

MICROTUBULES AS A TARGET FOR ANTICANCER DRUGS

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Highly dynamic mitotic-spindle microtubules are among the most successful targets for anticancer therapy. Microtubule-targeted drugs, including paclitaxel and *Vinca* alkaloids, were previously considered to work primarily by increasing or decreasing the cellular microtubule mass. Although these effects might have a role in their chemotherapeutic actions, we now know that at lower concentrations, microtubule-targeted drugs can suppress microtubule dynamics without changing microtubule mass; this action leads to mitotic block and apoptosis. In addition to the expanding array of chemically diverse antimetabolic agents, some microtubule-targeted drugs can act as vascular-targeting agents, rapidly depolymerizing microtubules of newly formed vasculature to shut down the blood supply to tumours.

Microtubules — key components of the cytoskeleton — are long, filamentous, tube-shaped protein polymers that are essential in all eukaryotic cells. They are crucial in the development and maintenance of cell shape, in the transport of vesicles, mitochondria and other components throughout cells, in cell signalling, and in cell division and mitosis. Microtubules are composed of α -tubulin and β -tubulin heterodimers (of dimensions 4 nm \times 5 nm \times 8 nm and 100,000 daltons in mass) arranged in the form of slender filamentous tubes that can be many micrometres long (FIGS 1,2). They are highly dynamic polymers and their polymerization dynamics are tightly regulated both spatially and temporally. The functional diversity of microtubules is achieved in several ways: through the binding of various regulatory proteins, including microtubule-associated proteins (MAPs), to soluble tubulin and to the microtubule surfaces and ends; by expression of different tubulin isotypes, which have different functions; and through several post-translational modifications of tubulin. For example, human tubulin isotypes (6 forms of α -tubulin and 7 forms of β -tubulin) are expressed at varying levels in different cells and tissues. These tubulins can be further modified post-translationally by polyglutamylolation, polyglycylation, phosphorylation, acetylation, detyrosination/tyrosination and removal of the penultimate glutamic-acid residue

of α -tubulins^{1,2}. There are many different MAPs, including the dynein and kinesin motor proteins, as well as many microtubule-regulatory proteins, such as **survivin**, **stathmin**, **TOG**, **MCAK**, **MAP4**, **EB1**, **dynactin 1** (also known as p150^{Glued}), **RAC1** and **FHIT**^{3–8}. Some of these differences predominate in certain cancer cells and some are associated with the development of drug resistance. The precise role of these changes in tumour sensitivity to microtubule-active agents is an area of intense investigation, and the degree to which the rapid progression of cancer cells through mitosis renders them selectively sensitive to antimetabolic microtubule-active compounds is an open question.

Why target microtubules?

Microtubules are extremely important in the process of mitosis, during which the duplicated chromosomes of a cell are separated into two identical sets before cleavage of the cell into two daughter cells. Their importance in mitosis and cell division makes microtubules an important target for anticancer drugs. Microtubules and their dynamics are the targets of a chemically diverse group of antimetabolic drugs (with various tubulin-binding sites) that have been used with great success in the treatment of cancer (TABLE 1). In view of the success of this class of drugs, it has been argued that microtubules represent the best cancer target to be identified so far, and it seems

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Summary

- Microtubules are highly dynamic cytoskeletal fibres that are composed of tubulin subunits. They show two types of non-equilibrium dynamics — treadmilling and dynamic instability — both of which are crucial to mitosis and cell division.
- Dynamic microtubules continue to be one of the most successful cancer chemotherapeutic targets. Many new drugs that target microtubules are in clinical trials and large numbers of microtubule-active compounds are being developed.
- Among the most successful microtubule-targeted chemotherapeutic drugs are paclitaxel and the *Vinca* alkaloids, which were previously thought to work through opposite mechanisms. We now recognize that their most potent actions are suppression of microtubule dynamics, rather than increasing or decreasing microtubule-polymer mass.
- Microtubule-active drugs generally bind to one of three main classes of sites on tubulin, the paclitaxel site, the *Vinca* domain and the colchicine domain. Drugs that bind to the colchicine domain are undergoing intensive investigation as vascular-targeting agents for cancer therapy.
- Development of resistance to microtubule-targeted drugs has several possible causes, some of which might involve changes in microtubule dynamics resulting from altered expression of tubulin isoforms, tubulin mutations, and altered expression or binding of microtubule-regulatory proteins.
- Microtubule-targeted drugs can synergize with one another.
- Understanding their modes of action might lead to improved dosing regimens and combinations with other microtubule-targeted drugs, as well as combinations with ‘molecularly targeted’ drugs.

likely that drugs of this class will continue to be important chemotherapeutic agents, even as more selective approaches are developed^{9,10}. Microtubules seem to be a favourite target of naturally occurring, presumably self-protective, toxic molecules that are produced by a large number of plants and animals — ranging from algae to sea hares — and most microtubule-targeted compounds have been discovered in large-scale screens of natural products (see TABLE 1 and below). Among the earliest developed were the *Vinca* alkaloids, which were isolated more than 40 years ago from periwinkle leaves (*Catharanthus roseus* (L.) G. Don, sometimes known as *Vinca rosea*).

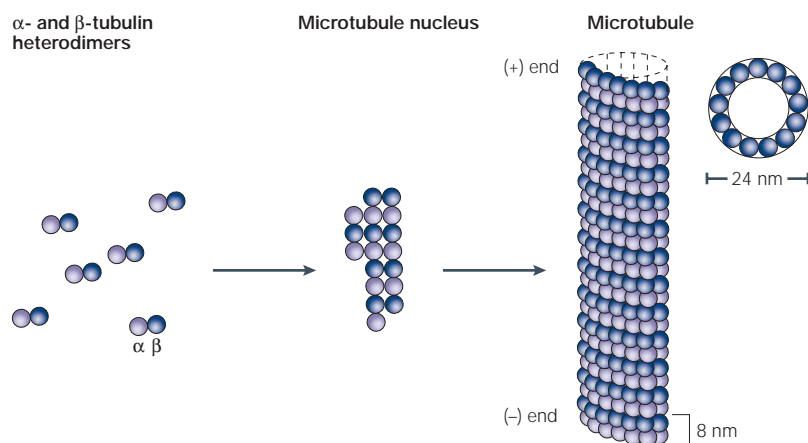


Figure 1 | **Polymerization of microtubules.** Heterdimers of α - and β -tubulin assemble to form a short microtubule nucleus. Nucleation is followed by elongation of the microtubule at both ends to form a cylinder that is composed of tubulin heterodimers arranged head-to-tail in 13 protofilaments. Each microtubule has a so-called plus (+) end, with β -tubulin facing the solvent, and a minus end (-), with α -tubulin facing the solvent.

These were serendipitously discovered to exert bone-marrow toxicity during the investigation of their reputed antidiabetic activity in folk medicine¹¹. Further development of the *Vinca* alkaloids since the introduction of the first two in this class — vincristine and vinblastine — has been motivated by our increased understanding of their mechanisms of action, their synergy in combination therapy, the desire to develop orally available analogues and the need to overcome their neurotoxicity and the development of resistance, which commonly occurs with these drugs.

Microtubules and their polymerization dynamics. The polymerization of microtubules occurs by a nucleation-elongation mechanism in which the relatively slow formation of a short microtubule ‘nucleus’ is followed by rapid elongation of the microtubule at its ends by the reversible, non-covalent addition of tubulin dimers. It is important to emphasize that microtubules are not simple equilibrium polymers. They show complex polymerization dynamics that use energy provided by the hydrolysis of GTP at the time that tubulin with bound GTP adds to the microtubule ends; these dynamics are crucial to their cellular functions. As described later, the correct movements of the chromosomes and their proper segregation to daughter cells require extremely rapid dynamics, making mitosis exquisitely sensitive to microtubule-targeted drugs.

The biological functions of microtubules in all cells are determined and regulated in large part by their polymerization dynamics^{12–15}. Microtubules show two kinds of non-equilibrium dynamics, both with purified microtubule systems *in vitro* and in cells (BOX 1; FIG 3). One kind of dynamic behaviour that is highly prominent in cells, called ‘dynamic instability’, is a process in which the individual microtubule ends switch between phases of growth and shortening¹⁶. The two ends of a microtubule are not equivalent; one end, called the plus end, grows and shortens more rapidly and more extensively than the other (the minus end). The changes in length with time at the ends of a group of microtubules due to dynamic instability are illustrated in FIG. 3a,b. The microtubules undergo relatively long periods of slow lengthening, brief periods of rapid shortening and periods of attenuated dynamics or pause, when the microtubules neither grow nor shorten detectably.

Dynamic instability is characterized by four main variables: the rate of microtubule growth; the rate of shortening; the frequency of transition from the growth or paused state to shortening (this transition is called a ‘catastrophe’); and the frequency of transition from shortening to growth or pause (called a ‘rescue’). Periods of pause are defined operationally, when any changes in microtubule length that might be occurring are below the resolution of the light microscope. The variable called ‘dynamicity’ is highly useful to describe the overall visually detectable rate of exchange of tubulin dimers with microtubule ends.

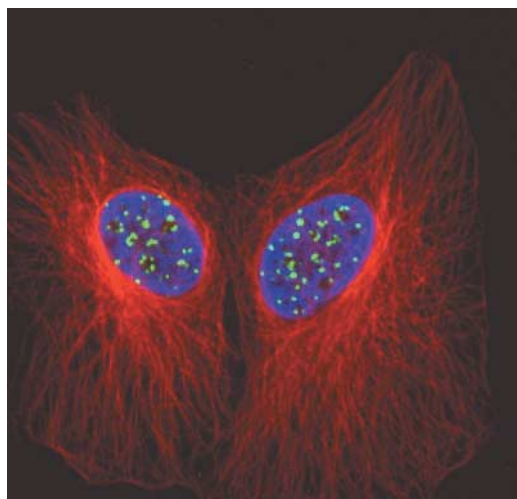


Figure 2 | **Microtubules in two human osteosarcoma cells in interphase of the cell cycle.** Microtubules are in red, chromatin is in blue, and centromeres are in green. Image reproduced with permission from Ref. 58 © (2002) Bentham Science Publishers.

The second dynamic behaviour, called 'treadmilling' (FIG. 3c), is net growth at one microtubule end and balanced net shortening at the opposite end^{17–21}. It involves the intrinsic flow of tubulin subunits from the plus end of the microtubule to the minus end and is created by differences in the critical subunit concentrations at the opposite microtubule ends. (The critical subunit concentrations are the concentrations of free tubulin subunits in equilibrium with the microtubule ends.) This behaviour occurs in cells as well as *in vitro*, and might be particularly important in mitosis²². Treadmilling and dynamic instability are compatible behaviours, and a specific microtubule population can show primarily treadmilling behaviour, dynamic-instability behaviour or some mixture of both. The mechanisms that control the degree to which a microtubule population shows one or the other behaviour are poorly understood, but probably involve the tubulin isotype composition of the microtubule population, the degree of post-translational modification of tubulin and, especially, the actions of regulatory proteins²³.

