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Review Article

Colchicine Binding Site Inhibitors as Anticancer Drugs

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Colchicine binding site inhibitors (CSBIs) target microtubules, a critical component of the cell cytoskeleton, by inhibiting its polymerization. To date, combretastatin, indibulin, crinobulin, plinabulin, etc., that bind to colchicine sites have been identified as promising anticancer agents. Compared to colchicine, its derivatives and other inhibitors binding at the same site are poor substrates for Pgp pumps, making them less prone to developing multiple drug resistance in tumor cells. Their high chemical stability with low neurotoxicity is an added advantage, making them suitable for entering clinical trials.These inhibitors dock onto the hydrophobic pocket of the colchicine binding site on tubulin via hydrophobic interactions. Hydrogen bonds and electrostatic interactions between the inhibitor molecule and the amino acid residues on tubulin are essential for stabilizing the inhibitor-tubulin complex and enhancing the binding specificity. This review discusses the clinical trials of colchicine-binding site inhibitors in cancer chemotherapy.

Introduction

Microtubules (MTs) regulate cell division, intracellular transport, and cellular integrity, rendering them one of the most promising targets for cancer treatment regimens.MTs have a cylindrical structure consisting of 13 protofilaments, alternating α- and b-tubulin subunits arranged in a helical pattern. The protofilaments are arranged in a circular pattern, forming a hollow tube with an outer diameter of about 25 nm and an inner diameter of about 15 nm. MTs are highly dynamic structures that can rapidly grow and shrink by adding and removing tubulin subunits[1]. The dynamics of microtubules are regulated by several proteins, including microtubule-associated proteins (MAPs) and motor proteins. Several tubulin-bindingor microtubule targeting agents (MTAs), such as paclitaxel, vinblastine, and colchicine, interfere with MT dynamics and inhibit cell division[2]. These agents also induce DNA damage and exhibit anti-angiogenetic, anti-metastatic, and anti-neurogenerative potentials. MTAs are predominantly classified into three sets. The first set (e.g., vincristine, vinblastine, vinorelbine, etc.) targets the vinca domain residing between two longitudinally arranged αβ tubulin heterodimers. The second one (e.g., colchicine, combretastatin A4, nocodazole, etc.) camps inside the tubulin dimer and primarily binds to β-tubulin (Fig. 1)[3].The third set (e.g., paclitaxel and epothilones) targets the taxane site on β-tubulin in the lumen of MTs[4].

The biological significance of microtubules in governing multiple cellular processes, especially in cell proliferation, has led to the identification of many MTAs as potential anti-cancer agents[5]. Several tubulin inhibitors have already been clinically approved for cancer treatment therapeutics, and many more are in pipelines and currently undergoing pre-clinical and clinical investigations. Tubulin inhibitors either act by binding to colchicine and vinblastine sitesand serving as promoters of tubulin disassembly or by binding to taxane sites such as paclitaxel and epothilone to enable tubulin assembly. In either of the two cases, they interfere with spindle-microtubule dynamics during mitosis, causing cell cycle arrest and apoptotic cell death. These MTAs distinguish between cancer cells, which are highly proliferative, and normal cells, which makes

Abbreviations

CSBIs -Colchicine site binding inhibitors, MTs - Microtubules, MTAs - Microtubule targeting agents, MAPs - Microtubule-associated proteins, SIMBA - Selective Immunomodulating Microtubule-binding agent, CIN - Chemotherapy-induced Neutropenia, NSCLC - Non-small cell lung carcinoma

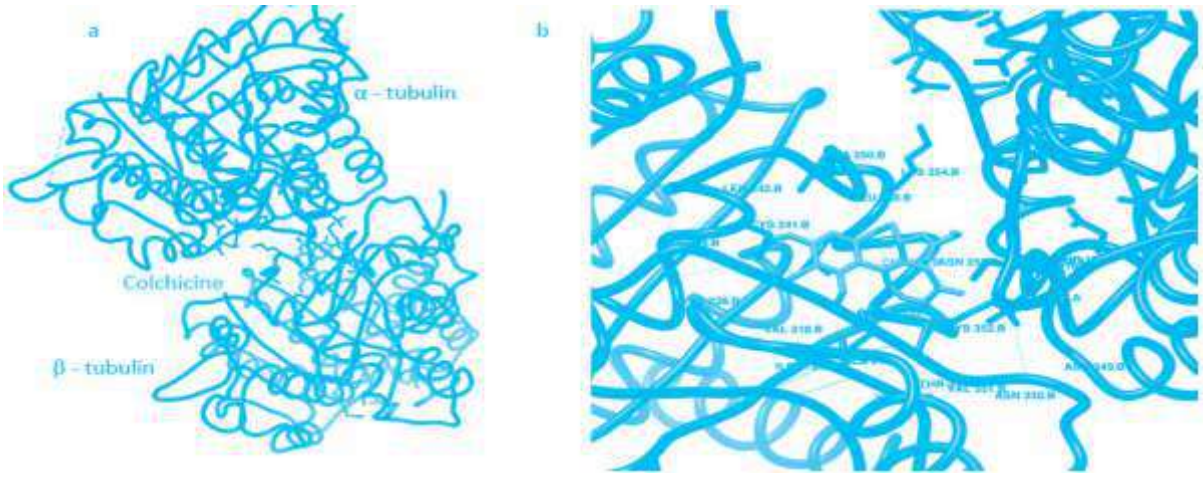

Figure 1: (a) Representative docked image of colchicine and tubulin dimer (PDB ID - 1SA0)(b) Zoomed-in image depicting the interacting amino acid residues with blue lines indicating the hydrogen bonds.

