Chapter 1. Overview of microtubule interactive agents

1.1 Introduction to tubulin and microtubules

Microtubules are essential polymers in the cytoplasm of eukaryotic cells and key components of the cytoskeleton.[1] They play crucial roles in the development and maintenance of cell shape, in cell transport and motility, in cell signaling, and in cell division and mitosis. Microtubules are long, hollow, tube-shaped protein aggregates, which are composed of α-tubulin and β-tubulin heterodimers. Each microtubule is formed by the parallel association of 13 linear protofilaments formed by tubulin heterodimers arranged head-to-tail. A microtubule tube is 24 nm in diameter and can be many μm long.

The assembly of tubulin to microtubules is through the nucleation-elongation pathway. In the nucleation phase, the tubulin heterodimers first assemble to form oligomers, which further aggregate to form a microtubule nucleus as small sheets of protofilaments.[2] In the next phase, tubulin adds rapidly to both ends of the nucleus, leading to the elongation, and then the sheets close up to form microtubules. In living cells, γ-tubulin that is localized at microtubule-organizing centers (MTOCs) forms ring structures, serving as templates for tubulin heterodimers to make small protofilament sheets.[3,4]

The two ends of microtubules are not equivalent either structurally or kinetically.[5] One end, called the plus end, is crowned by β-tubulin, whereas the other end with

α-tubulin facing outside is called the minus end. The plus end grows more rapidly and extensively than the minus end. *In vivo*, the minus end of the microtubule is associated with the centrosome, whereas the plus end is peripheral.

The functional diversity of microtubules relies on their high dynamics. Microtubules can switch between slow growing and fast shortening phases, which is called dynamic instability.[6] The transition from growing to shortening phases is referred to as catastrophe, and the opposite transition is called rescue. The dynamic instability of microtubules can be characterized by four factors: the growing rate, the shortening rate, the catastrophe frequency, and the rescue frequency. And it is highly related to the binding and hydrolysis of GTP by tubulin subunits. Generally, each tubulin monomer binds one GTP molecule. The GTP bound to α-tubulin is not exchangeable and cannot be hydrolyzed, while the one bound to β-tubulin is exchangeable.[7] At the time that the GTP-bound tubulin heterodimer adds to microtubule ends or shortly after that, GTP on β-tubulin is hydrolyzed to GDP, which provides energy for the assembly of tubulin subunits. The dynamic instability of microtubules can be explained by the GTP cap model.[5] In this model, the microtubule body made of GDP-tubulin subunits is believed to be unstable and its structure can be stabilized by a terminal cap composed of GTP-tubulin subunits. Once the GTP cap is gone, the microtubule depolymerizes rapidly. This is related to the conformational change of the tubulin subunit caused by hydrolysis of GTP to GDP. The GTP-bound tubulins assemble to form protofilaments with a straight conformation. When GTP is hydrolyzed

to GDP, the conformation of the tubulin subunit changes to a curved form, so that the tubulin locked in the microtubule core is conformationally constrained.[6a] When the GTP cap is present, the microtubule end is stabilized and the constrained tubulin subunits are locked and remain relatively stable. So the terminal GTP cap serves as a stabilizer of the GDP-containing microtubule body. Once the cap is lost, the curved-form GDP-tubulin subunits are exposed and dissociate from microtubules rapidly due to loss of the conformational constraint.

Besides dynamic instability, microtubules have a second dynamic behavior, called treadmilling,[8] which involves a net flow of tubulin subunits from the plus end to the minus end without a significant change in microtubule length. This results from a net growth at one end of the microtubule and a balanced net shortening at the other end, which is created by a difference in the critical subunit concentrations at opposite ends. Treadmilling might be particularly important in mitosis.[9]

Dynamic instability and treadmilling are compatible and related in the cell. A change from dynamic instability to treadmilling was observed in fibroblast cytoplasts lacking centrosomes. A particular microtubule population can primarily show dynamic instability behavior, treadmilling behavior, or mixed behaviors. The mechanism is not well understood. But the degree to which a microtubule population shows one or the other behavior probably depends on the tubulin isotype composition, the degree of post-translational modification of tubulin, and the interaction of microtubules with other

cellular factors, especially regulatory proteins.[10]

The assembly and stability of microtubules are regulated by the nucleotide state of tubulin. Further modifications can be achieved in the cell by interacting with cellular factors, which stabilize or destabilize microtubules through the expression of different tubulin isotypes, post-translational modification of tubulin subunits, and interaction with microtubule-associated proteins (MAPs) and a large number of other stabilizers and destabilizers in the cell.