The polymerization dynamics of microtubules are unusual compared with those of most polymers in nature. They are created by the gain and loss of a short region (perhaps no larger than a single layer of tubulin subunits) of tubulin–GTP or tubulin–GDP–inorganic phosphate (P_i) at the two microtubule ends, called a GTP cap (FIG. 4). Tubulin-bound GTP is hydrolysed to tubulin–GDP and P_i at the time that tubulin–GTP adds to the microtubule ends, or shortly thereafter. Ultimately, the cap dissociates from the microtubule, leaving a microtubule core consisting of tubulin with stoichiometrically bound GDP in β -tubulin. The tubulin–GDP remains non-dissociable and non-exchangeable until the tubulin subunit dissociates from the microtubule. A generally accepted model in the field is that tubulin–GTP polymerizes into protofilaments that have a straight

conformation, but that following GTP hydrolysis, the tubulin conformation changes to a curved form, such that the tubulin–GDP that is locked into the microtubule core is in a strained conformation²⁴. When the GTP or GDP– P_i cap is present, the end of the microtubule is stabilized and the microtubule can grow^{25,26}. When the cap is lost, the relatively unstable core of the microtubule is exposed and the end shortens rapidly. The precise chemical nature of the stabilizing cap is poorly understood and is under intense investigation^{27,28}. It seems clear, however, that at least two sets of association- and dissociation-rate constants govern tubulin addition at each microtubule end — a stabilized (capped) set, and an unstabilized (uncapped) set — and that modification of any of the rate constants at either end by drugs or regulatory proteins can have marked effects on polymerization dynamics²⁹ and microtubule function.

Dynamic instability and treadmilling behaviours can both be observed with purified microtubules *in vitro*. However, the rate and extent of both treadmilling and dynamic instability are relatively slow with purified microtubules compared with rates in cells. It is clear that microtubule dynamics in cells are regulated by a host of mechanisms: cells can alter their expression levels of the 13 tubulin isotypes; they can alter their levels of tubulin post-translational modifications; they can express mutated tubulin; and they can alter the expression and phosphorylation levels of microtubule-regulatory proteins^{1–8,30,31} that interact with the microtubule surfaces and ends. Although microtubule dynamics can be modulated by the interaction of regulatory molecules with soluble tubulin itself, the assembled microtubule is likely to be the primary target of cellular molecules that regulate microtubule dynamics. The many drugs that modulate microtubule dynamics might be mimicking the actions of the numerous natural regulators that control microtubule dynamics in cells^{3,23,30–36}.

Microtubule dynamics are crucial to mitosis. With the development of sophisticated methods for observing microtubule dynamics in living cells, it is now possible to visualize the dynamics of mitotic-spindle microtubules. With these advances it has become clear that microtubules in mitotic spindles have uniquely rapid dynamics that are crucial to successful mitosis^{37–39}. During interphase, microtubules turn over (exchange their tubulin with the soluble tubulin pool) relatively slowly, with half-times that range from several minutes to several hours^{38,40}. The interphase microtubule network disassembles at the onset of mitosis and is replaced by a new population of spindle microtubules that are 4–100 times more dynamic than the microtubules in the interphase cytoskeleton^{39,41}. Although there is variation among the various spindle-microtubule subpopulations, mitotic-spindle microtubules exchange their tubulin with tubulin in the soluble pool rapidly, with half-times on the order of 10–30 seconds³⁸. At least in some cells, the increase in dynamics seems to result from an increase in the catastrophe frequency and a reduction in the rescue frequency rather than from changes in the inherent rates of growth and shortening³⁹.

Table 1 | Antimitotic drugs, their diverse binding sites on tubulin and their stages of clinical development

| Binding domain | Related drugs or analogues | Therapeutic uses | Stage of clinical development | References | |
|--|---|--|--|---|--------------|
| Vinca domain | Vinblastine (Velban) | Hodgkin's disease, testicular germ-cell cancer | In clinical use; 22 combination trials in progress | 75–77,131 | |
| | Vincristine (Oncovin) | Leukaemia, lymphomas | In clinical use; 108 combination trials in progress | 132–134 | |
| | Vinorelbine (Navelbine) | Solid tumours, lymphomas, lung cancer | In clinical use; 29 Phase I–III clinical trials in progress (single and combination) | 135–137 | |
| | Vinflunine | Bladder, non-small-cell lung cancer, breast cancer | Phase III | 131,138 | |
| | Cryptophycin 52 | Solid tumours | Phase III finished | 139,140 | |
| | Halichondrins (such as E7389) | – | Phase I | 58,141–143 | |
| | Dolastatins (such as TZT-1027) | Potential vascular-targeting agent | Phase I; Phase II completed | 144 | |
| | Hemiasterlins (such as HTI-286) | – | Phase I | 145,146 | |
| | Colchicine domain | Colchicine | Non-neoplastic diseases (gout, familial Mediterranean fever) | Appears to have failed trials, presumably because of toxicity | 89–90 |
| | | Combretastatins (AVE8062A, CA-1-P, CA-4-P, <i>N</i> -acetylcolchicinol- <i>O</i> -phosphate, ZD6126) | Potential vascular-targeting agent | Phase I, II | 91,147 |
| 2-Methoxyestradiol | | – | Phase I | 148,149 | |
| Methoxybenzene-sulphonamide (such as ABT-751, E7010) | | Solid tumours | Phase I, II | 150 | |
| Taxane site | | Paclitaxel (Taxol), TL00139 and other analogues of paclitaxel | Ovarian, breast and lung tumours, Kaposi's sarcoma; trials with numerous other tumours | In clinical use; 207 Phase I–III trials in the United States; TL00139 is in Phase I trials | 82, 151–153 |
| | Docetaxel (Taxotere) | Prostate, brain and lung tumours | 8 trials in the United States (Phases I–III) | 154,155 | |
| | Epothilones (such as BMS-247550, epothilones B and D) | Paclitaxel-resistant tumours | Phases I–III | 156–159 | |
| | Discodermolide | – | Phase I | 160–164 | |
| | Other microtubule binding sites | Estramustine | Prostate | Phases I–III, in numerous combinations with taxanes, epothilones and <i>Vinca</i> alkaloids | 122, 165–168 |

Information on clinical trials was obtained from the National Institutes of Health Clinical Trials web site (www.clinicaltrials.gov), the European Organisation for Research and Treatment of Cancer web site (www.eortc.be) and the Proceedings of the American Association for Cancer Research meeting in 2003 (www.aacr.org). CA-4-P, combretastatin-A-4 3-*O*-phosphate; CA-1-P, combretastatin A-1-phosphate.

Mitosis in most cells progresses rapidly and the highly dynamic microtubules in the spindle are required for all stages of mitosis. First, for the timely and correct attachment of chromosomes at their kinetochores to the spindle during prometaphase after nuclear-envelope breakdown (FIG. 5a). Second, for the complex movements of the chromosomes that bring them to their properly aligned positions at the metaphase plate — called congression (FIG. 5b). Last, for the synchronous separation of the chromosomes in anaphase and telophase after the metaphase–anaphase checkpoint is complete (FIG. 5c,d). During prometaphase, microtubules emanating from each of the two spindle poles make vast growing and shortening excursions, essentially probing the cytoplasm until they 'find' and become attached to chromosomes at their kinetochores⁴². Such microtubules must be able to grow for long distances (typically 5–10 μm), then shorten almost completely, then re-grow again until they successfully become

attached. The presence of a single chromosome that is unable to achieve a bipolar attachment to the spindle is sufficient to prevent a cell from transitioning to anaphase; the cell then remains blocked in a prometaphase/metaphase-like state and eventually undergoes apoptosis (programmed cell death)⁴³. We have found that suppression of microtubule dynamics by drugs such as paclitaxel (Taxol) and *Vinca* alkaloids seems to be a common mechanism by which these drugs block mitosis and kill tumour cells. Human osteosarcoma cells after incubation with 10 nM paclitaxel and 50 nM vinflunine are shown in FIG. 5e,f, respectively. Many chromosomes are stuck at the spindle poles, unable to congress to the metaphase plate. At least one reason that cancer cells are relatively sensitive to these drugs compared with normal cells is that many cancer cells divide more frequently than normal cells and therefore frequently pass through a stage of vulnerability to mitotic poisons.

Box 1 | Measuring microtubule dynamic instability

With purified microtubules *in vitro* (generally purified from pig, cow or sheep brains, which are a rich source of microtubules), dynamic instability of individual microtubules is measured by computer-enhanced time-lapse differential interference-contrast microscopy. In living cells, individual fluorescent microtubules can be readily visualized in the thin peripheral regions of the cells after microinjection of fluorescent tubulin or by expression of GFP (green fluorescent protein)-labelled tubulin. The growing and shortening dynamics of the microtubules, which are prominent in this region of interphase cells, are recorded by time-lapse using a sensitive CCD (charge-coupled device) camera. To determine how microtubule length changes with time, both *in vitro* and in living cells, the ends of the individual growing and shortening microtubules are traced by a cursor on succeeding time-lapse frames, recorded, and their rates, lengths and durations of growing and shortening are calculated from the sequence of recorded *x-y* positions of the microtubule ends.

During metaphase in the absence of drugs (FIG. 5b), the duplicated chromosomes, which are attached to the microtubules at their kinetochores, oscillate back and forth under high tension in the spindle equatorial region in concert with growth and shortening of the attached microtubules^{44,45}. Superimposed on these oscillations, tubulin is continuously and rapidly added to microtubules at the kinetochores and is lost at the poles in a balanced fashion (that is, the microtubules treadmill)^{22,46,47}. The oscillations are believed to be required for proper functioning of the spindle. The absence of tension on the chromosomal kinetochores is also sufficient to block cell-cycle progress from metaphase to anaphase^{48–50}. In anaphase (FIG. 5c), microtubules that are attached to chromosomes must undergo a carefully regulated shortening at the same time that another subpopulation of spindle microtubules (the interpolar microtubules) lengthens.

Antimitotic drugs

Interestingly, a large number of chemically diverse substances bind to soluble tubulin and/or directly to tubulin in the microtubules. Most of these compounds are antimitotic agents and inhibit cell proliferation by acting on the polymerization dynamics of spindle microtubules, the rapid dynamics of which are essential to proper spindle function. The specific effects of individual microtubule-targeted drugs on the microtubule-polymer mass and on the stability and dynamics of the microtubules are complex. Microtubule-targeted antimitotic drugs are usually classified into two main groups. One group, known as the microtubule-destabilizing agents, inhibits microtubule polymerization at high concentrations and includes several compounds — such as the *Vinca* alkaloids (vinblastine, vincristine, vinorelbine, vindesine and vinflunine), cryptophycins, halichondrins, estramustine, colchicine and combretastatins — that are used clinically or are under clinical investigation for treatment of cancer (TABLE 1). In addition, this group includes a large number of compounds that have not undergone clinical development for cancer therapy, including the anti-tussive noscapine⁵¹, maytansine, rhizoxin, spongistatins, podophyllotoxin, steganacins and curacins⁵²; several herbicides that inhibit microtubule

polymerization⁵³; antifungal and antihelmintic agents⁵⁴; and some psychoactive drugs^{55–57}. The second main group is known as the microtubule-stabilizing agents. These agents stimulate microtubule polymerization and include paclitaxel (the first agent to be identified in this class), docetaxel (Taxotere), the epothilones, discodermolide, the eleutherobins, sarcodictyins, laulimalide, rhazinalam, and certain steroids and polyisoprenyl benzophenones^{58,59}.

