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# MICROTUBULE: A NOVEL TARGET FOR CANCER THERAPY

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**ABSTRACT**

For cancer therapy, microtubule network is an attractive cellular target. Main component of cell cycle is mitotic stage, particularly when the mitotic spindle separates the replicated chromosomes. The rapid microtubule dynamics also play a critical role for activities of the bipolar spindle. Microtubule targeting drugs act at binding site either by destabilizing or hyper-stabilizing microtubules. It results mitotic arrest at the prometaphase or metaphase to anaphase transition, then it leads to subsequent apoptotic cell death. Taxanes and Vinca alkaloids have been validated by the successful use for a wide variety of human cancer. Clinical development of colchicines for cancer therapy has not been successful till now because they produce toxic effect in normal tissues. Semi-synthetic analogue of taxane, docetaxal is more potent than paclitaxel against cancer cell proliferation. It is now used for the treatment of breast, prostate and non small cell lung cancer. Successful use of paclitaxel and docetaxel in cancer therapy has inspired the new microtubule targeting agents that bind to taxane site, like Epothilones, discodermolide, eleuthelobin, sarcodictyins are under clinical investigation. New treatment options are available to patient with metastatic breast cancer in the form of a new drug class – epothilone. But only ixabepilone from epothilones class is currently approved by the US. This review is specifically devoted to microtubules and their implications to cancer therapy.

**INTRODUCTION:** Among the oldest of the world's writings mentioning both benign and malignant tumors are several Egyptian papyrus scrolls, dating from approximately 1600 BC. Cancer is always emphasized as inappropriate cellular proliferation. Cancer cells disrupt the normal function of surrounding tissues (or distant tissues in the case of metastases), leading to eventual organ failure and death. Based on the notion that some tumor cells may proliferate more rapidly than normal cells, a common strategy for cancer chemotherapy has been to develop drugs that interrupt the cell cycle. A particularly attractive cellular target is the microtubule network, the main component of the mitosis stage of the cell cycle, during which the mitotic spindle (a bipolar apparatus constructed of microtubules) separates the replicated chromosomes.

Microtubules are major dynamic structural components of the cytoskeleton involved in a variety of cell functions important for the development and maintenance of cell shape, cell reproduction and division, cell signaling, intracellular transport and cell movement. The biological functions of microtubules are regulated for the most part by their polymerization dynamics. Microtubules are built by the self-association of individual α/β tubulin dimers. Tubulin is one of the most abundant cellular proteins accounting for 2-5% of total cell protein in most epithelial cells, while tubulin accounts for approximately 20% of total cell protein in the brain. The central role of tubulin in the cell division cycle, together with the fact that aberrant cell division is the hallmark of cancer has made tubulin and microtubules prime targets for cancer chemotherapy. In fact, microtubule-targeting drugs are the most effective class of anticancer agents. Among the most successful microtubule-targeted drugs, taxanes are arguably the most effective anticancer agents introduced in the clinic since cisplatin, due to their remarkable activity in a broad range of cancer malignancies. The list of compounds that bind tubulin is large and continues to expand. The overwhelming majority of them are natural products and their chemical structures are remarkably diverse. Microtubule-targeting drugs act cytotoxically by either destabilizing or hyperstabilizing microtubules, resulting in mitotic arrest at the prometaphase or metaphase to anaphase transition, leading to subsequent apoptotic cell death.

**STRUCTURE OF MICROTUBULES:** Microtubules are highly dynamic cytoskeletal fibers whose dynamic properties are based on their inherent structure and polarity. Microtubules are built by the self-association of α and β tubulin dimers that associate in a head to tail fashion to form proto-filaments. Thirteen proto-filaments (in most eukaryotic cells), interact with each other laterally by contacts between monomers of the same type to form a hollow helical cylindrical microtubule with an outer diameter of 25nm. [14]

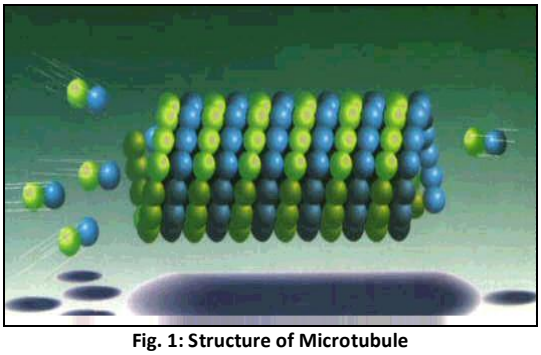

**Fig. 1: Structure of Microtubule**

The assembly of α- β tubulin heterodimers creates a polarity on the microtubule that greatly influences the polymerization rates of the two ends of the microtubule. The faster growing end is referred to the plus end and the slower growing end is referred to the minus end. The minus end is usually located near the microtubule organizing center near the nucleus, while the plus end is spread out through the cell. (Fig. 1) The αβ heterodimer is the basic structural unit of the microtubule. Each subunit has a binding site for one molecule of GTP. Once the αβ dimer is formed, the nucleotide in the alpha subunit (GTP) is buried at the intradimer interface, and it cannot be hydrolyzed. (Fig. 2) In contrast the nucleotide on the β-tubulin is partially exposed on the surface of the dimer and be hydrolyzed from GTP to GDP at the so-called exchangeable site (E-site). [14, 80]

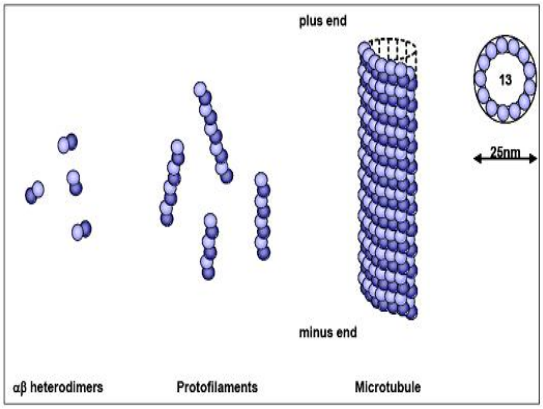


**Fig. 2: Formation of proto-filament and microtubule from heterodimers**

Microtubules are hollow cylindrical structures, built from two kinds of similar 50 kDa tubulin subunits, α and β-tubulin, which associate in a head-to-tail fashion to form a protofilament. The lateral association of protofilaments (usually 13) produces a microtubule with an outer diameter of 25 nm. Microtubules are polar structures with a dynamic plus end and a minus end that can be stabilized by embedding it in a microtubule-organizing center (MTOC).

