Drugs (2015) 75:1993–2016
DOI 10.1007/s40265-015-0489-4

REVIEW ARTICLE

Recent Advances in the Development of Antineoplastic Agents Derived from Natural Products

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Published online: 26 October 2015

Abstract Through years of evolutionary selection pressures, organisms have developed potent toxins that coincidentally have marked antineoplastic activity. These natural products have been vital for the development of multiagent treatment regimens currently employed in cancer chemotherapy, and are used in the treatment of a variety of malignancies. Therefore, this review catalogs recent advances in natural product-based drug discovery via the examination of mechanisms of action and available clinical data to highlight the utility of these novel compounds in the burgeoning age of precision medicine. The review also highlights the recent development of antibody-drug conjugates and other immunotoxins, which are capable of delivering highly cytotoxic agents previously deemed too toxic to elicit therapeutic benefit preferentially to neoplastic cells. Finally, the review examines natural products not currently used in the clinic that have novel mechanisms of action, and may serve to supplement current chemotherapeutic protocols.

Key Points

Natural products have potentiated many novel drug classes employed in cancer chemotherapy, including mechanistic target of rapamycin inhibitors, protein synthesis inhibitors, nucleic acid-directed agents, and microtubule-directed agents

Recent advances in immunotherapy have enabled highly cytotoxic natural products to be targeted towards specific tissues

There are still many natural products with mechanisms not currently seen in the clinical setting that could be very beneficial to the field of oncology

1 Introduction

The diversity of natural products currently used in the clinical setting to treat solid tumors, as well as disseminated cancers is truly extensive. Under the pressure of natural selection, various species produce cytotoxic secondary metabolites to combat potential predators, prey, or competition in the so-called "arms race" of evolution. Remarkably, some of these natural toxins appear to exhibit potent antineoplastic activity, and after years of research, have found their way from the ocean or soil to the highly heterogeneous environment of clinical oncology. The origins of cancer chemotherapy can be traced to human-made compounds, as Goodman, Gilman, and colleagues at Yale University began investigating the potential of nitrogen mustards in 1942 [1], which was shortly followed by

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Sidney Farber's use of the antifolate aminopterin to induce remissions among children with leukemia in 1947 [2, 3]. However, the institution of natural products and semisynthetic derivatives of these compounds in the latter part of the 20th century potentiated the idea of concomitant chemotherapy; using a variety of antineoplastic agents with different mechanisms of action to significantly perturb neoplastic development, and in some cases, produce long-term remissions.

Owing to recent advances in molecular biology, investigators have begun unraveling essential oncogenic pathways in carcinogenesis, potentiating an era of chemotherapy in which it is possible to theorize cancer-specific targets. This has launched the introduction of precision medicine in cancer chemotherapy in which clinicians now have the capability of selecting optimal therapies based on the genetic and phenotypic profile of the patient's malignancy in addition to traditional broad-spanning cytotoxic antineoplastic intervention. Despite these commendable advances in targeted therapy, natural products and their derivatives are still extensively relied upon against malignancies where finding cancer-specific targets has been less successful, and are often used in combination with these targeted approaches to generate more thorough treatment protocols. Further, novel natural product derivatives have shown notably efficacy against previously unresponsive malignancies at the clinical level, suggesting that natural product-based drug discovery still has considerable utility in the burgeoning era of personalized chemotherapy. Finally, natural products have the potential to improve novel immunotherapeutic strategies by conjugating monoclonal antibodies (mABs) or cytokines to highly cytotoxic compounds that have too low of a therapeutic index without an appropriate guidance mechanism.

This review catalogs recent advances in natural product drug discovery that have potentiated promising activity against aggressive malignancies, and have enabled a more precise delivery of highly cytotoxic, natural product-based agents to reduce unintended side effects. Specifically, this review covers the commendable advances in the development of microtubule-directed agents (eribulin and epothilones), mechanistic target of rapamycin (mTOR) inhibitors (everolimus and temsirolimus), protein synthesis inhibitors (omacetaxine mepesuccinate), nucleic acid-directed agents (trabectedin), engineered cytokine proteins (denileukin diftitox), and antibody-drug conjugates (ADCs; brentuximab vedotin, trastuzumab emtansine, calicheamicin conjugated monoclonal antibodies, and exotoxin conjugates). In addition, the review will highlight several novel natural products that act by mechanisms not currently seen in the clinic (cytochalasins and withanolides) to address their potential utility in cancer chemotherapy. Although this review provides an extensive coverage of novel natural product-based antineoplastic agents, additional agents have seen recent success in the clinical setting, and the reader is referred to the following reviews for further information [4–6]. In addition, the diversity of natural product-based antineoplastic agents and their derivatives currently approved by the US Food and Drug Administration (FDA) are highlighted in Table 1. They serve as a reminder of how important nature has been in the treatment of many, if not most types of malignancy.

2 Microtubule-Disrupting Eribulin

Eribulin is a fully synthetic, macrocyclic ketone analog of the marine sponge natural product halichondrin B (Fig. 1), a potent antimitotic initially isolated in 1986 from *Halichondria okadai* [7]. Although halicondrin B was designated for preclinical development after it was found to be highly cytotoxic against murine leukemia cells, difficulty in collecting sufficient material for developmental studies slowed its progress, and interest began to fade. However, the discovery that halocondrin B activity resides in the macrocyclic lactone C-1 to C-38 moiety [8] paved the way for development of a simplified synthetic analog, culminating in the design of eribulin.

As with vinca alkaloids, eribulin exerts its cytotoxic effects by interfering with microtubule dynamics, and inhibiting polymerization [9, 10]. In addition, eribulin also works through an end-poisoning mechanism, resulting in the inhibition of microtubule growth, and even sequesters tubulin into nonfunctional aggregates, promoting G_2/M phase arrest and apoptosis [11]. However, the two drug classes contrast in that eribulin does not bind the sides of tubulin polymers, and therefore does not markedly potentiate depolymerization [12, 13]. It does suppress spindle microtubule tension by interfering with centromere dynamics, as seen with some vinca alkaloids (particularly vinorelbine and vinflunine), but does so by inhibiting relaxation rates and the time spent stretching and relaxing, without the corresponding suppressive effects on stretching rates observed with the other agents [14]. Further, eribulin inhibits tubulin polymerization by binding the interdimer interface or the β-tubulin subunit alone contrary to other microtubule-directed agents, including epothilones and taxanes [12]. Interestingly, eribulin demonstrates significant activity against βIII-tubulin, an isotype that is overexpressed in cells resistant to microtubule inhibitors [15, 16], indicative of its unique clinical utility.

In regard to its clinical pharmacologic profile, eribulin demonstrates linear pharmacokinetics with rapid systemic distribution, but has a $t_{1/2}$ of 40 h and around 49–65 %

Table 1 US Food and Drug Administration (FDA) approved uses of natural products in cancer chemotherapy

Agent	Drug classification/species of origin	Mechanism of action	FDA approved use
Omacetaxine mepesuccinate (Synribo®)	Alkaloid ***Cephalotaxus harringtonia***	Inhibits protein synthesis and is independent of direct Bcr-Abl binding	Chronic- or accelerated-phase CML with resistance and/or intolerance to two or more TKIs
Daunorubicin (Cerubidine)	Anthracycline *Streptomyces peucetius*	Topo II inhibitor, intercalating agent	Remission induction in adult AML or in both children and adults for ALL
DaunoXome®		Citrate liposome formulation	Advanced HIV-associated Kaposi's sarcoma
Doxorubicin (Adriamycin®)	Anthracycline ***Streptomyces peucetius***	Topo II inhibitor, intercalating agent	ALL, AML, Wilms tumor, neuroblastoma, soft tissue and bone sarcoma, breast, ovarian, thyroid, bronchiogenic, gastric and transitional cell bladder carcinomas, HL, NHL
Doxil®		Liposome formulation	AIDS-related Kaposi's sarcoma, ovarian carcinoma, multiple myeloma
Epirubicin (Ellence®)	Anthracycline ***Streptomyces peucetius***	Topo II inhibitor, intercalating agent	Axillary node-positive breast carcinoma
Idarubicin (Idamycin®)	Anthracycline ***Streptomyces peucetius***	Topo II inhibitor, intercalating agent	Adults with AML classified M1 to M7 (French-American-British system)
Valrubicin (Valstar®)	Anthracycline ***Streptomyces peucetius***	Topo II inhibitor, intercalating agent	Carcinoma in situ of the urinary bladder
Mitoxantrone (Novantrone®)	Anthracycline ***Streptomyces peucetius***	Topo II inhibitor, intercalating agent	Adult AML, symptomatic hormone-refractory prostate adenocarcinoma
Brentuximab vedotin (Adcetris®)	Antibody-drug conjugate, dolastatin, MMAE is derived from peptides found in ***Dolabella auricularia***	MMAE enters cells expressing CD30, potentiating microtubule inhibition in addition to the antineoplastic effects of brentuximab	HL after failure of ASCT or after failure of two prior multiagent chemotherapeutic regimens in those who are not ASCT candidates, sALCL
Trastuzumab emtansine (Kadcyla®)	Antibody-drug conjugate, macrolide, DM1 is derived from maytansine, which can be extracted from plants of the genus ***Maytenus***	DM1 enters cells expressing HER2/neu receptor, potentiating microtubule inhibition in addition to the antineoplastic effects of trastuzumab	HER2+ breast carcinoma, metastatic gastric or gastroesophageal adenocarcinoma with HER2 overexpression
Mitomycin	Aziridine *Streptomyces caespitosus* or *Streptomyces lavendulae*	Alkylating agent, crosslinks DNA	Disseminated gastric adenocarcinoma, disseminated pancreactic adenocarcinoma
Irinotecan (Camptosar®)	Camptothecin ***Camptotheca acuminata***	Topo I inhibitor	Metastatic colorectal carcinoma
Topotecan (Hycamtin®)	Camptothecin ***Camptotheca acuminata***	Topo I inhibitor	Cervical carcinoma, metastatic ovarian carcinoma, SCLC
Denileukin diftitox (Ontak®)	Engineered cytokine protein *Corynebacterium diphtheriae*	Composed of diphtheria toxin fragments linked to IL-2 sequences, interacts with IL-2 cell surface receptors before inhibiting protein synthesis	Persistent or recurrent CTCL in patients who express the CD25 component of the IL-2 receptor
Asparaginase (Elspar®, Erwinase®)	Enzyme Elspar: *Escherichia coli* Erwinase: *Erwinia chrysanthemi*	Depletes asparagine, an amino acid required by some leukemias	Component of a multiagent induction regimen for ALL
Pegasparagase®		Pegylated version	Patient has hypersensitivity to asparaginase
Ixabepilone (Ixempra®)	Epothilone ***Sorangium cellulosum***	Stabilizes formed microtubules	Metastatic or locally advanced breast carcinoma after failure of an anthracycline and a taxane
Bleomycin (Blenoxane®)	Glycopeptide *Streptomyces verticillus*	Unresolved, but does induce DNA strand breaks	Squamous cell carcinomas, NHL, testicular cancers, HL, malignant pleural effusions
Etoposide (Vepesid®)	Lignan ***Podophyllum peltatum***	Topo II inhibitor	Testicular cancers, SCLC