Table - Summary of CSBIs with their status of clinical trial

CSBIs	Status of Clinical trial	References
Mivobulin / CI-980	Failed at Phase II for melanoma.	[13–16]
Indibulin / D-24851	Incomplete Phase I/II for metastatic breast cancer, advanced solid tumors in combination with Erlotinib.	[19–21, 23]
Crolibulin / Crinobulin / EPC2407	Phase I/II for anaplastic thyroid cancer	[27]
CA-4-P / Fosbretabulin	Phase II for ovarian cancer, solid tumors, and NSCLC in combination with carboplatin and paclitaxel, Phase III for anaplastic thyroid cancer	[34–36, 38]
Ombrabulin / AVE8062	Phase II for solid tumors in combination with paclitaxel and carboplatin, ovarian cancer, Phase III for soft tissue sarcoma	[44, 48]
ABT-751 / E7010	Phase II for breast cancer, NSCLC, prostate cancer, neuroblastoma	[50, 51, 53, 70]
ZD6126	Phase II for renal cell carcinoma, for colorectal cancer in combination with oxaliplatin and leucovorin	[55, 56]
Lisavanbulin / BAL101553	Phase II for solid tumors, glioblastoma	[59, 60]
Plinabulin / NPI-2358	Phase III for advanced solid tumors, for NSCLC in combination with docetaxel	[65, 66, 71]
Tivantinib / ARQ197	Phase III for hepatocellular carcinoma, for NSCLC in combination with Erlotinib	[68, 69]

them less susceptible to their effects[6, 7]. Over the last decade, many MTAs have been developed either by de-novo drug designing or by modification of existing lead molecules. They are beingtested for their anti-cancer potential,followed by effective absorption, distribution, metabolism, excretion, and toxicity (ADMET) to be potentially used at the clinical level[8].

Many of the MTAs targeting the taxane or vinca site have been shown to display multi-drug resistance and are substrates of P-glycoprotein (Pgp) pumps[9]. Colchicine itself is a substrate of Pgp[10]. To our interests, colchicine-site-binding inhibitors (CSBIs) demonstrated the capacity to circumvent drug resistance because of their distinct cavity structure, which is readily accessible to small molecule inhibitors[11, 12]. Many of these small molecules also exhibited high chemical stability and low neurotoxicity. This has gathered attention, and several CSBIs are currently under active investigation (Fig. 2, 3). This review focuses on

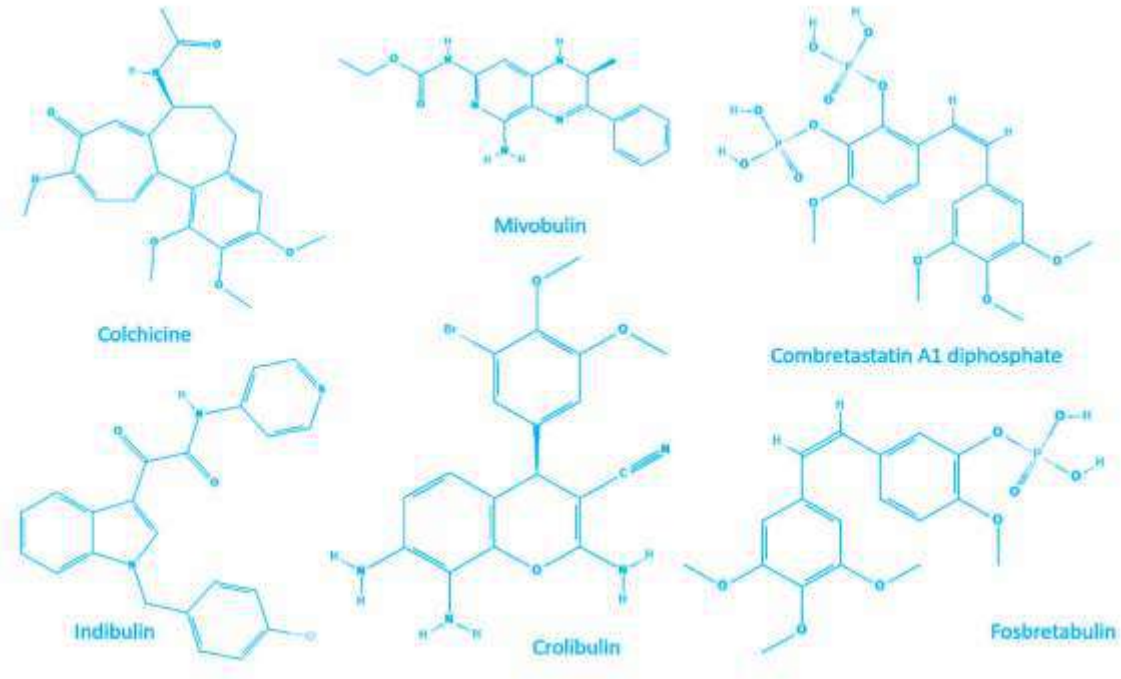

Figure 2: Chemical structures of colchicine (PubChem CID - 6167), mivobulin (PubChem CID - 182762), indibulin (PubChem CID - 2929), crolibulin (PubChem CID - 23649181), combretastatin A1 diphosphate (PubChem CID - 6918546) and fosbretabulin (PubChem CID - 5351387).