The energy released after the hydrolysis of the GTP at the β-tubulin is used to polymerize the αβ dimers and assemble them into microtubules. After hydrolysis, the nucleotide at the β-tubulin E-site is buried at the interdimer interface and becomes non-exchangeable. [14]

**Microtubule dynamics:**

**Dynamic instability:** The biological functions of microtubules in all cells are determined and regulated in large part by their polymerization dynamics. Microtubules are in a state of dynamic instability, in which individual microtubules are either growing or shrinking and stochastically switch between two states. (Fig. 3) The switch from growth to shrinkage is called a catastrophe, and the switch from shrinkage to growth is called a rescue.

Dynamic instability is due to the structural differences between the growing and the shrinking ends. If the nucleotide hydrolysis proceeds more rapidly than the subunit addition, the GTP cap is lost and the microtubule beings to shrink. But GTP-subunits can still be added to the shrinking end, and if enough subunits are added to form a new cap, then the microtubule growth resumes. In an intact microtubule, proto-filaments made from GDP are forced into a linear conformation by the many lateral bonds within the microtubule wall. At the end of the microtubule there is a stable GTP cap.

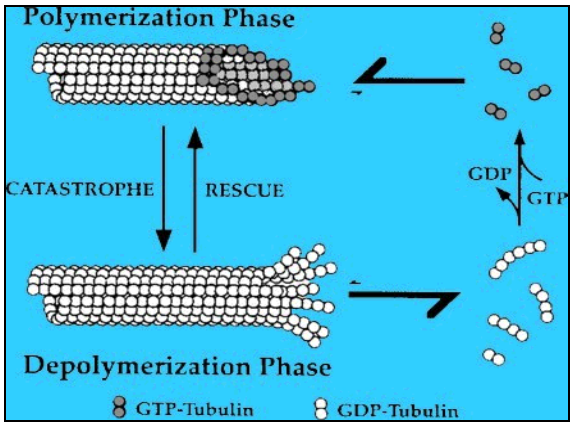


**Fig. 3: GTP-Cap Model for Microtubule Dynamic Instability**

The polymerization phase is thought to be stabilized by a thin cap of tubulin dimers at the microtubule plus end that have a GTP molecule associated with the β-tubulin subunit. Stochastic loss of the GTP cap, due to hydrolysis or subunit loss, results in a transition to the depolymerizing phase known as catastrophe. Several parameters have been used to characterize the dynamics of microtubule assembly: growth rate, shortening rate, frequency of transition from growth to shortening (catastrophe frequency), frequency of transition from shortening to growth or an attenuated (pause) state (rescue frequency, [1] and the duration of the attenuated state when neither microtubule growth nor shortening can be detected. [2] Overall microtubule dynamics due to dynamic instability is best described as "dynamicity," which measures the sum of visually detectable tubulin dimer exchange per unit time at the ends of microtubules. These dynamic properties are crucial for microtubules to carry out many of their cellular functions such as reorientation of the microtubule network when cells undergo migration or morphological changes and the dramatic microtubule rearrangement at the onset of mitosis. [3] Mitotic microtubules are 10-100 times more dynamic than interphase microtubules; they exchange their tubulin with the soluble tubulin pool with half-times of ~15 s during mitosis as compared with 3 min to several hours in interphase. [4] The rapid microtubule dynamics in mitosis is thought to be critical for both the morphogenesis and activities of the bipolar spindle, which directs the alignment of chromosomes at the metaphase plate and their final segregation into two daughter cells.

**Microtubule dynamics:**

**Treadmilling:** In addition to the dynamic instability, microtubules have another kind of dynamic behavior, called treadmilling, which is net growth at one microtubule end and balanced net shortening at the opposite end. In 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 (Fig. 4).

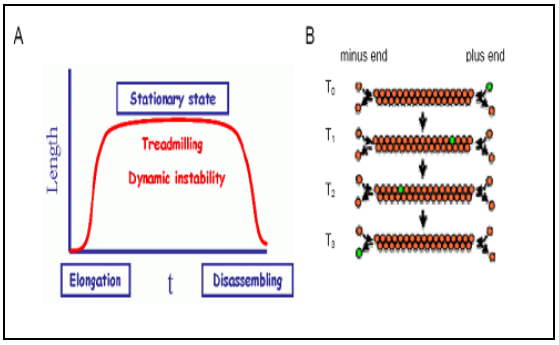


**Fig. 4: Dynamic Instability and Treadmilling in Microtubules**

- Microtubules are in a state of dynamic instability, in which individual microtubules are either growing or

shrinking and stochastically switch between two states. Microtubules grow and shorten by the reversible non-covalent addition and loss of tubulin dimers at their ends.

- Treadmilling at steady state in vitro showing a unidirectional flow (or flux) of tubulin subunits from plus to minus ends. Shown are consecutive ‘‘snapshots’’ of a microtubule exhibiting net growth at its plus end and equivalent net shortening at its minus end; the green subunits represent a marked segment and the microtubule remains at a constant length. $T_0$ = zero time; $T_1$, $T_2$ and $T_3$ = equal arbitrary increments of time.

This behavior occurs in cells as well as in vitro, and might be particularly important in mitosis, and in transport of organelles throughout the cell.

**Microtubule Assembly:** Assembly of microtubules in vivo occurs at the microtubule-organizing center (MTOC). In most animals cells there is a single, well-defined MTOC called the centrosome, located in the cytoplasm near the nucleus. The centrosome is a complex structure organized by a pair of perpendicular centrioles surrounded by pericentriolar material containing gamma tubulin in a large complex that includes other proteins (collectively known as grips) that form the gamma-tubulin ring complex (gamma-TuRC). The pericentriolar material contains several copies of a gamma-tubulin ring complex (gamma- TuRC). In most cells, microtubules are organized into single array with their minus ends associated with the centrosome. Nucleation plays a fundamental role in the function and intracellular dynamics of microtubules by preventing spontaneous polymerization of microtubules in the cytoplasm and thereby a random spatial organization of microtubules. This gives a cell a defined polarity, with the minus end of the microtubules located near the nucleus in the center of the cell and their plus ends toward the cell periphery near the plasma membrane.

