their plus ends to the cell periphery [15, 16].

Microtubules are a component of the cytoskeleton. This description is a bit misleading however, since it suggests microtubules as static structures. Instead, microtubules are intrinsically dynamic polymers that grow and shorten by the reversible non-covalent association and disassociation of α/β-tubulin heterodimers at their two ends [17]. The α-tubulin and β-tubulin subunits each has a GTP binding site, referred to as the nonexchangeable site in α-tubulin and the exchangeable site in β-tubulin [18, 19]. During the association of α/β-tubulin heterodimer to the ends of microtubules, GTP in β-tubulin is hydrolyzed to GDP and the resulting GDP in β-tubulin is unable to exchange. When the microtubule depolymerizes, the α/β-tubulin heterodimers are released and the GDP in β-tubulin is now able to exchange to GTP. In contrast, although α-tubulin also binds a GTP molecule, the GTP is bound at the nonexchangeable site and not able to be hydrolyzed to GDP during the addition of tubulin heterodimer to the ends of microtubules [20, 21].

The unique GTP binding and hydrolysis property on α-tubulin and β-tubulin gives microtubules two unusual dynamic properties, dynamic instability [22], and treadmilling [23-25]. Dynamic instability refers to the stochastic switching of a microtubule between episodes of growth and shortening, and treadmilling describes the net growth of a microtubule at one end and net shortening at the other end.

The dynamic properties of microtubules are crucial for many cellular functions, especially for proper spindle function during mitosis [26-28]. In fact, spindle microtubules are 10-100 fold more dynamic than interphase microtubules to enable efficient capturing, alignment and segregation of chromosomes [26, 28]. As a result, suppression of microtubule dynamics impairs successful chromosome attachment and movement, and subsequently blocks cell cycle progression at mitosis through engaging the spindle checkpoint. The spindle checkpoint monitors both the proper attachment of chromosomes at their kinetochores to spindle microtubules, and the tension exerted across paired kinetochores by the kinetochore microtubules [29]. A growing body of evidence has shown that even subtle alteration of microtubule dynamics by microtubule-targeting drugs can cause improper attachment of chromosomes and impair the kinetochore tension, which in turn signal the spindle checkpoint to prevent anaphase onset and chromosome segregation [30-33]. The cell eventually exits mitosis aberrantly and undergoes apoptosis [34-36].

MECHANISMS OF ACTION OF MICROTUBULE-TARGETING DRUGS

Chemical compounds targeting microtubules exert their inhibitory effects on cell proliferation primarily by blocking mitosis, which requires an exquisite control of microtubule dynamics. Microtubule-targeting drugs are therefore also frequently referred to as a group of anti-mitotic drugs, and their actions on microtubule stability and dynamic parameters differ from each other. At relatively high concentrations, these drugs either inhibit microtubule polymerization, destabilizing microtubules and decreasing microtubule polymer mass, or promote microtubule polymerization, stabilizing microtubules and increasing the polymer mass [37, 38]. Based on these dramatic effects, microtubule-targeting agents are divided into two traditional categories: microtubule-destabilizing agents such as the vinca alkaloids (vinblastine, vincristine, etc.) and colchicine, and microtubule-stabilizing agents such as the taxanes (paclitaxel and docetaxel) (Fig. **1**). The anti-mitotic and anti-cancer activities of microtubule-targeting drugs have been thought to result from their actions on microtubule stability and polymer mass.

However, at low but clinically relevant concentrations, both microtubule-stabilizing and -destabilizing drugs potently suppress microtubule dynamics without affecting microtubule polymer mass; however, they retain their ability to block mitotic progression and induce apoptosis [30, 32-34, 39]. Thus, it is reasonable to argue that the anti-mitotic and anti-cancer activities of microtubule-targeting agents may be largely due to their suppression of microtubule dynamics, instead of their effects on microtubule polymer mass, as previously thought.