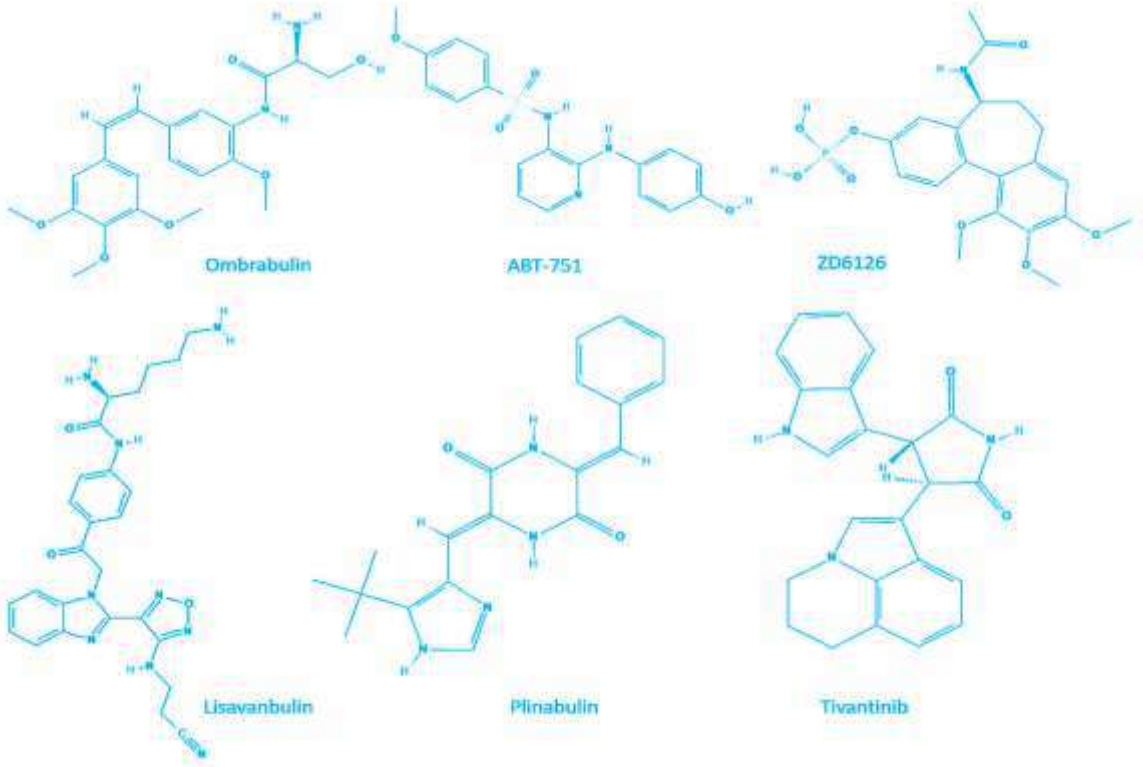

Figure 3: Chemical structures of ombrabulin (PubChem CID - 6918405), ABT-751 (PubChem CID - 3035714), ZD6126 (PubChem CID - 9896434), lisavanbulin (PubChem CID - 45259014), plinabulin (PubChem CID - 9949641), tivantinib (PubChem CID - 11494412).

CSBIs undergoing clinical evaluation and discusses their pros and cons as anti-cancer chemotherapeutic agents.

CSBIs under clinical development

Mivobulin

Mivobulin, or CI-980, is a synthetic colchicine analog and acts as a mitotic inhibitor by binding to the colchicine site of tubulin. At the pre-clinical level, it showed broad-spectrum activity by inhibiting the polymerization of microtubules and arresting cellular division in metaphase. CI-980 could cross the blood-brain barrier being useful in melanoma cases. It also augmented in vivo cytotoxic effects on both murine and human tumors. However, it failed to produce any objective response with 95% CI in phase II clinical level due to high hematologic toxicity, especially myelosuppression and granulocytopenia[13–15]. It caused leukopenia and anemia, with nausea, vomiting, and weakness. Mivobulin was found to be ineffective in phase II trials for advanced melanoma and refractory prostate cancer [16].

Indibulin

Indibulin is an indole-based MT inhibitor that disrupts MT polymerization by docking onto the colchicine site.It dampens the MT dynamics and inhibits the cell division at the G2/M phase. It activates the spindle assembly checkpoint by augmenting the levels of Mad2 and BubR1, thereby causing apoptosis-mediated cell death in multiple cell lines[17]. At pre-clinical levels, it synergizes with vinblastine to function as a potential anti-cancer agent. It induces minimal neurotoxicity in animal models and is less cytotoxic to differentiated neurons than undifferentiated ones[17, 18]. It has shown promising clinical activity and a favorable safety profile against various types of cancer, including metastatic breast cancer[19] and advanced solid tumors [20, 21]. In phase I/II dose determining and escalating clinical study, target plasma concentrations above IC_{50} doses have been achieved without any significant toxicity. Indibulin may not be a substrate of Pgp pumps, as it maintained its efficacy in multidrug-resistant cell lines resistant to taxol, vincristine, or doxorubicin[22]. It also exhibits low neural toxicity and hasthe advantage of oral bioavailability, making it a promising anti-cancer agent to further study for Phase III studies[23].

Crolibulin

Crolibulin, also known as crinobulin and EPC2407, is a chromene analog. It inhibits MT polymerization by binding to the colchicine site on β-tubulin, and causes mitotic cell arrest. It also inhibits neovascularization in tumors, leading to reduced blood flow and ischemic necrosis in the tumors[24, 25]. Clinically, it has been found effective in monotherapy for advanced tumor types, including pancreatic, colon, and prostate. Phase I/II dose-determining and escalating studies reported that crolibulin had an acceptable side effect profile with a transient increase in blood pressure. There were no reports of pulmonary adverse events, significant myelosuppression, or hepatic or renal toxicities[26]. It is synergistic with cisplatin for anaplastic thyroid cancer. In addition, it caused no infusion reactions or renal or hepatic dysfunctions and was well tolerated for up to 8 cycles over 6 months [27, 28].