Microtubules are crucial in cell division and mitosis, which makes them excellent targets for anticancer chemotherapy. With the development of observation methods in living cells, it has become clear that their rapid dynamics are essential to successful mitosis.[11] In the early stages (G1 phase and S phase) of interphase in the cell cycle, microtubules that constitute the cytoskeleton exchange their tubulin subunits with tubulin in the soluble pool slowly, with half-times ranging from several minutes to several hours.[11b,12] In the G2 phase of interphase, these low dynamic microtubule networks are broken and replaced by high dynamic spindle microtubules that turn over 4-100 times more rapidly than cytoskeleton microtubules, with half-times of 10-30 seconds.[11c,13] The spindle microtubules are attached to each of the spindle poles (centrosome) at their minus ends and the free plus ends will attach to chromosomes in the following stages.

During mitosis (M phase), the spindle microtubules play crucial roles at almost all stages. In prometaphase, the nuclear envelope breaks into fragments and disappears, and

the spindle microtubules grow and shorten very fast to search the cytoplasm until they reach and become attached to the duplicated chromosomes at kinetochores. The ability of microtubules to grow and shorten rapidly is a key to allow the chromosomes to become attached to spindle microtubules, which is essential in the prometaphase stage.[14] The presence of a single chromosome unable to attach to a spindle is sufficient to stop the transition to the next stage, and leads to the arrest of the cell cycle and ultimately apoptosis.

In metaphase, the chromosomes attached to spindles need to move and align to their position at the metaphase plate, which also requires the high dynamics of spindle microtubules.[15] In addition, the tubulins in the soluble pool are continuously and rapidly added to microtubules at the kinetochores and lost at the centrosome end in a balanced fashion (treadmilling),[9,16] which generates a high tension to allow chromosomes to oscillate back and forth in the spindle equatorial region. The oscillations of the chromosomes created by microtubule treadmilling are required for the spindle to function properly. The cell cycle progress from metaphase to anaphase will be blocked in the absence of this oscillation. In anaphase and telophase, the duplicated chromosome pair needs to be taken apart and finally divided into two chromosomes along with the division of the parent cell, which requires the microtubules attached to the chromosomes to achieve finely regulated shortening and the microtubules that directly connect two spindle poles to lengthen at the same time. Any disturbance to microtubule dynamics at

this stage will result in apoptosis.

Since microtubules are extremely important for mitosis, they have attracted interest as an anticancer target. It was found that suppression of microtubule dynamics can be a common mechanism by which a certain drug can block cancer cell mitosis and kill cancer cells. For example, paclitaxel, a clinically used anticancer drug, can bind to β-tubulin and change microtubule dynamics by promoting tubulin assembly and stabilizing microtubules. In human osteosarcoma cells after incubation with paclitaxel, the chromosomes are stuck at two spindle poles and unable to move to the metaphase plate region, which blocks cell mitosis.[17] Generally, cancer cells are more sensitive to this drug action mechanism than normal cells, because cancer cells divide more rapidly and therefore are more vulnerable to antimitoic agents.

1.2 Tubulin binding agents

A large number of tubulin binding substances have been found to display antimitotic activity and inhibit cell proliferation by suppressing spindle microtubule dynamics. Although they are chemically and structurally diverse, these substances all bind to β-tubulin at one of the three common sites: the Vinca site, the colchicine site, and the paclitaxel site. According to the effects of these substances on microtubules, tubulin interactive substances are usually classified into two groups: microtubule polymerization

inhibitors and microtubule polymerization promoters. The microtubule polymerization inhibitors destabilize spindle microtubules and promote microtubule depolymerization, while the microtubule polymerization promoters affect spindle microtubules in the opposite way. Importantly, it seems that both of the two groups suppress treadmilling and dynamic instability of spindle microtubules, leading to the reduction of spindle tension, slowing or preventing cell cycle progression from metaphase into anaphase, and eventually inducing apoptosis.[18]

1.2.1 Microtubule polymerization inhibitors

This group of tubulin binding substances includes the Vinca alkaloids, colchicine, combretastatins, cryptophycins, halichondrins, and estramustine. Some of them are in clinical use or under clinical investigation for treatment of cancer.