The classification of drugs as microtubule ‘stabilizers’ or ‘destabilizers’ is overly simplistic and can lead to confusion. The reason, as described further below, is that drugs that increase or decrease microtubule polymerization at high concentrations powerfully suppress microtubule dynamics at 10–100-fold lower concentrations and, therefore, kinetically stabilize the microtubules, without changing the microtubule-polymer mass. In other words, the effects of the drugs on dynamics are often more powerful than their effects on polymer mass. It was previously thought that the effects of the two classes of drugs on microtubule-polymer mass were the most important actions responsible for their chemotherapeutic properties. However, the drugs would have to be given and maintained at very high dosage levels to act primarily and continuously on microtubule-polymer mass. It now seems that the most important action of these drugs is the suppression of spindle-microtubule dynamics, which results in the slowing or blocking of mitosis at the metaphase–anaphase transition and induction of apoptotic cell death.

The microtubule-targeted drugs affect microtubule dynamics in several different ways. To suppress microtubule dynamics for a significant time, the drugs must bind to and act directly on the microtubule. For example, a drug that suppresses the shortening rate at microtubule ends must bind directly to the microtubule, either at its end or along its length. However, many of the drugs also act on soluble tubulin, and the relative ability of a given drug to bind to soluble tubulin or directly to the microtubule, and the location of the specific binding site in tubulin and the microtubule, greatly affect the response of the microtubule system to the drug.

The differences in the ways that the various drug classes modulate dynamics seem to specify to what extent and how the proliferation of a tumour cell will be inhibited. Suppression of spindle-microtubule treadmill and dynamic instability by antimitotic drugs seems to reduce spindle tension and slows or prevents progression from metaphase into anaphase^{43,60}. Drug-blocked cells (FIG. 5e,f) might eventually exit mitosis, often aberrantly⁶¹. Importantly for the efficacy of these drugs in cancer chemotherapy, mitotically blocked or mitotically slowed cells eventually die by apoptosis⁴³. So, the rapid dynamics of spindle microtubules are crucial for proper spindle function, and it is the requirement for rapid dynamics to ensure timely and accurate chromosome movement that make mitosis so exquisitely sensitive to paclitaxel, vinblastine and other antimitotic drugs^{42,60,62–65}.

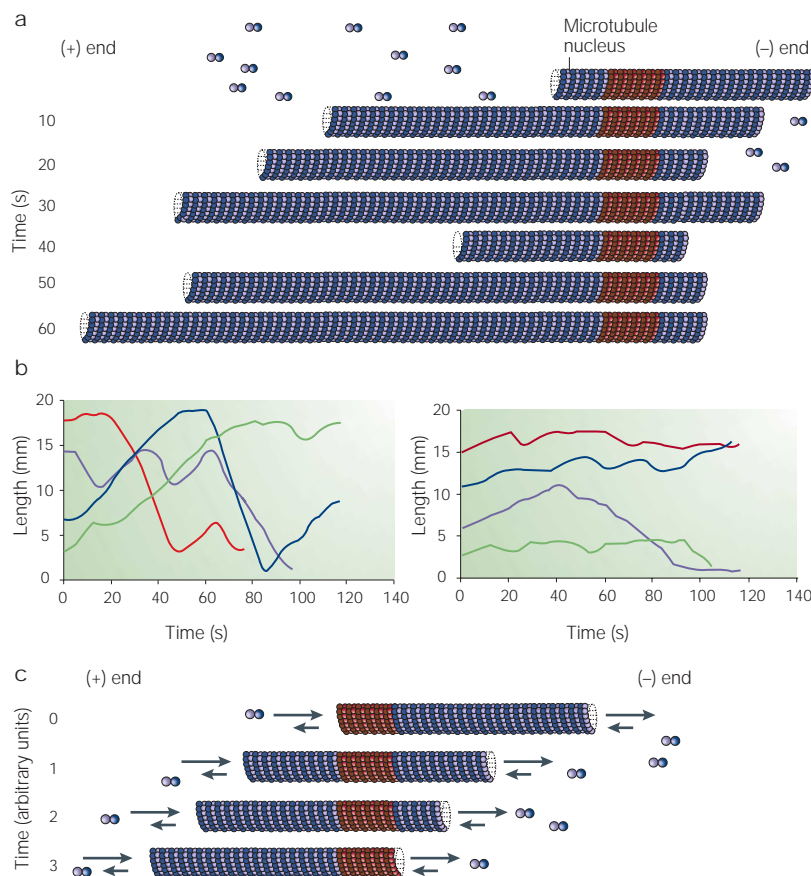


Figure 3 | Antimitotic drugs suppress dynamic instability of microtubules. a | Changes in the length of a single microtubule over time in a control cell (no drug). Microtubule ends grow and shorten stochastically over time by addition and loss of tubulin subunits from their ends. Changes in length at the plus ends are greater than at the minus ends. Microtubules also undergo phases of pause or attenuated dynamics. **b** | Life-history traces of the lengths of four individual microtubules in the absence of drug (left) and in the presence of a microtubule-targeted drug (right). The microtubules were assembled from purified bovine brain tubulin and the changes in length were traced by differential interference-contrast time-lapse microscopy. In the absence of drugs, dynamics are fast, with many length changes. In the presence of a drug such as paclitaxel, dynamics are suppressed. **c** | Treadmilling microtubule. Tubulin heterodimers are added at the plus end of the microtubule at time 0, treadmill through the microtubule and are lost from the minus end of the microtubule at time 3. The length of the microtubule is unchanged. Treadmilling is brought about by the different tubulin critical concentrations at the opposite ends.

As we shall see below, despite some important differences in their actions on tubulin and microtubules, the underlying antimitotic mechanisms of the three classes of drugs are similar.

Specific drug mechanisms

The Vinca alkaloids. The *Vinca* alkaloids have been responsible for many chemotherapeutic success stories since their introduction into the clinic 40 years ago. As indicated earlier, the naturally occurring members of the family — vinblastine and vincristine — were isolated from the leaves of the periwinkle plant *Catharanthus roseus* (L.) G. Don. The leaves of the periwinkle plant have been used for their medicinal properties since the seventeenth century. In the late 1950s, their antimitotic and, therefore, cancer chemotherapeutic potential was discovered by groups both at Eli Lilly

Research Laboratories and at the University of Western Ontario^{66,67}, and they came into widespread use for the single-agent treatment of childhood haematological and solid malignancies and, shortly after, for adult haematological malignancies. In fact, they were considered ‘wonder drugs’ because of their success in the treatment of childhood leukaemia. Since that time, their clinical efficacy in several combination therapies has led to the development of various novel semi-synthetic analogues, including vindesine, vinorelbine and vinflunine. Peripheral neuropathy and reversible myelosuppression are their principle side effects⁶⁸. The causes of the neurotoxicity are poorly understood⁶⁹, but undoubtedly involve the effects of the drugs on microtubules, which are a key component of neurons. Neuropathy might result from disruption of axonal flow⁷⁰, conceivably resulting from loss (or, with paclitaxel, bundling) of microtubules; from steric hindrance of motor-protein binding to microtubules; or from effects of altered microtubule dynamics in axonal processes or on transport in growth cones. Neurotoxicity might also result from neuronal retraction⁷⁰ and reduced arborization due to microtubule destabilization or to suppression of microtubule dynamics; from reduced responsiveness of neurons to incoming signals; or from demyelination⁶⁹. The causes of myelosuppression derive from blockage of mitosis and proliferation of the rapidly cycling bone-marrow cells.

Tubulin and microtubules are the main targets of the *Vinca* alkaloids⁷¹, which depolymerize microtubules and destroy mitotic spindles at high concentrations (for example, 10–100 nM in HeLa cells⁷¹), therefore leaving the dividing cancer cells blocked in mitosis with condensed chromosomes. At low but clinically relevant concentrations, vinblastine does not depolymerize spindle microtubules, yet it powerfully blocks mitosis (for example, IC₅₀ 0.8 nM in HeLa cells⁷¹) and cells die by apoptosis. Studies from our laboratory on the mitotic-blocking action of low *Vinca* alkaloid concentrations in living cancer cells indicate that the block is due to suppression of microtubule dynamics rather than microtubule depolymerization⁵⁸.

Vinblastine binds to the β-subunit of tubulin dimers at a distinct region called the *Vinca*-binding domain⁷². Various other novel chemotherapeutic drugs also bind at this domain (TABLE 1). The binding of vinblastine to soluble tubulin is rapid and reversible^{73,74}. Importantly, binding of vinblastine induces a conformational change in tubulin in connection with tubulin self-association^{75–77}. The ability of vinblastine to increase the affinity of tubulin for itself probably has a key role in the ability of the drug to stabilize microtubules kinetically.

Vinblastine also binds directly to microtubules. *In vitro*, vinblastine binds to tubulin at the extreme microtubule ends (FIG. 6a) with very high affinity, but it binds with markedly reduced affinity to tubulin that is buried in the tubulin lattice^{78,79}. Remarkably, the binding of one or two molecules of vinblastine per microtubule plus end is sufficient to reduce both treadmilling and dynamic instability by ~ 50%, without causing appreciable microtubule depolymerization. For example, the

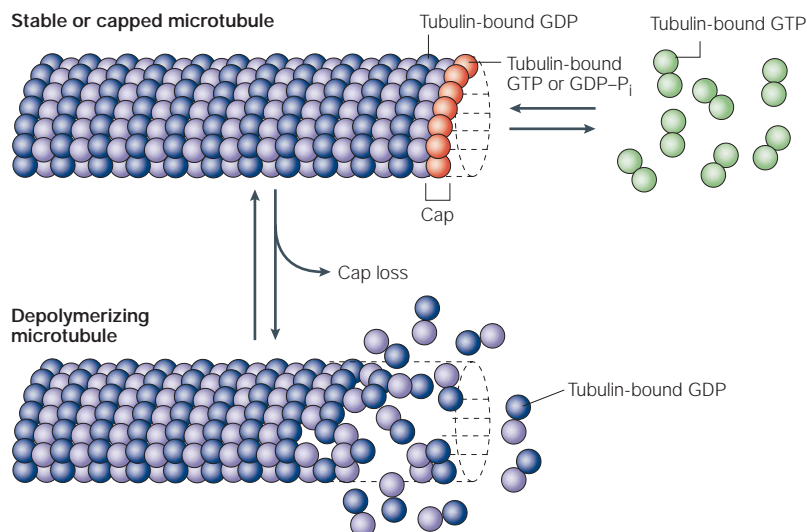


Figure 4 | Polymerization dynamics and the GTP cap. Tubulin-bound GTP is hydrolysed to tubulin-GDP and inorganic phosphate (P_i) at the time that tubulin adds to the microtubule ends, or shortly thereafter. Ultimately, the P_i dissociates from the microtubule, leaving a microtubule core consisting of tubulin with stoichiometrically bound GDP. A microtubule end containing tubulin-bound GTP or GDP- P_i is stable, or 'capped', against depolymerization. Hydrolysis of tubulin-bound GTP and the subsequent release of P_i induces conformational changes in the tubulin molecules that destabilize the microtubule polymer, resulting in catastrophe and shortening of the microtubule.

drug strongly reduces the rate and extent of microtubule growth and shortening and increases the percentage of time the microtubules spend in an attenuated or paused state, neither growing nor shortening detectably (FIG. 3b). In mitotic spindles, slowing of the growth and shortening and/or treadmilling dynamics of the microtubules blocks mitotic progression. This suppression of dynamics has at least two downstream effects on the spindle: it prevents the mitotic spindle from assembling normally and it reduces the tension at the kinetochores of the chromosomes. Mitotic progress is delayed in a metaphase-like state with chromosomes often stuck at the spindle poles, unable to congress to the spindle equator (FIG. 5e,f). The cell-cycle signal to the anaphase-promoting complex to pass from metaphase into anaphase is blocked and the cells eventually die by apoptosis.

