Vinflunine: a new vision that may translate into antiangiogenic and antimetastatic activity

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Microtubules and tubulin are major dynamic and structural cellular components that play a key role in several cell functions, including division, signalling and intracellular trafficking. Normal epithelial cells have a highly structured, rigid cytoskeletal network that is compatible with cell motility. Thus, tubulin and microtubules are compelling cellular targets for chemotherapy. In fact, among anticancer agents, those that target microtubules constitute one of the most effective classes of chemotherapeutics in cancer. The list of compounds that target either tubulin or microtubules is extensive and consists of chemically unique compounds that bind to the tubulin dimers and destabilize microtubules (*Vinca* alkaloids) and those that bind to the microtubule polymer and stabilize microtubules (taxanes). Tumour-induced angiogenesis, the formation of new capillaries from existing blood vessels, and epithelial–mesenchymal transition are two steps that are critical for both tumour growth and metastatic spread. Three possible mechanisms of action are described with vinflunine, the new-generation *Vinca* alkaloid to arrive in clinical practice are as follows: it acts against tubulin and microtubules, disrupts newly formed blood vessels and seems to be able to reduce the metastatic process as shown in preclinical studies. These findings support the hypothesis that vinflunine, by blocking microtubule functions that contribute to cell shape, polarization, migration and other processes, might be responsible not only for tumour-cytostatic but also for specific antiangiogenic or antiepithelial–mesenchymal transition effects. *Anti-Cancer Drugs* 23:1–11 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2012, **23**:1–11

Keywords: angiogenesis, epithelial–mesenchymal transition, mechanisms of action, microtubule-targeting drug, vinflunine

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Received 2 May 2011 **Revised form accepted** 14 September 2011

Introduction

Microtubules are dynamic, structural cellular components of great importance in several cell functions including division, signalling and intracellular trafficking. Thus, tubulin and microtubules are compelling cellular targets for chemotherapy as these functions are often dysregulated in many types of cancer. In fact, among anticancer drugs, those that target microtubules constitute one of the most effective classes of chemotherapy. Vinflunin (VFL) is one of the extensive list of compounds that bind to tubulin or microtubules. There are compounds that bind to the tubulin dimers and destabilize microtubules (*Vinca* alkaloids) and others that bind to the microtubule polymer and stabilize microtubules (taxanes). Recently, two new drugs have been added to the list of available treatment for advanced solid tumours: cabazitaxel, which acts as a tubulin-binding taxane drug with antitumour activity in docetaxel-resistant cancers [1], and eribulin, which is a nontaxane microtubule dynamics inhibitor with tubulin-based antimitotic activity and chemotherapeutic effects in heavily pretreated patients with breast cancer [2].

Many currently used antineoplastic agents are derived from natural products originally isolated from plants. One of the best-known classes of these agents is the *Vinca* alkaloid, which is found in the periwinkle plant, *Catharanthus roseus* [3]. Dimeric alkaloids from *C. roseus* form an important class of antitumour agents widely used in combination chemotherapy regimens for treating solid tumours.

Vinca alkaloids have been extensively used in cancer for over 30 years and four of them are currently available in daily clinical practice: vinblastine, vincristine, vindesine and vinorelbine (VRB) [4]. Vinblastine, the first alkaloid with antiproliferative properties, was discovered in extracts from *Vinca rosea* plant leaves. However, among the numerous derivatives synthesized, only vindesine and VRB, semisynthetic analogues of vinblastine, are in clinical use. More recently, a new *Vinca* alkaloid, known as VFL, with substitutions in the little-exploited region of the catharanthine moiety, was obtained from the reaction of an electrophilic agent with VRB in superacid media (Fig. 1) [5].

VRB and VFL, the second generation of *Vinca* alkaloids, affect microtubule dynamics in a manner very different from vinblastine. Vinblastine suppresses the rate and extent of microtubule shortening when tested in in-vitro culture cells, whereas VRB and VFL suppress the rate and extent of microtubule growth and enlargement events [6].

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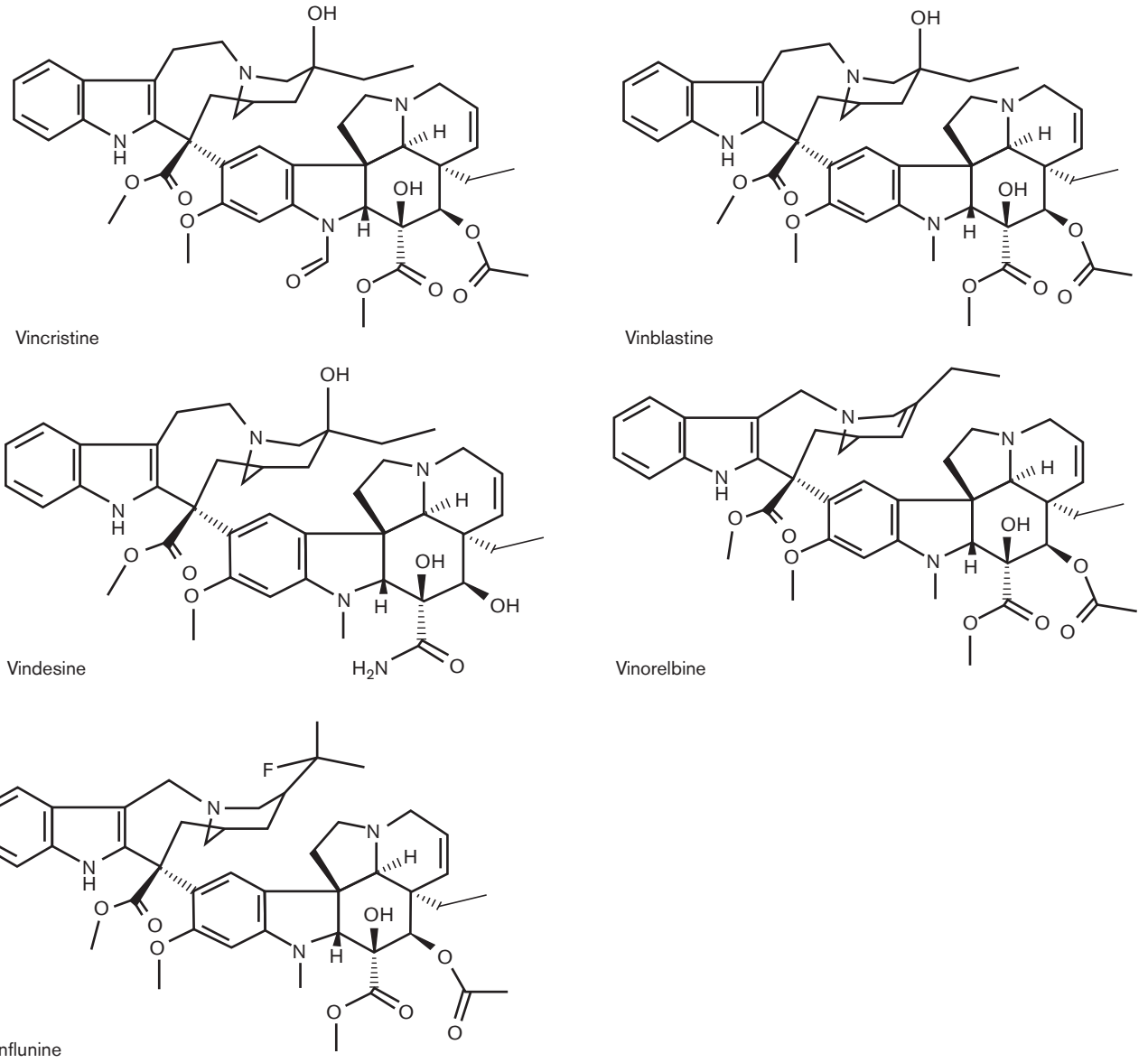
Fig. 1



Formulas of the *Vinca* alkaloid derivatives.