1.2.1.1 The Vinca alkaloids

Vincristine and vinblastine (Figure 1.1) have been used clinically for treatment of neoplastic diseases for over four decades. They were originally isolated from the leaves of the periwinkle plant *Catharanthus roseus* (L.) G. Don, and their antimitotic activity was established in the late 1950s by groups at Eli Lilly Research Laboratories and at the University of Western Ontario, which made them attractive potential cancer chemotherapeutic agents.[19] Shortly after, these two candidates began clinical use for single-agent treatment of haematological and solid malignancies, and they were so

successful in treatment of childhood leukemia that they were considered “wonder drugs” at that time. A large number of efforts towards improving their efficacy resulted in various analogs, including vindestine, vinorelbine, and vinflunine. Among them, vinorelbine has been widely used in the clinic for treatment of solid tumours, lymphomas, and lung cancer. Vinfunine is now in phase III clinical trial for treatment of bladder cancer, non-small-cell lung cancer, and breast cancer.

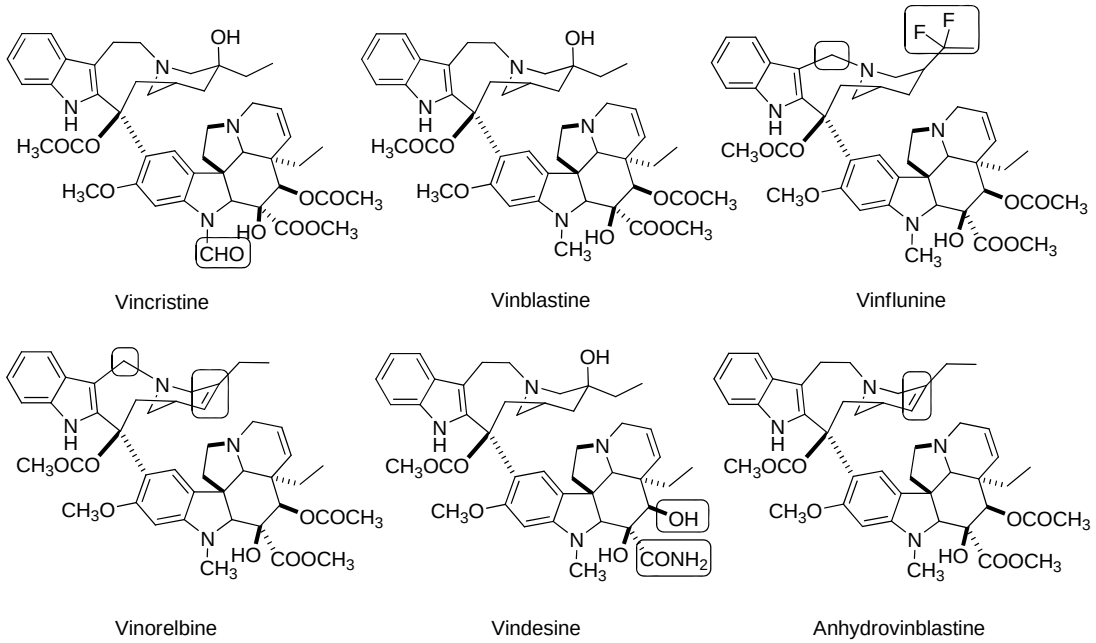

Figure 1.1 Structures of Vinca alkaloids

The mechanism of drug action involves the interaction of Vinca alkaloids with tubulin.[20] At high concentrations, Vinca alkaloids induce depolymerization of microtubules, break up the mitotic spindles in the cell, block cell mitosis, and ultimately lead to cell death by apoptosis. At low concentrations, Vinca alkaloids do not

depolymerize microtubules, but can still block cell mitosis through suppressing microtubule dynamics.

It was found that Vinca alkaloids bind to β-tubulin at a distinct region called the Vinca-binding site.[21] The rapid and reversible binding of Vinca alkaloids with tubulin in the soluble pool introduces a conformational change in tubulin, which decreases the ability of tubulin self-assembly. Remarkably, Vinca alkaloids bind directly to microtubules at the terminal ends with much higher affinity than to the tubulin within the microtubule body. More importantly, only a small amount of a Vinca alkaloid bound to microtubule ends is sufficient to suppress microtubule dynamics, without changing the mass of microtubules, or in other words, without inducing microtubule depolymerization. The suppression of microtubule dynamics prevents the mitotic spindle from forming normally, and reduces the tension of microtubules at the kinetochores of the chromosomes in metaphase, which blocks mitotic progression from metaphase to anaphase and leads to apoptosis.