Paclitaxel and related drugs. Paclitaxel and its semi-synthetic analogue docetaxel were among the most important new additions to the chemotherapeutic arsenal in the late twentieth century. Paclitaxel, a complex molecule that was isolated from the bark of the yew tree in 1967 by Monroe Wall and Mansukh Wani⁸⁰, underwent slow development until, in 1979, Peter Schiff and Susan Horwitz made the surprising discovery that, unlike the *Vinca* alkaloids, paclitaxel stimulated microtubule polymerization⁸¹. Even then, its development for clinical use was impeded by limited supplies of the natural compound until procedures for its semi-synthesis made its production feasible⁸². By 1995, it was approved for clinical use and it is now widely used to treat **breast and ovarian cancer, non-small-cell lung cancer and Kaposi's sarcoma**. Its principal side effects, like the *Vinca* alkaloids, are neurotoxicity and myelosuppression^{83,84}.

The taxanes bind poorly to soluble tubulin itself, but instead bind directly with high affinity to tubulin along the length of the microtubule (FIG. 6c). The binding site for paclitaxel is in the β -subunit, and its location, which is on the inside surface of the microtubule, is known with precision because determination of the electron crystal structure of tubulin was carried out with tubulin complexed with paclitaxel⁸⁵. Paclitaxel is thought to gain access to its binding sites by diffusing through small openings in the microtubule or fluctuations of the microtubule lattice²⁴. Binding of paclitaxel to its site on the inside microtubule surface stabilizes the microtubule and increases microtubule polymerization, presumably by inducing a conformational change in the tubulin that, by an unknown mechanism, increases its affinity for neighbouring tubulin molecules²⁴. There is one paclitaxel-binding site on every molecule of tubulin in a microtubule and the ability of paclitaxel to increase microtubule polymerization is associated with nearly 1:1 stoichiometric binding of paclitaxel to tubulin in approximately 10,000 tubulin molecules, then the ability of paclitaxel to increase microtubule polymerization requires the binding of $\sim 5,000$ paclitaxel molecules per microtubule. However, in contrast with the large numbers of taxane molecules that are required to increase microtubule polymerization, we found that the binding of a very small number of paclitaxel molecules powerfully stabilizes the dynamics of the microtubules without increasing microtubule polymerization⁸⁶. For example, just one paclitaxel molecule bound per several hundred tubulin molecules in a microtubule can reduce the rate or extent of microtubule shortening by $\sim 50\%$. Suppression of microtubule dynamics by paclitaxel leads to mitotic block in the absence of significant microtubule bundling⁶². In HeLa cells, mitosis is half-maximally blocked at 8 nM paclitaxel, whereas there is no increase in microtubule-polymer mass below 10 nM paclitaxel⁶⁰. In addition, the polymer mass is half-maximally increased at 80 nM paclitaxel⁶⁰. As with the *Vinca* alkaloids, the suppression of spindle-microtubule dynamics prevents the dividing cancer cells from progressing from metaphase into anaphase and the cells eventually die by apoptosis^{43,62,87}.

The clinical success of the taxanes has led to a search for other drugs that enhance microtubule polymerization, yielding several promising compounds, including the epothilones, discodermolide, the sarcodictyins, eleutherobin and laulimalide. Some of these compounds compete with paclitaxel for binding to microtubules and are said to bind at or near the taxane site (epothilones, discodermolide, eleutherobins and sarcodictyins), but others, such as laulimalide, seem to bind to unique sites on microtubules⁸⁸.

Colchicine. Colchicine is used clinically in the treatment of gout, but neither colchicine nor compounds that bind to the colchicine site on tubulin have yet found significant use in cancer treatment. The reasons for this are not clear, but might result from their potent toxicity. Several compounds that bind in the colchicine domain

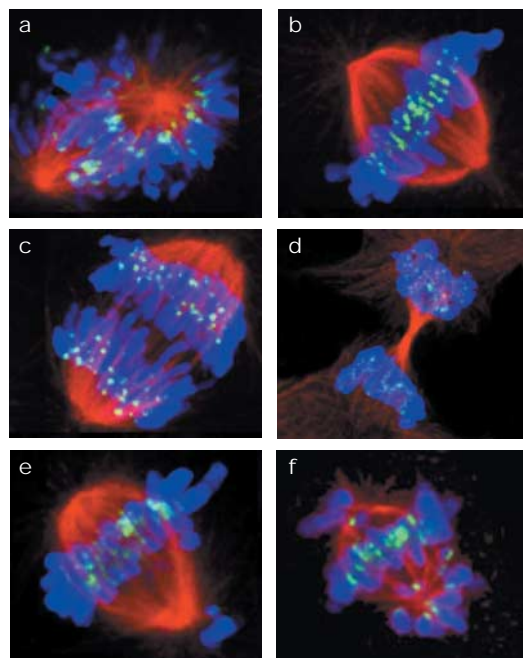


Figure 5 | Human osteosarcoma cells in different stages of the cell cycle with and without addition of antimitotic drugs. Microtubules are shown in red, chromosomes in blue and kinetochores in green. **a** | At prometaphase, the nuclear envelope has broken down, chromosomes are condensed and dynamic microtubules probe the cytoplasm until they contact a chromosome. **b** | In early metaphase, most chromosomes have congressed to the equator to form the metaphase plate. **c** | In anaphase, the duplicated chromosomes have separated and are moving towards the spindle poles to form the two daughter cells. **d** | In telophase, the separated chromosomes have reached the spindle poles and the cell is dividing to form two daughter cells. **e** | In the presence of 10 nM paclitaxel, some chromosomes remain at the spindle poles and have not congressed to the metaphase plate. **f** | Similarly, in the presence of 50 nM vinflunine, some chromosomes remain at the spindle poles. In the presence of antimitotic drugs the reduced dynamic movements of chromosomes reduces the tension on the kinetochores, centromeres and the conjoined chromosomes. These changes are associated with the blocking of mitosis at the metaphase–anaphase transition. Panel **f** reproduced with permission from Ref. 138 © (2003) American Association for Cancer Research. Panels **a, b, c** and **e** reproduced with permission from Ref. 87 © (2003) American Association for Cancer Research.

(many of which are derived from natural products; for example, the combretastatins), are now under investigation for cancer treatment (see below). The interaction of colchicine with tubulin and microtubules presents yet another variation in the mechanisms by which microtubule-active drugs inhibit microtubule function. As with the *Vinca* alkaloids, colchicine depolymerizes microtubules at high concentrations and stabilizes microtubule dynamics at low concentrations. Colchicine inhibits microtubule polymerization substoichiometrically (at concentrations well below the concentration of the tubulin that is free in solution) (for a review, see REF. 13), indicating that it inhibits microtubule polymerization by binding to microtubule

ends rather than to the soluble-tubulin pool. However, free colchicine itself probably does not bind directly to microtubule ends. Instead, it first binds to soluble tubulin, induces slow conformational changes in the tubulin and ultimately forms a poorly reversible final-state tubulin–colchicine complex^{13,89}, which then copolymerizes into the microtubule ends in small numbers along with large numbers of free tubulin molecules⁹⁰ (FIG. 6b). The ends remain competent to grow but their dynamics are suppressed.

Tubulin–colchicine complexes might have a conformation that disrupts the microtubule lattice in a way that slows, but does not prevent, new tubulin addition. Importantly, the incorporated tubulin–colchicine complex must bind more tightly to its tubulin neighbours than tubulin itself does, so that the normal rate of tubulin dissociation is reduced. Finally, because tubulin–colchicine complexes strongly reduce the catastrophe frequency and increase the rescue frequency, the tubulin–colchicine complex can modulate the mechanism responsible for gain and loss of the stabilizing GTP or GDP–P_i cap.

So, despite the differences between the effects at high concentrations of the *Vinca*/colchicine-like drugs and the taxane-like drugs, nearly all of the microtubule-targeted antimitotic drugs stabilize microtubule dynamics at their lowest effective concentrations. Stabilization of microtubule dynamics correlates with blocking of the cell cycle at mitosis and in sensitive tumour cells, ultimately resulting in cell death by apoptosis. Therefore, the most potent mechanism of nearly all of the microtubule-targeted drugs seems to be stabilization of dynamics of mitotic-spindle microtubules.

Antivascular effects

The tumour vasculature is a relatively attractive new target for cancer therapy. The vasculature is easily accessible to blood-borne therapeutic agents, and tumour cells generally die rapidly unless they are supplied with oxygen and nutrients through the blood. There are two types of approaches to inhibiting vascular function. One, which has received much attention, is the search for agents that inhibit the process of angiogenesis — the formation of new blood vessels. However, more recently, the ability of several compounds, especially microtubule-targeted agents, to rapidly shut down existing tumour vasculature has been recognized⁹¹. Since the late 1990s, the combretastatins and *N*-acetylcolchicinol-*O*-phosphate, compounds that resemble colchicine and bind to the colchicine domain on tubulin, have undergone extensive development as antivascular agents. Several of them (combretastatin-A-4 3-*O*-phosphate (CA-4-P), combretastatin A-1-phosphate (CA-1-P), ZD6126 and AVE8062A), as well as TZT-1027, which binds in the *Vinca* domain, are in clinical trials (TABLE 1).

When vascular-targeting agents such as CA-4-P are added to cultures of endothelial cells (at 0.1–1 μM), the microtubules rapidly depolymerize, the cells become round within minutes, undergo blebbing and detachment from the substrate, actin stress fibres form

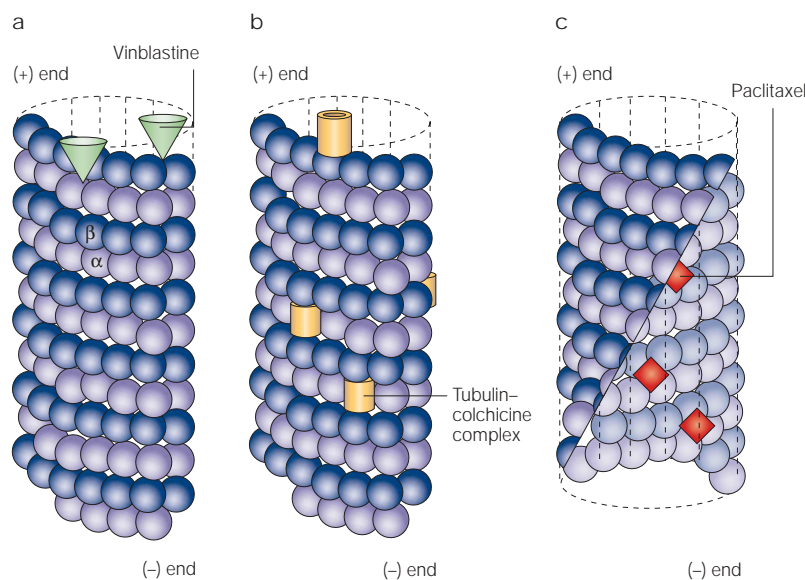


Figure 6 | Antimitotic drugs bind to microtubules at diverse sites. **a** | A few molecules of vinblastine bound to high-affinity sites at the microtubule plus end suffice to suppress microtubule dynamics. **b** | Colchicine forms complexes with tubulin dimers and copolymerizes into the microtubule lattice, suppressing microtubule dynamics. **c** | A microtubule cut away to show the interior surface is shown. Paclitaxel binds along the interior surface of the microtubule, suppressing its dynamics.