Table 1 continued

Agent	Drug classification/species of origin	Mechanism of action	FDA approved use
Etoposide phosphate (Etophos®)		Ester derivative that increases water solubility	Testicular cancer, SCLC
Teniposide (Vumon®)	Lignan ***Podophyllum peltatum***	Topo II inhibitor	Refractory childhood ALL
Eribulin (Halaven®)	Macrolide ***Halichondria okadai***	Inhibits the growth phase of microtubules without affecting the shortening phase, sequesters tubulin into nonproductive aggregates	Metastatic breast carcinoma that has received at least two prior chemotherapy regimens for late-stage disease, including both anthracycline- and taxane-based chemotherapies
Everolimus (Afinitor®, Afinitor Disperz®)	mTOR inhibitor ***Streptomyces hygroscopicus***	Inhibits mTOR by binding FKBP-12	Postmenopausal women with advanced hormone receptor+ and ER− breast carcinoma, PNET, RCC, renal angiomyolipoma, pediatric and adult SEGA
Temsirolimus (Torisel®)	mTOR inhibitor ***Streptomyces hygroscopicus***	Inhibits mTOR by binding FKBP-12	Advanced RCC
Streptozotocin (Zanosar®)	Nitrosourea *Streptomyces achromogenes*	Alkylating agent	Metastatic islet cell carcinoma of the pancreas
Dactinomycin (Cosmegen®)	Polypeptide various species of the genus *Streptomyces*	Binds DNA at the transcription initiation complex and prevents elongation of RNA chain by RNA polymerase	Wilms tumor, pediatric rhabdomyosarcoma, Ewing's sarcoma, metastatic and nonseminomatous testicular cancer, gestational trophoblastic neoplasia, locally recurrent or locoregional solid malignancies
Paclitaxel (Taxol®)	Taxane *Taxus brevifolia*	Stabilizes formed microtubules	Ovarian carcinoma, breast carcinoma, NSCLC, AIDS-related Kaposi's sarcoma
Abraxane®		Protein bound, conjugated to albumin	Metastatic breast carcinoma, locally advanced or metastatic NSCLC
Docetaxel (Taxotere®)	Taxane ***Taxus brevifolia***	Stabilizes formed microtubules	NSCLC, breast carcinoma, prostate adenocarcinoma, gastric adenocarcinoma, head and neck carcinomas
Cabazitaxel (Jevtana®)	Taxane ***Taxus brevifolia***	Stabilizes formed microtubules	Hormone-refractory prostate adenocarcinoma
Vincristine (Oncovin®)	Vinca alkaloid *Catharanthus roseus*	Inhibits tubule polymerization	Acute leukemias, HL, NHL, neuroblastoma, Wilms tumor, rhabdomyosarcoma
Marqibo®		Liposome formulation	Philadelphia chromosome+ALL
Vinblastine (Velban®)	Vinca alkaloid *Catharanthus roseus*	Inhibits tubule polymerization	Testicular cancers, HL, NHL, mycosis fungoides, Kaposi's sarcoma, histiocytic lymphoma, Letterer-Siwe disease (histiocytosis X), breast carcinoma, choriocarcinoma
Vinorelbine (Navelbine®)	Vinca alkaloid ***Catharanthus roseus***	Inhibits tubulin polymerization	NSCLC

Underline indicates different formulations of the agent. Bold indicates that the compound is a synthetic or semisynthetic derivative of the original natural product

AIDS acquired human immunodeficiency syndrome, *ALL* acute lymphoid leukemia, *AML* acute myeloid leukemia, *ASCT* autologous stem cell transplant, *CD* cluster of differentiation, *DM1* mertansine, *CML* chronic myeloid leukemia, *CTCL* cutaneous T-cell lymphoma, *ER* estrogen receptor, *FKBP12* 12 kDa FK506 binding protein, *HIV* human immunodeficiency virus, *HL* Hodgkin's lymphoma, *IL-2* interleukin-2, *MMAE* monomethyl auristatin E, mTOR mechanistic target of rapamycin, *NHL* non-Hodgkin's lymphoma, *NSCLC* non-small-cell lung carcinoma, *PNET* neuroendocrine tumors of pancreatic origin, *RCC* renal cell carcinoma, *sALCL* systemic anaplastic large-cell lymphoma, *SEGA* subependymal giant-cell astrocytoma, *SCLC* small-cell lung carcinoma, *Topo* DNA topoisomerase, *TKI* tyrosine kinase inhibitor

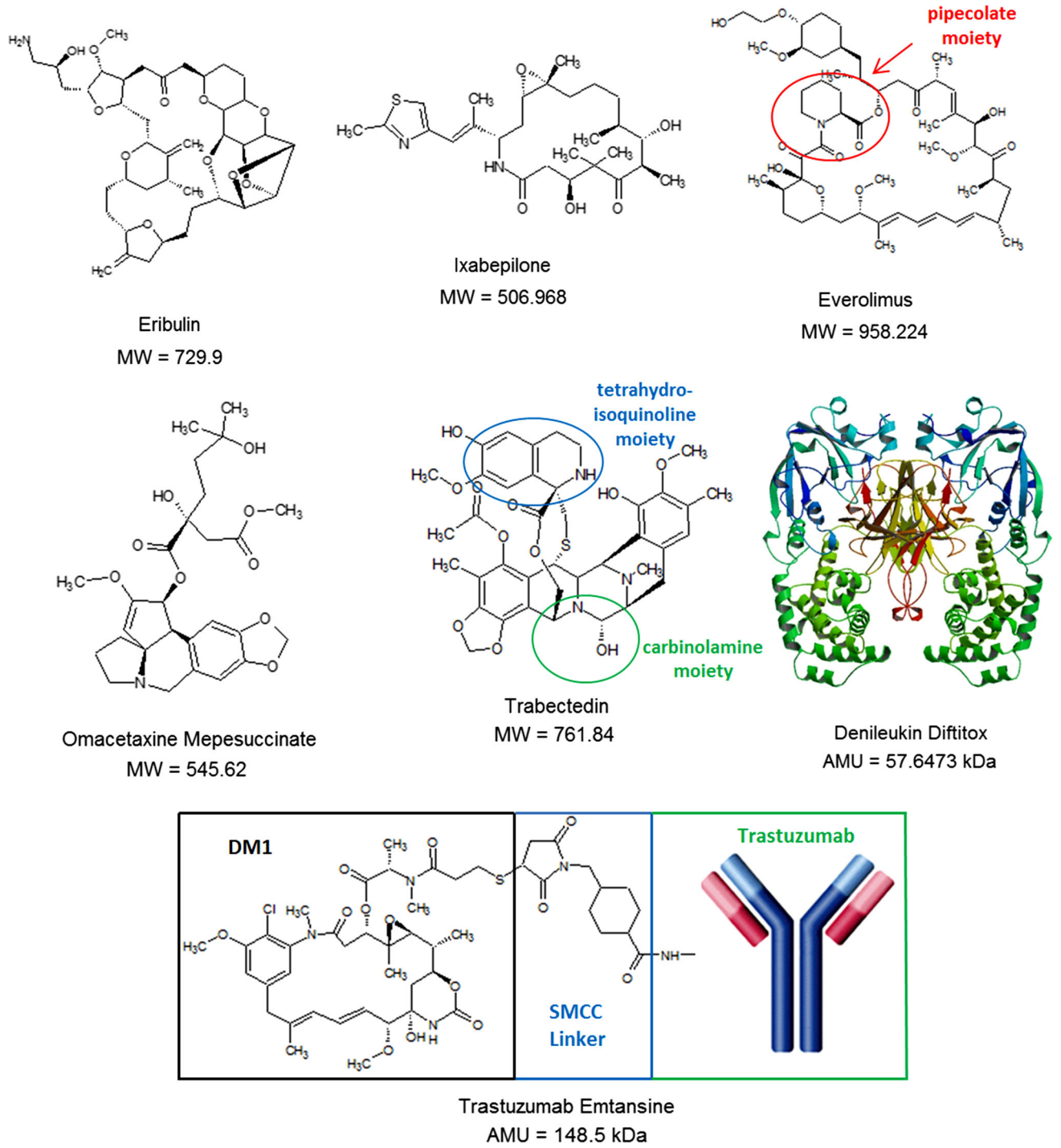

Fig. 1 Molecular diversity of antineoplastic agents derived from natural products. *AMU* atomic mass unit, *DM1* mertansine, *MW* molecular weight, *SMCC* succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate

remains protein bound in circulation [3, 17]. The majority of the agent is eliminated through bile emulsification and fecal excretion. Eribulin is manufactured at 0.5 mg/mL concentrations, and no routine premedication is needed. One of the most common dosing schedules is 1.4 mg/m^2 over 2–5 min on days 1 and 8 of a 21-day cycle, either undiluted or diluted in 100 mL of 0.9 % normal saline [17]. Although this schedule is typically well tolerated, eribulin is known to potentiate notable neutropenia (>grade 3 toxicity is ~57 %), elevate transaminases, and induce peripheral neuropathy (~8 % for grade 3 neuropathy and 0.4 % for grade 4 neuropathy).