Currently there are three well established drug binding sites on β-tubulin, the vinca domain, the taxane site and the colchicine site [40]. The vinca domain is located adjacent to the exchangeable GTP binding site in β-tubulin at the plus end interface [41, 42]. The taxane site resides in a deep hydrophobic pocket at the lateral interface between adjacent protofilaments, within the lumen of the microtubule [43-47]. Finally, the colchicine site is located at the intra-dimer interface between β-tubulin and α-tubulin [48-50]. In addition to these three well characterized drug-binding sites, there is another binding site on _-tubulin that is occupied by laulimalide (Fig. **1**), a microtubule-stabilizing drug isolated from the marine sponge *Cacospongia mycofijiensis*; however, the exact location of this binding site remains elusive [51, 52]. This is the first microtubule-stabilizing drug shown to bind at a site distinct from the taxane site on tubulin.

Agents That Bind to the Vinca Domain

The vinca alkaloids, vinblastine and vincristine, were originally extracted over 40 years ago from the leaves of the Madagascar periwinkle, formerly known as *Vinca rosea* but reclassified as *Catharanthus roseus*. These compounds were

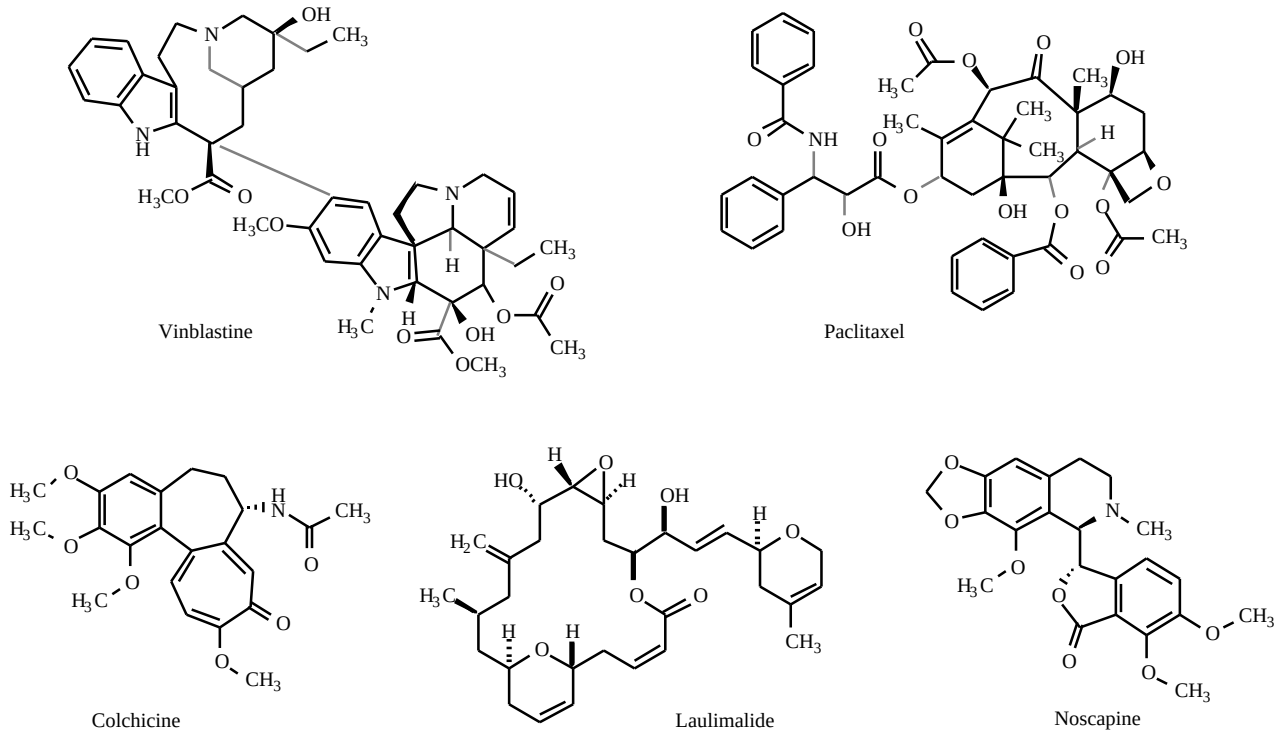

Fig. (1). Chemical structures of vinblastine, paclitaxel, colchicine, laulimalide, and noscapine.

initially studied because of the hypoglycemic activities, but were discovered to have anti-leukemic effects and cause bone marrow suppression [53, 54]. Since then they have been widely used clinically for the treatment of leukemias, lymphomas, and some solid malignancies. The clinical success of vinblastine and vincristine together with the elucidation of their mechanism of action on cellular microtubules, have facilitated the development of several semi-synthetic derivatives notably vindesine, vinorelbine and vinflunine, which are now used in the clinic for the treatment of cancer [55].