(presumably as a result of signalling from the depolymerizing microtubule cytoskeleton) and the cells die with no evidence of apoptosis⁹².

The process of vascular shutdown can be observed in rats through windowed chambers that are implanted subcutaneously. This indicates that a primary and marked effect of vascular-targeting agents is an extremely rapid reduction of blood flow to the interior of solid tumours, often within 5 minutes of administration of the drug to the animal. Within 1 hour, the red-cell velocity might drop to <5% of the starting value⁹³. Small blood vessels disappear, blood flow slows, red blood cells aggregate in stacks or 'rouleaux', haemorrhaging from peripheral tumour vessels occurs, vascular permeability increases and the interior tumour cells die by necrosis (for a review, see REF. 91).

Importantly, the vascular-targeting agents that are now under development seem to damage tumour vasculature without significantly harming normal tissues⁹⁴. The source of this specificity is not known, but has been suggested to be attributable to differences between the mature vasculature of normal tissues and the immature or forming vasculature of tumours⁹³. There are suggestions that endothelial cells of immature vasculature could have a less well-developed actin cytoskeleton that might make the cells more susceptible to collapse⁹⁵. In addition, more sluggish or more variable blood flow in tumour vasculature might make the tumour vessels particularly susceptible to damaging agents. Differences in rates of endothelial-cell proliferation, in post-translational modifications of tubulin, and in interactions between actin and microtubules might also contribute to the

specificity of vascular-targeting agents⁹³. As the targeted endothelial cells are non-tumour cells, they should be less susceptible to the development of resistance to these drugs than genetically unstable tumour cells.

Many microtubule-targeted agents, including colchicine, vincristine and vinblastine, induce similar deleterious effects at high concentrations on endothelial cells in culture and damage tumour vasculature in animals. The difference between these classical antiproliferative microtubule-targeted agents and the novel agents that are undergoing clinical testing as vascular-targeting agents might be that the effects of potential vascular-targeting agents are rapidly reversible and that their effects occur at concentrations that are well below their maximum tolerated doses⁹¹. The efficacy of a microtubule-targeted agent as either an antiproliferative agent or as a vascular-targeting agent might reside in its pharmacodynamic and pharmacokinetic characteristics, the reversibility of its binding to tubulin and its lack of long-term retention in cells. Those agents that enter cells rapidly, that rapidly and reversibly bind to tubulin or microtubules, that rapidly depolymerize microtubules and that are rapidly metabolized or excreted might act best as antivascular agents. Those that are retained in cells and induce long-term mitotic block might work best as antiproliferative agents that induce apoptotic cell death.

Tumour sensitivity and resistance

Among the most important unsolved questions about the antitumour activities of microtubule-targeted drugs concern the basis of their tissue specificities and the basis for the development of drug resistance to these agents. For example, it is not known why paclitaxel is so effective against ovarian, mammary and lung tumours, but essentially ineffective against many other solid tumours, such as kidney or colon carcinomas and various sarcomas. Similarly, for the *Vinca* alkaloids, it is unclear why they are frequently most effective against haematological cancers, but often ineffective against many solid tumours. There are clearly many determinants of sensitivity and resistance to antimitotic drugs, both at the level of the cells themselves and at the level of the pharmacological accessibility of the drugs to the tumour cells⁹⁶. Ultimate failure or inherent resistance to chemotherapy with antimitotic drugs often results from overexpression of a class of membrane transporter proteins known as ABC-transporters (ATP-dependent drug efflux pumps or ATP-binding cassettes). These membrane pumps produce decreased intracellular drug levels and lead to cross-resistance (multidrug resistance (MDR)) to drugs of different chemical structures, such as paclitaxel and *Vinca* alkaloids. The first of many identified was P-glycoprotein, the product of the human *MDR1* gene⁹⁷. Considerable efforts are underway to understand these mechanisms of resistance, to develop P-glycoprotein inhibitors and to develop microtubule-targeted drugs that are not removed by these pumps⁹⁷⁻¹⁰⁰.

However, in addition, cells also have many microtubule-related mechanisms that confer resistance or determine intrinsic insensitivity to antimetabolic drugs^{96,100–107}. Microtubule-polymer levels and dynamics are regulated by a host of factors, including expression of regulatory proteins, post-translational modifications of tubulin and expression of different tubulin isotypes. The levels of each of these isotypes differ among tissue and cell types, and there are numerous examples of changes in their levels that correlate with development of resistance to paclitaxel or *Vinca* alkaloids and other microtubule-targeted drugs^{4,96,102,108–117}.

One of the 'hot' debates among researchers is whether it is changes in microtubule-polymer levels or changes in microtubule dynamics that offer the most 'successful' and prevalent means by which cancer cells evade the effects of antimetabolic drugs. On the side of those arguing for the importance of microtubule-polymer levels, we know that the total mass of microtubule polymer is important to cells because many cell components move along microtubules. So, the ability of a cell to retain long microtubules during the onslaught from high concentrations of microtubule-depolymerizing drugs will give the cell a selective advantage during treatment with high concentrations of vinblastine or similar drugs. On the other hand, if a drug like paclitaxel induces the formation of an excessive amount of tightly packed microtubules in bundles, it is likely that the transport-motor molecules (kinesins and dyneins) will not be able to support intracellular transport. With paclitaxel, expression of endogenous microtubule-depolymerizing factors might favour the development of drug resistance. So, microtubule-regulatory molecules that counter the effects of high concentrations of antimetabolic drugs by altering microtubule-polymer levels can be important determinants of drug resistance.

However, on the side of the arguments for the importance of microtubule dynamics as a source of resistance, we now know that subtle suppression of microtubule dynamics by paclitaxel, vinblastine or other antimetabolic drugs, without any attendant change in the microtubule-polymer mass, prevents progress through the cell cycle from metaphase to anaphase in sensitive cells. Changes in microtubule dynamics can lead to altered sensitivity to microtubule-targeted drugs. In one well-studied case of paclitaxel resistance, resistant and paclitaxel-dependent A549 lung cancer cells had inherently faster microtubule dynamics following withdrawal of paclitaxel than sensitive cells (increased by 57% and 167% in two resistant cell lines)¹¹¹. In the absence of paclitaxel, the paclitaxel-resistant/dependent cells with the faster microtubule dynamics were unable to progress from metaphase to anaphase and their spindles became disorganized. So, these cells were resistant to paclitaxel and also dependent on paclitaxel to slow their dynamics and allow them to go through mitosis successfully. The inherent sensitivity of cells to subtle changes in microtubule dynamics means that there are numerous ways for cells to become resistant to microtubule-targeted drugs. In the case of the paclitaxel-resistant A549 cells discussed above, the mechanisms of increased dynamics seem to involve several changes. The resistant cells overexpress one of the

isotypes of tubulin, β III-tubulin^{118–120}. In addition, they have a heterozygous point mutation in α -tubulin and they overexpress the active form of the microtubule-destabilizing protein stathmin and the inactive form of the putative microtubule-stabilizing protein MAP4. The location of the α -tubulin mutation is the putative site of interaction with MAP4 and stathmin¹²⁰.

In addition, drug resistance might involve some of the other forms of tubulin (γ -, δ - and ϵ -tubulin) that associate with the centrosomes in interphase and with the spindle poles in mitotic cells. Although we know that centrosome structure is very sensitive to low levels of *Vinca* alkaloids¹²¹, there is very little known about how microtubule-targeted drugs interact with centrosomal proteins and whether they might be involved in drug efficacy, regulation of microtubule dynamics and mitotic progress, and the development of drug resistance. So, the development of drug resistance is complex and there are reasons to believe that both changes in microtubule-polymer mass and changes in microtubule dynamics are used by cells in the process of becoming drug resistant. Conceivably, if one knew the tubulin isotype and microtubule-regulatory protein composition of a specific tumour cell, one could design or choose drugs to selectively target that tumour. In addition, an important point is that microtubule function is affected by many endogenous cellular regulatory proteins. Therefore, there are many unexplored potential targets involving microtubules for the design of novel chemotherapeutic drugs.

The surprising synergy of microtubule drugs
The fact that antimetabolic drugs bind to many diverse sites on tubulin and microtubules means that clinical combinations of two or more of these drugs have the potential to improve efficacy and reduce the side effects of therapy. For example, estramustine, which binds to a novel site in tubulin¹²², can act synergistically or additively in its antiproliferative effects when combined with either vinblastine or paclitaxel in cells^{123–125}. Its ability to suppress microtubule dynamic instability is also additive with that of vinblastine¹²². In addition, vinorelbine plus paclitaxel and vinorelbine plus docetaxel are superior^{126–128} to either drug alone (in each combination), as are docetaxel and the colchicine analogue CI-980 (REF. 129), paclitaxel and vinblastine¹³⁰, and paclitaxel and discodermolide¹¹⁹. The discovery of the synergism of paclitaxel with discodermolide is particularly interesting, as both drugs bind to the same or overlapping sites on tubulin or microtubules¹¹⁹. Interestingly, following examination of the mechanism of synergism between paclitaxel and discodermolide in these cells, we found that they synergistically suppressed microtubule dynamics (S. Honore, K. Kamath, D. Braguer, S. Horwitz, L.W. and M.A.J., unpublished observations). Although this synergistic effect on dynamics is not understood, and is very surprising, it is clear that the potential for use of microtubule-targeted drugs that work by similar mechanisms is an untapped source of chemotherapeutic potential. These discoveries have led to the initiation of several clinical trials involving

combinations of two or more microtubule-targeted drugs. These include Phase I, II and III trials of estramustine in combination with paclitaxel, docetaxel, vinorelbine or epothilone derivatives, and Phase I and II trials of vinorelbine in combination with paclitaxel, docetaxel or vinblastine (TABLE 1). The use of combinations of drugs that stabilize microtubule dynamics by different mechanisms holds great promise for enhancing antitumour activity without inducing the deleterious side effects that are associated with the use of high concentrations of a single agent.

What lies ahead?

Future challenges in the use of microtubule-targeted agents lie in increasing the understanding of their basic mechanisms and improving their clinical effectiveness. For example, microtubule-targeted drugs could be used in combination therapy at much lower doses than are now used — at their biologically effective doses (that suppress microtubule dynamics) rather than at their maximum tolerated doses. Furthermore, relatively weak microtubule-targeted drugs that suppress dynamics (for example, griseofulvin, coumarins and benomyl) could be used as adjuvants in chemotherapy to attain efficacy with decreased toxicity. The maintenance of low concentrations of microtubule-targeted drugs in tumour tissue for long durations could be more important in effective tumour-cell killing than the rapidly rising and falling drug concentrations that

are associated with bolus administration at maximum tolerated doses. Finally, because of the involvement of microtubules in so many different cellular processes, they could be combined very effectively with 'molecularly targeted' drugs. With regard to improving the basic knowledge of these drugs, we need to understand the relationship between drug-induced mitotic block and cell death, as well as the interactions of the microtubule-targeted drugs with the centrosome or mitotic-spindle pole, where other forms of tubulin are focused. In addition, the sources of resistance to microtubule-targeted drugs, such as expression of tubulin isotypes, tubulin mutations and microtubule-regulatory proteins, are areas of intense research. To understand and design microtubule-targeted drugs that overcome MDR and neuropathy are extremely important clinical goals. Understanding why some drugs, such as the taxanes work well in ovarian and breast cancer, whereas the *Vinca* alkaloids often work well in blood cancers is important. In addition, designing the best combinations of agents — ultimately, to take advantage of tumour-tissue expression typing — will be of increasing importance. Nature has presented us with a validated, highly successful anticancer target in the microtubule, one that we have only begun to understand and to use efficaciously. In combination with drugs against other targets and with other microtubule-targeted drugs, this class of drugs promises to be a successful mainstay of cancer therapy long into the future.