Owing to the unique mechanisms by which eribulin inhibits microtubule dynamics, the agent is FDA approved for metastatic breast carcinoma refractory or relapsed on at least two prior treatment protocols for late-stage disease, including both anthracycline- and taxane-based chemotherapies [18]. This approval stems in large part from a phase III open-label study ($n = 762$) in which eribulin improved overall survival [median of 13.1 months, 95 % confidence interval (CI) 11.8–14.3] in comparison to treatments of the physicians' choice (median of 10.6 months, 9.3–12.5; hazard ratio = 0.81, 95 % CI 0.66–0.99; $p = 0.041$) [19]. Peripheral neuropathy was the most common adverse event leading to discontinuation from eribulin, occurring in 24 (5 %) of 503 patients. In addition, eribulin has also been investigated for use in a variety of other solid tumors, including non-small-cell lung carcinoma, head and neck carcinoma, pancreatic adenocarcinoma, prostate adenocarcinoma, and various sarcomas. Published data indicate that non-small-cell lung carcinoma patients receive some benefit from eribulin, but the agent does not appear to be effective in the treatment of head and neck or pancreatic malignancies [20]. In addition, eribulin appears to demonstrate activity against metastatic castration-resistant prostate adenocarcinoma and advanced soft-tissue sarcoma with a relatively favorable toxicity profile being observed [21, 22].

3 Microtuble-Stabilizing Epothilones

Epothilones are 16-member macrolide microtubule-stabilizing agents initially isolated in 1987 from the So ce90 strain of the myxobacterium *Sorangium cellulosum* [23, 24]. Originally investigated for their antimycotic activity, samples of epothilones A and B sent to the National Cancer Institute in 1994 demonstrated potent antineoplastic activity in multiple cancer cell lines that was comparable and sometimes superior to paclitaxel. However, a critical difference between epothilones and other bulky natural products or derivatives is that overexpression of ATP-binding cassette (ABC) transporters does not significantly alter the cytotoxicity of epothilones, as these congeners have minimal substrate affinity for these proteins [25]. Epothilones A and B have marked anti-proliferative activity in neoplastic cells with elevated levels of permeability glycoprotein (P-gp) [26, 27], and tumor samples obtained from patients that respond to ixabepilone have shown significantly elevated levels of ABC transporters P-gp and multidrug resistance-associated protein 1 [27]. It should be noted that multidrug-resistance protein 7 is capable of effluxing a variety of antineoplastic agents, including epothilone B [28]. Although drug resistance to taxanes is often associated with β-III subunit overexpression, epothilones appear to be equally as potent against cells demonstrating this phenotype [29, 30]. Whereas paclitaxel is less effective in suppressing the growth rate and catastrophe frequency of purified α/βIII tubulin, ixabepilone markedly suppresses the dynamic instability of α/βIII dimers [31]. Further, ixabepilone has exhibited substantially higher activity than either taxanes or vinca alkaloids against neoplastic cells in vitro and in vivo in which taxane resistance is associated with βIII overexpression [32–34]. In regards to its structure-activity relationship with tubulin, epothilones appear to bind the taxane pocket of β-tubulin and promote structuring of the M-loop into a short helix, as demonstrated by epothilone A [35]. Consequently, the M-loop establishes lateral tubulin contacts in microtubules, thereby potentiating microtubule assembly and stability.

Modifications to the macrolide ring have been shown to alter both the antineoplastic activity and pharmacologic properties of epothilones. Second- and third-generation congeners have been synthesized that possess higher potency and enhanced water solubility compared with the original natural products [32, 33]. Ixabepilone, a second-generation semisynthetic analog of epothilone B has nitrogen substituted at position 16 of the macrolide ring instead of oxygen, making it a lactam [34] (Fig. 1). This substitution increases water solubility and plasma stability in comparison to epothilones B and D, but also reduces cytotoxicity by one-fold [36]. In addition to ixabepilone, a second semisynthetic derivative of epothilone B, BMS-310705, a congener synthesized by the substitution of a hydroxyl group with an amino group at C-21 of the methylthiazole side chain, is 10-fold more water soluble than epothilone B and is more cytotoxic than epothilone D in multiple human neoplastic cell lines [37, 38]. Several other recent novel epothilone derivatives have gained notable preclinical and clinical interest. 20-Desmethyl-20 methylsulfanyl epothilone B (ABJ-879) is a second-generation derivative that exhibits more cytotoxicity than epothilone B [39]. Sagopilone (ZK-EPO) is the first fully synthetic, third-generation epothilone B derivative that exhibits greater potency in vitro relative to the other epothilones, retains activity in multidrug-resistant malignant cells not observed in other congeners, and even crosses the blood–brain barrier, indicating the potential for penetration into the central nervous system [40]. The preclinical data have been so compelling that sagopilone has been investigated at the clinical level against advanced solid tumors and melanoma, with the agent demonstrating favorable pharmacokinetic data, a feasible toxicity profile, and anti-tumor activity against melanoma not elicted by other congeners [41, 42]. Finally, a second-generation epothilone D analog, KOS-1584, exhibits 3- to 12-fold increased potency compared with epothilones B and D, enhanced

neoplastic tissue penetration, and reduced central nervous system toxicity [43].

Despite the isolation and characterization of epothilones A–F, the only agent of this class to reach FDA approval is ixabepilone. Although ixabepilone has better aqueous solubility than paclitaxel, Kolliphor EL is still typically used as an excipient, and is contraindicated in patients who are hypersensitive to the vehicle [44, 45]. Most clinical investigations of ixabepilone have used a dosing schedule of 32–50 mg/m^2 infused over 1 or 3 h on day 1 of a 21-day cycle. Nevertheless, a 3-h infusion time is recommended, as more prominent neurotoxicity is often observed with shorter infusion times, and the FDA-approved dose and schedule is 40 mg/m^2 intravenously (i.v.) over 3 h q3w [46]. Multiple phase I/II trials have revealed that ixabepilone exposure is not significantly affected by patient characteristics (age, sex, renal function, body weight, body surface area, race) [44, 47]. As with many mitotic inhibitors, the most common grade 3 or 4 toxicity associated with standard single-agent dosing schedules is neutropenia (grade 3 neutropenia is observed in the range of 10–33 %, while grade 4 neutropenia is observed in 7–32 % of patients, the wide range likely being a reflection of the number and type of prior therapies [46, 48–50].

Similar to eribulin, ixabepilone is currently indicated in metastatic or locally advanced breast carcinoma that is refractory or has relapsed on an anthracycline and a taxane, and is typically administered in combination with capecitabine [48, 50]. Interestingly, ixabepilone has shown notable efficacy against triple-negative breast carcinoma (TNBC), producing a pathologic complete response rate of 26 % in TNBC patients in comparison to 15 % in the non-triple-negative population [51]. Concomitant administration of ixabepilone and capecitabine has resulted in higher overall response rates than monotherapy, and a phase III trial of ixabepilone plus capecitabine produced a median progression-free survival significantly longer for TNBC patients treated with ixabepilone plus capecitabine (4.2 months) in comparison to treatment with capecitabine alone (1.7 months) [51]. In addition to breast carcinoma, ixabepilone has been investigated against non-Hodgkin's lymphoma and pancreatic adenocarcinoma, both as a stand-alone agent and in concomitant chemotherapy [52–54].

4 Mechanistic Target of Rapamycin Inhibitors

Although medicinal chemists have made progress in perturbing the PI3K/AKT/mTOR (phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B/mechanistic target of rapamycin) pathway through the development of synthetic compounds, the only mTOR inhibitors currently FDA approved for chemotherapeutic intervention are analogs of the macrolide rapamycin, a natural product initially isolated from *Streptomyces hygroscopicus* in 1975 within the soils of Easter Island (also referred to as Rapa Nui, giving rise to the name of the agent) [55–57]. Although currently used as an immunosuppressive agent, rapamycin has also demonstrated marked cytostatic activity against several cancer types [58]. Its unique pharmacokinetic profile has prevented rapamycin from being further developed as an antineoplastic agent, but its potent mechanisms of action have inspired the development of temsirolimus, a novel soluble rapamycin derivative that has a favorable toxicity profile in mammalian models, thereby potentiating the use of mTOR inhibitors in chemotherapy. As of now, only temsirolimus and the later developed analog everolimus have received FDA approval as antineoplastic agents.

Rapamycin is a complex 21-member macrolide lactone that contains a pipecolate moiety in the upper left region of the molecule. The agent inhibits mTOR primarily by crosslinking FKBP-12 (12 kDa FK506 binding protein) via its methoxy functional group [59, 60]. The high affinity of rapamycin to FKBP-12 is mediated in part by its pipecolate region, which hydrogen bonds at two different hydrophobic binding pockets, as revealed by X-ray crystallography [61–63]. Once bound, the rapamycin/FKBP-12 complex blocks the binding of the accessory protein RAPTOR (regulatory-associated protein of mTOR) to mTOR, necessary for downstream phosphorylation of S6K1 and 4EBP1. Consequently, S6K1 dephosphorylates, which reduces protein synthesis and decreases cell motility and size [64–66]. In addition, rapamycin induces dephosphorylation of 4EBP1. Such activity potentiates increases in p27, and decreases in cyclin D1 expression, invoking late blockage of G_1/S during the cell cycle [66]. Although it is clear that rapamycin induces apoptosis in neoplastic cells, the molecular mechanism of apoptosis has not yet been fully resolved.