Combretastatins

Combretastatins are water-soluble natural phenols and they inhibit tubulin polymerization and also exhibit anti-angiogenic potential. Combretastatin A1 diphosphate (OXi4503) and Combretastatin A-4 phosphate (CA-4P), named fosbretabulin, are the two combretastatin analogs that have entered clinical trials. OXi4503 has shown promising results in phase I as monotherapy in hepatic tumors, other advanced solid tumors, and myeloid leukemia [29–31]. It has also been tested with cytarabine as a Phase I/II trial for Acute Myelogenous Leukemia and Myelodysplastic Syndromes [32].

CA-4P or fosbretabulin has shown highly encouraging results and is currently in Phase III trials for platinum-resistant ovarian cancer [33], anaplastic thyroid cancer [34, 35], and Phase I/II trials for other advanced solid tumors [36, 37], and recurrent high-grade gliomas [38]. It has been tested in over 500 patients as monotherapy or in combination with paclitaxel and bevacizumab. CA-4P has been well tolerated and exhibited no significant toxicity during dose escalation trials. Given combretastatin's promising potential as an anti-cancer agent, many synthetic analogs of it are currently in pre-clinical studies. Amongst other synthetic analogs of combretastatin, C12 (5-Quinolin-3-yl and 4-(3,4,5-trimethoxyphenyl) selectively targets both mitotic and non-mitotic cancer cells than non-cancerous cells. It elicits multiple modes of cell death via targeting the MT dynamics and activating the spindle assembly checkpoint. It has been found to reduce tumor volume in MCF-7 xenograft in NOD-SCID mice[39–41], holding positive expectations at the clinical stage in future investigations.

Ombrabulin

Ombrabulin,or AVE8062 is a water-soluble combretastatin analog that inhibits tubulin polymerization by docking onto the colchicine site.

It has been reported as a vascular disruptor causing arterial constriction along with its anti-tumor activity. It hampers the tumor blood flow, causing extensive necrosis. It acts synergistically with docetaxel for endothelial and tumor cell lines[42, 43]. It has been clinically tested as monotherapy and in combination with cisplatin, docetaxel, and carboplatin on solid tumors, non-small cell lung cancer, and ovarian cancer. This drug passed a couple of phase I/ II trials[44–47], however, failed to meet the expectations due to significant cardiovascular toxicity at higher doses[48]. It also did not show a significant objective response with the taxane-platinum regimen versus placebo in non-small cell lung carcinoma [47].

ABT-751

ABT-751 or E7010 is an orally bioavailable, sulfonamide tubulin inhibitor. It blocks the cell cycle at the G2-M phase by lodging into the colchicine site of β-tubulin, thereby resulting in cellular apoptosis. ABT-751 engenders broad-spectrum anti-tumor activity extending to drug-resistant lines. Parallelly, it also reduced tumor perfusion and hampered tumor vasculature[49]. It is currently under phase II trials for breast cancer[50], non-small cell lung cancer[51], metastatic prostate cancer that failed hormonal therapy [52], and recurrent neuroblastoma[53]. The event-free survival upon ABT-751 treatment is longer in neuroblastoma children compared to other diagnoses. Although the results are encouraging, the drug is well tolerated with an acceptable toxicity (abdominal pain, fatigue, and constipation) profile and no myelosuppression, the objective response has not been significantly better than other active compounds and current treatments.

ZD6126

ZD6126 is an N-acetylcolchinol tubulin-binding agent. It characteristically disrupts the endothelial cell tubulin cytoskeleton, causing selective changes in cell shape. The immature tumor vasculature is disrupted, culminating in nutrient deprivation and tumor necrosis. In pre-clinical mice models, ZD6126 caused a delay in tumor growth alone and combination with cisplatin[54]. At the clinical level, the dose escalation trials are currently halted owing to their gastrointestinal (abdominal pain, vomiting) and cardiovascular toxicity at higher doses. At tolerated doses, it failed to produce any objective response in patients. It is being tested at Phase II levels as monotherapy in metastatic renal cell carcinoma [55] and in combination with oxaliplatin and leucovorin in metastatic colorectal cancer[56].

Lisavanbulin

Lisavanbulin or BAL101553, a prodrug of avanbulin or BAL27862, is causing tumor cell death by MT destabilization and activating the spindle assembly checkpoint. The anti-tumor activity of lisavanbulin, which binds to the colchicine site of tubulin heterodimer, is higher in tissues expressing end-binding protein (EB1), MT associated protein that regulates MT dynamics. It has completed its phase I trials via oral infusions with a favorable safety and tolerability profile. It exhibits significant tolerance and no vascular toxicity, even though at doses above 70mg/m^2, some symptoms of hypotension and neutropenia were observed[57]. It demonstrates benefits in GBM PDX models alone and in combination with radiation therapy and temozolomide [58]. It is currently under phase II trials for solid tumors [59] and high-grade, recurrent glioblastomas[60].