The diverse actions of VFL on microtubules are likely to exert different effects on mitotic spindle functions, leading to modifications of cell-cycle progression and cell killing [7]. As with other *Vinca* alkaloids, VFL is a specific inhibitor of tubulin that prevents microtubule assembly during mitosis [8] and induces apoptosis [9]. VFL also exerts effects on microtubule dynamic instability, suppressing the rate and extent of microtubule growth events [6]. The affinity profile of VFL shows features that suggest a greater effect on mitotic rather than axonal tubulin [10]; this is why VFL shows a reduced rate of neurotoxicity compared with other *Vinca* alkaloid agents. VFL participates in P-glycoprotein-mediated drug resistance, sharing this function with other compounds of the same family [11].

Epithelial–mesenchymal transition (EMT) and angiogenesis are essential mechanisms that guide proper development during several phases of embryogenesis and the early stages of newborns. In both processes, cells can change from an epithelial to a mesenchymal state by losing their characteristic epithelial traits and gaining mesenchymal cell properties. In adult organs, the activation of angiogenesis may result in some physiological activities but may also promote pathological conditions. Angiogenesis and EMT are implicated in

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tumour development and metastasis and are considered critical targets for anticancer strategies.

There are several reviews related to the clinical aspects and mode of action of VFL [12–14]. Here, we focus on a different aspect of the drug, attempting to summarize the knowledge behind the utility of VFL as antiangiogenic and as an anti-EMT process agent.

Mechanisms of action

The antineoplastic properties of *Vinca* alkaloids arise from their interaction with tubulin, the major component of microtubules in mitotic spindles. These drugs diminish microtubule dynamics and assembly, resulting in the arrest of cell division in the metaphase [15–18]. As antimitotic drugs, their affinity for tubulin has an impact on drug efficacy and toxicity. The order for the overall affinity for tubulin is vincristine > vinblastine > VRB > VFL [19]. VRB-induced, vinblastine-induced and vincristine-induced tubulin self-association occurs through a similar mechanism for all three drugs [19]. Consistent with these findings, it has been shown that VFL has a much lower binding affinity for tubulin than VRB and, consequently, induces smaller spirals with a more rapid relaxation time. As shown by various pharmacological studies defining its mitotic arresting and tubulin-interacting properties, VFL seems to act as a specific inhibitor of tubulin, while exhibiting quantitatively distinctive tubulin-binding properties [8].The *Vinca* alkaloids as a class participate in the classical multidrug resistance [20]. Among the tubulin-interacting agents, *Vinca* alkaloids and their derivatives have generally shown common cross-resistance patterns, and this is especially true of PgP-mediated resistance [21]. VFL, like other *Vinca* alkaloids, participates in Pgp-mediated multidrug resistance [11].

In addition to other *Vinca* alkaloids, the molecular mechanisms of cell killing by VFL also include a series of events leading to apoptotic cell death [22,23]. Apoptosis-inducing concentrations of VFL caused caspase 3/7 activation and the cleavage of one of its specific substrates, the poly-(ADP-ribose) polymerase. VFL also activated c-Jun N-terminal kinases, whose activation has been shown to occur in response to diverse stress stimuli including cellular treatment with microtubule inhibitors [24]. Furthermore, several studies have proposed a role for Bcl-2 phosphorylation in the apoptotic response of tumour cells to microtubule-damaging agents [25]. The capacity of VFL to cause Bcl-2 phosphorylation might depend on the cellular type. The apoptosis mechanisms induced by VFL involve caspases 3/7 and c-Jun N-terminal kinase 1 activation, but do not require Bcl-2 phosphorylation.

Mitochondria play an important role in crossroads for intracellular signalling pathways induced by microtubule-targeting drugs (MTDs) [26–29]. Inactivation of Bcl-2, through hyperphosphorylation [30], leads to mitochondrial membrane permeability and apoptosis triggered by MTDs, including VFL [26]. A relationship has been established between Bcl-2 overexpression and clinical resistance to MTDs [31]. Nevertheless, there is also contradictory evidence showing that VFL effectiveness was largely decreased in the absence of Bcl-2, whereas Bcl-x_L and Bax expressions were unchanged [32], and that it was in part restored by reintroducing Bcl-2 [33]. Like other MTDs, VFL directly affects isolated mitochondria [34], leading to the release of apoptotic factors. The antiproliferative effects of VFL in tumour cells, either as p53-dependent, postmitotic G1 arrest or a cell-cycle block at G2/M, converge at the mitochondria for the induction of apoptosis (Fig. 2). A p53-dependent transactivation of proapoptotic Bax in the mitochondria is considered to shift the cellular fate in favour of apoptosis, when cells are treated with low concentrations of VFL, despite the presence of functional Bcl-2 [35].

Tubulin is detected on mitochondrial membranes and Bcl-2 has been shown to specifically bind both mitochondrial tubulin and the voltage-dependent anion channel [28,32,36]. Such a protein complex could regulate the direct initiation of the mitochondrial signalling pathway by MTDs.

Microtubule disruption

The characteristic block of cell proliferation during mitosis induced by all *Vinca* alkaloids may be attributable to a specific action on mitotic spindle microtubules. The mechanism of action of the *Vinca* alkaloids was initially thought to be involved in the depolymerization of spindle microtubules and induction of paracrystalline tubulin–*Vinca* alkaloid arrays. At relatively high concentrations (μmol/l levels), all these drugs inhibit microtubule polymerization in reconstituted microtubule systems. In cells, alternative mechanisms, involving drug–tubulin oligomers, also exist. It is known that the *Vinca* alkaloids bind to free tubulin and to tubulin spiral oligomers, as well as to microtubules. VFL, VRB and vinblastine bind to guanosine triphosphate (GTP)–tubulin subunits with similar affinities. Evidence was found that VFL, like vinblastine, vincristine and VRB, appeared to interact with the *Vinca* alkaloid-binding domains on tubulin, as judged by proteolytic cleavage patterns, and induced tubulin structural changes favouring an inhibition of GTP hydrolysis. However, more detailed studies revealed that VFL expressed some distinctive features in terms of its binding properties to free tubulin and its ability to compete for binding with the other *Vincas* [37].

Tubulin

Microtubules, a major component of the cytoskeleton, are macromolecular filaments composed of the tubulin protein; the filaments provide structural and functional support in vesicle/organelle transport, cell morphogenesis and mitosis [38]. The proper assembly of tubulin

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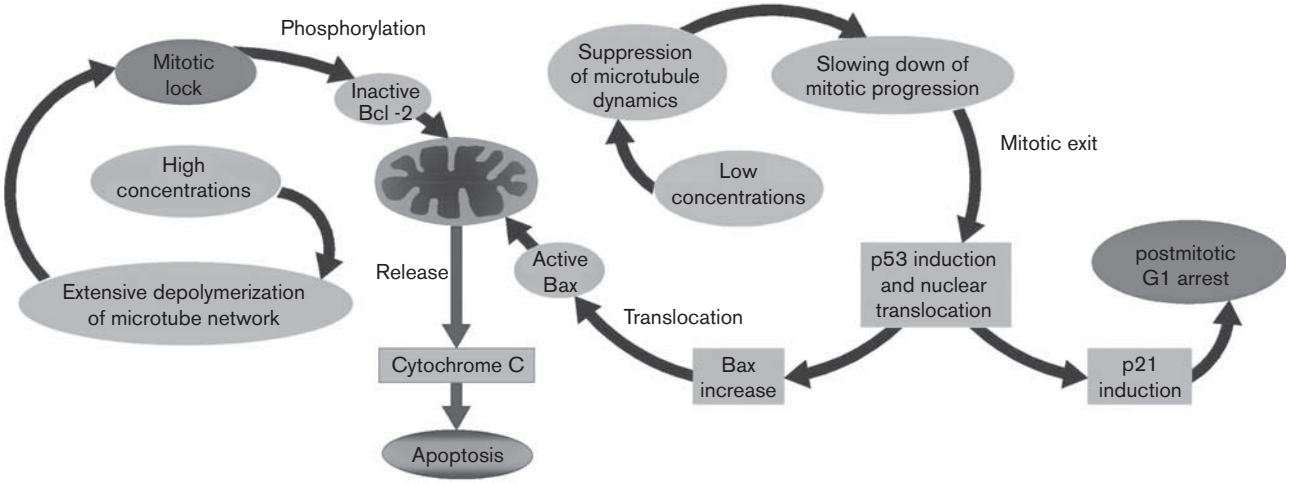
Fig. 2



Vinflunine directly affects the isolated mitochondria, leading to the release of apoptotic factors. The antiproliferative effects of VFL in tumour cells, either as p53-dependent, postmitotic G1 arrest or as a cell-cycle block at G2/M, converge at the mitochondria for the induction of apoptosis.

subunits (heterodimers of α-tubulin and β-tubulin) into microtubule protofilaments is energy dependent, requiring hydrolysis of the GTP bound to the β-tubulin subunit in the heterodimer. Polymerization of the tubulin subunits into linear polymers occurs through a mechanism of nucleation-elongation, a process initiated with a relatively slow synthesis of a short microtubule nucleus, followed by the rapid elongation of the microtubule at its ends by the reversible, noncovalent addition of tubulin dimers.

