# Contributed Reviews

## Tubulin-Interactive Natural Products as Anticancer Agents[1]

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This review provides an overview of the discovery, structures, and biological activities of anticancer natural products that act by inhibiting or promoting the assembly of tubulin to microtubules. The emphasis is on providing recent information on those compounds in clinical use or in advanced clinical trials. The vinca alkaloids, the combretastatins, NPI-2358, the halichondrin B analogue eribulin, dolastatin 10, noscapine, hemiasterlin, and rhizoxin are discussed as tubulin polymerization inhibitors, while the taxanes and the epothilones are the major classes of tubulin polymerization promoters presented, with brief treatments of discodermolide, eleutherobin, and laulimalide. The challenges and future directions of tubulin-interactive natural products-based drug discovery programs are also discussed briefly.

### Introduction

Natural products have proven to be the most reliable single source of new and effective anticancer agents. Thus Newman and Cragg have shown that 63% of anticancer drugs introduced over the last 25 years are natural products or can be traced back to a natural products source,[2] and similar observations have been made by many others.[3−7] A recent review by Butler lists 79 natural products or natural product analogues that entered clinical trial as anticancer agents in the 2005−2007 time frame.[8] Natural products have not only yielded new and effective drugs but have also provided insight into new mechanisms of action, and cancer treatment would be immeasurably poorer without the insights and the compounds provided from Nature. The reasons for the effectiveness of natural products are at least twofold. In the first place, there is a high correlation between the properties of drugs and those of natural products.[9,10] Second, natural products usually have built-in chirality and are thus uniquely suited to bind to complex proteins and other three-dimensional biological receptors.

Among the various mechanisms of action of natural products, that of interaction with the cellular protein tubulin is one of the most important, and over 25% of the new clinical candidates listed by Butler operate by this general mechanism.[8] Two major classes of anticancer drugs owe their effectiveness to this mechanism; the first class is that of the tubulin polymerization inhibitors, and the second is that of tubulin polymerization promoters. This review covers natural products or modified natural products that interact with tubulin and that are in clinical use or are in advanced development toward clinical use.

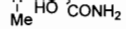
The cellular protein tubulin is a crucial protein for cellular replication. The cell cycle involves the replication of DNA and the packaging of the resulting replicated chromosomes into two daughter cells. The separation of the daughter chromosomes in mitosis is brought about by microtubules, which are formed by the with the centromeres of daughter chromosomes and generate the necessary aligned chromosomes at metaphase. The normal functioning of tubulin assembly and disassembly is thus crucial to cell division, and any interference with this process will disrupt cell division and cause cell death by apoptosis.

Although the most dramatic effect of the tubulin-interactive drugs is that of changing the extent of microtubule polymer mass, either decreasing it for the tubulin polymerization inhibitors or increasing it for the tubulin polymerization promoters, cancer cell growth can be inhibited at concentrations significantly lower than those necessary to exert these macroscopic effects. This fact can be explained by the observation that cell growth inhibition at low concentrations is caused by the suppression of microtubule dynamics.[11]

The structure of the tubulin heterodimer has been solved by electron diffraction.[12] Both the vinca alkaloids and the taxane drugs bind to $\beta$-tubulin, but at different locations on the protein; the vinca alkaloids bind to $\beta$-tubulin between amino acids 175 and 213,[13] while paclitaxel binds both to an N-terminal unit of $\beta$-tubulin[14] and to the region bounded by amino acids 217−231.[15] Colchicine, which is not a clinically used drug for cancer but which has been studied extensively, binds between the two subunits. The epothilones also bind at the paclitaxel site.[16]

### Inhibitors of Tubulin Polymerization

**The Vinca Alkaloids.** The first natural products to enter clinical use were the bisindole alkaloids vinblastine (**1**) and vincristine (**2**). These complex compounds were isolated from the Madagascar periwinkle *Catharanthus roseus* (L.) G. Don (previously known as *Vinca rosea* L.) in the late 1950s and early 1960s by two independent groups. Their discovery makes an interesting story, because one of the groups working on them, that of Robert Noble



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Vinblastine and vincristine have been used in clinical oncology for almost 50 years, and their use has been reviewed.[20] Vincristine is used in combination chemotherapy of acute lymphoblastic leukemias and lymphomas, while vinblastine is used in combination chemotherapy to treat bladder and breast cancers. Perhaps the most significant impact of vinblastine has been as part of the curative regimen for Hodgkin's disease.