1.2.1.2 Colchicine

Colchicine (Figure 1.2) was originally isolated from the poisonous plant meadow saffron (*Colchicum autumnale L.*) and related species in 1820 by two French chemists P. S. Pelletier and J. Caventou. The plant extracts were known since antiquity for their

therapeutic properties in gout and their toxicity. Colchicine is an important bioactive compound to treat various diseases and currently is used clinically for treatment of acute gout and familial Mediterranean fever.

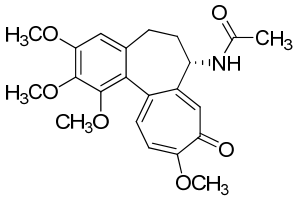

Figure 1.2 Structure of colchicines

The antimitotic activity of colchicine was recognized about sixty years after it was isolated. Colchicine is able to bind to β-tubulin at the colchicine site, and the binding is relatively slow, but extremely tight. Unlike other microtubule destabilizers such as the Vinca alkaloids, colchicine interacts with tubulin and inhibits microtubule functions by a different mechanism. Interestingly, colchicine depolymerizes microtubules at high concentrations, and stabilizes microtubule dynamics at low concentrations. Moreover, colchicine inhibits microtubule polymerization even at concentrations much lower than the tubulin concentration, indicating that colchicine interacts with the tubulin subunits in microtubules rather than the tubulin in the soluble pool. However, it was found that colchicine itself cannot directly bind to microtubules. It is very possible that colchicine first binds to soluble tubulin, induces conformational changes in the tubulin, and finally forms a stabilized tubulin-colchicine complex that is poorly reversible.[22] Then the

complex is incorporated to the microtubule ends in small numbers along with a large amount of free tubulin. The microtubules that contain the tubulin-colchicine complex can still grow, but their dynamics are suppressed. The reason might be the disruption to the microtubule structure by the complex, which slows down the addition of new tubulin, instead of totally blocking it.

Clinical use of colchicine in cancer therapy has been hampered by its general cytotoxicity of colchicine. A large amount of work has been done to modify the colchicine structure and prepare colchicine analogs in order to reduce its general cytotoxicity while retaining its antimitotic activity. It turns out that it is difficult to reduce the general cytotoxicity, but the unique mechanism by which colchicine suppresses microtubule dynamics is still attractive, and compounds that interact with tubulin at the colchicine binding site are of pharmaceutical interest for cancer therapy.

Combretastatin A4 (Figure 1.3) is a competitive inhibitor of colchicine binding and an inhibitor of tubulin polymerization. Combretastatins were isolated from the stem bark of the South African tree *Combretum caffrum* and their structure elucidated in 1982.[23] Combretastatin A4 shows both antimitotic activity and antivascular effects inside tumors, which is a new mechanism for cancer therapy. Antivascular agents can rapidly shut down existing tumor vasculature so that the tumor cannot be supplied with oxygen and nutrients through blood, leading to rapid cell death. Interestingly, combretastatin A4 seems to damage tumor vasculature without harming normal tissue too much, which

results in some efforts to develop combretastatin A4 analogs for cancer therapy. At this date, several combretastatin derivatives are in clinical trials for treatment of cancer.

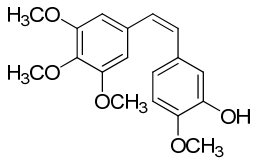

Figure 1.3 Structure of combretastatin A4

1.2.2 Microtubule polymerization promoters

The microtubule polymerization promoters form a very important group of antimitotic agents that have achieved significant success in cancer chemotherapy. Generally, microtubule polymerization promoters bind to microtubules with high affinity and promote microtubule polymerization by inducing conformational changes on the bound tubulin subunits and increasing their affinity for the neighbor tubulin subunits. Among them, paclitaxel, as well as its semi-synthetic analog, docetaxel, is among the most successful anticancer drugs in the market today (Figure 1.4). Several other paclitaxel derivatives also display good anticancer potential and some of them are under clinical investigation. Their specific mechanism of action and the structure-activity relationships will be discussed in detail later in Chapter 2.