The dynamic behaviour of microtubules can be modulated by the differential expression of tubulin isotypes, posttranslational modifications and interaction with microtubule-regulatory proteins. Spatial and temporal regulation of microtubule functions occurs at several levels. Differential expressions of human tubulin isotypes (six forms of α-tubulin and seven forms of β-tubulin), in a cell/tissue-specific manner, confer a functional diversity to the microtubules [39,40].

Mitotic arrest

During mitosis, chromosomes that are attached in a bipolar manner to the metaphase spindle oscillate about the spindle equator. The kinetochores of sister chromatids are under tension and are periodically stretched (separation) apart by shortening (relaxation) of dynamic microtubules, and then relax back together as tension is relieved. The signal to progress from metaphase to anaphase seems to involve the development of sufficient tension or the attachment of an adequate number of microtubules to kinetochores [41,42].

Tension on kinetochores may also be provided by the treadmilling of kinetochore microtubules, a process that transports tubulin subunits along the lengths of the microtubules from their attachment at the kinetochores towards the spindle poles [43,44]. *Vinca* alkaloids affect centromere dynamics similarly. They markedly decrease centromere dynamicity, primarily by increasing the time centromeres remain in a paused state. In addition, vinblastine, vincristine and VRB suppress the relaxation rate, decrease the stretching duration and decrease the transition frequencies. The abnormalities mostly fall into a series of increasingly aberrant types that are categorized [6]. Abnormal spindle types I and II consist of bipolar spindles with one or more uncondensed chromosomes, respectively (Fig. 3). Type III includes monopolar spindles enclosed in chromosome balls.

Tubulin-interacting protein

The tubulin isotype composition of microtubules is one of the factors that may modulate the action of *Vinca* alkaloids against α-tubulins and β-tubulins that exist in the form of isotopes and are distinguished by slightly different amino acid sequences [45]. Six α-isotypes and seven β-isotypes of tubulin have been described [46]. Several in-vitro studies have shown that β-tubulin isotype composition affects microtubule dynamics [47], stability [48] and sensitivity to *Vinca* alkaloid*s* [49].

The tubulin isotype composition of microtubules is an important factor, but *Vinca* alkaloid interactions should be considered, and not only versus purified tubulin isotypes as, in cells, microtubules are composed of a backbone of tubulin dimers and microtubule-associated proteins (MAPs) [50].

Tubulin, in addition to differential isotype expression, can be further posttranslationally modified by polyglutamylation, polyglycylation, phosphorylation and acetylation that regulate tubulin function [51]. Interactions with various

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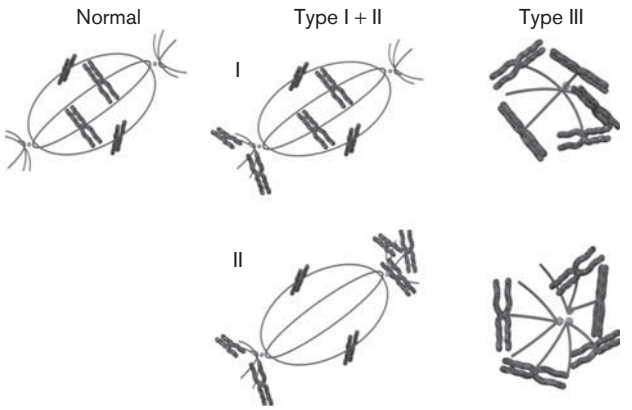
Fig. 3



Abnormal spindle types I, II and III.

microtubule-regulatory proteins such as MAP4, MCAK, TOG, survivin and stathmin [52–55] can stabilize or destabilize microtubules at different stages in the cell cycle [56–58].

Microtubule dynamics

Within cells, most microtubule (+) ends are oriented towards, and interact with, the cell cortex, whereas the (–) ends are usually stably capped and often anchored at the centrosome, which is the main organelle responsible for the nucleation of new microtubules.

Microtubules display two types of unusual behaviour, dynamic instability and treadmilling, which appear to be important for progression through mitosis and the cell cycle.

Dynamic instability is a stochastic switching of microtubule ends between phases of relatively slow growth and rapid shortening [59].

Dynamic instability is important for condensation of the chromosomes to the equatorial metaphase plate during prometaphase [60]. It also plays an important role in the tension-associated oscillations of the chromosomes at prometaphase and metaphase [61].

Treadmilling is the net addition of tubulin subunits at one end of a microtubule [the (+) end] and the balanced net loss from the opposite (–) end [62–64]. Treadmilling has been postulated to play a role in inducing tension on the kinetochores during mitosis [59], a condition that may be critical to shift from metaphase to anaphase [42]. Treadmilling, or poleward tubulin flux [65], may have an important function during metaphase by mediating the transport of signalling molecules from the kinetochores to the spindle poles and also by creating tension [59]. The magnitude of the intercentromere distance may depend, in part, on microtubule shortening and/or treadmilling and/or motor proteins.

The treadmilling inhibition rate of VRB was four-fold higher than that produced by VFL, whereas the inhibition rate of vinblastine was seven-fold higher. These potency differences in treadmilling may be an important determinant of antitumour activity. Using sedimentation velocity [19], it was found that VFL had a three-fold to 16-fold lower overall affinity for tubulin than VRB, whereas VRB had a lower overall affinity than vinblastine [19]. This behaviour suggested that the lower affinity of VFL for tubulin, might, at least in part, explain the high concentrations of drug required to block mitosis and cell proliferation [10].

Vinblastine has been shown to stabilize microtubule (+) ends and to destabilize (–) ends [64].

The dynamics of microtubules are coordinated with the actions of an undefined number of molecular motors to induce the equipartitioning of chromosomes to the two daughter cells by the mitotic spindle.

Antiangiogenesis

Considering drug-based approaches to vascular targeting, it is the tubulin-binding agents that have emerged as major players. Previously, *Vinca* alkaloid members, such as vincristine and vinblastine, had been shown to mediate their antitumour activities through an antivascular mechanism [66]. Novel derivatives of these compounds, VRB and VFL, have been shown to display significant antitumour activity [23,34,67,68]. As a member of the *Vinca* alkaloid family, VFL was at least as active as vincristine, with antivascular effects seen at doses much lower than the maximum tolerated dose. Their antitumour effects can be attributed to shutting down the tumour vasculature [34]. It has also been hypothesized that the effect of VFL on the tumour vasculature is due to the presence of dividing endothelial cells in newly formed tumour blood vessels [23]. VFL exerts an antitumour effect, and is at least as effective as vincristine and VRB when administered as single-dose schedules [34]. Endothelial cell shape change, rather than apoptosis, may be the primary event resulting in the rapid vascular shutdown seen in in-vivo tumours after treatment with VFL. In summary, VFL could act by the following antivascular mechanisms: vascular-disrupting effects, endothelial cell morphology changes, newly formed deficient capillary-like structures, inhibition of endothelial cell motility and proliferation and preventing endothelial cells from correctly aligning to form capillary-like structures.

A series of studies [69,70] also identified a dose-dependent inhibitory effect of VFL on fibroblast growth factor (bFGF)-induced angiogenesis.