Vindesine (**3**) was the first analogue of vinblastine to enter clinical use. It differs from vinblastine in having an amide function rather than a methyl ester on the vindoline ring and in lacking an acetyl group on this ring system. It has a somewhat higher hematological toxicity than vincristine, but it has been incorporated into several effective combination regimens for treatment of leukemia, lymphoma, and non-small cell lung cancer (NSCLC).[21,22]

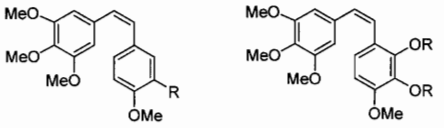
Vinorelbine (**4**) is a semisynthetic derivative of vinblastine in which the bridge linking the indole ring to the piperidine nitrogen has been shortened by one carbon, and water has been eliminated from the piperidine ring. It was launched in 1989 by Pierre Fabre for the treatment of nonmetastatic breast cancer and NSCLC, and it is available both in iv and oral formulations.[23−25]

Vinflunine (Javlor, **5**) is a dihydrodifluoro derivative of vinorelbine. It can be prepared by treatment of vinorelbine (**4**) with HF/$SbF_5$ in $CHCl_3$; the proposed mechanism involves chlorination of the cation generated by protonation of the cyclohexenyl double bond and isomerization. The chloro compound then loses a hydride ion and is fluorinated to a chlorofluoro compound, which is finally converted to vinflunine.[26] Vinflunine interacts with tubulin in a qualitatively similar way to vinblastine, but detailed studies indicate that it has quantitatively different properties from the classic vinca alkaloids.[27] It is in phase III trials at Pierre Fabre for the treatment of bladder cancer and NSCLC, based on the observation of clinically significant activity in phase II studies for the treatment of bladder, non-small cell lung, and breast cancers,[26,28] and it is also being evaluated for second-line chemotherapy in hormone refractory prostate cancer (HRPC) and for HER2-overexpressing metastatic breast cancer (with Trastuzumab),[29] and for ovarian cancer.[30]

Anhydrovinblastine ((Hydravin, KRX-0403, **6**) is an analogue of vinblastine that differs from its parent by one molecule of water. It can also be considered a homologue of vinorelbine with an additional carbon in the indole-piperidine bridge. It entered phase I trial for the treatment of advanced solid tumors, including NSCLC, soft tissue sarcoma, and colorectal cancer. Stable disease was noted in one patient with metastatic sarcoma to the lungs and in three patients with metastatic NSCLC.[31] Keryx discontinued development in 2005, but trials may still be ongoing by Prescient Neuropharma.[8]

**Combretastatins and Analogues.** The vinca alkaloids are the only inhibitors of tubulin polymerization in clinical use, but several compounds with this mechanism of action are in advanced clinic trials and will be discussed here.

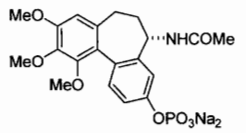
Combretastatin A-4 (**7**, CA4) was originally isolated by Pettit al. from the root bark of the *Combretum caffrum* tree, also know as the Cape Bushwillow.[32] It has been shown to target t microtubule, inhibiting the polymerization of tubulin to microt bules. However, it is much more cytotoxic than its activity again tubulin would seem to warrant, and four explanations have be proposed to explain this discrepancy: (i) CA4 targets, in vivo, subpopulation of tubulin; (ii) CA4, alongside tubulin, recognizes yet unidentified relevant target; (iii) the interaction between tubul and CA4 is qualitatively different; (iv) CA4, for as yet unknow reasons, rapidly and preferentially disrupts in vivo cellular process in the endothelium that require tubulin assembly.[33] Combretastat A4 phosphate (CA4P, **8**), is a simple derivative of the natur product that was prepared to increase water solubility.[34] CA4P h also been shown to be a vascular disrupting agent, and data fro phase I studies have established that it can selectively reduce tum blood flow at well-tolerated doses.[35] Its vascular effect can explained by its disruption of the endothelial cytoskeleton.[36] CA is in phase III clinical trials sponsored by Oxigene, Inc. for treatme of cervical, colorectal, NSC lung, prostate, ovarian, and thyro cancers; reviews of the clinical results to date have appear recently.[37,38]