The vinca alkaloids bind to both tubulin and microtubules, and their actions are highly dependent on the drug concentration [56]. At relatively high concentrations, they cause microtubule depolymerization, dissolve spindle microtubules and arrest cells at mitosis, and at even higher concentrations (μM), they induce the aggregation of tubulin into paracrystalline arrays [41, 57, 58]. In contrast, at low concentrations, the vinca alkaloids suppress microtubule dynamics without depolymerizing spindle microtubules, but remain able to arrest mitosis and induce apoptosis [39]. The mechanisms of action of the vinca alkaloids were unclear when they were initially used in the treatment of leukemia. In a case report published in 1968, where high doses of intrathecal vincristine sulfate caused the death of a leukemia patient, post-mortem staining of the spinal cord revealed the presence of argentophilic strands and rhombohedral crystals [59]. The authors suggested that the mechanism by which vincristine produced these neuronal changes might lie in the decrease in DNA synthesis or RNA synthesis. Since then, our understanding of the mechanisms of action of these drugs has significantly deepened.

Several other naturally occurring microtubule-targeting compounds that bind to the vinca domain on β-tubulin have been identified, including halichondrins (isolated from the marine sponges *Halichondria okadai*, *Axinella sp.*, *Phakellia carteri*, and *Lissodendoryx sp.*) [60], hemiasterlins (isolated from the marine sponge *Cymbastela sp.*) [61, 62], spongistatin (isolated from the marine sponge *Spirastrella spinispirulifera*) [63], dolastatins (isolated from the sea hare *Dolabella auricularia*) [64], and cryptophycins (isolated from the blue-green algae *Nostoc sp.*) [65]. All of these compounds block mitotic progression and induce apoptosis in cancer cells, and they are currently at various stages of clinical development for the treatment of cancer [55, 66].

Agents That Bind to the Taxane Site

Isolated originally in the 1960s from the bark of the Pacific yew *Taxus brevifolia*, paclitaxel did not receive much attention until it was discovered to possess microtubule-stabilizing activity [67]. This drug is now in widespread use for the treatment of breast, ovarian, prostate and non-small-cell lung cancer, as well as Kaposi's sarcoma. Its semi-synthetic analog, docetaxel, is synthesized from a precursor isolated from the needles of the European yew *Taxus baccata*. Docetaxel is more water-soluble than paclitaxel, and is also more active than paclitaxel against cancer cell proliferation, and is now used clinically for the treatment of breast, prostate and non-small-cell lung cancer [68].

At relatively high concentrations, the taxanes promote microtubule polymerization and stabilize microtubules [67, 68]. In addition, high concentrations of taxanes produce microtubule bundles, although the biological significance of

this phenomenon remains unclear [67, 69-71]. At lower concentrations, similar to the vinca alkaloids, the taxanes suppress microtubule dynamics without affecting microtubule polymer mass, but retain their capability of inducing mitotic arrest and subsequent apoptotic cell death [30, 32-34].

The success of paclitaxel and docetaxel in cancer therapy has inspired the discovery of new microtubule-targeting agents that bind to the taxane site and have similar mechanisms of action, including discodermolide (isolated from the marine sponge *Discodermia dissoluta*) [72-74], epothilones (isolated from the myxobacterium *Sorangium cellulosum*) [75], eleutherobin (isolated from the marine soft coral *Eleutherobia sp.*) [76], and sarcodictyins (isolated from the Mediterranean stoloniferan coral *Sarcodictyon roseum*) [77]. These agents block mitosis and induce cell death downstream of their anti-microtubule effects, and their cancer chemotherapeutic potential is also under clinical investigations [78].

