

Beyond Taxanes: A Review of Novel Agents That Target Mitotic Tubulin and Microtubules, Kinases, and Kinesins

Michael R. Harrison, MD, Kyle D. Holen, MD, and Glenn Liu, MD

Dr. Harrison is a Medical Oncology Fellow at the University of Wisconsin Hospital and Clinics and Dr. Holen and Dr. Liu are Associate Professors at the University of Wisconsin, Paul P. Carbone Comprehensive Cancer Center in Madison, Wisconsin.

Address correspondence to:

Glenn Liu, MD

University of Wisconsin

Paul P. Carbone Comprehensive

Cancer Center, 600 Highland Avenue,

Madison, WI 53792

Email: gxl@medicine.wisc.edu

Fax: 608-265-8133

Phone: 608-265-8689

Abstract: Until recently, development of chemotherapeutic agents that target mitosis has centered on inhibiting the mitotic spindle through interactions with microtubules. The taxanes, while significantly advancing the treatment of many types of cancer, suffer from problems of hematopoietic and neurologic toxicities, development of resistance, and an inconvenient formulation. Novel microtubule inhibitors currently in clinical trial and in clinical use have the main advantage of overcoming resistance. Still, they have side effects related to the inhibition of microtubules in normal host cells. Novel antimetabolites, which target the mitotic spindle through interactions with nonmicrotubule mitotic mediators like mitotic kinases and kinesins, have been identified and are now in clinical trial. They offer the prospect of surmounting more of the problems inherent with taxanes and the hope of improving upon their broad antitumor efficacy. This review will concentrate on novel agents in later clinical development that target both the spindle microtubule and nonmicrotubule constituents of mitosis.

Introduction

Cancer is a disease of uncontrolled mitosis, which results in cells that grow, divide, and invade beyond the normal limits. Normally, mitosis is tightly regulated and culminates in the assembly of the mitotic spindle, which segregates replicated chromosomes into daughter cells. In a preparative stage of mitosis, prophase, the cell's DNA is modified in a process called resolution. Then, formation of the mitotic spindle is initiated by triggering centrosomes to move to opposite poles and the nuclear envelope breaks down. In prometaphase, spindle microtubules become attached to the kinetochore of each chromosome through a search and capture mechanism. During metaphase, chromosomes congress on the metaphase plate, equidistant from the centrosomes. Kinetochore microtubules shorten during anaphase, separating sister chromatids and moving them towards opposite poles, in a process known as segregation. Finally, the cell is pinched in 2 during telophase and cytokinesis, resulting in 2 daughter cells. The rapid dynamics of microtubules are essential throughout mitosis.

(Continued on page 55, following the Clinical Roundtable Monograph)

Keywords

Microtubule-inhibitor, antimetabolite, epothilones, mitotic kinases, mitotic kinesins

(Harrison et al, continued from page 54)

Microtubules play an important part in an array of cellular functions besides mitosis, including movement of organelles, vesicles, and proteins; development and maintenance of cell shape; and growth and signaling. Polymers composed of heterodimers of α -tubulin and β -tubulin microtubules are thin (24 nm diameter) filamentous tubes that may be many micrometers long. They readily polymerize and depolymerize in cells and exhibit 2 kinds of dynamic behaviors: dynamic instability and treadmilling.¹ The complex dynamics of microtubules are highly regulated and exquisitely sensitive to manipulation. If bipolar spindle dynamics are compromised, mitotic block or slowing occurs at the metaphase-anaphase transition, eventually leading to apoptosis.²

Agents that disrupt microtubule dynamics play key roles in both curative and palliative cytotoxic chemotherapeutic regimens. Taxanes and vinca alkaloids are the mainstays of this class of drugs, known as microtubule inhibitors, that act to either destabilize or stabilize the dynamic process of microtubule polymerization. The widespread clinical use of these drugs represents an important advance in cancer treatment.