**7** R = OH
**8** R = $OPO_3Na_2$
**11** R = $NHCOCH(NH_2)CH_2OH$



**9** R = H
**10** R = $OPO_3Na_2$

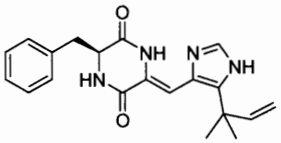


**12**

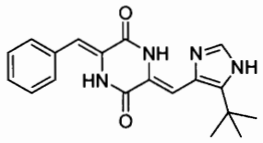
Combretastatin A-4 is accompanied in the Cape Bushwillow wi numerous congeners, differing in the ring substitutions (CA serie and by reduction of the internal stilbene double bond to give t CB series.[39] Combretastatin A-1 (**9**) is a simple hydroxyl derivati of combretastatin A-4, and it is also in phase I clinical developme as its diphosphate derivative OXi4503 (**10**).[8] A study of its effe on mice indicated that substantial microvascular damage to liv tumors and minimal normal liver injury occurred, and it w concluded that "a combination of OXi4503 with other chemothe peutic modalities might achieve complete tumor eradication a improve long-term survival".[40]

The relative simplicity of the combretastatin skeleton has pawned the synthesis of numerous analogues. The medicinal hemistry of these compounds is beyond the scope of this review, ut it is covered in detail in recent reviews.[41−43] Two of the many halogues that have been prepared are in advanced development. he serinamide derivative AVE8062A (**11**, AC-7700)[44] is in clinical ials in Europe and the United States, and its primary therapeutic ffect is to reduce blood flow to the tumor.[36,45] The compound D-6126 (ANG453, **12**) can be considered as an analogue of both ombretastatin and colchicine, and it is rapidly converted in vivo to *N*-acetylcolchinol. It too is a vascular disrupting agent.[46] Its hase II trials were halted by AstraZeneca due to problems with he method of administration, but Angiogene has solved this roblem.[8] The development of combretastatin A-4 and these halogues as anticancer agents has been reviewed.[43] Although it is o early yet to tell which (if any) of these combretastatin analogues ill enter clinical use, it is safe to predict that at least one and robably more than one drug based on the combretastatins will ter clinical use over the next few years.

**NPI-2358.** Fungi have yielded relatively few tubulin inhibitors, ut one such is the diketopiperazine halimide (**13a**). NPI-2358 (**13b**) a simple analogue of halimide, which is active as a tubulin-epolymerizing agent.[47] It is in phase I trials at Nereus.[8,48]

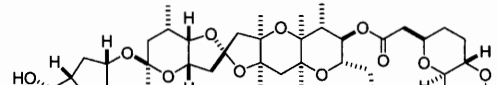


**13a**



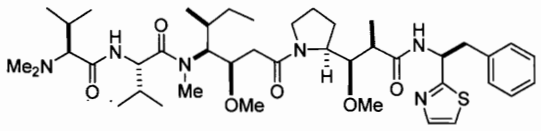
**13b**

**Halichondrin B and Eribulin.** Complex marine natural roducts of the halichondrin class were isolated by Uemura and irata from the western Pacific sponge *Halichondria okadai*[49] and ter by Pettit et al. from an *Axinella* sp.[50] Several members of the lass showed strong cytotoxicity, with the most potent being omohalichondrin B and halichondrin B (**14**). These compounds ere shown to bind to tubulin and to inhibit tubulin polymerization. alichondrin B is a noncompetitive inhibitor of vinblastine binding tubulin and has no effect on colchicine binding.[51] It showed ubnanomolar activity in the NCI 60-cell line panel[52] and excellent ctivity in various animal models,[49] and it was thus a clear candidate r clinical development. The major obstacle to clinical development as the issue of compound supply, since it was obtained only in iniscule amounts from its marine source.