Temsirolimus has been an important addition to chemotherapeutic protocols indicated for renal cell carcinoma (RCC) owing to the importance of mTOR in carcinogenesis; in RCC tumors, activated mTOR further exacerbates accumulation of HIF-1α (hypoxia-inducible factor 1-α) by increasing synthesis of this transcription factor and its angiogenic target gene products [67]. As such, rapamycin and its analogs have demonstrated notable antiangiogenic activity [68], indicating the potential of combining these congeners with agents that elicit similar effects on neoplastic vasculature, including bevacizumab, sorafenib, and sunitinib [69, 70]. In addition to its utility in RCC therapy, temsirolimus is being clinically evaluated against other carcinomas known to have elevated mTOR activity, including malignancies of the breast and lung [71, 72]. Everolimus (Fig. 1) is an orally (p.o.) administered 40-*O*-(2-hydroxyethyl) derivative of

rapamycin that is currently indicated for postmenopausal women with advanced hormone receptor positive and estrogen receptor negative breast carcinoma, as well as neuroendocrine tumors of pancreatic origin, RCC, renal angiomyolipoma, and both pediatric and adult subependymal giant cell astrocytoma [18]. The agent has also been clinically evaluated against gastric adenocarcinoma, hepatocellular carcinoma, and in multiple types of lymphoma [73–75].

5 Protein Synthesis Inhibitor Omacetaxine Mepesuccinate

Omacetaxine mepesuccinate (homoharringtonine) is a natural ester of the alkaloid cephalotaxine, a compound initially isolated and characterized in 1969 from *Cephalotaxus harringtonia* (Japanese plum yew) [76] (Fig. 1). Although cephalotaxine itself does not exhibit antineoplastic activity, fractionations of extracts obtained from several variants of *C. harringtonia* produced a series of cephalotaxine esters that demonstrated antineoplastic activity. One of these compounds, homoharringtonine (later renamed omacetaxine mepesuccinate), was shown to influence the progression of acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) in China during the 1970s, with later studies in the United States confirming these findings [77, 78]. However, the clinical development of omacetaxine mepesuccinate was halted after the development of imatinib, which has since shown remarkable activity in patients with Philadelphia chromosome positive CML and acute lymphoid leukemia (ALL). Nevertheless, reemergence in the investigation of omacetaxine mepesuccinate quickly resumed once it was realized that a subset of indicated leukemias are either refractory or develop resistance to imatinib or related tyrosine kinase inhibitors (TKIs), particularly T315I subtypes.

Omacetaxine mepesuccinate elicits its antineoplastic effects through inhibition of protein synthesis. Specifically, the agent prevents aminoacyl-tRNA from binding the ribosomal acceptor site, thereby preventing peptide bond formation at the early stage of protein elongation [79]. In addition, omacetaxine mepesuccinate inhibits the elongation phase of translation by preventing substrate binding to the acceptor site on the 60s ribosome subunit, leading to the blockade of aminoacyl-tRNA binding and peptide bond formation [77, 80]. Further, it has been demonstrated that the agent blocks protein synthesis by competing with the amino acid side chains of incoming aminoacyl-tRNAs for binding at the A-site of formed ribosomes [81]. Interestingly, omacetaxine mepesuccinate has shown notable activity against leukemic stem cells (LSCs) of CML origin with the agent having a similar inhibitory effect on BCR-ABL T315I-expressing LSCs in comparison to non-mutant BCR-ABL-expressing LSCs [82], indicating that the agent may be able to markedly inhibit clonal expansion in select CML patients.

Unlike most antineoplastic agents, omacetaxine mepesuccinate is administered subcutaneously (s.c.). with the standard regimen being 1.25 mg/m^2 s.c. b.i.d., days 1–14 every 28 days, with a maintenance treatment of 1.25 mg/m^2 s.c. b.i.d. for 7 days every 28 days [79]. Omacetaxine mepesuccinate disappears rapidly from plasma after cessation of infusion, with an observed α-$t_{1/2}$ of 5 h, a β-$t_{1/2}$ of 9.3 h, and a mean steady-state terminal $t_{1/2}$ of 7 h with biexponential decay observed. The agent undergoes a rapid metabolism with urinary excretion representing about 12–15 % of the administered dose. In addition, omacetaxine mepessucinate has a favorable toxicity profile with effects on liver and cardiovascular function being the most prominent [79–81, 83]. Owing to its efficacy in TKI-resistant cells, omacetaxine mepesuccinate is FDA approved for both chronic- and blast-phase CML [18], and clinical trials are currently ongoing to determine optimal agents to use in combination with the protein synthesis inhibitor. Interestingly, not as much interest has been paid towards the potential of omacetaxine mepesuccinate in Philadelphia chromosome-positive ALL that is unresponsive to TKI therapy, and may be an avenue of future clinical interest.

6 Nucleic Acid-Directed Trabectedin

Trabectedin is a novel antineoplastic agent that was isolated from the sea squirt *Ecteinascidia turbinate* in 1984 [84, 85], and has a relatively complex structure; three tetrahydroisoquinoline moieties, eight rings including one 10-membered heterocyclic ring containing a cysteine residue, and seven chiral centers (Fig. 1). While the *E. turbinate* extract that trabectedin is derived from was shown to have antineoplastic activity as early as 1969 [84], separation and characterization of the active molecules was not feasible until the development of sufficiently sensitive techniques. Further delaying its development was the fact that yields from *E. turbinate* are extremely low; it takes 1000 kg of animals to isolate 1 g of trabectedin. It was not until the development of synthetic methods of preparation that actual clinical investigation was feasible. The current supply of trabectedin is based on a semisynthetic process starting from Safracin B, an antibiotic obtained by fermentation of the bacterium *Pseudomonas fluorescens* [85].

Trabectedin is extremely potent, requiring only picomolar to low nanomolar concentrations to initiate cell death in various cancer types in vitro [86–88]. This notable cytotoxicity is attributed to at least two separate actions; DNA alkylation and inhibition of transcription. The agent

binds the minor groove of DNA, showing a preference for GG- and GC-rich regions, and then alkylates the exocyclic N-2 on guanine [89, 90]. This alkylation step is dependent on the dehydration of the carbinolamine (also referred to as hemiaminal) group found on trabectedin, as this potentiates the formation of an electrophilic iminium intermediate that attracts nucleophilic DNA bases. Two subunits of trabectedin form the primary contacts with DNA, while another subunit protrudes out of the minor groove [91]. It is this subunit that has been associated with the inhibition of transcription, as interaction between the subunit and transcription factors has been observed. Alkylation of DNA produces the standard monoalkylating single-strand breaks that proceed to double-strand breaks when the adduct is recognized by the transcription-coupled nucleotide excision repair (TC-NER) complex [92, 93]. These in vitro observations suggest that patients with BRCA mutations may receive benefit from trabectedin, as BRCA1 locates DNA damage and attracts the TC-NER complex to repair DNA breaks, while BRCA2 mediates homologous recombination by loading other proteins to the double-strand break sites and stalled DNA replication forks [94, 95]. This postulation has been confirmed in select patients with ovarian carcinoma [93], making trabectedin particularly attractive for certain subtypes of breast, lung, and ovarian carcinoma with BRCA mutations.

Separating trabectedin from other alkylating agents is its ability to inhibit the expression of potentially oncogenic transcription factors, including those that code for oncogene products, c-myb, maf, and myc) and cell-cycle related factors (E2F and SRF), and general transcription factors (SCR, NF-Y, SXR, and Sp1) [96]. Its inhibition of P-gp and heat shock protein 70 (Hsp70) expression through NF-Y (as well as SXR, which is also responsible for P-gp transcription) interaction are of particular intrigue [97, 98], as both are integral to neoplastic drug resistance. Further, trabectedin has profound activity on the tumor microenvironment, exerting effects on quiescent tumor-promoting monocytes and macrophages that is atypical of a DNA-directed alkylating agent [96].

Trabectedin has linear pharmacokinetics when administered as a 24-h i.v. infusion as is seen in the standard dosing schedule of 1.5 mg/m^2 over 24 h every 3 weeks [99]. In addition, trabectedin is extensively bound to human serum albumin, and the concentration of unbound trabectedin in the plasma at clinically relevant trabectedin plasma concentrations is in the picomolar range. Drug metabolism presides mainly in the liver, with a large number of metabolites being produced by cytochrome P450 isozyme 3A4 and to a lesser extent by other cytochrome P450 isozymes and phase II enzymes [99].

Trabectedin is approved in Europe (including Russia) and South Korea for the treatment of advanced soft-tissue sarcoma. This clinical indication has been validated by multicenter phase II trials and a number of noncomparative phase II trials [99, 100]. In addition, the European Commission and the FDA have granted orphan drug status to trabectedin for soft-tissue sarcomas and ovarian carcinoma. The effects of trabectedin against ovarian carcinoma are particularly intriguing; coadministration of trabectedin and pegylated liposomal doxorubicin was associated with a significantly longer (6 weeks; $p = 0.019$) median progression-free survival than pegylated liposomal doxorubicin monotherapy in patients with recurrent ovarian carcinoma after progression on platinum-based chemotherapy [101]. In addition, concomitant administration of trabectedin and pegylated liposomal doxorubicin was associated with a relative risk reduction (RRR) in disease progression or death of 21 % (HR 0.79; 95 % CI 0.65, 0.96) in comparison to the doxorubicin monotherapy. These findings are encouraging due to the fact that ovarian carcinomas usually portend a poor prognosis (overall 5-year survival rate is 44 %, while stage IV invasive ovarian carcinoma has a 5-year survival rate of 17 %) [102]. The dearth of available treatment options for these patients is apparent, warranting further investigation of trabectedin and other novel therapeutic strategies. The agent has also been investigated in breast carcinoma, lung carcinoma, prostate adenocarcinoma [103–105], and various pediatric sarcomas (Ewing's sarcoma, and rhabdomyosarcoma) [106].