**Isotypes of tubulin:** Tubulin is encoded by a multigene family that produces a distinct set of gene products, or isotypes, of both α and β tubulin subunits. In mammals, there are at least six isotypes of α-tubulin and seven isotypes of β-tubulin. (Table 1) The reason for the existence of such a large number of tubulin isotypes is not very clear, although they are differentially expressed in cells and tissues and there is evidence that the tubulin isotypes play an important role during embryogenesis. However, the proto-filaments of an individual microtubule can easily contain α and β tubulin subunits from different tubulin isotypes. [49]

**Table 1: Tubulin Isoforms**

| | Class | Human gene | Expression |
|---|---|---|---|
| α-tubulin | 1 | TUBA1 | Widely expressed |
| | 1 | TUBA3 | Mainly in brain |
| | 3 | TUBA2 | Testis-specific |
| | 4 | TUBA4 | Brain; muscle |
| | 6 | TUBA6 | Widely expressed |
| | 8 | TUBA8 | Heart; muscle; testis |
| β-tubulin | I | HM40 | Constitutive; predominant isotype in many cells |
| | II | Hβ9 | Major isotype of neurons |
| | III | Hβ4 | Neurons; Sertoli cells of testis |
| | IVa | Hβ5 | Brain-specific |
| | IVb | Hβ2 | Constitutive high levels in testis |
| | V | ND | ND |
| | VI | Hβ1 | Hematopoiesis-specific cell types |
| | 4Q | TUBB8 | ND |

The different isotypes of α and β tubulin are highly conserved and most of the sequence variation among them is found in the last 10-15 carboxyl terminal (C-terminus) amino acids. The C-termini of tubulin are the primary binding location of the microtubule associated proteins (MAPs). While the C-terminal regions are highly variable among the isotypes within a species, the same regions are highly conserved within a single isotype, among species are diverse as human, mouse and chicken. Variations among tubulin isotypes are expected to affect primarily the association of accessory proteins on the surface of the microtubule rather than the microtubule polymerization per se. Some MAPs are more highly expressed in certain tissues and cells bind with higher affinity to certain tubulin isotypes, possibly explaining the array of tubulin isotypes that exist. [5] In the case of cancer chemotherapy drugs, in vitro studies have shown that alteration of alpha and beta tubulin isotype composition has the potential to affect the sensitivity of tubulin to microtubule targeting drugs, especially taxol. [6] Furthermore, it has also been proposed that altered expression of different tubulin isotypes represents another mechanism underlying the resistance of cancer cells to microtubule-targeting drugs.

However, later studies in ovarian cancer xenograft models suggested that altered expression of β-tubulin isotypes does not influence taxol's sensitivity in vivo, arguing against a role for tubulin isotype composition in chemo-resistance in the clinical setting. [7] In addition to the alpha and beta tubulin gene families, recent genetic analyses and database searches have added four new members of the tubulin superfamily, which now includes α, β, γ, δ, ε, ζ, η tubulin. Gamma and eta tubulin seem to be associated mainly with flagella and cilia motility, [8] whereas sigma and epsilon tubulin were discovered by database searches, and their cellular functions have yet to be established[9]. Gamma tubulin was first identified in the filamentous fungus Aspergillus idulans [10] and it is approximately 30% identical to alpha and beta tubulin, and likely to be present in all eukaryotes. Gamma tubulin is located in centrosomes where it plays an essential role in initiation of microtubule assembly.

**Microtubule Regulation:** Microtubule dynamics can be regulated by different mechanism in-vitro, including the expression of different α and β tubulin isoforms, post translational modifications and interaction, with cellular factors that stabilise or destabilize microtubule, which operate in both spatially and temporally specific ways to generate different microtubule assemblies during the cell cycle.

**Microtubule stabilizing proteins:** Proteins known to regulate microtubule polymerization called microtubule associated proteins or MAPs. MAPs are proteins that bind to microtubules in a nucleotide insensitive manner to be microtubule lattice and they stabilize microtubules. Classical MAPs include MAP1, MAP2 and tau in neurons and MAP4 in non neuronal cells. The binding of MAPs to microtubules is predominantly electrostatic involving the highly acidic carboxyl terminal domain of α and β tubulin. MAPs are negatively regulated by phosphorylation, as the more phosphorylated forms exhibit reduced

affinity for the microtubule lattice, presumably by weakening this electrostatic interaction. Inactivation of MAPs reduces the frequency of rescue and is one of the mechanism by which microtubule turnover can be increased in-vivo. [14, 80]

Other MAPs, such as the highly conserved XMAPs215/Stu2p/TOG family, may be enriched on a subset of microtubules. XMAp215 affects microtubule dynamics by strongly increasing the polymerization rate of pure tubulin, but only at the microtubule plus ends. XMAP215 also increase the rate of rapid depolymerisation at the minus ends decreasing the rescue frequency, thereby increases the microtubule turnover. Microtubule end binding MAPs, such as CLIP-170 and EB1,copolymerize with new tubulin subunits and selectively bind to a special conformation of the microtubule end, serving as attachments for microtubules to kinectochores or cellular membranes through interaction with adaptor proteins such as the APC (adenomatous polyposis coli) protein and CLASPs (CLIP-associated proteins). [14]

**Microtubule destabilizing proteins:** Destabilizing proteins, in contrast to MAPs, reduce the net assembly and increase the microtubule turnover. The microtubule destabilizing protein katanin, functions as a servering Factors, generating new ends lacking a GTP cap. Katanin localizes at centrosomes and is responsible for the majority of M phase severing activity in Xenopus while is essential for releasing microtubules from the neuronal centrosome. [14] Depolymerizing kinesis of the KinI family, such as XKCM1, XKIF2 and MCAK, bind to both microtubule ends and distort the microtubule lattice, forcing pro-filament peeling. These kinesin like proteins are required for the formation and maintenance of the dynamic nature of the mitotic spindle. Op18/Stathmin increases the catathophe rate of microtubules, presumably by either sequestering tubulin dimmers by promoting GTP hydrolysis at the E site. [14, 80]

**Role of Microtubule Dynamics in Cell Division:** During eukaryotic cell division, in order for each daughter cell to inherit one and only one copy of each chromosome, the mother cell must replicate its chromosomes exactly once in the synthetic phase, and then must separate the replicated chromosomes evenly at the end of the mitotic phase to the two daughter cells. Defects in the coordination of chromosome replication and chromosome segregation can have severe consequences leading to genetic instability and aneuploidy, and eventually fostering tumor malignancy. [11]

To ensure faithful transmission of chromosomes during cell division, eukaryotic cells have evolved cellular regulatory mechanisms termed cell cycle checkpoints. The checkpoints prevent or delay cell cycle progression if certain cellular processes or proteins are disrupted, to gain time to repair the damage before cell division occurs. When the damage is irreparable, the cell undergoes apoptosis through the triggering of specific biochemical pathways. However, cancer cells often harbor defective cell cycle checkpoints allowing for uncontrolled cell proliferation, even when cell division does not occur properly. Therefore, effective

cancer treatment can be achieved by drugs that target certain processes or proteins impinging on the cell cycle machinery. [13] In particular, chemical compounds that target microtubules and inhibit the normal function of the mitotic spindle, have proven to be one of the best classes of chemotherapy.