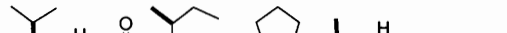


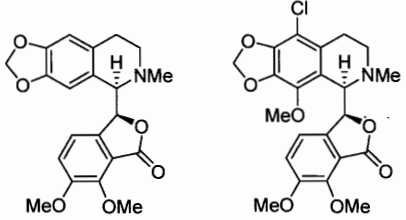
at Eisai Research Institute for activity. This led to the realization that several truncated halichondrins had significant bioactivity, and so a research program was initiated to develop a simplified and thus synthetically accessible anticancer agent based on the halichondrin B skeleton. The truncated halichondrin B analogue eribulin (**15**, E7389) was discovered as a result of this program, and its synthesis was effected in a highly convergent manner, although this still required over 70 steps.[54,55] The synthesis of this highly cytotoxic compound on an industrial scale had its own unique challenges, but these were successfully overcome so that eribulin mesylate could enter clinical trials. Like its parent compound, halichondrin B, eribulin acts as an inhibitor of tubulin polymerization.[56] Eribulin mesylate is in phase III trials for the treatment of prostate, sarcoma, breast, NSCL, bladder, head and neck, and ovarian cancers. Its discovery and development have been reviewed,[57] and its clinical potential has also been reviewed.[58,59] An encouraging overall response rate of 15% was observed in a phase II trial in NSCLC patients, and the compound also showed promising results in treatment of breast cancer. One significant advantage over other tubulin-interactive agents is that eribulin appears to have a lower neurotoxicity than the other agents. It is concluded that "E7389 would probably be a very welcome addition to the available agents used to treat women with advanced breast cancer".[59]

**Dolastatins.** Dolastatin 10 (**16**) was isolated from the sea hare *Dolabella auricularia* by Pettit as part of an extensive series of investigations; it is the most potent member of a fairly large class of related compounds.[60,61] It binds to tubulin at a distinct site for peptide antimitotic agents near the exchangeable nucleotide and vinca alkaloid sites, inhibiting tubulin polymerization.[62] The relative simplicity of the structure and the difficulty of sourcing the natural product made chemical synthesis the preferred approach, and dolastatin 10 has been synthesized by the Pettit group[63] and others.[64] Dolastatin 10 entered phase I clinical trials in the 1990s, with the finding that 40% of patients developed moderate peripheral neuropathy.[65] It then progressed to phase II trials under the auspices of the National Cancer Institute (NCI) for the treatment of several solid tumors, including liver, bile duct, gallbladder cancer, pancreatic cancer, and advanced kidney cancer, but the results of these trials were not encouraging, as summarized in two recent reviews.[37,66]



**16**





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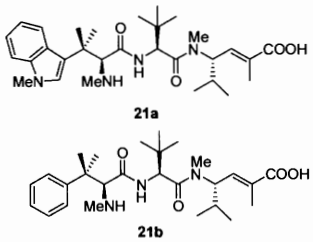
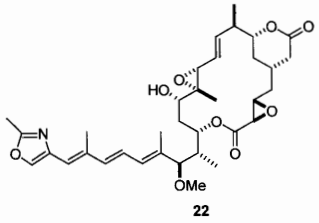
**Hemiasterlin.** Hemiasterlin (**21a**) is a tripeptide that was first isolated by Kashman from the sponge *Hemiasterella minor*, with reported activity against the P388 murine leukemia cell line.[77] It was later reisolated by Andersen, who reported that it had antitubulin activity, producing abnormal mitotic spindles at low concentrations and microtubule depolymerization at higher concentrations.[78] Synthetic studies identified the phenylalanine analogue HTI-286 (**21b**) as a more accessible and more potent analogue,[79] and both HTI-286[80] and hemiasterlin[8] are in clinical trials.[81]



21a



21b



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**Rhizoxin.** Rhizoxin (**22**) was isolated in 1984 together with several congeners from the plant pathogenic fungus *Rhizopus chinensis*.[82] It is an inhibitor of tubulin polymerization[83] and is more potent than maytansine against human and murine tumor cells.[84] It has been synthesized[85] and has been evaluated in clinical trials,[86] but has not yet entered clinical use.