Plinabulin

Plinabulin or NPI-2358, is a synthetic analog of halimide derived from marine Aspergillus sp. It is a CSBI and works effectively both as an anti-tumor and anti-vascular agent[61]. Plinabulin triggers early mitotic arrest and causes JNK-mediated apoptosis in multiple myeloma cells [62]. It has been tagged as a selective immunomodulating microtubule-binding agent (SIMBA) and is actively investigated for the prevention of chemotherapy-induced neutropenia (CIN). Plinabulin can also induce macrophage to attain an M1-like tumor inflammatory phenotype [63]. In the PD-1 non-responsive immune-competent animal model, Plinabulin in combination with PD-1 antibody and radiation (IR) – triple IO combination – induced~ 80% tumor reduction. It has been tested globally in over 1400 patients and 14 clinical trials. It displayed effective dose tolerance and safety profile in phase I and II trials and is currently under phase III trials for CIN[64] and non-small cell lung cancer. In Phase I, plinabulin in combination with PD-1 and CTLA-4 antibodies produced significant benefitsin SCLC patients who failed platinum and PD-1/PD-L1 antibodies [65]. It is also tested with radiation and immunotherapy for advanced bladder carcinoma in phase I/II trials [66].

Tivantinib

Tivantinib or ARQ197 perfectly fits onto the colchicine site of beta-tubulin, rendering disrupted MTs. It was initially evaluated as a selective MET inhibitor; however, it inhibits cell proliferation in both MET-dependent and independent manner. It disrupts MTs and induces arrest of the cells in the G2-M phase. Tivantinib can induce apoptosis via both extrinsic-death receptor and intrinsic – mitochondrial pathway. ABC transporters also do not impede its

anti-apoptotic activity,thus showing similar pre-clinical results in both parent and MDR cell lines[67]. In phase II studies for hepatocellular carcinomas, tivantinib doubled the overall survival rate. It has been investigated in Phase III trials globally on about 1500 patients with liver [68] and non-small cell lung cancer[69]. The multiple mechanisms of action and adverse hematological toxicity of tivantinib have limited its further investigation at the clinical level,and more pre-clinical studies are necessary to elucidate its cellular action.

Conclusion

This review highlightsthe clinical developmentprogress ofCSBIs.In addition to their anti-mitotic effects, many CSBIs bear anti-vascular and anti-angiogenic properties. As discussed, many of the CSBIs, such as CA-4P, OXi4503, Crinobulin, and indibulin, continue to advance in the clinical development process, whereas some are halted due to their severe toxicity in the patients during the trials.

CSBIs such as Ombrabulin and ZD6126 exert anti-tumor effects by vascular disruption. They cause contraction in proliferating endothelial cells, leading to shutdown of tumor blood flow and extensive necrosis in tumor models. These drugs fail to produce immediate tumor size reduction at the clinical level, however, their combination with standard-of-care chemotherapies could devise a multi-targeted line of therapy. Plinabulin has shown a significant objective response with a platinum-PD-1/PD-L1 immunotherapy regimen.

Currently, many CSBIs are under pre-clinical studies, showing encouraging evidence for treating MDR malignancies. They hold promises for their journey from pre-clinical to clinical investigations in the near future.

References

1 Mitchison, T. and Kirschner, M. (1984) Dynamic instability of microtubule growth. *Nature*, Nature **312**, 237–242 https://doi.org/10.1038/312237A0

2 Singh, P., Rathinasamy, K., Mohan, R. and Panda, D. (2008) Microtubule assembly dynamics: An attractive target for anticancer drugs. *IUBMB Life*, John Wiley & Sons, Ltd **60**, 368–375 https://doi.org/10.1002/IUB.42

3 Ravelli, R. B. G., Gigant, B., Curmi, P. A., Jourdain, I., Lachkar, S., Sobel, A., et al. (2004) Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature***428**, 198–202 https://doi.org/10.1038/nature02393

4 Wilson, L., Panda, D. and Jordan, M. A. (1999) Modulation of microtubule dynamics by drugs: a paradigm for the actions of cellular regulators. *Cell Struct Funct*, Cell Struct Funct**24**, 329–335 https://doi.org/10.1247/CSF.24.329

5 Kumari, A. and Panda, D. Chapter 27 Monitoring the Disruptive Effects of Tubulin-Binding Agents on Cellular Microtubules https://doi.org/10.1007/978-1-0716-1983-4_27

6 Jordan, M. A. and Wilson, L. (2004) Microtubules as a target for anticancer drugs. *Nat Rev Cancer*, Nat Rev Cancer **4**, 253–265 https://doi.org/10.1038/NRC1317

7 Èermák, V., Dostál, V., Jelínek, M., Libusová, L., Kováø, J., Rösel, D., et al. (2020) Microtubule-targeting agents and their impact on cancer treatment. *Eur J Cell Biol*, Urban & Fischer **99**, 151075 https://doi.org/10.1016/J.EJCB.2020.151075

8 Segall, M. (2014) Advances in multiparameter optimization methods for de novo drug design. *http://dx.doi.org/10.1517/17460441.2014.913565*, Taylor & Francis **9**, 803–817 https://doi.org/10.1517/17460441.2014.913565

9 Dumontet, C. and Sikic, B. I. (1999) Mechanisms of action of and resistance to antitubulin agents: microtubule dynamics, drug transport, and cell death. *J Clin Oncol*, J Clin Oncol **17**, 1061–1070 https://doi.org/10.1200/JCO.1999.17.3.1061

10 Silva, R., Carmo, H., Vilas-Boas, V., Barbosa, D. J., Palmeira, A., Sousa, E., et al. (2014) Colchicine effect on P-glycoprotein expression and activity: in silico and in vitro studies. *Chem Biol Interact*, Chem Biol Interact **218**, 50–62 https://doi.org/10.1016/J.CBI.2014.04.009

11 Bhattacharyya, B., Panda, D., Gupta, S. and Banerjee, M. (2008) Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin. *Med Res Rev*, Med Res Rev **28**, 155–183 https://doi.org/10.1002/MED.20097

12 Arnst, K. E., Wang, Y., Hwang, D. J., Xue, Y., Costello, T., Hamilton, D., et al. (2018) A potent, metabolically stable tubulin inhibitor targets the colchicine binding site and overcomes taxane resistance. *Cancer Res*, American Association for Cancer Research Inc. **78**, 265–277 https://doi.org/10.1158/0008-5472.