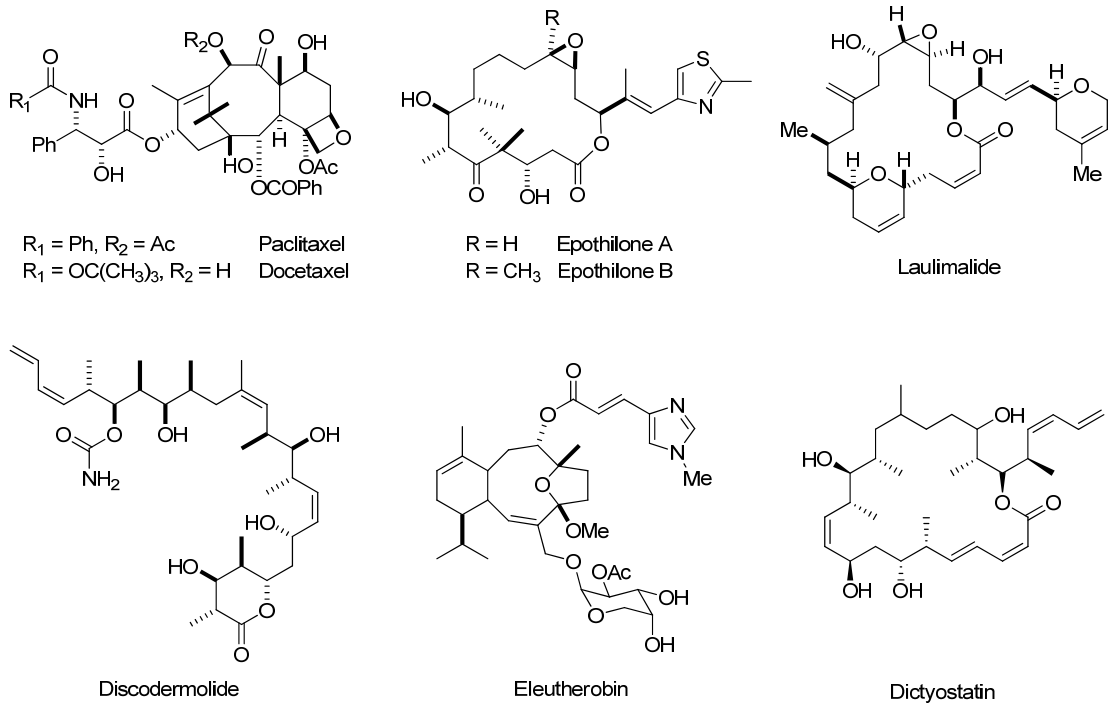

Figure 1.4 Microtubule polymerization promoters

The clinical success of taxanes led to a search for other drugs from this group. Some promising substances including discodermolide, epothilones, sarcodictyins, dicyostatin, eleutherobin and laulimalide, have been identified (Figure 1.4). Although they bind to tubulin at various sites, these compounds share a similar mechanism of action and show good antimitotic activity. Among them, discodermolide, eleutherobin, and epothilones bind to tubulin at the paclitaxel binding site, whereas others, like laulimalide, bind to distinct sites on microtubules. Epothilones and eleutherobin were observed to inhibit the binding of paclitaxel to microtubules, indicating that they share the same binding site as paclitaxel. Laulimalide and paclitaxel bind to microtubules simultaneously with a molar

ratio of 1:1, which enhances microtubule assembly. Interestingly, although discodermolide binds to tubulin at the paclitaxel binding site, the combination of discodermolide and paclitaxel shows better antimitotic activity than either of them alone.

1.2.3 Drug resistance

Different microtubule interactive agents display antiproliferative activity towards different cancer cells specifically, and the reason why one agent is efficient for some types of cancer, but totally inactive for other types is not well understood. It might result from either the nature of the cancer cell itself or the pharmacological accessibility of the drugs to the cancer cells.[24]

One big problem of microtubule interactive drugs in cancer chemotherapy is drug resistance. It often results from overexpression of membrane transporter proteins, for example, P-glycoprotein.[25] These membrane transporter proteins are able to decrease the intracellular drug levels by pumping the drug out of the cell, and lead to multidrug resistance (MDR). In addition, since all these drugs induce apoptosis by suppressing microtubule dynamics that are regulated by a host of factors such as expression of the microtubule associated proteins, post-translational modification of tubulin, and expression of different tubulin isotypes, cancer cells have many microtubule-related mechanisms to confer drug resistance.[18,24,26] The development of drug resistance might

also involve some of the tubulin isotypes associated with the centrosomes and spindle poles. But the mechanism of how drugs interact with the tubulin at centrosomes and how this interaction affects microtubule dynamics is not clear, so whether they are related to drug resistance is still an unsolved question. The development of drug resistance is complicated and there is very little known about it currently. Considerable efforts are underway to recognize various microtubule regulatory proteins, to understand how these proteins regulate microtubule formation and dynamics, to understand how particular cancer cells develop drug resistant, and to figure out a way to overcome drug resistance.