7 Finding Novel Utility in Natural Products through Improved Drug Delivery

The natural products discussed so far are potent antineoplastic agents, but are all inherently limited by their untargeted cytotoxic mechanisms. Although malignant cells are preferentially damaged as a result of increased proliferation rates and increased uptake of the given agent, normal tissue is also perturbed, preventing higher concentrations that could elicit more antitumor activity from being administered. Further, there are natural products too potent for clinical use because their activity is not specific enough for neoplastic cells to garner any therapeutic benefit. Finding a delivery system that discriminates between neoplastic and normal cells, and then transports a cytotoxic agent across the plasma membrane of aberrant cells to induce apoptosis would therefore be an ideal situation. As it turns out, the idea of preferentially delivering highly cytotoxic natural products to neoplastic tissue has made commendable progress in recent years owing to advances in immunotherapy. By using epitopes highly expressed on the cell surface of malignant cells, investigators have been able to develop methods capable of transporting a drug

payload to the intended target, substantially reducing the toxicity of these agents had they not been conjugated to the delivery protein. This targeted drug delivery can be accomplished through two mechanisms; engineered cytokine proteins and ADCs.

7.1 Engineered Cytokine Proteins

Cytokines are small proteins (~5–20 kDa) important for various paracrine and autocrine signaling throughout the body, but are most associated with their role in the immune system. These proteins enable leukocytes to communicate with one another to generate a coordinated, robust, but self-limited response to a target antigen [107, 108]. Cytokines themselves have been used for years in chemotherapy because of their ability to directly stimulate immune effector cells and stromal cells at the tumor site, and enhance neoplastic cell recognition by cytotoxic effector cells [108–110]. Interleukin-2 (IL-2) is particularly appealing in hematological malignancies because the IL-2 receptor (IL-2R) is selectively expressed on activated T lymphocytes, B cells, and natural killer cells [111]. In a normal patient, IL-2R is expressed mostly at a low level in less than 5 % of normal circulating peripheral blood mononuclear cells [112]. However, many transformed leukocytes have a high expression of high or intermediate affinity IL-2R isoforms, particularly in cutaneous T-cell lymphoma (CTCL) in which approximately 50 % of cases express this phenotype as demonstrated by immunohistochemical staining [112, 113]. Therefore, fusing a highly cytotoxic agent to IL-2 may be an effective method by which to target CTCL, while eliciting minimal toxicity to non-hematological tissue.

7.2 Denileukin Diftitox

Denileukin diftitox is a fusion protein in which the receptor binding domain of diphtheria toxin is exchanged for that of IL-2 (Fig. 1) [114, 115]. Diphtheria toxin is produced naturally in the pathogen *Corynebacterium diphtheriae*, and is a single polypeptide chain of 535 amino acids consisting of two subunits linked by disulfide bonds, known as an A–B toxin [116, 117]. The less stable B unit binds the cell surface of a target cell, enabling the more stable A subunit to penetrate the plasma membrane. Once internalized, the diphtheria toxin catalyzes the transfer of NAD^+ to a diphthamide residue in eukaryotic elongation factor-2 (eEF2) through ADP-ribosylation, resulting in its deactivation (the same function of endogenous NAD^+-diphthamide ADP-ribosyltransferase) [118]. In essence, this action halts translation, as eEF2 facilitates the movement of the peptidyl tRNA-mRNA complex from the A site of the ribosome to the P site during protein synthesis; thereby potently inhibiting the production of new proteins. Consequently, diphtheria toxin is extraordinarily potent with the average human lethal dose being ~0.1 μg/kg [118].

Through human innovation, one of the deadliest toxins in nature can be harnessed for cancer therapy once it is given the appropriate delivery system. Denileukin diftitox contains the full-length sequence of IL-2, as well as protein fragments of diphtheria toxin; 97 amino acids from the native part of the toxin containing the disulfide bond are removed to increase the half-life and affinity of the compound to its target receptor [119, 120]. After diphtheria toxin and IL-2 are fused together, the agent seeks out CD25-bearing cells, as CD25 represents the high-affinity α-subunit of the IL-2 receptor. CTCL cells internalize the agent via receptor-mediated endocytosis after it binds IL-2R, and is subsequently acidified inside the vesicle. This process releases diphtheria toxin fragment A into the cytoplasm, enabling the internalized fragment A to catalyze the transfer of ADP to eEF2. Premature ADP-ribosylation of eEF-2 inhibits further protein synthesis, ultimately potentiating apoptosis [121, 122].

There are limitations to the delivery mechanism by which denileukin diftitox acts. In addition to the expected immunosuppression and potential for bacterial infections, the agent is known to cause acute hypersensitivity-type reactions, asthenia, and nausea/vomiting [123]. Further, the use of IL-2 as a method to selectively transfer diphtheria toxin into CTCL cells is inherently limited. IL-2Rs are classified into three subtypes based on their affinity for IL-2. Each subtype is composed of a combination of subunits: α (CD25 and p 55), β (CD122 and p 75), and γ (CD132 and p 64). Intermediate and high-affinity receptors are composed of IL-2R-β/γ (CD122 and CD132) and IL-2R-α/β/γ (CD25, CD122, and CD133) subunits, respectively, and both perpetuate internalization and signal transduction. By contrast, low-affinity receptors consist of the IL-2R-α/γ (CD25 and CD132) subunits that bind IL-2, but do not cause internalization or activation [112, 124, 125]. Therefore, the presence of the β and γ (CD122 and CD132) subunits is essential for sensitivity and internalization of the fusion toxin. If CTCL cells are lacking appropriate receptors, denileukin diftitox will be ineffective, paving the way for potential drug resistance.

Nevertheless, denileukin diftitox has elicited notable responses in patients, and the agent is currently FDA approved for persistent or recurrent CTCL in patients expressing the critical CD25 component of IL-2R [18]. Because the expression of IL-2R is vital for the T-cell targeting of denileukin diftitox, strategies to upregulate IL-2R expression are of particular clinical interest. Retinoids are a particularly attractive concomitant agent, as they have immunomodulatory function and have been shown to

increase IL-2R expression on T-cells [126, 127]. It has been demonstrated that both bexarotene and alitretinoin are modulators of high affinity IL-2R expression, as the agents increase the expression of CD25 and CD75 in CTCL cells. These CTCL cells can be subsequently exposed to denileukin diftitox, resulting in a 50–70 % decrease in protein synthesis [126]. This is particularly exciting because bexarotene is already standard therapy for patients with early- and advanced-stage CTCL. The concomitant use of denileukin diftitox and retinoids is currently being clinically investigated [128, 129], and may potentiate a novel avenue of chemotherapy for CTCL patients.

7.3 Antibody-Drug Conjugates

Immunoconjugates are a relatively novel class of chemotherapeutic agents that are gaining increasing interest because of their efficacy and reduced side effects in comparison to some traditional cytotoxic agents. If traditional chemotherapy is equivalated to indiscriminate bombing, immunoconjugates are roughly analogous to modern day missile guidance systems. These agents consist of three main components; the monoclonal antibody (mAb; guidance system), the degradable linker (delivery mechanism), and the cytotoxic agent (warhead) that can either be a toxin or a radioisotope. While not completely specific to neoplastic tissue, immunoconjugates target a given epitope based on the mAb that is chosen, enabling the use of cytotoxic agents that would likely elicit too broad of a toxicity profile in a typical patient. Although the scope of this review limits commentary to ADCs, radioimmunotherapy has shown efficacy in a variety of cancers, and the reader would benefit from the following reviews [130–132].

7.4 Brentuximab Vedotin

Brentuximab vedotin is a conjugate of a humanized CD30-targeting mAB and monomethyl auristatin E (MMAE) linked via a cathepsin cleavable linker (valine-citrulline) and a para-aminobenzylcarbamate (PABC) spacer (the name vedotin refers to MMAE plus its linking structure to the mAB) [133–136]. MMAE is a potent mitotic inhibitor derived from the naturally occurring dolastatin 10 that was isolated from *Dolabella auricularia* (wedge sea hare) in 1987 [137]. Several related molecules have since been produced through total syntheses, establishing the drug class auristatin [138]. As with vinca alkaloids, the auristatins exert antineoplastic activity by inhibiting tubulin polymerization. However. these agents are much more toxic (MMAE is 200 times more potent than vinblastine) [139], substantially limiting their clinical utility. MMAE was subsequently developed with a built-in functionality for stable linker attachment, and still retains high potency in addition to exhibiting water solubility and stability under physiological conditions [133]. The ability to conjugate MMAE to mABs and potentially other proteins has considerably increased the utility of auristatins, enabling these once intolerable agents to be directed towards the intended target, thereby dramatically reducing unintended toxicity.

CD30 is an ideal target for ADCs because the antigen is a tumor necrosis factor receptor that stimulates apoptosis, and is highly expressed in Hodgkin's lymphoma, systemic anaplastic large cell lymphoma, CTCL, and other selected lymphoid tumors [140, 141], as well as in some non-lymphoid malignancies including germ cell tumors [141, 142]. Further, cross-reactivity of CD30 on normal tissues is very low, with some expression on activated T and B lymphocytes; this expression is not observed on resting T and B lymphocytes [141]. In addition to the CD30-targeting mAB, brentuximab vedotin employs a protease-cleavable linker because protease activity is abundant in lysosomes, where ADCs are often directed [133]. In addition, protease activity is significantly reduced outside of cells owing to secreted protease inhibitors [141]. MMAE is attached to suitable degradable peptides through the built-in *N*-terminal amine functionality via the self-immolative spacer (PABC). The spacer is required so that the cleavable peptide is situated away from MMAE to allow facile proteolysis. Val-Cit (the clevable peptide) is stable in plasma, but is rapidly hydrolyzed via proteolysis by cathepsin in lysosomes [143]. Upon peptide cleavage, the PABC group rapidly fragments, releasing the highly cytotoxic MMAE into the cytoplasm.