- Ludueno, R. F. Multiple forms of tubulin: different gene products and covalent modifications. *Int. Rev. Cytology* **178**, 207–275 (1998).
Comprehensive review of tubulin isotypes and post-translational modifications.
- Verdier-Pinard, P. *et al.* Direct analysis of tubulin expression in cancer cell lines by electrospray ionization mass spectrometry. *Biochemistry* **42**, 12019–12027 (2003).
Describes analysis of tubulin isotypes, mutations and post-translational modifications by liquid chromatography/electrospray-ionization mass spectrometry in paclitaxel-sensitive and -resistant cell lines.
- Ligon, L. A., Shelly, S. S., Tokito, M. & Holzbaur, E. L. The microtubule plus-end proteins EB1 and dynactin have differential effects on microtubule polymerization. *Mol. Biol. Cell* **14**, 1405–1417 (2003).
- Galmarini, C. M. *et al.* Drug resistance associated with loss of p53 involves extensive alterations in microtubule composition and dynamics. *Br. J. Cancer* **88**, 1793–1799 (2003).
- Giodini, A. *et al.* Regulation of microtubule stability and mitotic progression by survivin. *Cancer Res.* **62**, 2462–2467 (2002).
- Cassimeris, L. The oncoprotein 18/stathmin family of microtubule destabilizers. *Curr. Opin. Cell Biol.* **14**, 18–24 (2002).
- Spittle, C., Charasse, S., Larroque, C. & Cassimeris, L. The interaction of TOGp with microtubules and tubulin. *J. Biol. Chem.* **275**, 20748–20753 (2000).
- Maney, T., Wagenbach, M. & Wordeman, L. Molecular dissection of the microtubule depolymerizing activity of mitotic centromere-associated kinesin. *J. Biol. Chem.* **276**, 34753–34758 (2001).
- Jordan, M. A. & Wilson, L. Microtubules and actin filaments: dynamic targets for cancer chemotherapy. *Curr. Opin. Cell Biol.* **10**, 123–130 (1998).
- Giannakakou, P., Sackett, D. & Fojo, T. Tubulin/microtubules: still a promising target for new chemotherapeutic agents. *J. Natl Cancer Inst.* **92**, 182–183 (2000).
- Gerzon, K. in *Anticancer Agents Based on Natural Product Models* (eds Cassidy, J. M. & Douros, J. D.) 271–317 (Academic, New York, 1980).
- Wordeman, L. & Mitchison, T. J. in *Microtubules* (eds Hyams, J. S. & Lloyd, C. W.) 287–302 (Wiley-Liss, New York, 1994).
- Wilson, L. & Jordan, M. A. in *Microtubules* (eds Hyams, J. S. & Lloyd, C. W.) 59–84 (Wiley-Liss, New York, 1994).
- McIntosh, J. R. in *Microtubules* (eds Hyams, J. S. & Lloyd, C. W.) 413–434 (Wiley-Liss, New York, 1994).
- Waterman-Storer, C. & Salmon, E. D. Microtubule dynamics: treadmill comes around again. *Curr. Biol.* **7**, 369–372 (1997).
- Mitchison, T. J. & Kirschner, M. Dynamic instability of microtubule growth. *Nature* **312**, 237–242 (1984).
- Margolis, R. L. & Wilson, L. Opposite end assembly and disassembly of microtubules at steady state *in vitro*. *Cell* **13**, 1–8 (1978).
- Margolis, R. L. & Wilson, L. Microtubule treadmill: what goes around comes around. *Bioessays* **20**, 830–836 (1998).
- Rodionov, V. I. & Borisy, G. G. Microtubule treadmill *in vivo*. *Science* **275**, 215–218 (1997).
- Shaw, S. L., Kamyar, R. & Ehrhardt, D. W. Sustained microtubule treadmill in *Arabidopsis* cortical arrays. *Science* **300**, 1715–1718 (2003).
- Panda, D., Miller, H. P. & Wilson, L. Rapid treadmill of MAP-free brain microtubules *in vitro* and its suppression by tau. *Proc. Natl Acad. Sci. USA* **96**, 12459–12464 (1999).
- Chen, W. & Zhang, D. Kinetochore fibre dynamics outside the context of the spindle during anaphase. *Nature Cell Biol.* **6**, 227–231 (2004).
Demonstration of importance of treadmill in anaphase-chromosome poleward movement and the independence of treadmill from the spindle matrix.
- Wilson, L., Panda, D. & Jordan, M. A. Modulation of microtubule dynamics by drugs: a paradigm for the actions of cellular regulators. *Cell Struct. Funct.* **24**, 329–335 (1999).
- Nogales, E. Structural insights into microtubule function. *Annu. Rev. Biophys. Biomol. Struct.* **30**, 397–420 (2001).
- Wilson, L. & Jordan, M. A. Microtubule dynamics: taking aim at a moving target. *Chem. Biol.* **2**, 569–573 (1995).
- Lodish, H. *et al.* *Molecular Cell Biology* (W. H. Freeman, New York, 1999).
- Panda, D., Miller, H. & Wilson, L. Determination of the size and chemical nature of the stabilizing cap at microtubule ends using modulators of polymerization dynamics. *Biochemistry* **41**, 1609–1617 (2002).
- Caplow, M. & Fee, L. Concerning the chemical nature of tubulin subunits that cap and stabilize microtubules. *Biochemistry* **42**, 2122–2126 (2003).
- Panda, D., Miller, H. P. & Wilson, L. Rapid treadmill of brain microtubules free of microtubule-associated proteins *in vitro* and its suppression by tau. *Proc. Natl Acad. Sci. USA* **96**, 12459–12464 (1999).
- Wittmann, T., Bokoch, G. & Waterman-Storer, C. Regulation of leading edge microtubule and actin dynamics downstream of Rac1. *J. Cell Biol.* **161**, 845–851 (2003).
- Alli, E., Bash-Babula, J., Yang, J.-M. & Hait, W. N. Effect of stathmin on the sensitivity to antimicrotubule drugs in human breast cancer. *Cancer Res.* **62**, 6864–6869 (2002).
- McNally, F. Microtubule dynamics: new surprises from an old MAP. *Curr. Biol.* **13**, 597–599 (2003).
- Ohi, R., Coughlin, M. L., Lane, W. S. & Mitchison, T. J. An inner centromere protein that stimulates the microtubule depolymerizing activity of a Kln1 kinesin. *Dev. Cell* **5**, 309–321 (2003).
- Carvalho, P., Timauer, J. & Pellman, D. Surfing on microtubule ends. *Trends Cell Biol.* **13**, 229–237 (2003).
- Hergovich, A., Lisztwan, J., Barry, R., Ballschiemier, P. & Krek, W. Regulation of microtubule stability by the von Hippel-Lindau tumour suppressor protein pVHL. *Nature Cell Biol.* **5**, 64–70 (2003).
- Komarova, Y., Akhmanova, A., Kojima, S., Galjart, N. & Borisy, G. Cytoplasmic linker proteins promote microtubule rescue *in vivo*. *J. Cell Biol.* **159**, 589–599 (2002).
- Mitchison, T. J. Microtubule dynamics and kinetochore function in mitosis. *Annu. Rev. Cell Biol.* **4**, 527–549 (1988).
- Saxton, W. M. *et al.* Tubulin dynamics in cultured mammalian cells. *J. Cell Biol.* **99**, 2175–2186 (1984).
- 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- α tubulin. *Mol. Biol. Cell* **12**, 971–980 (2001).
- Pepperkok, R., Bre, M. H., Davoust, J. & Kreis, T. E. Microtubules are stabilized in confluent epithelial cells but not in fibroblasts. *J. Cell Biol.* **111**, 3003–3012 (1990).