13 Thomas, J. P., Moore, T., Kraut, E. H., Balcerzak, S. P., Galloway, S. and Vandre, D. D. (2002) A phase II study of CI-980 in previously untreated extensive small cell lung cancer: an Ohio State University phase II research consortium study. *Cancer Invest*, Cancer Invest **20**, 192–198 https://doi.org/10.1081/CNV-120001146

14 Whitehead, R. P., Unger, J. M., Flaherty, L. E., Eckardt, J. R., Taylor, S. A., Didolkar, M. S., et al. (2001) Phase II trial of CI-980 in patients with disseminated malignant melanoma and no prior chemotherapy: A Southwest Oncology Group study. *Invest New Drugs*, Kluwer Academic Publishers **19**, 239–243 https://doi.org/10.1023/A:1010624702340

15 Sklarin, N. T., Lathia, C. D., Benson, L., Grove, W. R., Thomas, S., Roca, J., et al. (1997) A phase I trial and pharmacokinetic evaluation of CI-980 in patients with advanced solid tumors. *Invest New Drugs*, Springer **15**, 235–246 https://doi.org/10.1023/A:1005854510468/METRICS

16 Ryan, C. W., Shulman, K. L., Richards, J. M., Kugler, J. W., Sosman, J. A., Ansari, R. H., et al. (2000) CI-980 in advanced melanoma and hormone refractory prostate cancer. *Invest New Drugs*, Invest New Drugs **18**, 187–191 https://doi.org/10.1023/A:1006382014403

17 Kapoor, S., Srivastava, S. and Panda, D. (2018) Indibulin dampens microtubule dynamics and produces

synergistic antiproliferative effect with vinblastine in MCF-7 cells: Implications in cancer chemotherapy. *Sci Rep*, Sci Rep **8** https://doi.org/10.1038/S41598-018-30376-Y

18 Kumari, A., Prassanawar, S. S. and Panda, D. (2023) â-III Tubulin Levels Determine the Neurotoxicity Induced by Colchicine-Site Binding Agent Indibulin. *ACS Chem Neurosci*, American Chemical Society **14**, 19–34

19 https://ClinicalTrials.gov/show/NCT01113970. Study of a Novel Indibulin Dosing Schedule for the Treatment of Metastatic Breast Cancer

20 https://ClinicalTrials.gov/show/NCT00591136. Phase I Study of Indibulin in Patients With Solid Tumors

21 https://ClinicalTrials.gov/show/NCT00591383. Phase I Study of Indibulin in Combination With Erlotinib in Advanced Solid Tumors

22 Bacher, G., Nickel, B., Emig, P., Vanhoefer, U., research, S. S.-C. and 2001, undefined. D-24851, a Novel Synthetic Microtubule Inhibitor, Exerts Curative Antitumoral Activity in Vivo, Shows Efficacy toward Multidrug-resistant Tumor Cells, and Lacks. *AACR*

23 Kuppens, I. E. L. M., Witteveen, P. O., Schot, M., Schuessler, V. M., Daehling, A., Beijnen, J. H., et al. (2007) Phase I dose-finding and pharmacokinetic trial of orally administered indibulin (D-24851) to patients with solid tumors. *Invest New Drugs*, Invest New Drugs **25**, 227–235 https://doi.org/10.1007/S10637-006-9027-2

24 Anthony, S. P., Hoff, D. Von, Whisnant, J. K. and Tseng, B. Y. (2007) EPC2407, a new beta-tubulin vascular disrupting agent with potent apoptosis and cell growth inhibition. *https://doi.org/10.1200/jco.2007.25.18_suppl.14043*, American Society of Clinical Oncology **25**, 14043–14043 https://doi.org/10.1200/JCO.2007.25.18_SUPPL.14043

25 Anthony, S. P., Read, W., Rosen, P. J., Tibes, R., Park, D., Everton, D., et al. (2008) Initial results of a first-in-man phase I study of EPC2407, a novel small molecule microtubule inhibitor anticancer agent with tumor vascular endothelial disrupting activity, American Society of Clinical Oncology **26**, 2531–2531 https://doi.org/10.1200/JCO.2008.26.15_SUPPL.2531

26 Lorza, A. M. A., Ravi, H., Philip, R. C., Galons, J. P., Trouard, T. P., Parra, N. A., et al. (2020) Dose-response assessment by quantitative MRI in a phase 1 clinical study of the anti-cancer vascular disrupting agent crolibulin. *Sci Rep*, Sci Rep **10** https://doi.org/10.1038/S41598-020-71246-W

27 https://ClinicalTrials.gov/show/NCT00423410. A Safety Study of EPC2407, An Anti-Cancer Drug With Vascular Disrupting Activity: In Patients With Advanced Cancer

28 Gramza, A. W., Balasubramaniam, S., Fojo, A. T., Ward, J. and Wells, S. A. (2013) Phase I/II trial of crolibulin and cisplatin in solid tumors with a focus on anaplastic thyroid cancer: Phase I results. American Society of Clinical Oncology **31**, 6074–6074 https://doi.org/10.1200/JCO.2013.31.15_SUPPL.6074