The principal events typical of animal cell division can briefly be summarized as follows. During 'prophase', interphase chromatin condenses into well-defined chromosomes and previously duplicated centrosomes migrate apart, thereby defining the poles of the future spindle apparatus. Concomitantly, centrosomes begin nucleating highly dynamic microtubules that probe space in all directions, and the nuclear envelope breaks down. During 'prometaphase', microtubules are captured by kinetochores (specialized proteinaceous structures associated with centromere DNA on mitotic chromosomes).

Monopolar attachments of chromosomes are still unstable; the eventual interaction of paired sister chromatids with microtubules emerging from opposite poles results in a stable and bipolar attachment. Chromosomes congress to an equatorial plane, the metaphase plate, where they continue to oscillate throughout 'metaphase', suggesting that a balance of forces keeps them under tension. After all the chromosomes have undergone a proper bipolar attachment, a sudden loss in sister-chromatid cohesion triggers the onset of 'anaphase'. Sister chromatids are then pulled towards the poles (anaphase A) and the poles themselves separate further towards the cell cortex (anaphase B). Once the chromosomes have arrived at the poles, nuclear envelopes reform around the daughter chromosomes, and chromatin decondensation begins ('telophase'). Finally, an actomyosin-based contractile ring is formed and 'cytokinesis' is completed. [13]

**Microtubule Targeting Drugs:** Microtubules play a crucial role in cell division makes them a very suitable target for the development of chemotherapeutic drugs against the rapidly dividing cancer cells. The effectiveness of microtubule targeting drugs has been validated by the successful use of several vinca alkaloids and taxanes for the treatment of a wide variety of human cancers. Their clinical success has prompted a worldwide search for compound with similar mechanisms of action but improved characteristics. This search has resulted in the discovery of a number of novel microtubule targeting drugs, the majority of which are natural products. Their natural sources and chemical structures are remarkably diverse, making microtubules the only target for which such a diverse group of anti-cancer agents has been identified. Microtubule targeting agents are divided into two traditional categories: [13]

1. Microtubule destabilizing agents such as the vinca alkaloids (vinblastine, vincristine, etc.) colchicines
2. Microtubule stabilizing agents such as the taxanes (paclitaxel and docetaxel)

Chemical compounds targeting microtubules exert their inhibitory effects on cell proliferation primarily by blocking mitosis, which requires an exquisite control of microtubule dynamics. Microtubule-targeting drugs are therefore

also frequently referred to as a group of anti-mitotic drugs, and their actions on microtubule stability and dynamic parameters differ from each other. At relatively high concentrations, these drugs either inhibit microtubule polymerization, destabilizing microtubules and decreasing microtubule polymer mass, or promote microtubule polymerization, stabilizing microtubules and increasing the polymer mass. [13, 14]

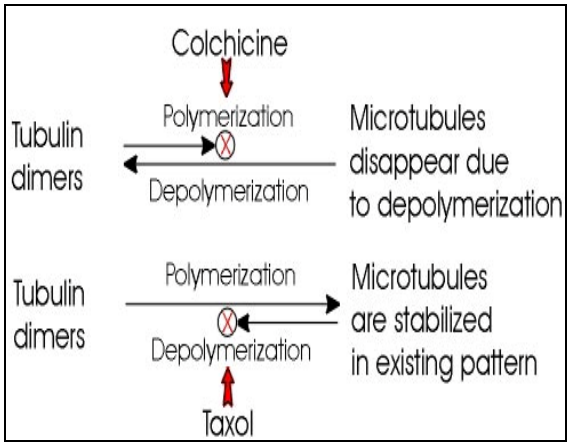


**Fig. 5: Effect of novel inhibitors on Microtubules**

The anti-mitotic and anti-cancer activities of microtubule-targeting drugs have been thought to result from their actions on microtubule stability and polymer mass. However, at low but clinically relevant concentrations, both microtubule-stabilizing and -destabilizing drugs potently suppress microtubule dynamics without affecting microtubule polymer mass; however, they retain their ability to block mitotic progression and induce apoptosis. [15, 16] Thus, it is reasonable to argue that the anti-mitotic and anti-cancer activities of microtubule-targeting agents may be largely due to their suppression of microtubule dynamics, instead of their effects on microtubule polymer mass, as previously thought (Fig. 5).

Currently there are three well established drug binding sites on β-tubulin;

- the vinca domain,
- the taxane site
- And the colchicine site [17]

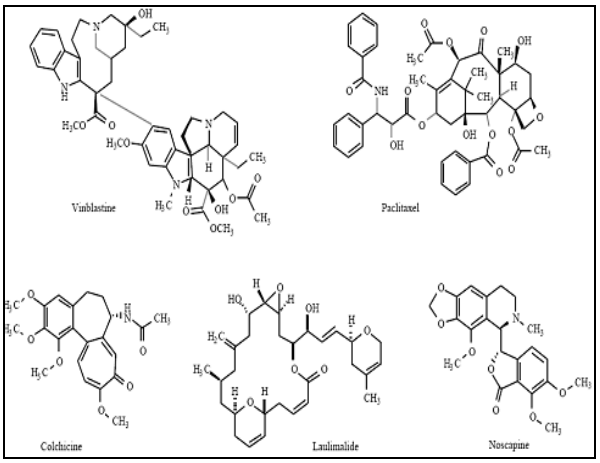


**Fig. 6: Chemical structures of vinblastine, paclitaxel, colchicine, laulimalide and noscapine**

The vinca domain is located adjacent to the exchangeable GTP binding site in β-tubulin at the plus end interface. [18, 19] The taxane site resides in a deep hydrophobic pocket at the lateral interface between adjacent protofilaments, within the lumen of the microtubule. [20] Finally, the colchicine site is located at the intradimer interface between β- tubulin and α-tubulin. [21] In addition to these three well characterized drug-binding sites, there is another binding site on β- tubulin that is occupied by laulimalide, a microtubule-stabilizing drug isolated from the marine sponge Cacospongia mycofijiensis. [22, 23] This is the first microtubule-stabilizing drug shown to bind at a site distinct from the taxane site on tubulin. Agents that Bind to the Vinca Domain are the vinca alkaloids, vinblastine and vincristine, were originally extracted over 40 years ago

from the leaves of the Madagascar periwinkle, formerly known as Vinca rosea but reclassified as Catharanthus roseus. These compounds have anti-leukemic effects and cause bone marrow suppression (Fig. 6). [24]