1.2.4 Synergy

Figure 1.5 Colchicine binding inhibitor CI-980

There are many diverse binding sites on tubulin and microtubules, besides the three common tubulin binding domains mentioned above. One tubulin interactive agent can bind exclusively and specifically to tubulin at one site to affect tubulin dynamics. Theoretically it is possible that more than one tubulin interactive drug could bind to tubulin at different sites simultaneously to have an additional advantage. Many examples have indicated that the clinical combinations of two or more tubulin interactive drugs

have the potential to improve efficacy, overcome drug resistance and reduce the side effects of cancer therapy. The combination of vinorelbine (Figure 1.1) and paclitaxel or docetaxel (Figure 1.4) shows better antimitotic activity than each drug alone.[27] The same synergistic effects have been observed with the combination of docetaxel and the colchicine binding inhibitor CI-980 (Figure 1.5),[28] the combination of paclitaxel and vinblastine,[29a] the combination of estramustine (Figure 1.6) and vinblastine or paclitaxel.[29b,c] Surprisingly, paclitaxel and discodermolide that share the same or overlapping binding sites on tubulin and have similar mechanisams of action were found to act synergistically in the suppression of microtubule dynamics and their antimitotic effect.[30] The synergism of paclitaxel and discodermolide is not well understood, but it is of great value, since the potential for the use of microtubule interactive drugs with similar mechanisms is still an untapped source of anticancer chemotherapeutic potential.

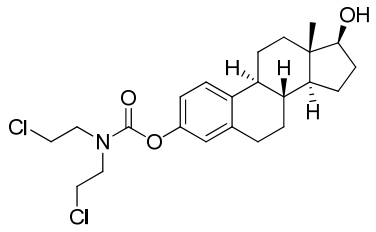

Figure 1.6 Estramustine

Several clinical trials involving the combination of two or more microtubule interactive drugs are in progress. The combinations of estramustine (Figure 1.6) with paclitaxel, docetaxel, vinorelbine or epothilone analogs are in clinical trials in various

phases.[18] In addition, 22 combination trials of vinblastine, 108 combination trials of vincristine, and combination trials of vinorelbine for treatment of cancer are underway.[18] It is hoped that efficient combinational chemotherapy with fewer side effects will be found in the near future.

1.3 Dissertation overview

This dissertation consists of two parts, linked by the common theme of the study of microtubule polymerization promoting agents. The first part involves the design, synthesis and biological evaluation of androgen receptor targeted taxane analogs for treatment of hormone refractory prostate cancer (Chapter 2 and 3). The second part consists of an introduction to discodermolide (Chapter 4), and the conformational studies on how discodermolide binds to microtubules, including the design and total synthesis of fluorescently labeled discodermolide derivatives (Chapter 5), and the design and total synthesis of conformationally constrained bridged discodermolide derivatives (Chapter 6).

References

1. Alberts, B.; Johnson, A.; Lewis, J.; Raf, M.; Roberts, K.; Walter, P., *Molecular Biology of the Cell* 4th ed.; Garland Publishing: New York, 2002.
2. (a) Palmer, G. R.; Clark, D. C.; Bayley, P. M.; Satelle, D. B., A quasi-elastic laser light scattering study of tubulin and microtubule protein from bovine brain. *J. Mol. Biol.* **1982,** *160*, 641-658; (b) Spann, U.; Renner, W.; Mandelkow, E. M.; Bordas, J.; Mandelkow, E., Tubulin oligomers and microtubule assembly studied by time-resolved X-ray scattering: separation of prenucleation and nucleation events. *Biochem* **1987,** *26*, 1123-1132; (c) Carlier, M. F.; Didry, D.; D., P., Hydrolysis of GTP associated with the formation of tubulin oligomers is involved in microtubule nucleation. *Biophys. J.* **1997,** *73*, 418-427; (d) Caudron, N.; Valiron, O.; Usson, Y.; Valiron, P.; Job, D., A reassessment of the factors affecting microtubule assembly and disassembly in vitro. *J. Mol. Biol.* **2000,** *297*, 211-220.
3. Erickson, H. P.; Stoeffler, D., Protofilaments and rings, two conformations of the tubulin family conserved from bacterial FtsZ to alpha/beta and gamma tubulin. *J. Cell Biol.* **1996,** *135*, 5-8.
4. Moritz, M.; Braunfeld, M. B.; Sedat, J. W.; Alberts, B.; Agard, D. A., Microtubule nucleation by tubulin-containing rings in the centrosome. *Nature* **1995,** *378*, 638-640.
5. Nogales, E., Structural insights into microtubule function. *Annu. Rev. Biochem.* **2000,** *69*, 277-302.