29 https://ClinicalTrials.gov/show/NCT00960557. Safety Study of Increasing Doses of Combretasatin A1 Diphosphate (OXi4503) as Monotherapy in Subjects With Hepatic Tumor Burden

30 https://ClinicalTrials.gov/show/NCT00977210. Safety Study of OXi4503 (a Vascular Disrupting Agent) Given by 3 x Weekly Intravenous Infusions to Patients With Advanced Solid Tumors

31 https://ClinicalTrials.gov/show/NCT01085656. A Phase I Clinical Trial of OXi4503 for Relapsed and Refractory AML and MDS

32 https://ClinicalTrials.gov/show/NCT02576301. Dose Escalation of OXi4503 as Single Agent and Combination With Cytarabine w/Subsequent Ph 2 Cohorts for AML and MDS

33 https://ClinicalTrials.gov/show/NCT02641639. FOCUS: PCC + Bevacizumab + CA4P Versus PCC + Bevacizumab + Placebo for Subjects With Platinum Resistant Ovarian Cancer

34 https://ClinicalTrials.gov/show/NCT01701349. Fosbretabulin or Placebo in Combination With Carboplatin/Paclitaxel in Anaplastic Thyroid Cancer

35 https://ClinicalTrials.gov/show/NCT00507429. Study of Combretastatin and Paclitaxel/Carboplatin in the Treatment of Anaplastic Thyroid Cancer

36 https://ClinicalTrials.gov/show/NCT00003768. Combretastatin A4 Phosphate in Treating Patients With Advanced Solid Tumors

37 https://ClinicalTrials.gov/show/NCT00395434. Safety Study of Increasing Doses of Combretastatin in Combination With Bevacizumab (Avastin) in Patients With Advanced Solid Tumors

38 https://ClinicalTrials.gov/show/NCT01052363. OXC401-PO1s/WVU 1309 - A Pilot Study of FosbretabulinWith Bevacizumab in Recurrent High-Grade Gliomas

39 Hura, N., Sawant, A. V, Kumari, A., Guchhait, S. K. and Panda, D. (2018) Combretastatin-Inspired Heterocycles as Antitubulin Anticancer Agents. *ACS Omega***3**, 9754–9769 https://doi.org/10.1021/acsomega.8b00996

40 Kumari, A., Srivastava, S., Manne, R. K., Sisodiya, S., Santra, M. K., Guchhait, S. K., et al. (2019) C12, a combretastatin-A4 analog, exerts anticancer activity by targeting microtubules. *BiochemPharmacol*, Elsevier Inc. **170**, 113663 https://doi.org/10.1016/j.bcp.2019.113663

41 Chaudhary, V., Venghateri, J. B., Dhaked, H. P. S., Bhoyar, A. S., Guchhait, S. K. and Panda, D. (2016) Novel Combretastatin-2-aminoimidazole Analogues as Potent Tubulin Assembly Inhibitors: Exploration of Unique Pharmacophoric Impact of Bridging Skeleton and Aryl Moiety. *J Med Chem***59**, 3439–3451 https://doi.org/10.1021/acs.jmedchem.6b00101

42 von Pawel, J., Gorbounova, V., Reck, M., Kowalski, D. M., Allard, A., Chadjaa, M., et al. (2014) DISRUPT: a randomised phase 2 trial of ombrabulin (AVE8062) plus a taxane-platinum regimen as first-line therapy for metastatic non-small cell lung cancer. *Lung Cancer*, Lung Cancer **85**, 224–229 https://doi.org/10.1016/J.LUNGCAN.2014.05.013

43 Eskens, F. A. L. M., Tresca, P., Tosi, D., Van Doorn, L., Fontaine, H., Van Der Gaast, A., et al. (2014) A phase I pharmacokinetic study of the vascular disrupting agent ombrabulin (AVE8062) and docetaxel in advanced solid tumours. *British Journal of Cancer 2014 110:9*, Nature Publishing Group **110**, 2170–2177 https://doi.org/10.1038/bjc.2014.137

44 https://ClinicalTrials.gov/show/NCT01193595. Dose-escalation, Safety, Pharmacokinetics Study of AVE8062 Combined With Bevacizumab in Patients With Advanced Solid Tumors

45 https://ClinicalTrials.gov/show/NCT01293630. Dose-escalation Study of Ombrabulin in Combination With Paclitaxel and Carboplatin in Patients With Advanced Solid Tumors

46 https://ClinicalTrials.gov/show/NCT01332656. Study of Ombrabulin in Patients With Platinum-Sensitive Recurrent Ovarian Cancer Treated With Carboplatin/ Paclitaxel

47 https://ClinicalTrials.gov/show/NCT01263886. Trial of Ombrabulin (AVE8062) in Combination With Taxane and Platinum in Patients With Non-small Cell Lung Cancer

48 https://ClinicalTrials.gov/show/NCT00699517. A Study of AVE8062 in Advanced-stage Soft Tissue Sarcoma After Failure of Anthracycline and Ifosfamide Chemotherapies

49 Luo, Y., Hradil, V. P., Frost, D. J., Rosenberg, S. H., Gordon, G. B., Morgan, S. J., et al. (2009) ABT-751, a novel tubulin-binding agent, decreases tumor perfusion and disrupts tumor vasculature. *Anticancer Drugs*, Anticancer Drugs **20**, 483–492 https://doi.org/10.1097/CAD.0B013E32832C0ACF

50 https://ClinicalTrials.gov/show/NCT00063102. A Study of ABT-751 in Patients With Recurrent Breast Cancer After Taxane (Taxol or Taxotere) Therapy.