The taxanes, paclitaxel and docetaxel in particular, have been extensively used because of their efficacy in a wide variety of tumor types. However, their effectiveness has been limited by toxicities related to the role of microtubules in normal, nontumor cells. Hematopoietic and neurologic toxicities have been problematic. Resistance to taxanes has emerged through the expression of multidrug-resistance (MDR) proteins and of tubulin isotypes, as well as mutations in tubulin. In addition, premedication to avoid hypersensitivity reactions is required before administration due to formulation in polyethylated castor oil (Cremophor EL). Thus, the search began for natural products that target microtubules without encountering these problems, with the hope of greater therapeutic indices and wider antitumor spectra of activity.

As mitosis has been further studied and better understood, the distinct biochemical mediators of mitosis have been identified. Targeting these proteins and kinases with specific functions in mitosis is a rational continuation of successful attempts at targeting microtubules. The Aurora family of protein kinases is required for multiple events during mitosis. Aurora A is required for spindle assembly and Aurora B is required for phosphorylation of histone H3 (during resolution), chromosome segregation, and cytokinesis.³ Polo-like kinase 1 is involved in centrosome maturation and formation of the mitotic spindle, and is also required for exit from mitosis and the separation of sister chromatids during anaphase.⁴ Kinesin spindle proteins are motor proteins essential in the formation of the mitotic spindle during early mitosis.⁵ Centromeric protein E is required for accurate congression during metaphase.⁶ A better understanding of these

mitotic mediators and their roles in tumorigenesis has led to the broadening of efforts to target mitosis in other ways besides disruption of the mitotic spindle through binding microtubules.

With the intense research focus on targeted agents as anticancer therapies, attention has now turned to nonmicrotubule elements of mitosis, such as kinases and kinesins, as possible targets. This review will focus on novel agents that target the spindle microtubule elements of mitosis, as well as those that target the nonmicrotubule effectors of mitosis. Discussion will center on those agents showing promise in late clinical development (ie, phase II and III clinical trials).

Epothilones

Epothilones as a whole are the farthest along in clinical development of the new class of antimetabolites. Their mechanism of action and biologic activity have been well reviewed elsewhere.⁷ These 16-member ring macrolides with a methylthiazole side chain were isolated from the myxobacterium *sorangium cellulosum*. Naturally occurring epothilones are classified as epoxides (A, B, E, F) or olefins (C and D).⁸ They compete with paclitaxel for binding to microtubules and appear to suppress microtubule dynamics much the same way as paclitaxel.⁹⁻¹¹ With IC₅₀ concentrations in the low- to sub-nanomolar range, epothilones possess much greater cytotoxic potency than taxanes.^{7,11,12} Multiple drug resistance mechanisms, including tubulin mutations and overexpression of multidrug-resistance proteins or β III tubulin, confer only a low level resistance against epothilones.^{7,13-16} In an effort to improve antitumor efficacy, epothilone analogs have been synthesized. Modifications, as with the synthetic forms, alter both their pharmacologic and biologic properties including antitumor activity and solubility.^{17,18} Epothilone B (patupilone, EPO906), a natural product, and several of its synthetic derivatives, including ixabepilone (aza-epothilone B, BMS-247550), BMS-310705, ZK-EPO (ZK-219477), and epothilone D (desoxy-epothilone B, KOS-862) are in clinical development for cancer treatment.

Patupilone

Patupilone (EPO906, Novartis) is twice as potent as epothilone A or paclitaxel at inducing tubulin polymerization in vitro.^{7,11} Diarrhea was the dose-limiting toxicity (DLT) in the 3 schedules of administration evaluated in phase I studies,^{19,20} in contrast to other epothilones. Fatigue and nausea and vomiting were less common, and significant neuropathy was uncommon. Because patupilone is metabolized by carboxylesterase-1, with the P-450 system playing a minimal role, tissue esterase activity likely plays an important role in determining its toxicity profile.^{7,21}

In phase II studies (Table 1), promising activity has been shown in lung (non-small cell, including a population with brain metastases),²²⁻²⁴ ovarian,²⁵ and renal cancers.²⁶ However, although there was a high rate of stable disease, no response to ixabepilone was seen in neuroendocrine tumors.²⁷ Minimal response was seen in colorectal,^{28,29} hepatocellular,³⁰ and gastric tumors.³¹ A phase III study versus doxorubicin is underway in ovarian, fallopian tube, and peritoneal cancers.