6. (a) Mitchison, T. J.; Kirschner, M., Dynamic instability of microtubule growth. *Nature* **1984,** *312*, 237-242; (b) Desai, A.; Mitchison, T. J., Microtubule polymerization dynamics. *Annu. Rev. Dev. Biol.* **1997,** *13*, 83-117.

7. Nogales, E., Structural insight into microtubule function. *Annu. Rev. Biophys. Biomol. Struct.* **2001,** *30*, 397-420.

8. (a) Margolis, R. L.; Wilson, L., Opposite end assembly and disassembly of microtubules at steady state in vitro. *Cell* **1978,** *13*, 1-8; (b) Margolis, R. L.; Wilson, L., Microtubule treadmilling: what goes around comes around. *BioEssays* **1998,** *20*, 830-836; (c) Shaw, S. L.; Karnyar, R.; Ehrhardt, D. W., Sustained microtubule treadmilling in arabidopsis cortical arrays. *Science* **2003,** *300*, 1715-1718; (d) Panda, D.; Miller, H. P.; Wilson, L., Rapid treadmilling of MAP-free brain microtubules in vitro and its suppression by tau. *Proc. Natl. Acad. Sci. USA* **1999,** *96*, 12459-12464.

9. Chen, W.; Zhang, D., Kinetochore fibre dynamics outside the context of the spindle during anaphase. *Nature Cell Biol.* **2004,** *6*, 227-231.

10. Wilson, L.; Panda, D.; Jordan, M. A., Modulation of microtubule dynamics by drugs: a paradigm for the actions of cellular regulators. *Cell Struct. Funct.* **1999,** *24*, 329-335.

11. (a) Mitchison, T. J., Microtubule dynamics and kinetochore function in mitosis. *Annu. Rev. Cell Biol.* **1988,** *4*, 527-549; (b) Saxton, W. M.; Stemple, D. L.; Leslie, R. J.; Salmon, E. D.; Zavortink, M.; McIntosh., J. R., Tubulin dynamics in cultured mammalian cells. *J. Cell Biol.* **1984,** *99*, 2175-2186; (c) Rusan, N. M.; Fagerstrom, C.

J.; Yvon, A. M. C.; Wadsworth, P., Cell cycle-dependent changes in microtubule dynamics in living cells expressing green fluorescent protein-a tubulin. *Mol. Biol. Cell* **2001,** *12*, 971-980.

12. Pepperkok, R.; Bre, M. H.; Davoust, J.; Kreis, T. E., Microtubules are stabilized in confluent epithelial cells but not in fibroblasts. *J. Cell Biol.* **1990,** *111*, 3003-3012.
13. Zhai, Y.; Kronebusch, P. J.; Simon, P. M.; Borisy, G. G., Microtubule dynamics at the G2/M transition: abrupt breakdown of cytoplasmic microtubules at nuclear envelope breakdown and implications for spindle morphogenesis. *J. Cell Biol.* **1996,** *135*, 201-214.
14. Hayden, J. J.; Bowser, S. S.; Rieder, C., Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: direct visualization in live newt cells. *J. Cell Biol.* **1990,** *111*, 1039-1045.
15. (a) Rieder, C.; Schultz, A.; Cole, R.; Sluder, G., Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J. Cell Biol.* **1994,** *127*, 1301-1310; (b) Shelby, R. D.; Hahn, K. M.; Sullivan, K. F., Dynamic elastic behavior of α-satellite DNA domains visualized in situ in living human cells. *J. Cell Biol.* **1996,** *135*, 545-557.
16. (a) Mitchison, T. J., Poleward microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J. Cell Biol.* **1989,** *109*, 637-652; (b) Wilson, P. J.; Forer, A., Effects of nanomolar taxol on cranefly spermatocyte spindles indicate that acetylation of kinetochore microtubules can be used as a marker of poleward tubulin

flux. *Cell Motil. Cytoskeleton* **1997,** *37*, 20-32.

17. Kelling, J.; Sullivan, K.; Wilson, L.; Jordan, M. A., Suppression of centromere dynamics by taxol in living osteosarcoma cells. *Cancer Res.* **2003,** *63*, 2794-2801.