Brentuximab vedotin is currently indicated for Hodgkin's lymphoma after failure of an autologous stem cell transplant or after failure of two prior multiagent chemotherapeutic regimens in those who are not suitable for autologous stem cell transplantation, as well as systemic anaplastic large cell lymphoma. Due to the prevalence of CD30 in various malignancies, brentuximab vedotin is also being investigated in patients with CTCL, other CD30-positive hematological malignancies, and CD30-positive germ cell tumors [144].

7.5 Trastuzumab Emtansine

Trastuzumab emtansine is an ADC consisting of the humanized HER2/Neu-targeting mAB trastuzumab linked to mertansine (DM1) via SMCC (succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate), a heterobifunctional crosslinker (Fig. 1) [145, 146]. While trastuzumab is already effective against HER2-positive breast carcinomas, esophageal carcinomas, and gastric adenocarcinomas [147, 148], conjugating the mAB with the highly cytotoxic DM1 is an effective method to generate activity

in refractory or relapsed tumors [149–151]. DM1 refers to mertansine, a derivative of maytansine that has been chemically modified to have a terminal thiol for conjugation. Maytansine is a macrolide of the ansamycin type (contains an aromatic moiety bridged by an aliphatic chain) that was originally isolated in 1972 from the flowering plant *Maytenus ovatus* [152]. Similar to MMAE, maytansine is a highly potent microtubule inhibitor that by itself lacks the tumor specificity required to elicit therapeutic benefit [153, 154]. It was not until the development of ADCs that the extremely cytotoxic potential of maytansine could be harnessed in the form of conjugate-compatible DM1.

Trastuzumab is a logical choice for ADC enhancement because its target HER2/Neu is preferentially overexpressed in certain subtypes of breast carcinoma, esophageal carcinoma, and gastric adenocarcinoma. In particular, gain- and loss-of-function experiments, as well as immunohistochemistry analyses have indicated HER2 amplification as a driving event in the onset and progression of as much as 25–30 % of breast carcinomas [155, 156]. Further, this subtype of breast carcinoma is often very aggressive and resistant to traditional cytotoxic chemotherapy [157, 158]. Due to its apparent oncogene addiction, targeting HER2 positive breast carcinomas through this vital receptor has become an attractive approach for therapeutic intervention. Each molecule of trastuzumab emtansine consists of a single trastuzumab mAB with several molecules of DM1 attached; trastuzumab may be conjugated with up to 8 DM1 molecules (three to four on average) [159, 160]. SMCC contains two reactive functional groups, a succinimide ester and a maleimide. The succinimide group of SMCC reacts with the free amino group of a lysine residue in the trastuzumab molecule, and the maleimide moiety of SMCC links to the free sulfhydryl group of DM1, forming a covalent bond between the mAB and DM1 [160].

After binding HER2, trastuzumab emtansine gains entry to the cellular interior via receptor-mediated endocytosis [161]. Since the non-reducible SMCC linker is stable in circulation, as well as in the tumor microenvironment, DM1 release occurs only as a result of proteolytic degradation of the mAB part of trastuzumab emtansine in the lysosome. Following release from the lysosome, DM1-containing metabolites potently inhibit microtubule assembly, causing cell-cycle arrest at the G_2/M checkpoint, eventually triggering apoptotic mechanisms [162, 163]. Importantly, linkage of DM1 to trastuzumab does not affect the binding affinity of trastuzumab to HER2, nor does it reduce the inherent antineoplastic effects of trastuzumab [162, 164, 165]. Consequently, trastuzumab emtansine benefits from the mechanisms of both trastuzumab and DM1.

Trastuzumab emtansine is currently FDA approved for HER2-positive breast carcinoma, HER2-positive metastatic gastric adenocarcinomas, and gastroesophageal adenocarcinoma with HER2 overexpression [18]. In the USA, trastuzumab emtansine is typically reserved for the treatment of HER2-positive metastatic breast carcinoma in patients who have been treated previously with trastuzumab and a taxane (paclitaxel or docetaxel), and who have already been treated for metastatic breast cancer or developed tumor recurrence within 6 months of adjuvant therapy [166]. This is due in part to the expense of trastuzumab emtansine treatments, as protocols can cost patients US $9800 a month, or US $94,000 for a typical course of treatment. Since trastuzumab and pertuzumab (another HER2/Neu-targeting mAB) bind different epitopes [167, 168], clinical investigation has also been extended to concomitant chemotherapy consisting of both trastuzumab emtansine and pertuzumab against unresectable HER2-positive breast carcinoma that is locally advanced or metastatic [169].

7.6 Calicheamicin Conjugated Monoclonal Antibodies

The cytotoxic agents available for mAB conjugation are not limited to highly potent microtubule inhibitors. Perhaps the most notable example is calicheamicin conjugated mABs. Calicheamicins are a class of enediyne nucleic acid-directed agents that were initially characterized in 1989 from the fermentation broth of the bacterium *Micromonospora echinospora* [170]. Since then, calicheamicin γ1 and the related enediyne esperamicin have become known as two of the most potent antineoplastic agents in existence [171]. As is the case with most extremely cytotoxic agents, their utility would come not as stand-alone agents, but rather as components of epitope-targeting ADCs. The initial attempt to harness the potency of calicheamicins was with gemtuzumab ozogamicin, a humanized CD33-targeting mAB conjugated to a carbohydrate conjugate of *N*-acetyl-γ calicheamicin dimethyl hydrazide via a bifunctional AcBut linker (4-(4′-acetylphenoxy)butanoic acid) [172, 173]. Gemtuzumab ozogamicin was the first FDA-approved ADC, being approved for the treatment of AML in 2000. Unfortunately, this initial attempt also elicited marked unintended toxicity (most notably the potentiation of sinusoidal obstruction syndrome) [174–176], and was withdrawn from the market in 2010 after a randomized, phase III comparative controlled trial demonstrated that the agent increased patient death and added no benefit over conventional cancer therapies [177]. Nevertheless, an additional calicheamicin conjugated mAb has since been developed (inotuzumab

ozogamicin), and calicheamicin congeners are still being investigated for their antineoplastic potential.

The extreme potency of calicheamicins can be traced back to their unique mechanism of action. All calicheamicins appear to interact with cellular DNA and initiate double-strand cleavage by carbon-centered diradical hydrogen abstraction processes [178, 179]. Calicheamicins bind DNA in the minor groove, wherein they undergo a reaction analogous to a Bergman cyclization to generate a diradical species. This diradical, 1,4-didehydrobenzene, then abstracts hydrogen atoms from the deoxyribose backbone of DNA, ultimately resulting in irreversible strand scission [180]. The affinity of calicheamicin γ1 for the minor groove of DNA is due its aryltetrasaccharide domain [181, 182]. Specifically, calicheamicin γ1 binds the DNA minor groove with its aryltetrasaccharide domain in an extended conformation spanning TCCT/AGGA segments of DNA. Calicheamicin γ1 then inserts itself in an edgewise manner deep into the minor groove with the molecule wedged between the walls of the groove [182]. A range of intermolecular hydrophobic and hydrogen-bonding interactions are also observed, accounting for the sequence-specific recognition in the complex.

In addition to gemtuzumab ozogamicin, another calicheamicin-conjugated humanized mAB, inotuzumab ozogamicin has reached phase III clinical trials. Rather than targeting CD30, inotuzumab ozogamicin consists of a humanized mAb that recognizes the CD22 antigen. Nevertheless, this mAB is still conjugated to *N*-acetyl-γ-calicheamicin dimethyl hydrazide via the acid labile 4-(4′-acetylphenoxy)butanoic acid linker [183]. Since CD22 is a B-lymphocyte-restricted phosphoglycoprotein [184], inotuzumab ozogamicin has been clinically examined in B-cell leukemias and lymphomas with reversible thrombocytopenia as the main toxicity observed. [185, 186]. It should be noted that CD22 may be expressed in certain lung carcinomas, but this is still inconclusive, as the data have been conflicting [187, 188]. The ADC has shown notable efficacy against pediatric and adult ALL resistant to traditional cytotoxic chemotherapy [186, 189], and is indicative of the potential ADCs have in previously unresponsive cancers.

7.7 Exotoxin Conjugates

Antibody/toxin conjugates are not limited to small-molecule cytotoxic agents. In a manner similar to denileukin diftitox, mABs have now been successfully conjugated to large protein bacterial exotoxins, thereby increasing the diversity of available immunotoxins. The two most well-known examples are moxetumomab pasudotox and SS1P.

Moxetumomab pasudotox is a recombinant immunotoxin composed of the Fv fragment of a fully human CD22-targeting mAB fused to a 38-kDa fragment of Pseudomonas exotoxin A [190]. Moxetumomab pasudotox is actually an improved, more active form of a predecessor recombinant immunotoxin, BL22 (also called CAT-3888), which produced complete remission in relapsed/refractory hairy cell leukemia (HCL), but had a <20 % response rate in chronic lymphoid leukemia (CLL) and ALL, which are noted for containing much lower numbers of CD22 [191, 192]. Compared with BL22, moxetumomab pasudotox is up to 50-fold more active against CLL and HCL [193].