Antimetastatic activity

VFL demonstrated in-vitro antiproliferative effects in several murine and human tumour cell lines [71], and important in-vivo antitumour activities against subcutaneous tumour

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xenografts. In general, mice treated systemically with VFL showed significantly lower tumour growth and longer survival than those treated with VRB [8,68]. Using an orthotopic murine model of transitional cell carcinoma of the bladder, VFL demonstrated greater superiority than VRB. The number of mitotic figures in biopsies obtained from mice treated with VFL was significantly lower than that in controls. A tendency towards a higher apoptotic index could be seen as the doses of each drug increased [72]. Lower doses of VFL also reduce the number of experimental liver metastases by colon cancer cells [70].

Although this inhibitory effect could be explained, at least in part, on the basis of the reduced cell motility generated in cancer cells after treatment with VFL, other mechanisms involved in tumour invasion, such as EMT, were also shown to be affected.

With respect to the angiogenic process, VFL inhibited bFGF-induced angiogenesis in matrigel implants [72] at subtherapeutic doses considerably (20–40-fold) lower than its maximum tolerated dose. Fibroblast growth factor receptor 3 is somatically mutated in approximately 50% of bladder cancers [73,74]. These are oncogenic gain-of-function mutations.

In the clinical setting, VFL was approved by the European Medicines Agency in September 2009 for the treatment of adult patients with advanced or metastatic transitional cell carcinoma of the urothelial (TCCU) tract after the failure of a prior platinum-containing regimen. Three consecutive trials were the basis for approval. First, a multicentre phase II trial demonstrated the feasibility and promising efficacy of VFL as a second-line therapy in patients with advanced bladder cancer [75]. In this study, VFL therapy yielded an overall response rate of 18%, and 67% of patients achieved disease control (partial response and stable disease). The median duration of tumour response was 9.1 months (long-duration responses ranging from 4.2 to 15.0 months). The median overall survival was 6.6 months and the median progression-free survival was 3.0 months. Similar results were obtained in a later phase II study including patients with all types of advanced TCCU at this time [76]. Finally, a pivotal phase III trial compared VFL with the best supportive care as a second-line therapy conducted in patients with metastatic TCCU demonstrated a better objective response rate (8.6 vs. 0%), disease control rate (41.1 vs. 24.8%) and a median progression-free survival (3.0 vs. 1.5 months), which implied a significant clinical and statistical benefit for those patients treated with VFL [77]. Therefore, VFL was the first agent to show a survival advantage after the failure of a prior platinum-containing chemotherapeutic regimen in TCCU.

Clinical development of VFL did not stop in TCCU tumours and a broad clinical development was followed. VFL has shown activity in patients with refractory non-small-cell lung cancer (NSCLC) both as a combination with cisplatin [78] and as a single agent in the platinum-resistance setting [79,80]. When VFL was combined with cisplatin for the treatment of 62 advanced patients with NSCLC for their first-line treatment, the combination seemed to be feasible and partial response was observed in five patients (8%) with a 1-year survival rate of 43.4%. A noninferiority designed phase III trial of VFL compared with docetaxel in patients with NSCLC previously treated with platinum-containing chemotherapy showed that no significant differences were found in terms of efficacy, patient benefit and quality of life according to the Functional Assessment of Cancer-Therapy Lung questionnaire between both chemotherapeutic drugs. VFL has also been demonstrated to have potential clinical activity in two prospective nonrandomized trials in patients with metastatic breast cancer after failure to anthracycline and taxanes previous regimens [81,82]. In both trials, conducted in more than a hundred women, VFL given as a single agent achieved a clinical control of the disease in 51.2% of the patients and a progression-free survival longer than 2.6 months. VFL has also been combined with trastuzumab in patients with metastatic breast cancer with a human epidermal growth factor receptor-2-positive status. Unfortunately, the results did not appear to be as good as expected and no clear significant activity was observed compared with the activity expected for trastuzumab alone [83].

VFL also failed to demonstrate significant clinical activity in prostate [84], malignant pleural mesothelioma [85], melanoma [86] and renal cell carcinoma [87], among others.

Epithelial–mesenchymal transition

EMT is an essential mechanism that guides proper development during several phases of embryogenesis [88] but within the adult organism, it promotes pathological conditions such as tumour metastases [89]. During the EMT process, cells can change from an epithelial to a mesenchymal state. In this process, tumour cells lose their characteristic epithelial traits and gain the properties of mesenchymal cells instead. The molecular correlation of this transition consists in the loss of epithelial markers, such as E-cadherin, certain cytokeratins, occludin and claudin, and the gain of mesenchymal markers, such as N-cadherin, vimentin and fibronectin (Fig. 4). After the transition to a mesenchymal state, cells can also revert back to an epithelial state in a process known as mesenchymal–epithelial transition [90].

Tumour cell migration

Cell migration can be viewed as a periodically repeating sequence of events that include the formation of pseudopodial protrusions, attachment and translocation of the cell body in the direction of new adhesion sites [91]. Microtubules and actin filaments play a critical

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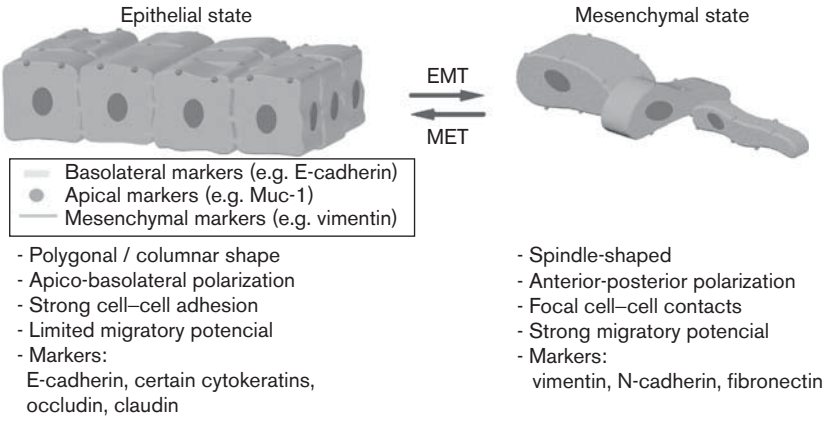
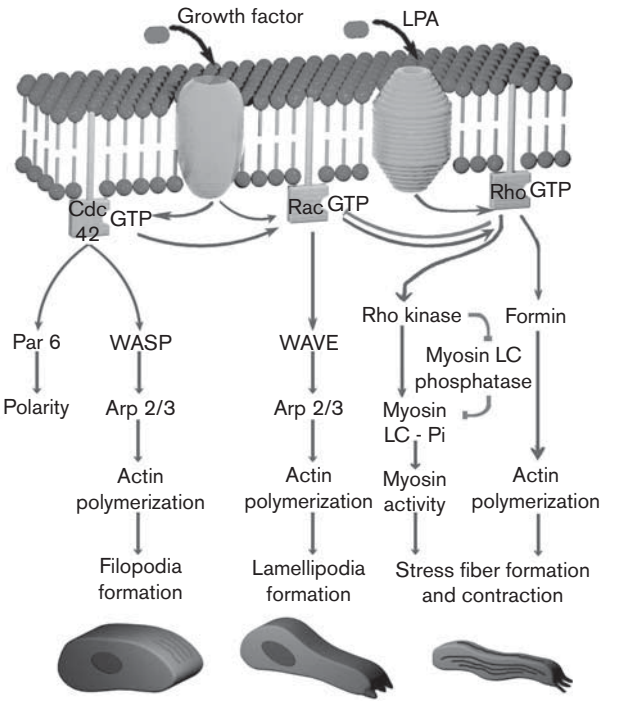
Fig. 4



Epithelial–mesenchymal transition (EMT) is an essential mechanism that guides pathological conditions such as tumour metastasis during the EMT process. Cells can shift from an epithelial to a mesenchymal state. MET, mesenchymal–epithelial transition.

role in intracellular trafficking, cell polarity and motility. These functions involve the ability of these polarized polymers to assemble and disassemble rapidly and to interact with proteins that regulate their dynamic properties, both temporally and spatially. Although the central role of the actin cytoskeleton in migration is largely known, more recent data support a significant role for microtubules in this process [92]. In fact, coordinated regulation of microtubules, actin cytoskeleton and adhesion sites are likely to be essential throughout the migration process [93]. Several putative mechanisms by which MTDs could inhibit cell migration have been proposed (Fig. 5). These include (a) impairment of microtubule-organizing centre reorientation towards the leading edge; (b) inhibition of Rho GTPases and their downstream effectors by altering the cycle of microtubule polymerization and depolymerization; (c) blockade of lamellipodia formation and cell polarization as a consequence of the inhibition of intracellular protein trafficking and vesicle transport; (d) inhibition of microtubule-mediated integrin clustering and increased integrin avidity; and (e) HSP90 degradation [94–96].