18. Jordan, M. A.; Wilson, L., Microtubules as a target for anticancer drugs. *Nat. Rev. Cancer* **2004,** *4*, 253-265.

19. (a) Johnson, I. S.; Wright, H. F.; Svoboda, G. H., Experimental basis ofr clinical evaluation of anti-tumor principles derived from Vinca rosea Linn. *J. Lab. Clin. Med.* **1959,** *54*, 830-837; (b) Noble, R. L.; Beer, C. T.; Cutts, J. H., Further biological activities of vincaleukoblastine: an alkaloid isolated from Vinca rosea (L.). *Biochem. Pharmacol.* **1958,** *1*, 347-348.

20. Jordan, M. A.; Thrower, D.; Wilson, L., Mechanism of inhibition of cell proliferation by Vinca alkaloids. *Cancer Res.* **1991,** *51*, 2212-2222.

21. Bai, R. B.; Pettit, G. R.; Hamel, E., Binding of dolastatin 10 to tubulin at a distinct site for peptide antimitotic agents near the exchangeable nucleotide and Vinca alkaloid sites. *J. Biol. Chem.* **1990,** *265*, 17141-17149.

22. Hastie, S. B., Interactions of colchicine with tubulin. *Pharmacol. Ther.* **1991,** *512*, 377-401.

23. Pettit, G. R.; Gragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P., Isolation and structure of combretastatin. *Can. J. Chem.* **1982,** *60*, 1374-1376.

24. Dumontet, C.; Sikic, B., Mechanisms of action of and resistance to antitubulin agents: microtubule dynamics, drug transport, and cell death. *J. Clin. Oncol.* **1999,** *17*,

1061-1070.

25. Ambudkar, S. V.; Kimchi-Sarfaty, C.; Sauna, Z. E.; Gottesman, M. M., P-glycoprotein: from genomics to mechanism. *Oncogene* **2003,** *22*, 7468-7485.

26. Orr, G. A.; Verdier-Pinard, P.; McDaid, H.; Horwitz, S. B., Mechanisms of taxol resistance related to microtubules. *Oncogene* **2003,** *22*, 7280-7295.

27. (a) Knick, V. C.; Eberwein, D.; Miller, C., Vinorelbine tartrate and paclitaxel combinations: enhanced activity against in vivo P388 murine leukemia cells. *J. Natl. Cancer Inst.* **1995,** *87*, 1072-1077; (b) Photiou, A.; Shah, P.; Leong, L.; Moss, J.; Retsas, S., In vitro synergy of paclitaxel (taxol) and vinorelbine (navelbine) against human melanoma cell lines. *Eur. J. Cancer* **1997,** *33*, 463-470; (c) Diéras, V.; Furnoleau, P.; Kalla, S.; Misset, J. L.; Azli, N.; Pouillart, P., Docetaxel in combination with doxorubicin or vinorelbine. . *Eur. J. Cancer* **1997,** *33*, 20-22.

28. Garcia, P.; Braguer, D.; Carles, G.; Briand, C., Simultaneous combination of microtubule depolymerizing and stabilizing agents acts at low doses. *Anticancer Drugs* **1995,** *6*, 533-544.

29. (a) Giannakakou, P.; Villalba, L.; Li, H.; Poruchynsky, M.; Fojo, T., Combinations of paclitaxel and vinblastine and their effects on tubulin polymerization and cellular cytotoxicity: characterization of a synergistic schedule. *Int. J. Cancer* **1998,** *75*, 57-63; (b) Hudes, G. R.; Greenberg, R.; Krigel, R. L.; Fox, S.; Scher, R.; Litwin, S.; Watts, P.; Speicher, L.; Tew, K.; Comis, R., Phase II study of estramustine and vinblastine, two microtubule inhibitors, in hormone-refractory prostate cancer. *J.*

Clin. Oncol. **1992,** *10*, 1754-1761; (c) Hudes, G. R.; Nathan, F. E.; Khater, C.; Greenberg, R.; Gomella, L.; Stern, C.; McAleer, C., Paclitaxel plus estramustine in metastatic hormone-refractory prostate cancer. *Semin. Oncol.* **1995,** *22*, 41-45.

30. Martello, L. A.; McDaid, H. M.; Regl, D. L.; Yang, C.-P. H.; Meng, D.; Pettus, T. R. R.; Kaufman, M. D.; Arimoto, H.; Danishefsky, S. J.; III, A. B. S.; Horwitz, S. B., taxol and discodermolide represent a synergistic drug combination in human carcinoma cell lines. *Clin. Cancer Res.* **2000,** *6*, 1978-1987.