Pseudomonas exotoxin is a highly cytotoxic product of the pathogenic bacterium *Pseudomonas aeruginosa* [194]. With a mechanism very similar to diphtheria toxin, the exotoxin potently inhibits eEF2 through ADP-ribosylation, thereby ceasing further elongation of polypeptides. Crystallographic studies have demonstrated that *Pseudomonas* exotoxin is made up of three major structural domains; domain Ia is the cell binding domain, domain Ib contains no known function, domain II contains a furin site necessary to release domain III from the cell binding domain, and domain III contains the ADP-ribosylating activity that inactivates eEF2 [194].

In recombinant immunotoxins targeting CD22 (BL22 and moxetumomab pasudotox), domain Ia of PE is removed and replaced by the Fv portion of a mAB reacting with CD22 [190]. Because BL22 was limited by the lower expression of CD22 in CLL and ALL, the mAB portion of the agent was improved. By mutating three residues in the heavy chain of the BL22 Fv (residues 100, 100a, and 100b), investigators were able to increase the affinity of the mAB to HCL by about 15-fold and cytotoxicity toward HCL and CLL cells by up to 50-fold [193]. This improvement in binding affinity and increased cytotoxicity did not interfere with pharmacokinetics or off-target toxicity, indicative of superior antineoplastic potential. Moxetumomab pasudotox has produced complete responses against CD22 positive leukemias in the clinical setting, demonstrating particular promise with relapsed and refractory HCL [195]. Phase III evaluations are currently underway to fully assess the efficacy of moxetumomab pasudotox in comparison to more established therapeutic approaches.

In addition to its potential with CD22-targeting mAB Fvs, the pseudomonas exotoxin has also been fused with an anti-mesothelin Fv to create SS1P. Mesothelin is a 40-kDa cell surface glycoprotein present on some normal mesothelial cells lining the pleura, peritoneum, and pericardium [196, 197]. However, mesothelin is also highly expressed in several malignancies, including epithelial mesotheliomas (~100 % of cases), lung carcinomas (~50 % of cases), ovarian carcinomas (~70 % of cases), and pancreatic/biliary adenocarcinomas (~100 % of cases) [198–201], thereby being an epitope of particular

antineoplastic interest. SS1P acts by the same mechanism as moxetumomab pasudotox and is very similar in structure, but is specific for mesothelin-positive cells. This epitomizes the utility of immunoconjugates, as the same highly toxic small molecule or protein can be directed towards different targets once linked to an appropriate effector. Demonstrating potent antitumor activity in preclinical in vivo models [202–204], SS1P has since progressed towards clinical examination. Owing to its response in mesothelioma patients during phase I trials [205–207], SS1P has gained particular attention in the treatment of this malignancy. Nevertheless, SS1P has also been clinically investigated in other cancers with high mesothelin expression, and the agent may gain traction as a multipurpose immunotoxin.

8 Natural Products with Mechanisms Not Currently Seen in the Clinical Setting

Although the diversity of natural products currently used in the clinical setting is considerably immense, it is not comprehensive. There are in fact many other potential targets inherent to cancer pathology that could be exploited through the implementation of novel natural products. One target of considerable importance is the cytoskeleton. Not only do many malignant cells have a perturbed cytoskeleton owing to the effects of dysplasia and subsequent anaplasia [208, 209], but the cytoskeleton has also been indicated in oncogenic signaling and metastatic progression [210–212]. While microtubule-directed agents have been a mainstay in traditional cytotoxic chemotherapy, they are inherently limited to one component of the cytoskeleton. The other potential targets, intermediate filaments and microfilaments, have remained as elusive clinical prospects.

Cytoskeletal filaments are indeed viable targets to exploit in chemotherapy. Microfilaments are inherently required for cell motility, cytokinesis, and many other processes vital for malignant cell stability [213–216]. Intermediate filaments such as keratins are often overexpressed in carcinomas as a result of the aberrant effects of associated oncogenes [217, 218], and vimentin has been shown to be vital for cell survival in numerous experiments [219–221]. Although there has yet to be a microfilament- or intermediate filament-directed agent approved for clinical use, nature offers potential solutions to these previously elusive targets. Through the course of evolution, a considerable assortment of small molecules has been developed by various organisms that perturb the dynamics of actin polymerization or intermediate filament formation, and have demonstrated profound preclinical antineoplastic activity. A comprehensive review of cytoskeletal filament-directed agents available for further preclinical investigation has already been compiled [222]. Therefore, the potential of exploiting each currently untargeted component of the cytoskeleton will be briefly highlighted by two drug classes; cytochalasins and withanolides.

8.1 Cytochalasins

As indicated by both in vitro and in vivo investigation, microfilaments are vital for the progression of many malignancies, and are therefore a suitable target for chemotherapy. One of the most studied drug classes of microfilament-directed agents has been the cytochalasins, which were initially characterized in 1967 as being biological metabolites of the molds *Helminthosporium dematiodeum* (cytochalasins A and B) and *Metarrhizium anisopliae* (cytochalasins C and D) [223]. It has since been discovered that these mycotoxins potently inhibit actin polymerization, affecting activities ranging from cell motility and adhesion to cytokinesis. Since their initial discovery, more than 60 different cytochalasins from several species of fungi have been classified into various subgroups based on the size of the macrocyclic ring and the substituent of the perhydroisoindolyl-1-one residue at the C-3 position [224]. While all cytochalasins demonstrate the propensity to bind filamentous (F)-actin and block polymerization, only cytochalasins B and D have been extensively studied for their chemotherapeutic potential. Both congeners bind the fast growing barbed end of microfilaments, essentially fulfilling the role of capping proteins that prevent further actin polymerization once the filament has grown to a sufficient length [222]. However, cytochalasin D is more potent than cytochalasin B, reflected by cytochalasin B being 20-fold less toxic in mice than cytochalasin D [225], a pharmacological property attributed to the affinity cytochalasin D also has for globular (G)-actin.

Although only a handful of laboratories have investigated the potential of cytochalasins as antineoplastic agents, enough studies have been performed to establish mechanisms by which these agents exert their antineoplastic effects (Fig. 2). In preclinical mammalian models of malignancy, cytochalasins B and D exhibit marked antitumor and antimetastatic activity in murine melanomas (B16BL6 and B16F10), lung carcinomas (LA4, Lewis Lung, and M109), leukemias (P388 and P388/ADR), and M5076 sarcoma administered intraperitoneally, intravenously, or subcutaneously [226–239]. In addition, the pharmacokinetics, tissue distribution, and potential toxicities of cytochalasin B and liposome-encapsulated cytochalasin B have been extensively characterized [228–231]. The only major toxicity consistently elicited by cytochalasin B is marked immunosuppression, which has

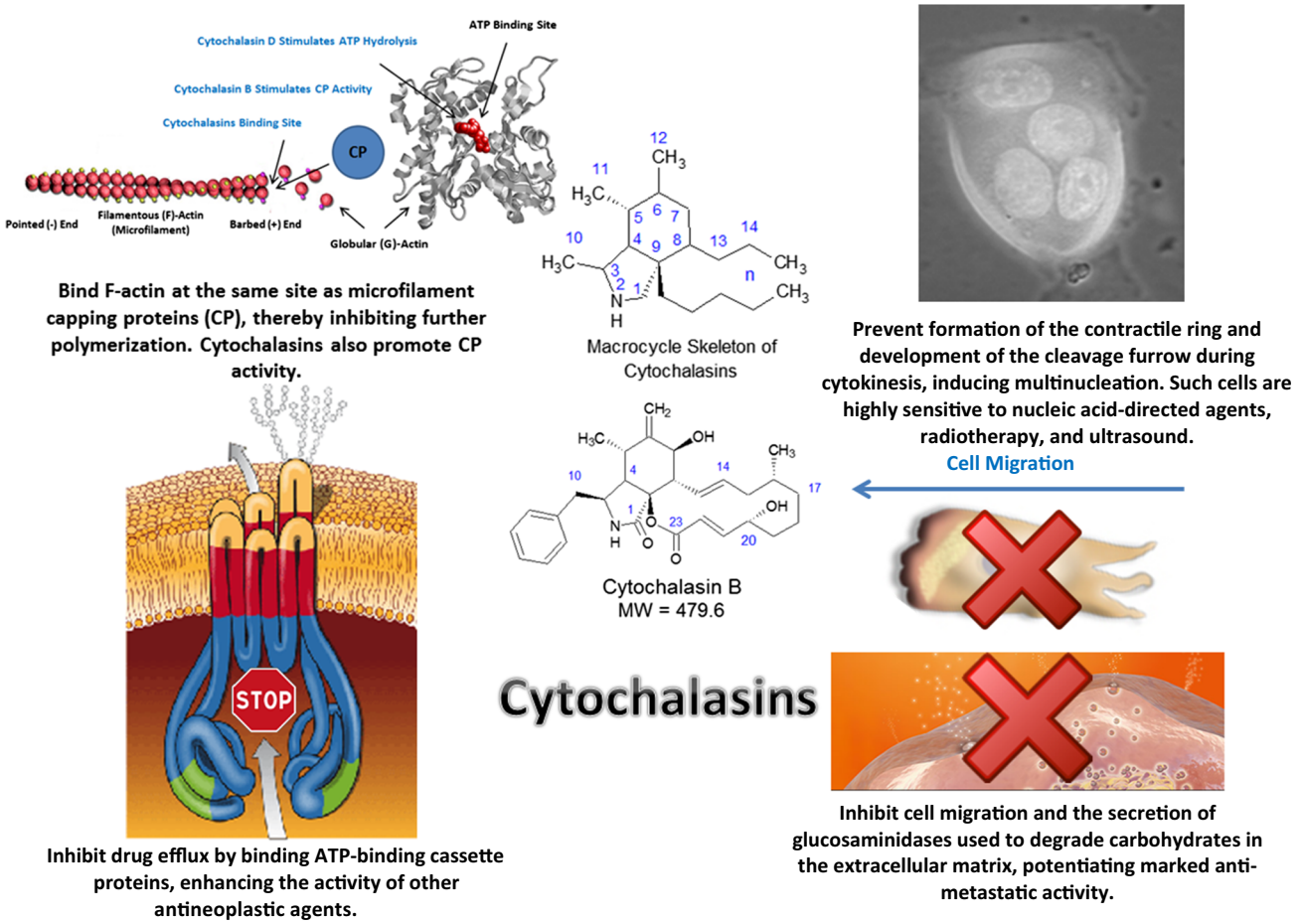

Fig. 2 Antineoplastic mechanisms of microfilament-disrupting cytochalasins. *MW* molecular weight

been characterized in multiple murine models [230, 231]. Nevertheless, this in vivo toxicity can be markedly reduced either through the administration of human recombinant IL-2, or liposome encapsulation [228, 229, 231]. Further, liposome encapsulated cytochalasin B shows equal, or even superior antitumor activity in M109 lung carcinoma in vivo when compared with the non-encapsulated compound [228]. It has also been demonstrated by Huang et al. [232] that a pegylated formulation of cytochalasin D is more water soluble, accumulates in tumor tissue more efficiently, and has a much longer $t_{1/2}$ than natural cytochalasin D (4 h vs. 10 min), while still retaining the antitumor activity of the natural compound.