Agents That Bind to the Colchicine Site

Drugs binding to the colchicine site typically induce micro-tubule depolymerization at high concentrations, similar to the vinca alkaloids, and they suppress microtubule dynamics at low concentrations similar to both the vinca alkaloids and taxanes [79]. Isolated from the meadow saffron *Colchicum autumnale*, colchicine is one of the earliest microtubule-targeting agents identified, and its mechanism of action has been extensively investigated. In fact, tubulin was first purified based on its high-affinity binding with colchicine and was referred to as a "colchicine-binding protein" [18, 80, 81]. The clinical development of colchicine for cancer treatment has not been successful to date probably because of the high toxicity to normal tissues.

However, development of agents binding to the colchicine site as potential cancer chemotherapeutic drugs has recently gained intense interest. Combretastatins, for example, are isolated from the South African willow *Combretum caffrum*, bind to tubulin, and exhibit potent anti-cancer activity by inhibiting cell cycle progression at mitosis and triggering apoptosis [82, 83]. Similarly, 2-methoxy-estradiol (2ME2), a naturally occurring metabolite of the hormone estradiol, appears to bind to the colchicine site and inhibits tumor growth [84]. Both the combretastatins and 2ME2 are now in clinical trials as cancer chemotherapeutic agents.

ANTI-ANGIOGENETIC ACTIVITY OF MICROTUBULE-TARGETING DRUGS

Besides their ability to inhibit tumor cell proliferation, microtubule-targeting drugs have recently been shown to possess varying degrees of activity against new blood vessel formation (angiogenesis), including the vinca domain agents such as vinblastine, vincristine, and vinflunine [85-88], the taxane site agents paclitaxel and docetaxel [85, 89-92], and the colchicine site agents combretastatins and 2ME2 [84, 93-95]. It remains largely unknown as to how the microtubule-targeting agents exert their anti-angiogenetic activity. It has been suggested that the varying anti-angiogenetic activity of these drugs may be related with their distinct tubulin binding mechanisms. Recently, it has been demonstrated that the inhibition of HIF-1α by 2ME2 is downstream of its anti-microtubule effect [84]. It will be interesting to investigate whether this applies to other microtubule-targeting drugs.

NOSCAPINE AND ITS DERIVATIVES: POTENTIAL ANTI-CANCER AGENTS

Noscapine (Fig. **1**) and its derivatives represent another group of microtubule-targeting agents that possess potent anti-cancer activity [96, 97]. Noscapine is a phthalideisoquinoline alkaloid that occurs in abundance in the opium plant, *Papaver somniferum*. This agent has been used medicinally as a cough suppressant in humans and in experimental animals, but the mechanism for its anti-tussive action remains largely unknown. Noscapine was initially discovered to possess anti-mitotic properties by a semi-rational cell-based screen of naturally occurring agents that are structurally similar to colchicine and colchicine analogs. This agent binds to tubulin stoichiometrically, but its binding site remains unclear [96]. Unlike the other microtubule-targeting drugs, noscapine does not appear to significantly change the microtubule polymer mass even at high concentrations [31]. Instead, this compound suppresses microtubule dynamics by increasing the time that microtubules spend in an attenuated (pause) state when neither microtubule growth nor shortening is detectable [31, 98]. The noscapine-induced suppression of microtubule dynamics, even though subtle, is sufficient to interfere with the proper attachment of chromosomes to kinetochore microtubules and to suppress the tension across paired kinetochores. Consequently, the spindle checkpoint is engaged to block cells at a metaphase-like state, similar to the actions of low concentrations of the vinca alkaloids and taxanes, at which chromosomes do not complete congression to the equatorial plane [31].

Noscapine effectively inhibits the progression of various cancer types both in cultured cells and in animal models with no obvious side effects [96-101]. This agent is currently undergoing phase I/II clinical trials at the University of Southern California for patients with low grade non-Hodgkin's lymphoma or chronic lymphocytic leukemia refractory to chemotherapy. Several derivatives of noscapine have been recently developed, possessing more potent anti-mitotic and anti-cancer activities in preclinical models, in comparison to noscapine [97]. Noscapine and its derivatives have also demonstrated anti-proliferative activity in cancer cells that are resistant to the conventional microtubule-targeting drugs [97, 102, 103]. Their potential as cancer chemotherapeutic agents merits thorough investigation.