## Promoters of Tubulin Polymerization

**The Taxanes.** Paclitaxel (**23**) was isolated by Wall and Wani in the 1960s from bark of the Pacific yew, *Taxus brevifolia*, and given the name taxol.[87] Its initial discovery was greeted with underwhelming enthusiasm, because of the obvious problems of compound supply and solubility, and because it showed only relatively modest in vivo activity against the then current antileukemic models at the NCI.[88] Fortunately, the B16 melanoma solid tumor assay was introduced in the early 1970s, and paclitaxel showed excellent and reproducible activity against this solid tumor, with an increase in life span (ILS) of 126%, 32%, and 86% in three separate experiments.[88] Even with these data the problems of developing paclitaxel loomed large, but fortunately the late Matthew Suffness, who had joined the NCI in 1976, recognized

assembly of microtubules;[89] this new mechanism was a crucia added factor in raising interest in this compound. The solubilit problem was overcome by the development of an emulsio formulation in Cremophor, a polyethoxylated castor oil, whic unfortunately caused allergic reactions in some patients. Thes problems were overcome by premedication with antihistamines an the use of extended intravenous infusions, which were initially fo 24 h but were later reduced to 3 h.[90]

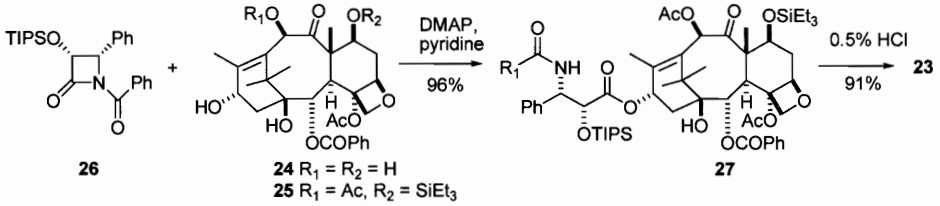
Paclitaxel, or taxol as it was then known, was found to hav clinical activity against ovarian cancer in 1989[91] and against brea cancer in 1991.[92] Further development was taken over by Bristo Myers Squibb (BMS) in 1991 under a Cooperative Research an Development Agreement (CRADA) with the NCI. BMS was ab to trademark the name Taxol for their formulation of the drug, an the generic name paclitaxel was applied to the chemical compoun formerly known as taxol. Taxol was approved by the FDA i December 1992 for the treatment of refractory breast cancer an refractory ovarian cancer and was launched on the U.S. market b Bristol-Myers Squibb the following year. It was later approved fo treatment of breast cancer after failure of combination chemotherap for metastatic disease, for second-line treatment of AIDS-relate Kaposi's sarcoma, and for NSCLC in combination with cisplatin. Extensive continuing clinical trials are evaluating combinations paclitaxel with other drugs for treatment of many other cancers.[94,

The enormous demand for paclitaxel caused by its excelle activity created a significant supply crisis, which was initially solve by aggressive collection of *T. brevifolia* bark. A viable semisynthet route was then developed. In this semisynthesis the more readi available 10-deacetylbaccatin III (**24**) is first converted to 7-triet ylsilylbaccatin III (**25**), and this is coupled with the protecte $\beta$-lactam **26** in the key step, yielding the protected paclitaxel **2** Final deprotection gives paclitaxel (**23**) in excellent overall yie (Scheme 1).[96] This synthetic route essentially ended the supp crisis. Paclitaxel is now also produced commercially by plant tiss culture methods.[97]

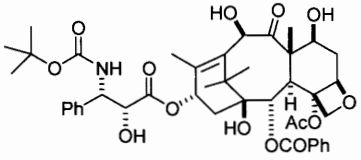
As noted above, paclitaxel acts by promoting the assembly tubulin into microtubules. This results in the inhibition of the norm dynamic reorganization of the microtubule network that is essenti for vital cellular functions and leads ultimately to cell death b apoptosis. The actual mechanism by which paclitaxel stabilizes t microtubule is still under investigation, but Xiao et al. specula that "Taxol induces a loss of flexibility in the involved regions th prevents a "roll out" of lateral contacts in microtubules that wou otherwise open up their wall".[98]

The success of paclitaxel has spurred enormous interest in findi improved analogues as well as improved formulations designed circumvent the problems associated with the Cremophor formul tion, and several analogues are currently in clinical trials. The on analogue approved for clinical use to date in the United States docetaxel (**28**), a semisynthetic analogue developed in France. Docetaxel was launched in 1995 and is the drug of choice in treati advanced NSCLC that is refractory to primary therapy.[100] It is al