8.2 Withanolides

Along with microfilaments, intermediate filaments are the other component of the cytoskeleton that has yet to be exploited in the clinical setting. It has been repeatedly demonstrated that a variety of malignancies have aberrant or an elevated expression of intermediate filaments keratin (type I or II), nestin (type VI), and vimentin (type III) [233–242]. Although no potential antineoplastic agent has been identified to specifically target aberrant keratin or nestin levels in malignant cells, withanolides have shown promise as a potent type III intermediate filament inhibitor.

The most promising clinical prospect of this drug class is withaferin A, a steroidal lactone that was initially isolated from *Withania somnifera* (winter cherry) in 1965 [243], and was the first member of the withanolides to be discovered. Withaferin A has been shown to potently inhibit a variety of proteins, with the most notable target being vimentin and other type III intermediate filaments [244–248] (Fig. 3). In addition to its affinity for intermediate filaments, withaferin A has been shown repeatedly to inhibit angiogenesis [247–251], with potent anti-angiogenic activity being exerted at doses as low as 7 μg/kg/day intraperitoneally in C57BL/6J mice [250].

Withaferin A also elicits antineoplastic activity in a considerable variety of cancer cell lines by directly inhibiting neoplastic growth, including carcinomas of the breast, head and neck, ovaries, and thyroid, as well as glioblastoma multiforme and melanoma [251–255]. Owing

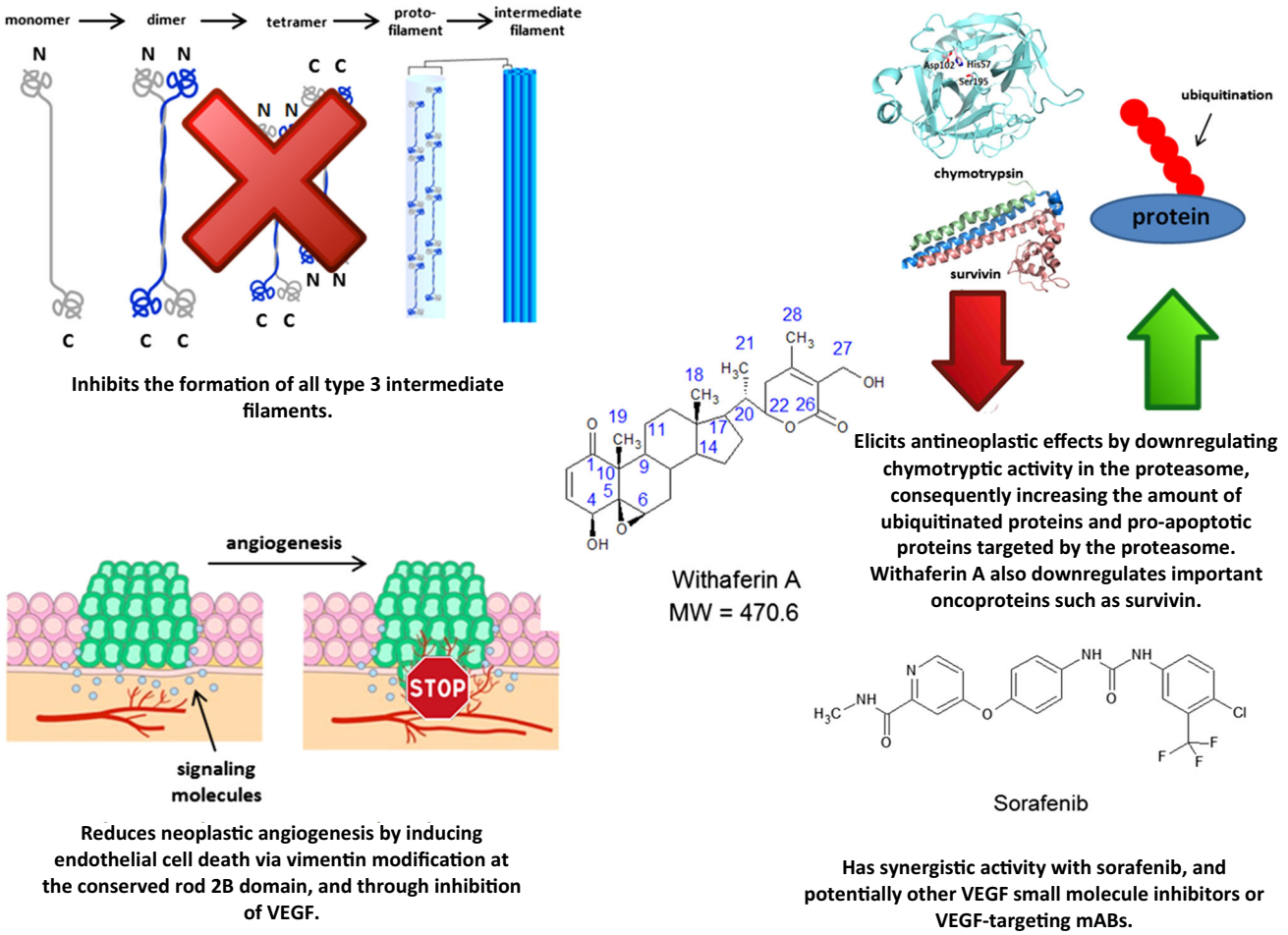

Fig. 3 Antineoplastic mechanisms of intermediate filament-disrupting withaferin A. *MW* molecular weight, *VEGF* vascular endothelial growth factor

to its notable anti-angiogenic activity, withaferin A has demonstrated significant synergistic effects with the multikinase inhibitor sorafenib [256], suggesting that withaferin A may be a viable supplement for renal cell carcinomas, and other malignancies notably affected by the inhibition of neoplastic angiogenesis. It should also be noted that withaferin A, withalongolide A (a 19-hydroxy derivative of withaferin A), and several other closely related congeners may influence signaling regulated by the PI3K/AKT/mTOR pathway. These congeners suppress RET and Akt phosphorylation and protein expression, as well as inhibit mTOR activity, the translational activity of 4EBP1, and protein synthesis mediated by p70S6 kinase activation in neoplastic cells [patient-derived medullary thyroid carcinoma cells, as well as human (U87 and U251) and murine (GL26) glioblastoma cells] in vitro [257, 258]. These data indicate a potential synergistic relationship between withanolides and mTOR inhibitors currently used in the clinical setting, and warrant further preclinical investigation.

9 Conclusion

Beginning with the VAMP (vincristine, amethopterin/methotrexate, 6-mercaptopurine, and prednisone) protocol that potentiated long-term survival in pediatric ALL for the first time in medical history [259], natural products have a long history as antineoplastic agents. They have repeatedly added to the mechanisms of action available to practicing clinicians, as well as inspire semi-synthetic derivations that demonstrate improved clinical utility. Although sometimes archaic by their less than ideal discrimination of neoplastic over normal tissue, these agents are still highly implemented in many forms of chemotherapy. In an age where clinicians are becoming ever more sophisticated in their ability to target solid tumors and disseminated cancers with small-molecule inhibitors and the rapidly expanding field of immunotherapy, we should not lose sight of the importance of natural products for the treatment of cancer. Epitomized by ADCs, natural products previously deemed too potent to elicit therapeutic benefit can now be

conjugated to an appropriate protein delivery system, thereby delivering highly cytotoxic and specific treatments to neoplastic tissue. This is an important lesson, as there are currently many cytotoxic mechanisms of action employed in nature that are not currently used in the clinical setting, such as the microfilament-targeting cytochalasins, or the intermediate filament-targeting withanolides.

While precision medicine has captivated the field of oncology in recent years and is gaining an increasing foothold in the clinic, we should also not discourage the continual investigation of natural products as antineoplastic agents. As demonstrated by current protocols, their unique mechanisms can be coupled with synthetic inhibitors or genetically modified proteins to create effective first-line therapies. Further, some derivatives of natural products such as the rapamycin analogs have the potential to be used in select cancers that demonstrate specific aberrant signaling cascades. Nature has already done the hard work through years of evolutionary pressures. Now, modern medicine can harness these complex, but often synthetically reproducible molecules, to develop ever more comprehensive and effective treatment modalities. As demonstrated by the countless lives they have prolonged or even saved, natural products have, are still, and will continue to function as vital components of cancer chemotherapy.

Acknowledgments The author extends his sincerest gratitude to all of the investigators who have been involved in the development of natural product-based antineoplastic agents, including his mentor, Dr. Thomas Fondy of Syracuse University. In addition, the author thanks the two anonymous reviewers whose suggestions helped improve the quality of the article.

Compliance with Ethical Standards

Funding information The author thanks Syracuse University for providing funds for research on microfilament-directed agents.

Conflict of interest The author declares no conflicts of interest.

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