51 https://ClinicalTrials.gov/show/NCT00073151. A Study of ABT-751 in Patients With Non-Small Cell Lung Cancer

52 https://ClinicalTrials.gov/show/NCT00471718. ABT-751 in Treating Patients With Metastatic Prostate Cancer That Did Not Respond to Hormone Therapy

53 https://ClinicalTrials.gov/show/NCT00436852. ABT-751 in Treating Children With Neuroblastoma That Has Relapsed or Not Responded to Previous Treatment

54 Blakey, D. C., Westwood, F. R., Walker, M., Hughes, G. D., Davis, P. D., Ashton, S. E., et al. (2002) Antitumor activity of the novel vascular targeting agent zd6126 in a panel of tumor models. *Clinical Cancer Research***8**, 1974–1983

55 https://ClinicalTrials.gov/show/NCT00065572. Exploratory Study to Assess the Biological Activity of ZD6126 in Subjects With Newly Diagnosed Metastatic Renal Cell Carcinoma (Stage IV)

56 https://ClinicalTrials.gov/show/NCT00065117. Safety, Tolerability and Efficacy of ZD6126 in Combination With Oxaliplatin, 5-Fluorouracil and Leucovorin in Subjects With Metastatic Colorectal Cancer

57 Joerger, M., Stathis, A., Metaxas, Y., Hess, D., Mantiero, M., Mark, M., et al. (2020) A Phase 1 study of BAL101553, a novel tumor checkpoint controller targeting microtubules, administered as 48-h infusion in adult patients with advanced solid tumors. *Invest New Drugs*, Invest New Drugs **38**, 1067–1076 https://doi.org/10.1007/S10637-019-00850-Z

58 Burgenske, D. M., Talele, S., Pokorny, J. L., Mladek, A. C., Bakken, K. K., Carlson, B. L., et al. (2022) Preclinical modeling in glioblastoma patient-derived xenograft (GBM PDX) xenografts to guide clinical development of lisavanbulin—a novel tumor checkpoint controller targeting microtubules. *Neuro Oncol*, Oxford Academic **24**, 384–395 https://doi.org/10.1093/NEUONC/NOAB162

59 https://ClinicalTrials.gov/show/NCT01397929. An Open-Label Study of Intravenous BAL101553 in Adult Patients With Solid Tumors

60 https://ClinicalTrials.gov/show/NCT02490800. Phase 1/2a Study of Oral BAL101553 in Adult Patients With Solid Tumors or Glioblastoma or High-grade Glioma

61 Singh, A. V., Bandi, M., Raje, N., Richardson, P., Palladino, M. A., Chauhan, D., et al. (2011) A novel vascular disrupting agent plinabulin triggers JNK-mediated apoptosis and inhibits angiogenesis in multiple myeloma cells. *Blood*, The American Society of Hematology **117**, 5692 https://doi.org/10.1182/BLOOD-2010-12-323857

62 Singh, A. V., Bandi, M., Raje, N., Richardson, P., Palladino, M. A., Chauhan, D., et al. (2011) A novel vascular disrupting agent plinabulin triggers JNK-mediated apoptosis and inhibits angiogenesis in multiple myeloma cells. *Blood*, The American Society of Hematology **117**, 5692 https://doi.org/10.1182/BLOOD-2010-12-323857

63 Natoli, M., Herzig, P., PishaliBejestani, E., Buchi, M., Ritschard, R., Lloyd, G. K., et al. (2021) Plinabulin, a Distinct Microtubule-Targeting Chemotherapy, Promotes M1-Like Macrophage Polarization and Anti-tumor Immunity. *Front Oncol*, Frontiers Media SA **11**, 644608 https://doi.org/10.3389/FONC.2021.644608

64 https://ClinicalTrials.gov/show/NCT03294577. Plinabulin vs. Pegfilgrastim in Prevention of TAC Induced Neutropenia

65 https://ClinicalTrials.gov/show/NCT02504489. Docetaxel + Plinabulin Compared to Docetaxel + Placebo in Patients With Advanced NSCLC

66 https://ClinicalTrials.gov/show/NCT04902040. Plinabulin in Combination With Radiation/Immunotherapy in Patients With Select Advanced Cancers After Progression on PD-1 or PD-L1 Targeted Antibodies

67 Aoyama, A., Katayama, R., Oh-hara, T., Sato, S., Okuno, Y. and Fujita, N. (2014) Tivantinib (ARQ 197) exhibits antitumor activity by directly interacting with tubulin and overcomes ABC transporter-mediated drug resistance. *Mol Cancer Ther*, Mol Cancer Ther**13**, 2978–2990 https://doi.org/10.1158/1535-7163.MCT-14-0462

68 https://ClinicalTrials.gov/show/NCT02029157. A Randomized Double-blind, Placebo-controlled Japanese Phase III Trial of ARQ 197 in Hepatocellular Carcinoma (HCC)

69 https://ClinicalTrials.gov/show/NCT01244191. Tivantinib Plus Erlotinib Versus Placebo Plus Erlotinib for the Treatment of Non-squamous, Non-small-cell Lung Cancer

70 https://ClinicalTrials.gov/show/NCT00735878. ABT-751 in Treating Patients With Metastatic Prostate Cancer That Did Not Respond to Hormone Therapy

71 https://clinicaltrials.gov/study/NCT00322608. *Study Details | Study of the Vascular Disrupting Agent NPI-2358 in Patients With Advanced Solid Tumors or Lymphoma | ClinicalTrials.Gov, n.d.)* https://clinicaltrials.gov/study/NCT00322608