41. 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.* **135**, 201–214 (1996).
42. 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.* **111**, 1039–1045 (1990).
- Classic demonstration of the role of microtubule dynamic instability in the attachment of chromosomes to the mitotic spindle.**
43. Jordan, M. A. *et al.* Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. *Cancer Res.* **56**, 816–825 (1996).
- Demonstration that mitotic block by low concentrations of paclitaxel leads to apoptosis.**
44. 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.* **127**, 1301–1310 (1994).
45. 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.* **135**, 545–557 (1996).
46. Mitchison, T. J. Poleward microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J. Cell Biol.* **109**, 637–652 (1989).
47. Wilson, P. J. & Forer, A. Effects of nanomolar taxol on crane-fly spermatocyte spindles indicate that acetylation of kinetochore microtubules can be used as a marker of poleward tubulin flux. *Cell Motil. Cytoskeleton* **37**, 20–32 (1997).
48. Li, X. & Nicklas, R. B. Mitotic forces control a cell-cycle checkpoint. *Nature* **373**, 630–632 (1995).
49. Nicklas, R. B., Ward, S. C. & Gorbsky, G. J. Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J. Cell Biol.* **130**, 929–939 (1995).
50. Gorbsky, G. J. Cell cycle checkpoints: arresting progress in mitosis. *BioEssays* **19**, 193–197 (1997).
51. Zhou, J. *et al.* Brominated derivatives of noscapine are potent microtubule-interfering agents that perturb mitosis and inhibit cell proliferation. *Mol. Pharmacol.* **63**, 799–807 (2003).
52. Hamel, E. & Covell, D. G. Antimitotic peptides and desipeptides. *Curr. Med. Chem. Anti-Canc. Agents* **2**, 19–53 (2002).
53. Hoffman, J. C. & Vaughn, K. C. Mitotic disrupter herbicides act by a single mechanism but vary in efficacy. *Protoplasma* **179**, 16–25 (1994).
54. Lacey, E. & Gill, J. H. Biochemistry of benzimidazole resistance. *Acta Trop.* **56**, 245–262 (1994).
55. Lobert, S., Ingram, J. & Correia, J. Additivity of dilantin and vinblastine inhibitory effects on microtubule assembly. *Cancer Res.* **59**, 4816–4822 (1999).
56. Cann, J. R. & Hinman, N. D. Interaction of chlorpromazine with brain microtubule subunit protein. *Molec. Pharmacol.* **11**, 256–267 (1975).
57. Boder, G. B., Paul, D. C. & Williams, D. C. Chlorpromazine inhibits mitosis of mammalian cells. *Eur. J. Cell Biol.* **31**, 349–353 (1983).
58. Jordan, M. A. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Curr. Med. Chem. Anti-Canc. Agents* **2**, 1–17 (2002).
59. Jimenez-Barbero, J., Amat-Guerri, F. & Snyder, J. P. The solid state, solution and tubulin-bound conformations of agents that promote microtubule stabilization. *Curr. Med. Chem. Anti-Canc. Agents* **2**, 91–122 (2002).
60. Jordan, M. A., Toso, R. J., Thrower, D. & Wilson, L. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Proc. Natl Acad. Sci. USA* **90**, 9552–9556 (1993).
61. Chen, J.-G. & Horwitz, S. B. Differential mitotic responses to microtubule-stabilizing and -destabilizing drugs. *Cancer Res.* **62**, 1935–1938 (2002).
62. Yvon, A.-M., Wadsworth, P. & Jordan, M. A. Taxol suppresses dynamics of individual microtubules in living human tumor cells. *Mol. Biol. Cell* **10**, 947–949 (1999).
- First demonstration that suppression of microtubule dynamics in living cells by low concentrations of paclitaxel correlates with mitotic block.**
63. Jordan, M. A. & Wilson, L. In *Methods in Cell Biology, in Mitosis and Meiosis* Vol. 61 (ed. Rieder, C. L.) 267–295 (Academic, New York, 1998).
64. Skibbens, R. V., Skeen, V. P. & Salmon, E. D. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J. Cell Biol.* **122**, 859–875 (1993).
65. Waters, J. C., Mitchison, T. J., Rieder, C. L. & Salmon, E. D. The kinetochore microtubule minus-end disassembly associated with poleward flux produces a force that can do work. *Mol. Biol. Cell* **7**, 1547–1558 (1996).
66. Johnson, I. S., Wright, H. F. & Svoboda, G. H. Experimental basis for clinical evaluation of anti-tubulin principles derived from *Vinca rosea* Linn. *J. Lab. Clin. Med.* **54**, 830–837 (1959).
67. Noble, R. L., Beer, C. T. & Cutts, J. H. Further biological activities of vincateubolastine: an alkaloid isolated from *Vinca rosea* (L.). *Biochem. Pharmacol.* **1**, 347–348 (1958).
68. Gidding, C. E., Kellie, S. J., Kamps, W. A. & de Graaf, S. S. Vincristine revisited. *Crit. Rev. Oncol. Hematol.* **29**, 267–287 (1999).
69. Quasthoff, S. & Hartung, H. P. Chemotherapy-induced peripheral neuropathy. *J. Neurol.* **249**, 9–17 (2002).
70. Sahenk, Z., Barohn, R., New, P. & Mendell, J. R. Taxol neuropathy. Electrodiagnostic and sural nerve biopsy findings. *Arch. Neurol.* **51**, 726–729 (1994).
71. Jordan, M. A., Thrower, D. & Wilson, L. Mechanism of inhibition of cell proliferation by *Vinca* alkaloids. *Cancer Res.* **51**, 2212–2222 (1991).
- First demonstration that antimitotic mechanism of Vinca alkaloids does not require microtubule depolymerization.**
72. 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.* **265**, 17141–17149 (1990).
73. Wilson, L., Jordan, M. A., Morse, A. & Margolis, R. L. Interaction of vinblastine with steady-state microtubules *in vitro*. *J. Mol. Biol.* **159**, 129–149 (1982).
74. Jordan, M. A. & Wilson, L. Kinetic analysis of tubulin exchange at microtubule ends at low vinblastine concentrations. *Biochemistry* **29**, 2730–2739 (1990).
75. Na, G. C. & Timasheff, S. N. Thermodynamic linkage between tubulin self-association and the binding of vinblastine. *Biochemistry* **19**, 1347–1354 (1980).
76. Na, G. C. & Timasheff, S. N. Stoichiometry of the vinblastine-induced self-association of calf brain tubulin. *Biochem. Soc. Trans.* **8**, 1347–1354 (1980).
77. Lobert, S. & Correia, J. In *Methods in Enzymology* Vol. 323, (ed. Johnson, M.) 77–103 (Academic Press, 2000).
78. Jordan, M. A., Margolis, R. L., Himes, R. H. & Wilson, L. Identification of a distinct class of vinblastine binding sites on microtubules. *J. Mol. Biol.* **187**, 61–73 (1986).
79. Singer, W. D., Jordan, M. A., Wilson, L. & Himes, R. H. Binding of vinblastine to stabilized microtubules. *Mol. Pharmacol.* **36**, 366–370 (1989).
80. Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P. & McPhail, A. T. *J. Am. Chem. Soc.* **93**, 2325–2327 (1971).
81. Schiff, P. B., Fant, J. & Horwitz, S. B. Promotion of microtubule assembly *in vitro* by taxol. *Nature* **277**, 665–667 (1979).
- Classic demonstration that paclitaxel enhances microtubule polymerization and stabilizes microtubules.**
82. Horwitz, S. B. How to make taxol from scratch. *Nature* **367**, 593–594 (1994).
83. Von Hoff, D. D. The taxoids: same roots, different drugs. *Semin. Oncol.* **24** (4 Suppl. 13), S13–S13-10 (1997).
84. Markman, M. Managing taxane toxicities. *Support Care Cancer* **11**, 144–147 (2003).
85. Nogales, E., Wolf, S. G., Khan, I. A., Luduena, R. F. & Downing, K. A. Structure of tubulin at 6.5 Å and location of the taxol-binding site. *Nature* **375**, 424–427 (1995).
- First high-resolution determination of the structure of polymerized tubulin and the paclitaxel-binding site.**
86. Derry, W. B., Wilson, L. & Jordan, M. A. Substoichiometric binding of taxol suppresses microtubule dynamics. *Biochemistry* **34**, 2203–2211 (1995).
- Mechanistic analysis of suppression of microtubule dynamics by paclitaxel.**
87. Kelling, J., Sullivan, K., Wilson, L. & Jordan, M. A. Suppression of centromere dynamics by taxol in living osteosarcoma cells. *Cancer Res.* **63**, 2794–2801 (2003).
88. Pryor, D. E. *et al.* The microtubule stabilizing agent laulimalide does not bind in the taxoid site, kills cells resistant to paclitaxel and epothilones, and may not require its epoxide moiety for activity. *Biochemistry* **41**, 9109–9115 (2002).
89. Hastie, S. B. Interactions of colchicine with tubulin. *Pharmacol. Ther.* **512**, 377–401 (1991).
90. Skoufias, D. & Wilson, L. Mechanism of inhibition of microtubule polymerization by colchicine: inhibitory potencies of unliganded colchicine and tubulin–colchicine complexes. *Biochemistry* **31**, 738–746 (1992).
91. Tozer, G. M., Kanthou, C., Parkins, C. S. & Hill, S. A. The biology of the combretastatins as tumour vascular targeting agents. *Int. J. Exp. Pathol.* **83**, 21–38 (2002).
- Review of mechanism of action of combretastatins and their therapeutic potential.**
92. Kanthou, C. & Tozer, G. M. The tumor vascular targeting agent combretastatin A-4-phosphate induces reorganization of the actin cytoskeleton and early membrane blebbing in human endothelial cells. *Blood* **99**, 2060–2069 (2002).
93. Tozer, G. M. *et al.* Mechanisms associated with tumor vascular shut-down induced by combretastatin A-4 phosphate: intravital microscopy and measurement of vascular permeability. *Cancer Res.* **61**, 6413–6422 (2001).
94. Prise, V. E., Honess, D. J., Stratford, M. R., Wilson, J. & Tozer, G. M. The vascular response of tumor and normal tissues in the rat to the vascular targeting agent, combretastatin A-4-phosphate, at clinically relevant doses. *Int. J. Oncol.* **21**, 717–726 (2002).
95. Davis, P. D. *et al.* ZD6126: a novel vascular-targeting agent that causes selective destruction of tumor vasculature. *Cancer Res.* **62**, 7247–7253 (2002).
96. Dumontet, C. & Skic, B. Mechanisms of action of and resistance to antitubulin agents: microtubule dynamics, drug transport, and cell death. *J. Clin. Oncol.* **17**, 1061–1070 (1999).
97. Ambudkar, S. V., Kimchi-Sarfaty, C., Sauna, Z. E. & Gottesman, M. M. P-glycoprotein: from genomics to mechanism. *Oncogene* **22**, 7468–7485 (2003).
98. Safa, A. R. Identification and characterization of the binding sites of P-glycoprotein for multidrug resistance-related drugs and modulators. *Curr. Med. Chem. Anti-Canc. Agents* **4**, 1–17 (2004).
99. Thomas, H. & Coley, H. M. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting P-glycoprotein. *Cancer Control* **10**, 159–165 (2003).
100. Geney, R., Ungreanu, M., Li, D. & Ojima, I. Overcoming multidrug resistance in taxane chemotherapy. *Clin. Chem. Lab. Med.* **40**, 918–925 (2002).
101. Orr, G. A., Verdier-Pinard, P., McDaid, H. & Horwitz, S. B. Mechanisms of taxol resistance related to microtubules. *Oncogene* **22**, 7280–7295 (2003).
- Comprehensive review of microtubule-related mechanisms of paclitaxel resistance.**
102. Kavaliris, M. *et al.* Multiple microtubule alterations are associated with *Vinca* alkaloid resistance in human leukemia cells. *Cancer Res.* **61**, 5803–5809 (2001).
103. Minotti, A. M., Barlow, S. B. & Cabral, F. Resistance to antimitotic drugs in Chinese hamster ovary cells correlated with changes in the level of polymerized tubulin. *J. Biol. Chem.* **266**, 3987–3994 (1991).
104. James, S. W., Sillow, C. D., Stroom, P. & Lefebvre, P. A. A mutation in the $\alpha 1$ -tubulin gene of *Chlamydomonas reinhardtii* confers resistance to anti-microtubule herbicides. *J. Cell Sci.* **106**, 209–218 (1993).
105. Lee, W.-P. Purification and characterization of tubulin from parental and vincristine-resistant HOB1 lymphoma cells. *Arch. Biochem. Biophys.* **319**, 498–503 (1995).
106. Ohta, S. *et al.* Characterization of a taxol-resistant human small-cell lung cancer cell line. *Jpn. J. Cancer Res.* **85**, 290–297 (1994).
107. Laing, N. M. *et al.* Amplification of the ATP-binding cassette 2 transporter gene is functionally linked with enhanced efflux of estramustine in ovarian carcinoma cells. *Cancer Res.* **58**, 1332–1337 (1998).
108. Burkhardt, C. A., Kavaliris, M. & Band Horwitz, S. The role of β -tubulin isotypes in resistance to antimitotic drugs. *Biochim. Biophys. Acta* **2**, O1–O9 (2001).
109. Dumontet, C. *et al.* Resistance to microtubule-targeted cytotoxins in a K562 leukemia cell variant is associated with altered tubulin expression. *Elect. J. Oncol.* **2**, 33–44 (1999).
110. Giannakakou, P. *et al.* A common pharmacophore for epothilone and taxanes: molecular basis for drug resistance conferred by tubulin mutations in human cancer cells. *Proc. Natl Acad. Sci. USA* **97**, 2904–2909 (2000).
111. Goncalves, A. *et al.* Resistance to taxol in lung cancer cells associated with increased microtubule dynamics. *Proc. Natl Acad. Sci. USA* **98**, 11737–11741 (2001).
- First demonstration of sensitivity of mitotic progression to precise regulation of microtubule dynamics and of the association of increased microtubule dynamics with paclitaxel resistance.**
112. Haber, M. *et al.* Altered expression of $\beta 2$, the class II β -tubulin isotype, in a murine J774.2 cell line with a high level of taxol resistance. *J. Biol. Chem.* **270**, 31269–31275 (1995).
113. Jaffrezou, J.-P. *et al.* Novel mechanism of resistance to paclitaxel in human K562 leukemia cells by combined selection with PSC833. *Oncology Res.* **7**, 517–527 (1995).
114. Kavaliris, M. *et al.* Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific β -tubulin isotypes. *J. Clin. Invest.* **100**, 1–12 (1997).
115. Poruchynsky, M. S. *et al.* Accompanying protein alterations in malignant cells with a microtubule-polymerizing drug-resistance phenotype and a primary resistance mechanism. *Biochem. Pharmacol.* **62**, 1469–1480 (2001).