LIMITING FACTORS FOR THE CLINICAL USE OF MICROTUBULE-TARGETING DRUGS

Side Effects

Adverse side effects affect the applicability of microtubule-targeting drugs in cancer therapy. Neurological and hematological side effects are the principal and often dose-limiting toxicities, but several other side effects also occur during the treatment with each individual drug [104-106]. Peripheral neuropathy is the most frequently encountered neurotoxicity typified by the loss of deep tendon reflex at the

ankle, numbness, and motor weakness. Cranial neuropathy may also occur after therapy with the vinca alkaloids and taxanes, resulting in jaw pain and vocal cord dysfunction. Autonomic neuropathy is another common symptom causing constipation, abdominal cramping, and urinary retention. Other central neurotoxicities include headache, dizziness, and mental depression. Neurotoxicity usually occurs after prolonged treatment with microtubule-targeting drugs, and is at least in part due to the inhibition of axonal microtubules, which are crucial for axonal transport in neurons [107]. Hematological toxicity is another major side effect caused by microtubule-targeting drugs, and is also frequently referred to as myelosuppression [104, 106]. Severe neutropenia, in particular, occurs early after treatment. The cause of myelosuppression may result from the inhibition of the rapidly dividing hematopoietic cells. In addition to the neurological and hematological toxicities, microtubule-targeting drugs may cause nausea, vomiting and diarrhea, but severe manifestations are uncommon.

Drug Resistance

Drug resistance, either intrinsic or acquired, is the major factor hampering the clinical applicability of microtubule-targeting agents. The molecular basis underlying drug resistance is an area of intensive investigation. One of the most extensively studied mechanisms involves the overexpression of drug efflux pumps, such as P-glycoprotein and multidrug resistance-associated protein 1 (MRP1), which are a family of ATP-dependent transporter proteins located in the cell membrane [108]. These drug efflux transporters can efficiently pump anti-cancer drugs out of the cells, thereby lowering intracellular drug concentrations. This is probably the most efficient mechanism for cancer cells to achieve resistance to many structurally and mechanistically unrelated drugs, a phenomenon known as multidrug-resistance (MDR) [108]. Drugs that are affected by this mechanism include the microtubule-targeting drugs vinca alkaloids and taxanes, as well as a broad range of other classes of anti-cancer agents [109].

Alterations in tubulin/microtubules, represents another major mechanism underlying the resistance of cancer cells to microtubule-targeting drugs. These mechanisms include acquired tubulin mutations at the drug binding sites [110-113], altered microtubule dynamics [114], altered expression of different tubulin isotypes [115], and changes in microtubule-regulatory proteins [116, 117].

It is worth pointing out, however, that the clinical significance of the drug efflux transporter-mediated mechanism and the microtubule-related mechanisms remains to be further defined. In addition, besides the above mentioned mechanisms, cancer cells may employ the dysfunctions in apoptosis pathways, defects in cell cycle checkpoints, and altered drug metabolisms, together with many other unknown mechanisms to evade the cytotoxic effects of microtubule-targeting chemotherapeutics.

CONCLUSIONS AND FUTURE DEVELOPMENTS

Given the wide clinical use of the vinca alkaloids and taxanes, it is reasonable to argue that microtubules represent the best target to date for cancer chemotherapy and will remain a promising target for new chemotherapeutic agents. Our knowledge of the mechanisms of action of microtubule-targeting agents has greatly evolved over the past years. We now appreciate that the chemotherapeutic actions of these agents may mainly rely on the suppression of microtubule dynamics, instead of their effects on microtubule polymer mass. In addition, chemical compounds that suppress microtubule dynamics without affecting microtubule polymer mass, such as noscapine, are expected to display reduced toxicity to normal tissues while retaining their anti-cancer activity. Strategies exploiting synergistic drug combinations have also shown a great potential in enhancing the anti-cancer activity of the conventional microtubule-targeting anti-cancer drugs. Microtubule-targeting drugs may be effectively used in combination with: 1) other microtubule-targeting drugs; 2) other classes of cancer chemotherapeutic agents; or 3) other treatment options such as immunotherapy. Nature has already provided us the vinca alkaloids, taxanes, and a number of other microtubule-targeting agents useful for cancer chemotherapy. Stay tuned. Many more remain to be discovered.

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