Since then they have been widely used clinically for the treatment of leukemias, lymphomas, and some solid malignancies. The clinical success of vinblastine and vincristine together with the elucidation of their mechanism of action on cellular microtubules, have facilitated the development of several semi-synthetic derivatives notably vindesine, vinorelbine and vinflunine, which are now used in the clinic for the treatment of cancer. Vinca alkaloids bind to both tubulin and microtubules, and their actions are highly dependent on the drug concentration. [24] At relatively high concentrations, they cause microtubule depolymerization, dissolve spindle microtubules and arrest cells at mitosis, and at even higher concentrations (mM), they induce the aggregation of tubulin into paracrystalline arrays. [18, 25, 26] In contrast, at low concentrations, the vinca alkaloids suppress microtubule dynamics without depolymerizing spindle microtubules, but remain able to arrest mitosis and induce apoptosis. [27] The mechanisms of action of the vinca alkaloids were unclear when they were initially used in the treatment of leukemia. [28]

The authors suggested that the mechanism by which vincristine produced these neuronal changes might lie in the decrease in DNA synthesis or RNA synthesis. Several other naturally occurring microtubule-targeting compounds that bind to the vinca domain on α, β-tubulin have been identified, including halichondrins (isolated from the marine sponges Halichondria okadai, Axinella sp., Phakellia carteri, and Lissodendoryx sp.), [29] hemiasterlins (isolated from the marine sponge Cymbastela sp.), [30] spongistatin (isolated from the marine sponge Spirastrella spinispirulifera), [31] dolastatins (isolated from the sea hare Dolabella auricularia), [32] and cryptophycins (isolated from the blue-green algae Nostoc sp.). [33] All of these compounds block mitotic progression and induce apoptosis in cancer cells, and they are currently at various stages of clinical development for the treatment of cancer.

**Agents that bind to the vinca site:** Vincristine and vinblastine are two complex bisindole alkaloids isolated from Catharanthus roseus G. Don (Madagascan periwinkle). Both agents inhibit the assembly of tubulin and are clinically used to treat various malignancies such as acute lymphoblastic leukaemia and Hodgkin's disease respectively. [34] Vincristine and vinblastine can tolerate only small changes to their structure without loss of activity. [35] Vinorelbine, a semi-synthetic vinca, has shown significant anti-tumour activity. [36] A more recent modification comprised the introduction of fluorine to give vinflunine. Vinflunine was identified in preclinical studies as having marked anti-tumour activity in vivo against a large panel of experimental tumour models, with tumour regressions being recorded in human renal and small cell lung cancer tumour xenografts. [37] Overall its level of activity was superior to that of vinorelbine in many of the experimental models and the agent is presently in Phase II clinical trials (Fig. 7). [38]

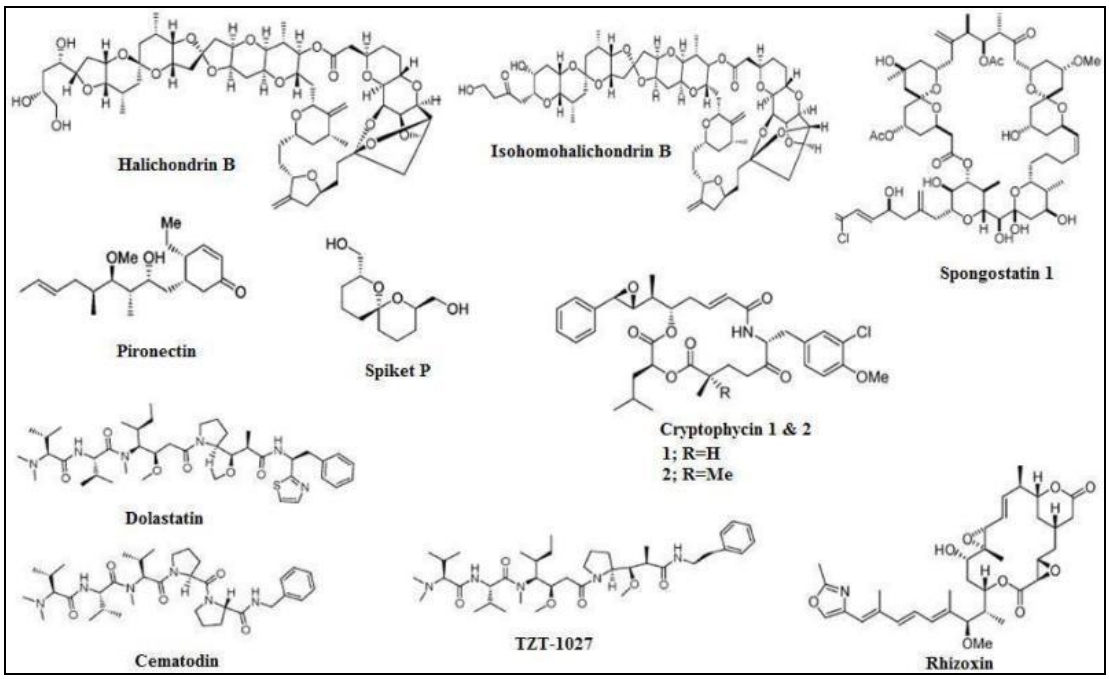


**Fig. 7: Chemical structures of Agents that bind to vinca site**

***Halichondrins B:*** *H*alichondrin B is one of a group of complex polyether macrolides isolated from the marine sponges Halichondria okadai, Axinella carteri, Phakellia carteri and Lissodendoryx n. sp. 1. Halichondrin B is a non-competitive inhibitor of the binding of vinblastine and is thought to bind in the vinca domain of tubulin. [29]

***Isohomohalichondrin*:** A related polyether, isohomohalichondrin B (isolated from Lissodendoryx n. sp. 1) has been shown to possess cytotoxic activity against multiple tumour types. Isohomohalichondrin B has shown high anti-proliferative activity in prostate cancer cell lines and is presently in preclinical evaluation. [40]

***Pironetin*:** Pironetin, a pyrone derivative isolated from Streptomyces sp. [41] Pironetin inhibits tubulin polymerisation by binding to the vincristine site while promoting the binding of colchicine to tubulin. It induces the disassembly of microtubules in a dose-dependent and reversible manner .This agent has also been shown to induce apoptosis via microtubule disassembly. [42]

***Spongistatin 1 /Spiket P*:** The spongistatins are a group of complex macrocyclic lactones isolated from the sponges Spirastrella spinispirulifera and Hyrtios erecta. Spongistatin 1 has no effect on the binding of colchicine to tubulin, but was a potent inhibitor of the binding of vinblastine and GTP to tubulin. Spongistatin 1 has shown particularly potent activity against several solid tumour cell lines including melanoma, lung cancer and colon cancers. [43] Recently a structurally simpler, rationally designed spiroketal subunit of the spongistatins, Spiket P, has been synthesised. Spiket P causes tubulin depolymerization in cell-free turbidity assays and exhibited potent cytotoxic activity against cancer cells as

evidenced by destruction of microtubule organization and prevention of mitotic spindle formation in human breast cancer cells. [44] The discovery of simpler anti-mitotic agents derived from complex natural products such as Spiket P should lead to synthetically more accessible second or third generation agents.