116. Ranganathan, S. *et al.* Increase of β III- and β IVa-tubulin isotypes in human prostate carcinoma cells as a result of estramustine resistance. *Cancer Res.* **56**, 2584–2589 (1996).
117. Verdier-Pinard, P. *et al.* Analysis of tubulin isotypes and mutations from taxol-resistant cells by combined isoelectrofocusing and mass spectrometry. *Biochemistry* **42**, 5349–5357 (2003).
118. Kavallaris, M., Burkhardt, C. A. & Horwitz, S. B. Antisense oligonucleotides to class III β -tubulin sensitize drug-resistant cells to Taxol. *Br. J. Cancer* **80**, 1020–1025 (1999).
119. Martello, L. A. *et al.* Taxol and discodermolide represent a synergistic drug combination in human carcinoma cell lines. *Clin. Cancer Res.* **6**, 1978–1987 (2000).
120. Martello, L. A. *et al.* Elevated levels of microtubule destabilizing factors in a taxol-resistant/dependent A549 cell line with an α -tubulin mutation. *Cancer Res.* **63**, 1207–1213 (2003).
121. Wendell, K. L., Wilson, L. & Jordan, M. A. Mitotic block in HeLa cells by vinblastine: ultrastructural changes in kinetochore-microtubule attachment and in centrosomes. *J. Cell Sci.* **104**, 261–274 (1993).
122. Panda, D., Miller, H., Islam, K. & Wilson, L. Stabilization of microtubule dynamics by estramustine by binding to a novel site in tubulin: a possible mechanistic basis for its antitumor action. *Proc. Natl Acad. Sci. USA* **94**, 10560–10564 (1997).
123. Seidman, A., Scher, H. I., Petrylak, D., Derrshaw, D. D. & Curley, T. Estramustine and vinblastine: use of prostate specific antigen as a clinical trial end point for hormone refractory prostatic cancer. *J. Urol.* **147**, 931–934 (1992).
124. Hudes, G. R. *et al.* Phase II study of estramustine and vinblastine, two microtubule inhibitors, in hormone-refractory prostate cancer. *J. Clin. Oncol.* **10**, 1754–1761 (1992).
125. Hudes, G. R. *et al.* Paclitaxel plus estramustine in metastatic hormone-refractory prostate cancer. *Semin. Oncol.* **22**, 41–45 (1995).
126. 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.* **87**, 1072–1077 (1995).
127. Photiou, A., Shah, P., Leong, L., Moss, J. & Rettsas, S. *In vitro* synergy of paclitaxel (Taxol) and vinorelbine (navelbine) against human melanoma cell lines. *Eur. J. Cancer* **33**, 463–470 (1997).
128. Dieras, V. *et al.* Docetaxel in combination with doxorubicin or vinorelbine. *Eur. J. Cancer* **33** (Suppl 7), 20–22 (1997).
129. Garcia, P., Braguer, D., Carles, G. & Briand, C. Simultaneous combination of microtubule depolymerizing and stabilizing agents acts at low doses. *Anticancer Drugs* **6**, 533–544 (1995).
130. 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* **75**, 57–63 (1998).
131. Duflos, A., Kruczynski, A. & Barret, J.-M. Novel aspects of natural and modified *Vinca* alkaloids. *Curr. Med. Chem. Anti-Canc. Agents* **2**, 55–70 (2002).
132. Plosker, G. L. & Figgitt, D. Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. *Drugs* **63**, 803–843 (2003).
133. Sandler, A. B. Chemotherapy for small cell lung cancer. *Semin. Oncol.* **30**, 9–25 (2003).
134. Armitage, J. O. Overview of rational and individualized therapeutic strategies for non-Hodgkin's lymphomas. *Clin. Lymphoma* **3**, S5–S11 (2002).
135. Jassem, J. *et al.* Oral vinorelbine in combination with cisplatin: a novel active regimen in advanced non-small-cell lung cancer. *Ann. Oncol.* **14**, 1634–1639 (2003).
136. Rossi, A. *et al.* Single agent vinorelbine as first-line chemotherapy in elderly patients with advanced breast cancer. *Anticancer Res.* **23**, 1657–1664 (2003).
137. Seidman, A. D. Monotherapy options in the management of metastatic breast cancer. *Semin. Oncol.* **30**, 6–10 (2003).
138. Okouneva, T., Hill, B. T., Wilson, L. & Jordan, M. A. The effects of vinflunine, vinorelbine, and vinblastine on centromere dynamics. *Mol. Cancer Ther.* **2**, 427–436 (2003).
139. Panda, D. *et al.* Interaction of the antitumor compound cryptophycin-52 with tubulin. *Biochemistry* **39**, 14121–14127 (2000).
140. Kerksek, K., Mejillano, M. R., Schwartz, R. E., Georg, G. I. & Himes, R. H. Interaction of cryptophycin 1 with tubulin and microtubules. *FEBS Lett.* **377**, 59–61 (1995).
141. Bai, R. B. *et al.* Halichondrin B and homohalichondrin B, marine natural products binding in the *Vinca* domain of tubulin. Discovery of tubulin-based mechanism of action by analysis of differential cytotoxicity data. *J. Biol. Chem.* **266**, 15882–15889 (1991).
142. Luduena, R. F., Roach, M. C., Prasad, V. & Pettit, G. R. Interaction of halichondrin B and homohalichondrin B with bovine brain tubulin. *Biochem. Pharmacol.* **45**, 421–427 (1993).
143. Towle, M. J. *et al.* *In vitro* and *in vivo* anticancer activities of synthetic macrocyclic ketone analogs of halichondrin B. *Cancer Res.* **61**, 1013–1021 (2001).
144. Hamel, E. Natural products which interact with tubulin in the *Vinca* domain: maytansine, rhizoxin, phomopsisin A, Dolastatins 10 and 15 and halichondrin B. *Pharmacol. Ther.* **55**, 31–51 (1992).
145. Bai, R., Durso, N. A., Sackett, D. L. & Hamel, E. Interactions of the sponge-derived antimitotic tripeptide hemiasterlin with tubulin: comparison with dolastatin 10 and cryptophycin 1. *Biochemistry* **38**, 14302–14310 (1999).
146. Loganzo, F. *et al.* HTI-286, a synthetic analogue of the tripeptide hemiasterlin, is a potent antimicrotubule agent that circumvents P-glycoprotein-mediated resistance *in vitro* and *in vivo*. *Cancer Res.* **63**, 1838–1845 (2003).
147. Hamel, E. *et al.* Antitumor 2,3-dihydro-2-(ary)-4(1H)-quinazolinone derivatives. Interactions with tubulin. *Biochem. Pharmacol.* **51**, 53–59 (1996).
148. Mubjeesh, N. J. *et al.* 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell* **3**, 363–375 (2003).
149. Lakhani, N. J., Sarkar, M. A., Venitz, J. & Figg, W. D. 2-Methoxyestradiol, a promising anticancer agent. *Pharmacotherapy* **23**, 165–172 (2003).
150. Yoshimatsu, K., Yamaguchi, A., Yoshino, H., Koyanagi, N. & Kitoh, K. Mechanism of action of E7010, an orally active sulfonamide antitumor agent: inhibition of mitosis by binding to the colchicine site of tubulin. *Cancer Res.* **57**, 3208–3213 (1997).
151. Manfredi, J. J., Parness, J. & Horwitz, S. B. Taxol binds to cell microtubules. *J. Cell Biol.* **94**, 688–696 (1982).
152. Parness, J. & Horwitz, S. B. Taxol binds to polymerized tubulin *in vitro*. *J. Cell Biol.* **91**, 479–487 (1981).
153. Diaz, J. F. & Andreu, J. M. Assembly of purified GDP-tubulin into microtubules induced by taxol and taxotere: reversibility, ligand stoichiometry, and competition. *Biochemistry* **32**, 2747–2755 (1993).
154. Belani, C. P., Langer, C. TAX 326 Study Group. First-line chemotherapy for NSCLC: an overview of relevant trials. *Lung Cancer* **38** (Suppl. 4), 13–19 (2002).
155. Fossella, F. V., Lynch, T. & Shepherd, F. A. Second line chemotherapy for NSCLC: establishing a gold standard. *Lung Cancer* **38**, 5–12 (2002).
156. Bollag, D. M. *et al.* Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. *Cancer Res.* **55**, 2325–2333 (1995).
157. Wartmann, M. & Allmann, K. H. The biology and medicinal chemistry of epothilones. *Curr. Med. Chem. Anti-Canc. Agents* **2**, 123–148 (2002).
158. Lee, F. Y. *et al.* BMS-247550: a novel epothilone analog with a mode of action similar to apclitaxel but possessing superior antitumor efficacy. *Clin. Cancer Res.* **7**, 1429–1437 (2001).
159. Kamath, K. & Jordan, M. A. Suppression of microtubule dynamics by epothilone B in living MCF7 cells. *Cancer Res.* **63**, 6026–6031 (2003).
160. ter Haar, E. *et al.* Discodermolide, a cytotoxic marine agent that stabilizes microtubules more potently than taxol. *Biochemistry* **35**, 243–250 (1996).
161. Honore, S. *et al.* Suppression of microtubule dynamics by discodermolide by a novel mechanism is associated with mitotic arrest and inhibition of tumor cell proliferation. *Mol. Cancer Ther.* **2**, 1303–1311 (2003).
162. Hung, D. T., Chen, J. & Schreiber, S. L. (+)-Discodermolide binds to microtubules in stoichiometric ratio to tubulin dimers, blocks taxol binding and results in mitotic arrest. *Chem. Biol.* **3**, 287–293 (1996).
163. Kavallaris, M., Verrills, N. M. & Hill, B. T. Anticancer therapy with novel tubulin-interacting drugs. *Drug Resist. Update* **4**, 392–401 (2001).
164. Kowalski, R. J. *et al.* The microtubule-stabilizing agent discodermolide competitively inhibits the binding of paclitaxel (Taxol) to tubulin polymers, enhances tubulin nucleation reactions more potently than paclitaxel, and inhibits the growth of paclitaxel-resistant cells. *Mol. Pharmacol.* **52**, 613–622 (1997).
165. Smaletz, O. *et al.* Pilot study of epothilone B analog (BMS-247550) and estramustine phosphate in patients with progressive metastatic prostate cancer following castration. *Ann. Oncol.* **14**, 1518–1524 (2003).
166. Kelly, W. *et al.* Dose escalation study of intravenous estramustine phosphate in combination with Paclitaxel and Carboplatin in patients with advanced prostate cancer. *Clin. Cancer Res.* **9**, 2098–2107 (2003).
167. Hudes, G. *et al.* Phase I clinical and pharmacologic trial of intravenous estramustine phosphate. *J. Clin. Oncol.* **20**, 1115–1127 (2002).
168. Dahloff, B., Billström, A., Cabral, F. & Hartley-Asp, B. Estramustine depolymerizes microtubules by binding to tubulin. *Cancer Res.* **53**, 4573–4581 (1993).

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Competing interests statement

The authors declare **competing financial interests**: see web version for details.

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