Fig. 5



Several putative mechanisms by which microtubule-targeting drugs (MTDs) could inhibit cell migration have been proposed. GTP, guanosine triphosphate; LC, light chain; LPA, lysophosphatidic acid.

Cytoskeleton and epithelial–mesenchymal transition

Despite the importance of cytoskeletal dynamics during both EMT and metastasis, few studies have examined the cytoskeleton of detached and circulating tumour cells. Specific posttranslational α-tubulin modifications are critical for adherent cell motility and implicated in cancer spread. It is known that EMT induced through the ectopic expression of twist or snail promotes α-tubulin detyrosination and the formation of tubulin-based microtentacles [97]. Mechanistically, EMT downregulates the tubulin tyrosine ligase enzyme, increasing α-tubulin detyrosination and promoting microtentacles that could enhance tumour cell dissemination.

It is now evident that microtubule involvement in cell migration requires interactions with both adhesion sites and actin cytoskeleton [93]. From this perspective, a growing list of proteins that interact with the microtubule (+) ends, called plus-end-tracking proteins (+TIPs), are critically important for many microtubule-regulated processes including cell migration. Among them, EB1

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regulates microtubule dynamic instability [98], the guidance of microtubule–actin cross-linkers and the selective microtubule stabilization at the cell cortex during cell migration [99].

Other specialized proteins that stabilize microtubules are the stable tubule only polypeptide (STOP) family that prevent microtubule disassembly [100,101] and play a key role in mitosis [102,103]. Moreover, calmodulin (CaM) is known to bind STOP proteins in a calcium-dependent manner and to regulate their microtubule-stabilizing function [104,105]. The colocalization of CaM with different STOP proteins has been demonstrated in the mitotic spindle [106,107]. This observation has led to the hypothesis that the Ca^{2+}-CaM-STOP complex plays a physiological role in numerous cell phenomena [102,103,108]. Its inactivation by Ca^{2+}-CaM enhances the microtubule dynamics and thus allows mitosis completion [6,107].

VFL inhibits adhesion site dynamics and induces stress fibre formation [109]. In VFL-treated cells, the cortical actin network and membrane ruffles disappear, and many thick and long-lived stress fibres are formed between static focal adhesions. The VFL effect demonstrated on CaM-STOP complex formation [110] suggests that the drug influences the microtubule dynamics not only through direct contacts with tubulin but also through other proteins involved in the network of microtubule stability regulation.

Discussion

VFL, a new bifluorinated microtubule inhibitor, is an innovative chemotherapeutic drug whose activity is directly linked to the disturbance of microtubular dynamics resulting from tubulin-binding affinity with free tubulin molecules and microtubule treadmilling inhibition.

Normal epithelial cells have a highly structured, rigid cytoskeletal network that is incompatible with cell motility. The dynamic cytoskeletal properties and greater deformability of transformed cells support successful metastases. Specific posttranslational α-tubulin modifications are critical for adherent cell motility. Stabilization of microtubules induces detyrosination that can inhibit microtubule disassembly, and contrary microtubule-destabilizing compounds prevent adhesion of circulating tumour cells.

Metastatic cancer cells express microtubule-stabilizing proteins, for example, MAP protein Tau, which induces increased reattachment of tumour cells and retention of circulating tumour cells in blood capillaries.

Perturbation with tubulin-depolymerizing agents, or MTDs, reduces both the frequency of micrometastasis and cellular reattachment to the extracellular matrix.

Activation of the EMT program, through the expression of diverse genes, significantly increases α-tubulin detyrosination by downregulating tubulin tyrosine ligase and alters microtubule stability and organization to promote plasma microtentacles in detached cells. Microtentacles are supported by the mesenchymal-associated intermediate filament protein, vimentin and also detyrosinated α-tubulin. This mechanism extends to metastatic human tumour cells, where the detyrosination and reorganization of microtubules depend on endogenous gene expression. Cancer cells exploit the EMT-mediated microtubule stabilization during escape from the primary tumour and subsequent invasion through the surrounding stroma.

The above rationale could provide evidence for an intricate mechanism by which VFL could stop the transition from epithelial cells to the invasive mesenchymal phenotype. We hypothesize that VFL exerts these effects through tubulin instability, which is one of the leading intracellular molecular process that drives EMT. In addition, VFL may induce upregulation in the expression levels of the E-cadherin repressors snail and slug. Both snail and slug cause a β-catenin/TCF-4-induced upregulation of TGFβ, which in turn is responsible for increases in LEF-1 expression. Initial E-cadherin repression through snail and slug reduces the level of substrate for β-catenin in the cell membrane. These β-catenin–LEF-1 transcription complexes then cause increased expression of target genes (vimentin, fibronectin, α-SMA) that drive the formation of the mesenchymal phenotype.

The most prominent effects of VFL on microtubule instability include a reduction of microtubule growth rate, an increase in the mean duration of a growth event and an increase in the percentage of time that microtubules spend in growth.

In addition to the suppression of microtubule dynamicity and mitotic spindle function, VFL leads to apoptosis at even lower concentrations through postmitotic G1 arrest; this is related to p53 and p21 upregulation and nuclear translocation. The antiproliferative effects of VFL converge at the mitochondria for the induction of apoptosis.

In addition, VFL showed antiangiogenic and vascular-disrupting activities at subcytotoxic doses and induced marked effects against experimental metastasis probably through EMT alteration. This can be attributed to the impairment of microtubule function at noncytotoxic concentrations.

Antiangiogenic approaches to cancer treatment are standard in many tumour types both as a combination with chemotherapeutic agents as it occurs in colorectal cancer or NSCLC with bevacizumab or as single agents as it occurs in renal cell cancer or hepatocellular carcinomas. The potency as an antiangiogenic inhibitor that VFL may display is probably less than the one obtained

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with selective new antivascular growth factor receptor-targeted agents. However, the reduction in blood vessel density that VFL induces may influence the overall tumour outcome and relapse of resistance.

EMT is universally accepted as one of the most important tumour stages and is believed to be critical in the process of metastasis. However, although there is an increasing amount of evidence describing the molecular pathways that drive this process of EMT, there is also a lack of data showing activity in the interruption of the process with drugs. A deeper understanding of the role that tubulin may play in the EMT process would allow determination of the clinical implication of VFL in the break of EMT induced by advanced tumours.

Currently, there is a potential basic rationale behind the utility of VFL in angiogenic and EMT field but translational trials showing this hypothesis in the clinic are lacking. Clinical combinations of VFL with other antiangiogenic agents like bevacizumab and tyrosine kinase inhibitors or with anti-EMT agents like new c-mesenchymal–epithelial transition inhibitors may offer, from a basic rationale perspective, a synergistic combination.

VFL has a solid clinical programme that supports its role as an antitumoral agent in different solid tumours. Currently, VFL is only approved for the treatment of patients with advanced or metastatic TCCU tract after failure of a prior platinum-containing regimen but further randomized trials in other solid tumour types like breast cancer are awaited. More in-depth research in any of the different cellular routes that VFL acts against would allow prospective trials to be carried out in an attempt to maximize the clinical activity of this drug to molecularly selected subgroups of patients.

Acknowledgements

The authors wish to thank Martin Hadley-Adams for assisting with the English language and preparation of the manuscript.

Conflicts of interest

There are no conflicts of interest.