***Cryptophycins*:** The cryptophycins are a class of cyclic depsipeptides whose parent cryptophycin 1 was isolated from the cyanobacterium Nostoc sp. It suppresses tubulin dynamics and induces apoptosis. The cryptophycins are thought to bind at the vinca site on tubulin or a site that overlaps with the vinca site. Several synthetic analogues have been prepared and cryptophycin 2 has been discovered as an extremely potent anti-proliferative agent showing activity in several drug resistant cell lines. [45] These exciting properties have led to cryptophycin 2 entering Phase II clinical trials. [46]

***Dolastatins*:** The dolastatins are a group of pseudopeptides isolated from the Indian Ocean shell-less mollusk Dolabella auricularia. These agents inhibit microtubule formation by binding to β tubulin near the vinca domain and induce apoptosis. Several dolastatins and derivatives are currently under clinical investigation. These includes dolastatin, cematodin (a water- soluble pentapeptide analogue of dolastatin), and TZT- 1027. This TZT- 1027 agent has shown activities similar or superior to vincristine, the ability to damage tumour vasculature and the ability to induce apoptosis. [47]

***Rhizoxin*:** Rhizoxin was identified as an antifungal agent from Rhizopus chinensis. This compound interacts at the vinca site on tubulin, although it has also been considered to have its own binding site which overlaps the vinca site. This agent is presently in Phase II clinical trials. [48]

**Agents That Bind to the Taxane Site:** Agents were isolated originally in the 1960s from the bark of the Pacific yew Taxus brevifolia, paclitaxel. [49] Its semi-synthetic analog, docetaxel, is synthesized from a precursor isolated from the needles of the European yew Taxus baccata. Docetaxel is more water-soluble than paclitaxel, and is also more active than paclitaxel against cancer cell proliferation, and is now used clinically for the treatment of breast, prostate and non-small-cell lung cancer. [50] At relatively high concentrations, the taxanes promote microtubule polymerization and stabilize microtubules. At lower concentrations, similar to the vinca alkaloids, the taxanes suppress microtubule dynamics without affecting microtubule polymer mass, but retain their capability of inducing mitotic arrest and subsequent apoptotic cell death [50]. The success of paclitaxel and docetaxel in cancer therapy has inspired the discovery of new microtubule-targeting agents that bind to the taxane site and have similar mechanisms of action, including discodermolide (isolated from the marine sponge Discodermia dissoluta), [52] epothilones (isolated from the myxobacterium Sorangium cellulosum , and sarcodictyins (isolated from the Mediterranean stoloniferan coral Sarcodictyon roseum). [53] These agents block mitosis and induce cell death downstream of their anti-microtubule effects, and their cancer hemotherapeutic potential is also under clinical investigations (Fig. 8). [51]

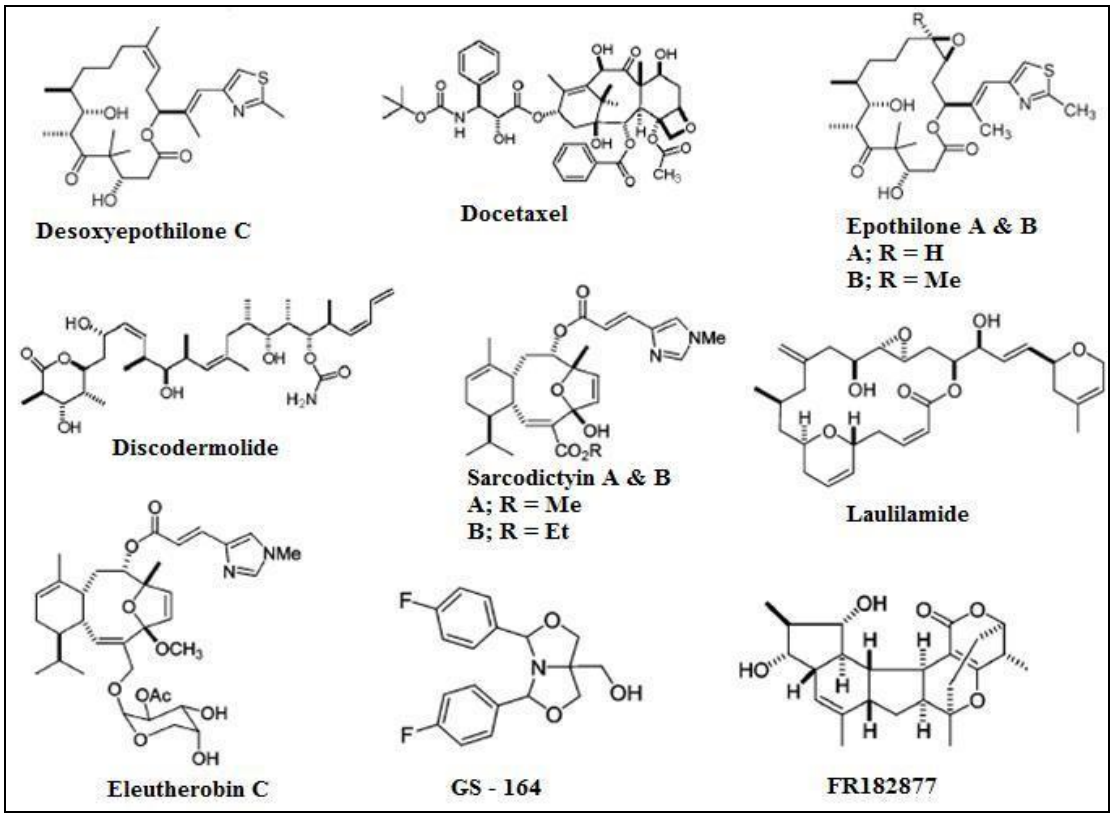


**Fig. 8: Chemical structures of Agents that bind to Taxane Site**

***Epothilones*:** The cytotoxic macrolide natural products, epothilone A and B, were isolated in 1993 from the myxobacterium Sorangium cellulosum. [54] It was not until 1995 that the paclitaxel-like mechanism of action of these compounds was discovered through induction of tubulin polymerisation and microtubule stabilisation. The epothilones A, B were also claimed to be 30 to 50 times more water soluble than paclitaxel and in contrast to paclitaxel, they inhibited the growth of MDR cell lines. [55] The very detailed structure-activity relationship studies led to the discovery of desoxyepothilone B, which lacks the epoxide ring. Its biological superiority relative to epothilone B and paclitaxel has been demonstrated in a variety of competitive in vitro and in vivo settings and as a result deoxyepothilone has recently entered a Phase I clinical trial. [56]