Ixabepilone

Ixabepilone (BMS-247550, Ixempra, Bristol-Myers Squibb) is a second-generation analog of epothilone B. Rational design by modification of a lactone to a lactam sidegroup results in greater metabolic stability by protecting it from degradation by human liver esterases. The first epothilone to successfully make its way to the clinic, ixabepilone is approved by the US Food and Drug Administration (FDA) for 2 indications in metastatic or locally-advanced breast cancer.

Phase I Studies Four dosing schedules have been studied in phase I trials.³²⁻³⁵ Ixabepilone is formulated in polyoxyethylated castor oil, which results in hypersensitivity reactions, requiring prophylactic antihistamines in all studies. The major DLT was neutropenia in 3 of 4 studies. Fatigue, the DLT in the fourth study, was the most common toxicity overall. Other side effects included gastrointestinal discomfort, diarrhea, stomatitis, anorexia, nausea and vomiting, hyponatremia, and neurotoxicity. Of note, neurotoxicity was predominantly grade 2 or less and thus was not dose-limiting.

Phase II Studies Ixabepilone has shown promising activity as monotherapy in a wide range of tumor types (Table 2), including early-stage,³⁶ locally-advanced, and metastatic breast cancer;³⁷⁻⁴¹ non-Hodgkin lymphoma (NHL)^{42,43}; non-small cell lung cancer⁴⁴; pancreatic cancer⁴⁵; prostate cancer^{46,47}; and renal cell cancer.⁴⁸ Many of these trials included patients with resistant or heavily pretreated tumors. Only modest responses were shown in bladder,⁴⁹ gastric,⁵⁰ gynecologic and breast,⁵¹ head and neck,⁵² and hepatobiliary⁵³ cancers, and sarcoma.⁵⁴ No responses have been seen in colorectal cancer⁵⁵ or metastatic melanoma.⁵⁶

Perez and colleagues⁴¹ conducted a phase II study (Table 2) in 126 patients with advanced breast cancer resistant to an anthracycline, a taxane, and capecitabine. The objective response rate based on independent radiologic review was 12.4% (95% confidence interval [CI], 6.9–19.9) with a median response duration of 6.0 months (95% CI, 5.0–7.6). In this study's heavily pretreated population (27% had grade 1 or 2 neuro-

pathy), 49% of patients developed grade 1 or 2 neuropathy during the study, but grade 3 or 4 neuropathy was reported in only 13% (1 grade 4 occurrence). Neuropathy was generally reversible with discontinuation of therapy and many patients were able to remain on dose-reduced therapy without worsening of neuropathy. Based on this study, ixabepilone is FDA approved as monotherapy for women with locally-advanced or metastatic breast cancer who are resistant or refractory to prior anthracyclines, taxanes, and capecitabine.

Ixabepilone in Combination Ixabepilone has been combined with other agents in breast, ovarian, and prostate cancers. In taxane- and anthracycline-pretreated women with metastatic breast cancer, ixabepilone combined with capecitabine had a response rate of 30%.⁵⁷ Ixabepilone has been combined with trastuzumab and carboplatin in women with HER2/neu positive, chemotherapy-naive, metastatic breast cancer with a response rate of 42.1%, median progression-free survival (PFS) of 8 months, and an acceptable toxicity profile.⁵⁸ In advanced breast and ovarian cancer, the combination of ixabepilone and polyethylene glycol (PEG)-liposomal doxorubicin has been evaluated in a phase I trial in which the DLTs were grade 3 mucositis and palmar-plantar erythrodysesthesia.⁵⁹ A phase II trial of this combination in platinum-refractory ovarian cancer is planned. Mitoxantrone and prednisone are also being combined with ixabepilone in hormone-refractory prostate cancer.⁶⁰