References

1 De Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet* 2010; **376**:1147–1154.
2 Cortes J, O'Shaughnessy J, Loesch D, Blum JL, Vahdat LT, Petrakova K. Eribulin monotherapy versus treatment of physician's choice in patients with metastatic breast cancer (EMBRACE): a phase 3 open-label randomised study. *Lancet* 2011; **377**:914–923.
3 Blasko G, Cordell GA. Isolation, structure elucidation and biosynthesis of the bisindole alkaloids of *Cantharanthus.* In: Brossi A, Suffness M, editors. *The alkaloids-antitumor bisindole alkaloids from Catharanthus roseus (L)*. San Diego, California: Academic Press, Inc. 1990; pp. 1–76.
4 Noble RL, Beer CT, Cutts JH. Role of chance observations in chemotherapy: *Vinca rosea. Ann N Y Acad Sci* 1958; **76**:882–894.
5 Fahy J, Hellier P, Breillout F, Bailly C. Vinflunine: discovery and synthesis of a novel microtubule inhibitor. *Semin Oncol* 2008; **35**:S3–S5.
6 Ngan VK, Bellman K, Hill BT, Wilson L, Jordan MA. Mechanism of mitotic block and inhibition of cell proliferation by the semisynthetic *Vinca* alkaloids vinorelbine and its newer derivative vinflunine. *Mol Pharmacol* 2001; **60**:225–232.
7 Ngan VK, Bellman K, Panda D, Hill BT, Jordan MA, Wilson L. Novel actions of the antitumor drugs vinflunine and vinorelbine on microtubules. *Cancer Res* 2000; **60**:5045–5051.
8 Kruczynski A, Barret JM, Etiévant C, Colpaert F, Fahy J, Hill BT. Antimitotic and tubulin-interacting properties of vinflunine, a novel *Vinca* alkaloid. *Biochem Pharmacol* 1998; **55**:635–648.
9 Etiévant C, Kruczinsky A, Chansard N, Cabrol N, Astruc J, Chazottes E, Hill BTL. Characterization of apoptosis induced by vinflunine, a novel fluorinated *Vinca* alkaloid, in sensitive and vinflunine resistant P388 tumor cells: concomitant cell cycle analysis. *Proc Am Assoc Cancer Res* 1998; **39**:166 (Abstract 1140).
10 Lobert S, Ingram JW, Hill BT, Correia JJ. A comparison of thermodynamic parameters for vinorelbine and vinflunie-induced tubulin self-association by sedimentation. *Mol Pharmacol* 1998; **53**:908–915.
11 Etiévant C, Barret JM, Kruczinsky A, Perrin D, Hill BT. Vinflunine (20′, 20′-difuoro- 33′-4′-dihydrovinorelbine), a novel *Vinca* alkaloid, which participates in P-glycoprotein (Pgp)-mediated multidrug resistance *in vivo* and *in vitro. Invest New Drugs* 1999; **16**:3–17.
12 Mamtani R, Vaughn DJ. Vinflunine in the treatment of advanced bladder cancer. *Expert Rev Anticancer Ther* 2011; **11**:13–20.
13 Gerullis H, Ecke T, Eimer C, Wishahi M, Otto T. Vinflunine as second-line treatment in platin-resistant metastatic urothelial carcinoma: a review. *Anticancer Drugs* 2011; **22**:9–17.
14 Frampton JE, Moen MD. Vinflunine. *Drugs* 2010; **70**:1283–1293.
15 Jordan MA, Himes RH, Wilson L. Comparison of the effects of vinblastine, vincrisine, vindesine, and vinepidine on microtubule dynamics and cell proliferation *in vitro. Cancer Res* 1985; **45**:2741–2747.
16 Jordan MA, Thrower D, Wilson L. Mechanism of inhibition of cell proliferation by *Vinca* alkaloids. *Cancer Res* 1991; **51**:2212–2222.
17 Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat Rev Drug Discov* 2010; **9**:790–803.
18 Toso RJ, Jordan MA, Farrell KW, Matsumoto B, Wilson L. Kinetic stabilization of microtubule dynamic instability in vitro by vinblastine. *Biochemistry* 1993; **32**:1285–1293.
19 Lobert S, Vulevic B, Correia JJ. Interaction of *Vinca* alkaloids with tubulin: a comparison of vinblastine, vincristine and vinorelbine. *Biochemistry* 1996; **35**:6806–6814.
20 Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Ann Rev Med* 1993; **62**:385–427.
21 Hill BT. Differing patterns of cross-resistance resulting from exposure to specific antitumour drugs or to radiation *in vitro. Cytotechnology* 1993; **12**:265–288.
22 Kruczynski A, Etiévant C, Chansard N, Cabrol N, Astruc J, Chazottes E, *et al.* Induction of apoptosis by vinflunine, a novel fluorinated *Vinca* alkaloid. *10th NCI-EORTC Symposium on New Drugs Cancer Ther* 1998; **A391**:102.
23 Kruczynski A, Etiévant C, Perrin D, Chansard N, Duflos A, Hill BT. Characterization of cell death induced by vinflunine, the most recent *Vinca* alkaloid in clinical development. *Br J Cancer* 2002; **86**:143–150.
24 Stone AA, Chambers TC. Microtubule inhibitors elicit differential effects on MAP kinase (JNK, ERK, and p38) signalling pathways in human KB-3 carcinoma cells. *Exp Cell Res* 2000; **254**:110–119.
25 Wang LG, Liu XM, Kreis W, Budman DR. The effect of anti-microtubule agents on signal transduction pathways of apoptosis: a review. *Cancer Chemother Pharmacol* 1999; **44**:355–361.
26 Pourroy B, Carré M, Honoré S, Bourgarel-Rey V, Kruczynski A, Briand C, *et al.* Low concentrations of vinflunine induce apoptosis in human SK-N-SH neuroblastoma cells through a postmitotic G_1 arrest and a mitochondrial pathways. *Mol Pharmacol* 2004; **66**:580–591.
27 André N, Carré M, Brasseur G, Pourroy B, Kovacic H, Briand C, *et al.* Paclitaxel targets mitochondria upstream of caspase activation in intact human neuroblastoma cells. *FEBS Lett* 2002; **532**:256–260.
28 Carré M, Braguer D. Microtubule damaging agents and apoptosis. In: Fojo T, editor. *Microtubules and disease*. Humana Press; 2006.
29 Pasquier E, Carré M, Pourroy B, Camoin L, Rebaï O, Briand C, *et al.* Antiangiogenic activity of paclitaxel is associated with its cytostatic effect, mediated by the initiation but not completion of a mitochondrial apoptotic signaling pathway. *Mol Cancer Ther* 2004; **3**:1301–1310.
30 Haldar S, Basu A, Croce CM. Bcl2 is the guardian of microtubule integrity. *Cancer Res* 1997; **57**:229–233.