***Discodermolide*:** The polyhydroxylated lactone discodermolide was isolated from the marine sponge Discodermia dissoluta in 1990 [57] and initially identified as a potential immune-suppressive agent. [58] Discodermolide is a microtubule stabilising agent. [52] Discodermolide competes with paclitaxel for the same binding site with higher affinity, is more potent than paclitaxel in stabilizing microtubules and like the epothilones, retains its growth inhibitory properties against MDR cell lines. [59]

***Sarcodictyins and Eleutherobin*:** Sarcodictyin A and B were first isolated

from the Mediterranean soft coral Sarcodictyon roseum and found to enhance tubulin assembly. [60] Later, eleutherobin C was isolated from an Eleutherobia sp. and also found to stabilise microtubules. [61]

***Laulimalide*:** Although isolated in 1988 from the Pacific sponge, Cacospongia mycofijiensis, laulimalide was only recently identified as a microtubule-stabilising agent. Like the epothilones, it was also found to retain potency in MDR cell lines. [62]

***GS- 164*:** The synthetic molecule GS-164 has been shown to cause cellular effects associated with microtubule stabilising agents, although its potency is significantly lower than paclitaxel. [63]

***FR182877*:** FR182877 is the most recent addition to the microtubule stabilising agents. It induces G2/M phase arrest and promotes microtubule assembly. It is recognised by the paclitaxel binding domain of β-tubulin or an overlapping binding site. [64]

**Agents That Bind to the Colchicine Site:** Drugs binding to the colchicine site typically induce microtubule depolymerization at high concentrations, similar to the vinca alkaloids, and they suppress microtubule dynamics at low concentrations similar to both the vinca alkaloids and taxanes. [65] Isolated from the meadow saffron Colchicum autumnale, colchicine is one of the earliest microtubule-targeting agents identified, and its mechanism of action has been extensively investigated. In fact, tubulin was first purified based on its high-affinity binding with colchicine and was referred to as a "colchicines binding protein". [67] The clinical development of colchicines for cancer treatment has not been successful to date probably because of the high toxicity to normal tissues. However, development of agents binding to the colchicine site as potential cancer chemotherapeutic drugs has recently gained intense estradiol, appears to bind to the colchicine site and inhibits tumor growth. Both the combretastatins and 2ME2 are now in clinical trials as cancer chemotherapeutic agents (Fig. 9). [66]

***Combretastatin A-4*:** The natural product combretastatin A-4 was isolated from the South African bush willow Combretum caffrum. [68] Combretastatin A-4 is highly cytotoxic to several cancer cell lines and shows potent antimitotic activity. Stilbene 2 inhibits in vitro tubulin polymerisation, stimulates tubulin dependent GTP hydrolysis and competitively inhibits the binding of colchicine to the protein. Like several other antimitotic agents, combretastatin A-4 can selectively damage tumour vasculature[71]. However, whereas most antimitotic agents are effective only at or close to their maximum tolerated dose (MTD) combretastatin A-4 is effective at 10% of its MTD. The most effective is the disodium phosphate salt (>20 mg/ml) and is the form of combretastatin A-4 presently in Phase II clinical trials. [69]

***ZD6126*:** ZD6126 is a prodrug phosphate derivative of N-acetylcolchinol. ZD6126 is a tubulin-binding agent; however it is also a vascular targeting agent that selectively damages tumour vasculature. ZD6126 shows anti-tumour effects when administered alone, but enhanced activity has been noted when used in combination with cisplatin. [73]

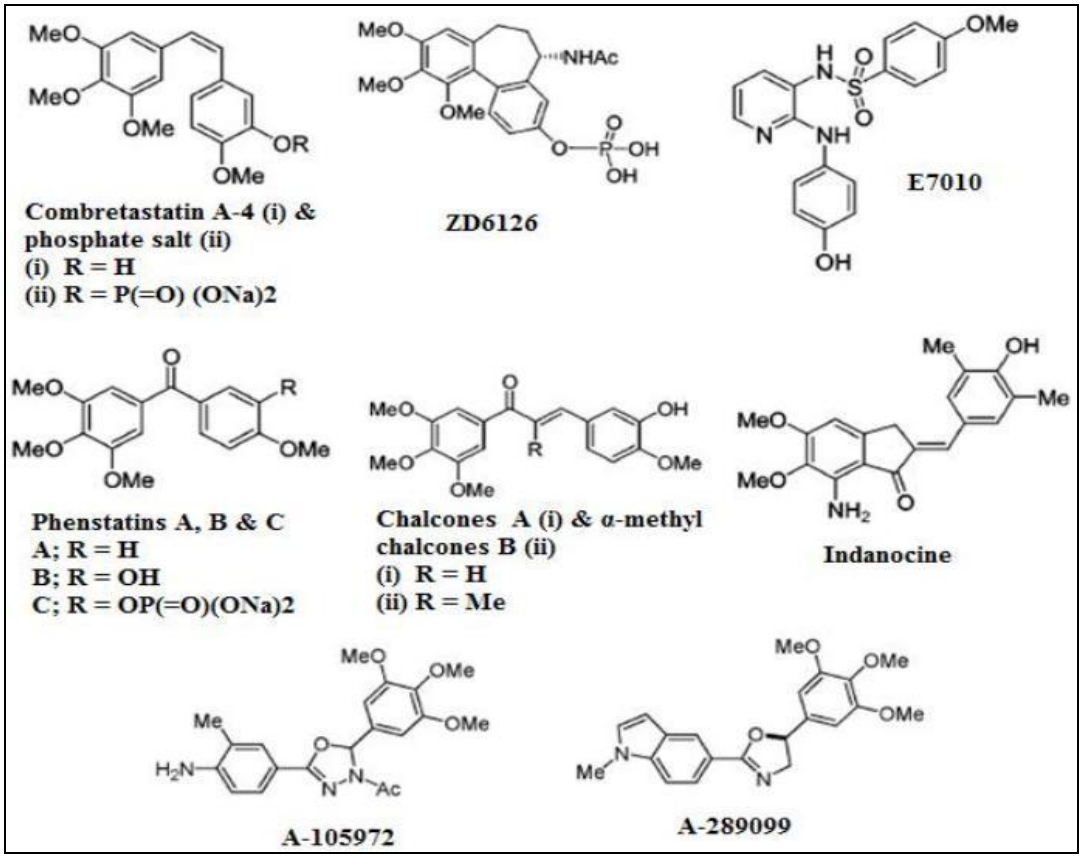


**Fig. 9: Chemical structures of Agents that bind to the Colchicine Site**

***Phenstatin*:** A phenolic derivative named phenstatin A was obtained unexpectedly from the Jacobsen oxidation of a combretastatin A-4 derivative. A water-soluble phosphate derivative has been synthesized and has shown equivalent potency to combretastatin A-4 in its ability to inhibit of tubulin polymerisation and to displace colchicine from its binding site on tubulin. [70]