**heme 1.** Semisynthesis of Paclitaxel



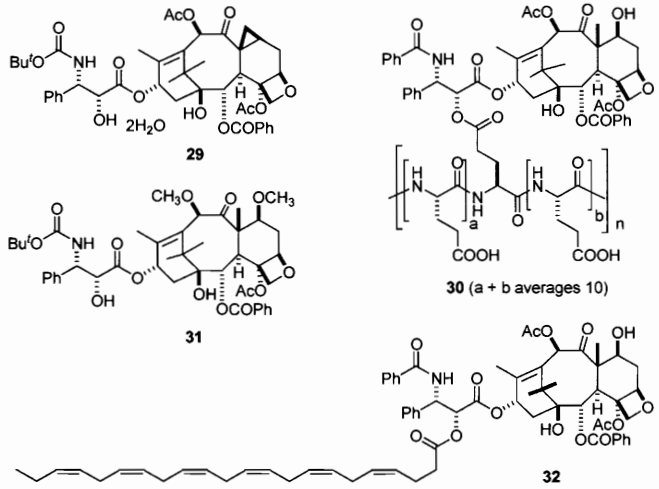

ed in the treatment of hormone refractory prostate cancer,[101,102] vanced breast cancer,[103] and other cancers including head and ck, stomach, and ovarian cancers.[104]




In addition to paclitaxel and docetaxel, the albumin nanoparticle ab) formulation of paclitaxel Abraxane was approved in 2005 in e United States for the treatment of advanced breast cancer. Since braxane consists only of albumin-bound paclitaxel nanoparticles, is free of Cremophor and requires no premedication. A recent view concludes "these studies have demonstrated that nab chnology has increased the therapeutic index of paclitaxel mpared with the conventional, solvent-based formulation",[105] hile another review states "Abraxane was safe, effective, induced gher response rate and longer time to progression compared with axol in patients with metastatic breast cancer".[106]

Although paclitaxel and docetaxel are the only taxanes currently proved for clinical use, there are many analogues in clinical trials, d the most important of these are summarized briefly below.

There are four taxanes in phase III clinical trials. Larotaxel hydrate (**29**, Sanofi-Aventis)[107] is an interesting derivative of cetaxel in which the methyl group at the C-8 position has formed cyclopropyl ring; the paclitaxel analogue of **29**, with a phenyl oup replacing the *tertiary*-butyloxy group in the side chain of **)**, was formed on treatment of 7-*epi*-paclitaxel with DAST.[108] arotaxel is in phase III trials for treatment of breast and pancreatic ncers, and a report from a phase II trial indicated that it has a vorable therapeutic index in women with taxane-pretreated etastatic breast cancer.[109] Paclitaxel poliglumex (**30**, Xyotax) is conjugate of paclitaxel with a biodegradable polyglutamic acid; is feature was designed to increase water solubility and improve s pharmacokinetic profile. It is in phase III trials by Cell herapeutics for the treatment of NSCLC and ovarian cancer.[110,111]




venting the P-glycoprotein drug efflux mechanism, and it is more potent than paclitaxel in paclitaxel-resistant tumors. Results of its phase I studies have been reported.[118,119] Ortataxel (**34**) was originally developed by Bayer, but the clinical trials encountered severe neutropenia[120] and were discontinued. The compound was then licensed exclusively to Spectrum Pharmaceuticals in 2007. It is currently in phase II clinical trials for the treatment of non-small cell lung cancer (NSCLC),[121] and it has also shown in vivo activity in animal models against head and neck squamous cell carcinoma (HNSCC).[122] Milataxel (**35**), which was developed by the new company Taxolog, is in phase II trials under Wyeth.[118] One recent report of a phase II study in colorectal cancer indicated that it had the side effect of neutropenic sepsis, necessitating close surveillance if the drug is to be used at its MTD.[123] Tesetaxel (**36**) was developed by Daiichi Sankyo Co. Ltd. for treatment of colorectal and gastric cancer. It was withdrawn from development in 2006 because of its failure to show clear benefit over existing agents,[124] but it was recently licensed by Genta, who hope to restart trials.[8] BMS-188797 (**37**) is a simple 4-carbonate derivative of paclitaxel that showed objective responses in four out of 16 patients in a phase I trial, including three complete remissions in ovarian and cervical cancer patients;[125] a phase I study of the drug in combination with

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