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31 Shore GC, Viallet J. Modulating the bcl-2 family of apoptosis suppressors for potential therapeutic benefit in cancer. *Hematology* 2005; **1**:1226–1230.
32 Ferlini C, Raspaglio G, Mozzetti S, Distefano M, Filippetti F, Martinelli E, *et al*. Bcl-2 down-regulation is a novel mechanism of paclitaxel resistance. *Mol Pharmacol* 2003; **64**:51–58.
33 Estève MA, Carré M, Bourgarel-Rey V, Kruczynski A, Raspaglio G, Ferlini C, *et al*. Bcl-2 down-regulation and tubulin subtype composition are involved in resistance of ovarian cancer cells to vinflunine. *Mol Cancer Ther* 2006; **5**:2824–2833.
34 Holwell SE, Hill BT, Bibby MC. Anti-vascular effects of vinflunine in the MAC 15: a transplantable adenocarcinoma model. *Br J Cancer* 2001; **84**:290–295.
35 Braguer D, Barret JM, McDaid H, Kruczinsky A. Antitumor activity of vinflunine: effector pathways and potential for synergies. *Semin Oncol* 2008; **35**:S13–S21.
36 Carré M, André N, Carles G, Borghi H, Brichese L, Briand C, *et al*. Tubulin is an inherent component of mitochondrial membranes that interacts with the voltage-dependent anion channel. *J Biol Chem* 2002; **277**:33664–33669.
37 Jordan MA, Wilson L. Kinetic analysis of tubulin exchange at microtubule ends at low vinblastine concentrations. *Biochemistry* 1990; **29**:2730–2739.
38 Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Macromolecules: structure, shape, and information. In: Alberts B, editor. *Molecular biology of the cell*. 4th ed. Oxford, UK: Garland Publishing. 2002; pp. 89–138.
39 Jordan MA, Wilson L. Microtubules as a target for anti-cancer drugs. *Nat Rev Cancer* 2004; **4**:253–265.
40 Jordan MA, Horwitz SB, Lobert S, Correia JJ. Exploring the mechanisms of action of the novel microtubule inhibitor vinflunie. *Semin Oncol* 2008; **35**:S6–S12.
41 Hays TS, Salmon ED. Poleward force at the kinetochore in metaphase depends on the number of kinetochore microtubules. *J Cell Biol* 1990; **110**:391–404.
42 Nicklas RB, Ward SC, Gorbsky GJ. Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J Cell Biol* 1995; **130**:929–939.
43 Mitchison TJ. Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J Cell Biol* 1989; **109**:637–652.
44 Waters JC, Mitchison TJ, Rieder CL, Salmon ED. The kinetochore microtubule minus-end disassembly associated with poleward flux produces a force that can do work. *Mol Biol Cell* 1996; **7**:1547–1558.
45 Sackett DL. Structure and function in the 'Tubulin dimer and the role of the acidic carboxyl terminus'. In: Biswas BB, Siddharthe R, editors. *Subcellular biochemistry–Proteins: structure, function and engineering*. New York: Plenum Press. 1995; pp. 255–302.
46 Luduena RF. Multiple forms of tubulin: different gene products and covalent modifications. *Int Rev Cytol* 1998; **178**:207–275.
47 Downing KH. Structural basis for the interaction of tubulin with proteins and drugs that affect microtubule dynamics. *Annu Rev Cell Dev* 2000; **16**:89–111.
48 Schwartz PM, Liggins JR, Ludueña RF. β-tubulin isotypes purified from bovine brain have different relative stabilities. *Biochemistry* 1998; **37**:4687–4692.
49 Sirotnak FM, Danenberg KD, Chen J, Fritz F, Danenberg PV. Markedly decreased binding of vincristine to tubulin in *Vinca* alkaloid-resistant Chinese hamster cells is associated with selective overexpression of alpha and beta tubulin isoforms. *Biochem Biophys Res Commun* 2000; **269**:21–24.
50 Maccioni RB, Cambiazo V. Role of microtubule-associated proteins in the control of microtubule assembly. *Physiol Rev* 1995; **75**:835–864.
51 Verdier-Pinard P, Wang F, Burd B, Angeletti RH, Horwitz SB, Orr GA. Direct analysis of tubulin expression in cancer cell lines by electrospray ionization mass spectrometry. *Biochemistry* 2003; **42**:12019–12027.
52 Cassimeris L. The oncoprotein 18/stahmin family of microtubule destabilizers. *Curr Opin Cell Biol* 2002; **14**:18–24.
53 Giodini A, Kallio MJ, Wall NR, Gorbsky GJ, Topnin S, Marchisio PC, *et al*. Regulation of microtubule stability and mitotic progression by survivin. *Cancer Res* 2002; **62**:2462–2467.
54 Maney T, Waagenbach M, Wordeman L. Molecular dissection of the microtubule depolymerizing activity of mitotic centromere-associated kinesin. *J Biol Chem* 2001; **276**:34753–34758.
55 Spittle C, Charrasse S, Larroque C, Cassimeris L. The interaction of TOGp with microtubules and tubulin. *J Biol Chem* 2000; **275**:20748–20753.
56 Desai A, Mitchison TJ. Microtubule polymerization dynamics. *Annu Rev Cell Dev Biol* 1997; **13**:83–117.
57 McNally FJ. Modulation of microtubule dynamics during the cell cycle. *Curr Opin Cell Biol* 1996; **8**:23–29.
58 Nogales E. Structural insight into microtubule function. *Annu Rev Biophys Biomol Struct* 2001; **30**:397–420.
59 Mitchison TJ, Kirschner M. Dynamic instability of microtubule growth. *Nature* 1984; **312**:237–242.
60 Hayden JJ, Bowser SS, Rieder C. Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: direct visualization in live newt cells. *J Cell Biol* 1990; **111**:1039–1045.
61 Skibbens RV, Skeen VP, Salmon ED. Directional instability of kinetochore mobility during chromosome congression and segregation in mitotic newt lung cells: a push–pull mechanism. *J Cell Biol* 1993; **122**:859–875.
62 Margolis RL, Wilson L. Microtubule treadmilling: what goes around comes around. *BioEssays* 1998; **20**:830–836.
63 Rodionov VI, Nadezhina E, Borisy GG. Centrosomal control of microtubule dynamics. *Proc Natl Acad Sci USA* 1999; **96**:115–120.
64 Panda D, Miller HP, Wilson L. Rapid treadmilling of MAP-free brain microtubules in vitro and its suppression by tau. *Proc Natl Acad Sci USA* 1999; **96**:12459–12464.
65 Mitchison TJ, Salmon ED. Poleward kinetochore fiber movement occurs during both metaphase and anaphase-A in newt lung cell mitosis. *J Cell Biol* 1992; **119**:569–582.
66 Hill SA, Longergan SJ, Denekamp J, Chaplin DJ. *Vinca* alkaloids: antivascular effects in a murine tumour. *Eur J Cancer* 1993; **29**:1320–1324.
67 Johnson SA, Harper P, Hortobagyi GN, Pouillart P. Vinorelbine: an overview. *Cancer Treat Rev* 1996; **22**:127–142.
68 Hill BT, Fiebig HH, Waud WR, Poupon MF, Colpaert F, Kruczinsky A. Superior in-vivo experimental antitumour activity of vinflunine, relative to vinorelbine, I a panel of human tumour xenografts. *Eur J Cancer* 1999; **35**:512–520.
69 Lavielle G, Hautefaye P, Schaeffer C, Boutin JA, Cudennec CA, Pierre AJ. New alpha-amino phosphonic acid derivatives of vinblastine: chemistry and antitumor activity. *Med Chem* 1991; **34**:1998–2003.
70 Kruczynski A, Poli M, Dossi R, Chazottes E, Berrichon G, Ricome C, *et al*. Anti-angiogenic, antivascular-disrupting and anti-metastatic activities of vinflunine, the latest *Vinca* alkaloid in clinical development. *Eur J Cancer* 2006; **42**:2821–2832.
71 Kruczinsky A, Colpaert F, Tarayre J-P, Mouillard P, Fahy J, Hill BT. Preclinical in vivo antitumor activity of vinflunine, a novel fluorinated *Vinca* alkaloid. *Cancer Chemother Pharmacol* 1998; **41**:437–447.
72 Bonfil RD, Russo DM, Binda MM, Delgado FM, Vicenti M. Higher antitumor activity of vinflunine than vinorelbine against an orthotopic murine model of transitional cell carcinoma of the bladder. *Urol Oncol* 2002; **7**:159–166.
73 Catalogue of somatic mutations in cancer. Available from: http://www.sanger.ac.uk/perl/genetics/CGP/cosmic.
74 Knowles MA. Role of FGFR3 in urothelial carcinoma: biomarker and potential therapeutic target. *World J Urol* 2007; **25**:581–593.
75 Culine S, Theodore C, De Santis M, Bui B, Demkow T, Lorenz J, *et al*. Phase II study of vinflunine in bladder cancer patients progression after line platinum-containing regimen. *Br J Cancer* 2006; **94**:1395–1401.
76 Vaughn DJ, Srinivas S, Stadler WM, Pili R, Petrylak D, Sternberg CN, *et al*. Vinflunine in platinum-pretreated patients with locally advanced or metastatic urothelial carcinoma. *Cancer* 2009; **115**:4110–4117.
77 Bellmunt J, Théodore C, Demkov T, Komyakov B, Sengelov L, Daugaard G, *et al*. Phase III trial of vinflunine plus best supportive care compared with best supportive care alone after a platinum-containing regimen in patients with advanced transitional cell carcinoma of the urothelial tract. *J Clin Oncol* 2009; **27**:4454–4461.
78 Souquet PJ, Krzakowski M, Ramlau R, Sun XS, Lopez-Vivanco G, Puozzo C, *et al*. Phase I/II and pharmacokinetic study of intravenous vinflunine in combination with cisplatin for the treatment of chemonaive patients with advanced non-small-cell lung cancer. *Clin Lung Cancer* 2010; **11**:105–113.
79 Bennouna J, Breton JL, Tourani JM, Ottensmeier C, O'Brien M, Kosmidis P, *et al*. Vinflunine – an active chemotherapy for treatment of advanced non-small-cell lung cancer previously treated with a platinum-based regimen: results of a phase II study. *Br J Cancer* 2006; **94**:1383–1388.
80 Krzakowski M, Ramlau R, Jassem J, Szczesna A, Zatloukal P, von Pawel J, *et al*. Phase III trial comparing vinflunine with docetaxel in second-line advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy. *J Clin Oncol* 2010; **28**:2167–2173.
81 Campone M, Cortes-Funes H, Vorobiof D, Martin M, Slabber CF, Ciruelos E, *et al*. Vinflunine: a new active drug for second-line treatment of advanced breast cancer. Results of a phase II and pharmacokinetic study in patients