***E7010***: E7010 is a synthetic diaryl 2, 3-diaminopyridine showing a broad spectrum of activity against various murine and human tumour cell lines and against human tumour xenografts. E7010 is an antimitotic agent which inhibits microtubule assembly and inhibits the binding of colchicine to tubulin. [73]

***Chalcones***: The chalcones are a series of biarylenones which show potent toxicity to several cancer cell lines and interact with tubulin at its colchicine-binding site. The most potent agents are those possessing a trimethoxy unit on one aryl ring. The α-methyl chalcones can adopt an s-trans conformation similar in shape to combretastatin A-4 whereas with a lack of methyl substitution, the chalcones prefer to adopt a less active s-cis conformation. [76]

***Indanocine***: Indanocine is potently cytotoxic to several tumour cell lines, but unlike other antimitotic agents, induces apoptotic cell death in stationary phase multidrug resistant (MDR) cancer cells at concentrations that do not impair the viability of normal non-proliferating cells.

Three MDR cell lines tested were more sensitive to growth inhibition on treatment with indanocine than in the parental cell lines. This sensitivity to MDR cancer cells suggests that indanocine should be considered as a lead compound in the search for agents to combat drug-resistant disease. [75]

***Indolyloxazolines***: The synthetic oxadiazoline A-105972 was recognized as a colchicine site binder and structural modifications have led to the discovery of indolyloxazolines as more stable and orally active antimitotic agents. A-289099 has been identified as the most active antimitotic indolyoxazoline against various cancer cell lines including those that express the MDR phenotype. [74]

**Noscapine and its derivatives: potential anti-cancer agents;** Noscapine and its derivatives represent another group of microtubule-targeting agents that possess potent anti-cancer activity. Noscapine is a phthalide isoquinoline alkaloid that occurs in abundance in the opium plant, Papaver somniferum. Noscapine was initially discovered to possess anti-mitotic properties. This agent binds to tubulin stoichiometrically, but its binding site remains unclear.

Unlike the other microtubule targeting drugs, noscapine does not appear to significantly change the microtubule polymer mass even at high concentrations. Instead, this compound suppresses microtubule dynamics by increasing the time that microtubules spend in an attenuated (pause) state when neither microtubule growth nor shortening is detectable. The noscapine-induced suppression of microtubule dynamics, even though subtle, is sufficient to interfere with the proper attachment of chromosomes to kinetochore microtubules and to suppress the tension across paired kinetochores. Consequently, the spindle checkpoint is engaged to block cells at a metaphase-like state, similar to the actions of low concentrations of the vinca alkaloids and taxanes, at which chromosomes do not complete congression to the equatorial plane. Noscapine effectively inhibits the progression of various cancer types both in cultured cells and in animal models with no obvious side effects. This agent is currently undergoing phase I/II clinical trials at the University of Southern California for patients with low grade non-Hodgkin's lymphoma or chronic lymphocytic leukemia refractory to chemotherapy. Several derivatives of noscapine have been recently developed, possessing more potent anti-mitotic and anti-cancer activities in preclinical models, in comparison to noscapine. Noscapine and its derivatives have also demonstrated anti-proliferative activity in cancer cells that are resistant to the conventional microtubule targeting drugs. [39]

**CONCLUSION:** Given the wide clinical use of the vinca alkaloids and taxanes, it is reasonable to argue that microtubules represent the best target to date for cancer chemotherapy and will remain a promising target for new chemotherapeutic agents. Our knowledge of the mechanisms of action of microtubule targeting agents has greatly evolved over the past years. We now appreciate that the chemotherapeutic actions of these agents may mainly rely on the suppression of microtubule dynamics, instead of their effects on microtubule

polymer mass. In addition, chemical compounds that suppress microtubule dynamics without affecting microtubule polymer mass, such as noscapine, are expected to display reduced toxicity to normal tissues while retaining their anti-cancer activity. Strategies exploiting synergistic drug combinations have also shown a great potential in enhancing the anticancer activity of the conventional microtubule targeting anti-cancer drugs. Microtubule-targeting drugs may be effectively used in combination with: Other microtubule targeting drugs; other classes of cancer chemotherapeutic agents; or other treatment options such as immunotherapy.

To minimize paclitaxel-induced neurotoxicity, peripheral neuropathy still remains an important toxicity that sometimes is dose limiting, as in the case of high dose Taxol. Investigations to improve understanding of the pathogenesis of paclitaxel-induced neuropathy have been ongoing. Since the concurrent administration of Taxol with neuroprotective agents has demonstrated little success in the clinical setting. Epothilones are also cytotoxic compounds that function in a similar fashion to paclitaxel and apoptotic cell death. However, their mechanism of microtubule binding is different from that of paclitaxel, which makes epothilones an attractive drug class for patients with taxane resistant malignancies. [77]

As taxane resistance remains a significant barrier in the treatment of a variety of cancers, it is important to understand epothilones and their indications. Several epothilone compounds, including ixabepilone (BMS-247550, aza-epothilone B, Ixempra), patupilone (EPO906, epothilone B), KOS-862 (desoxyepothilone B, epothilone D), BMS-310705, ZK-EPO (ZK-219477) and KOS-1584, have been tested for the treatment of a variety of solid tumor types. Clinical trials of epothilones are future directions for the use in cancer therapy, with a focus on the two most-studied epothilones to date: ixabepilone and patupilone. This compound is efficacious against a variety of human cancer cell lines including drug-resistant HCT-15 that over-expresses Pgp170. Biochemical studies show that A-432411 competes with the colchicines binding site on tubulin and inhibits microtubule polymerization. A-432411 causes $G_2$-M arrest and induces apoptosis. A-432411 destabilizes microtubules in cells. The compound disrupts normal spindle pole formation possibly through stabilization of microtubule dynamic at the concentration of 0.1µmol/L. Both structural and cellular properties of A-432411 make it an attractive candidate for further development.

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