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progressing after first-line anthracycline/taxane-based chemotherapy. *Br J Cancer* 2006; **95**:1161–1166.
82 Fumoleau P, Cortés-Funes H, Taleb AB, Chan S, Campone M, Pouget JC, *et al*. Phase 2 study of single-agent IV vinflunine as third-line treatment of metastatic breast cancer after failure of anthracycline-/taxane-based chemotherapy. *Am J Clin Oncol* 2009; **32**:375–380.
83 Yardley DA, McCleod M, Schreiber F, Murphy P, Patton J, Thompson DS, *et al*. A phase II trial of vinflunine as monotherapy or in combination with trastuzumab as first-line treatment of metastatic breast cancer. *Cancer Invest* 2010; **28**:925–931.
84 Hainsworth JD, Meluch AA, Lane CM, Spigel DR, Burris HA 3rd, Gandhi JG, *et al*. Single agent vinflunine in the salvage treatment of patients with castration-resistant prostate cancer: a phase II trial of the Sarah Cannon Research Consortium. *Cancer Invest* 2010; **28**:275–279.
85 Talbot DC, Margery J, Dabouis G, Dark G, Taylor H, Boussemart H, *et al*. Phase II study of vinflunine in malignant pleural mesothelioma. *J Clin Oncol* 2007; **25**:4751–4756.
86 Olver IN, Byrne MJ, Walpole E, Vorobiof D, Jacobs C, Maart K, *et al*. Phase II study of IV vinflunine in patients with chemotherapy naive metastatic malignant melanoma. *Eur J Cancer* 2007; **43**:1829–1832.
87 Goldstein D, Ackland SP, Bell DR, Olver IN, Davis ID, Rosenthal MA, *et al*. Phase II study of vinflunine in patients with metastatic renal cell carcinoma. *Invest New Drugs* 2006; **24**:429–434.
88 Hay ED. An overview of epithelio–mensenchymal transformation. *Acta Anat* 1995; **154**:8–20.
89 Thiery JP. Epithelial–mesenchymal transitions in tumor progression. *Nat Rev Cancer* 2002; **2**:442–454.
90 Kalluri R, Weinberg RA. The basis of epithelial–mesenchymal transition. *J Clin Invest* 2009; **119**:1420–1428.
91 Lauffenburger DA, Horwitz AF. Cell migration: a physically integrated molecular process. *Cell* 1996; **84**:359–369.
92 Watanabe T, Noritake J, Kaibuchi K. Regulation of microtubules in cell migration. *Trends Cell Biol* 2005; **15**:76–83.
93 Ballestrem C, Magid N, Zonis J, Shtutman M, Bershadsky A. Interplay between the actin cytoskeleton, focal adhesions, and microtubules. In: Ridley A, Peckham M, Clark P, editors. *Cell motility: from molecules to organisms*. Chichester: John Wiley and Sons, Ltd. 2004; pp. 75–99.
94 Liu SM, Magnusson KE, Sundqist T. Microtubules are involved in transport of macro-molecules by vesicles in cultured bovine aortic endothelial cells. *J Cell Physiol* 1993; **156**:311–316.
95 Waterman-Storer CM, Salmon E. Positive feedback interactions between microtubule and actin dynamics during cell motility. *Curr Opin Cell Biol* 1999; **11**:61–67.
96 Zhou X, Li J, Kucik DF. The microtubule cytoskeleton participates in control of β2 integrin avidity. *J Biol Chem* 2001; **76**:44762–44769.
97 Whipple RA, Matrone MA, Cho EH, Balzer EM, Vitolo MI, Yoon JR, *et al*. Epithelial-to-mesenchymal transition promotes tubulin detyrosination and microtentacles that enhance endothelial engagement. *Cancer Res* 2010; **70**:8127–8137.
98 Manna T, Honnappa S, Steinmetz MO, Wilson L. Suppression of microtubule dynamic instability by the +TIP protein EB1 and its modulation by the CAP-Gly domain of p150(Glued). *Biochemisty* 2008; **47**:779–786.
99 Lansbergen G, Akhmanova A. Microtubule pus end: a hub of cellular activities. *Traffic* 2006; **7**:499–507.
100 Margolis RL, Rauch CT, Job D. Purification and assay of a 145-kDa protein (STOP 145) with microtubule-stabilizing and motility behavior. *Proc Natl Acad Sci USA* 1986; **83**:639–643.
101 Pabiom M, Job D, Margolis RL. Sliding of STOP proteins on microtubules. *Biochemistry* 1984; **23**:6642–6648.
102 Bosc C, Frank R, Denarier E, Ronjat M, Schweitzer A, Wehland J, *et al*. Identification of novel bifunctional calmodulin-binding and microtubule-stabilizing motifs in STOP proteins. *J Biol Chem* 2001; **276**: 30904–30913.
103 Bosch C, Oenarier E, Andrieux A, Job D. Stop proteins. *Cell Struct Funct* 1999; **24**:393–399.
104 Pirollet F, Derancourt J, Haiech J, Job D, Maregolis RL. Ca^{2+}-calmodulin regulated effectors of microtubule stability in bovine brain. *Biochemistry* 1992; **31**:8849–8855.
105 Pirollet F, Margolis RL, Job D. Ca^{2+}-calmodulin regulated effectors of mirotubule stability in neural tissues. *Biochim Biophys Acta* 1992; **1160**:113–119.
106 Erent M, Pagakis S, Browne JP, Bayley PM. Association of calmodulin with cytoskeletal structures at different stages of HeLa cell division, visualized by a calmodulin-EGFP fusion protein. *Mol Cell Biol Res Commun* 1999; **1**:209–215.
107 Li CJ, Heim R, Lu P, Pu Y, Tsien RY, Chang DC. Dynamic redistribution of calmodulin in HeLa cells during cell division as revealed by a GFP-calmodulin fusion protein technique. *J Cell Sci* 1999; **112**: 1567–1577.
108 Bouvier D, Vanhaverbeke C, Simorre J, Arlaud GJ, Bally I, Forge V, *et al*. Unusual Ca^{2+}-calmodulin binding interactions of the microtubule-associated protein F-STOP. *Biochemistry* 2003; **42**:11484–11493.
109 Honoré S, Pagano A, Gautier G, Bourgaret-Rey V, Verdier-Pinard P, Civilett K, *et al*. Antiangiogenic vinflunine effects EB1 localization and microtubule targeting to adhesion sites. *Mol Cancer Ther* 2008; **7**: 2080–2089.
110 Makarov AA, Tsvetkov PO, Villard C, Esquqieu D, Pourroy B, Fahy J, *et al*. Vinflunine, a novel microtubule inhibitor, suppresses calmodulin interaction with the microtubule-associated protein STOP. *Biochemistry* 2007; **46**:14899–14906.

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