Phase III Studies In a pivotal randomized phase III trial, ixabepilone (40 mg/m² over 3 hours every 3 weeks) plus capecitabine (2,000 mg/m² for 14 of 21 days) was compared to capecitabine alone (2,500 mg/m² for 14 of 21 days) in 752 patients with metastatic breast cancer who had progression of disease after treatment with an anthracycline and a taxane.⁶¹ PFS, the primary end point, was prolonged in the combination group (5.8 vs 4.2 months; $P=0.0003$) as assessed by independent radiologic review. In addition, the overall response rate was superior in the combination arm (35% vs 14%; $P<0.0001$). More frequent toxicities in the combination group included grade 3 or 4 neutropenia (68% vs 11%), neuropathy (21% vs 0%), fatigue (9% vs 3%), and toxicity-related death (3% vs 1%). Patients with baseline liver dysfunction (\geq grade 2) were at greater risk for toxicity-related death, and all deaths were attributable to neutropenia. Neuropathy was generally reversible with 6 weeks mean time to resolution. Both groups had similar rates of capecitabine-related toxicities. Of note, this is the first study to show, in subgroup analysis, significantly improved PFS in so-called "triple negative" (negative for estrogen, progesterone, and HER2/neu receptors) breast cancers.⁶¹

Table 1. Efficacy of Patupilone in Phase II Trials

Study	Tumor Type	Pretreatment/Resistance	Evaluable Patients	Response Rate
Poplin et al ²⁹	Colorectal, advanced	Fluoropyrimidine, irinotecan, and/or oxaliplatin pretreated	91	4%
Casado et al ²⁸	Colorectal, advanced	≤4 prior chemotherapy regimens	43	8%
Hsin et al ³¹	Gastric, advanced local or metastatic	Unknown	22	9%
Venook et al ³⁰	Hepatocellular, unresectable and/or metastatic	Unknown; Child-Pugh A	24	4%
Anthony et al ²⁷	Neuroendocrine, metastatic	Stable dose required if on octreotide	14	0%; (71% SD after 3 cycles)
Sanchez et al ²⁴	NSCLC, unresectable locally advanced or metastatic	28% taxane-, 78% other-, and 100% platinum-pretreated	47	11%
Abrey et al ²²	NSCLC, recurrent or progressive brain metastases	Failed or recurred after prior chemotherapy, surgery, and/or radiation to brain	13	38%
Smit et al ²⁵	Ovarian, advanced	All platinum-resistant; 90% taxane-pretreated	19 (RECIST); 24 (CA-125)	16% by clinical or RECIST; 29% with 50% reduced CA-125
Thompson et al ²⁶	Renal, advanced	68% immunotherapy-pretreated, 9% prior chemotherapy, 25% treatment-naive	52	4%; (46% SD for 4 cycles)

NSCLC=non-small cell lung cancer; SD=stable disease.

Other Epothilones in Clinical Studies

ZK-EPO (Bayer Schering Pharma AG) is a fully synthetic, third generation epothilone that crosses the blood brain barrier and is polyethylated castor oil-based in its formulation. In vitro, it exhibits greater potency compared to other epothilones and retains activity even in multidrug resistant tumor cells.⁸ In one phase I study, the DLTs were neuropathy and ataxia.⁶² In a phase II study in platinum-resistant ovarian cancer, there was a 31% (4/13) objective response rate in 1 arm and 60% (9/15) had grade 2/3 neuropathy.⁶³ Phase II studies are ongoing in recurrent platinum-sensitive ovarian cancer (monotherapy and in combination with carboplatin), metastatic hormone-refractory prostate cancer in combination with prednisone, small cell lung cancer with cisplatin, non-small cell lung cancer, metastatic breast cancer with prior anthracycline and taxane administration, breast cancer metastatic to the brain, and recurrent glioblastoma multiforme.

KOS-862 (epothilone D or desoxyepothilone B, Roche/Kosan) is an epothilone D analog that showed at least equivalent potency and less toxicity overall compared with taxanes and epothilone B analogs in preclinical

studies.^{11,64} Several dosing schedules have been evaluated in 3 phase I studies,⁶⁵⁻⁶⁷ in which a polyethylated castor oil-based formulation of KOS-862 has been used. The DLT was neurologic in all studies (central neurotoxicity, including impaired gait and cognition). Other notable side effects included fatigue, nausea and vomiting, and neuropathy. Three phase Ib studies⁶⁸⁻⁷⁰ have combined KOS-862 with gemcitabine, carboplatin, and trastuzumab. In one phase II study of 35 patients with non-small cell lung cancer, the response rate was 3%.⁷¹ There are no current clinical trials with KOS-862.⁷²

BMS-310705 (Bristol-Myers Squibb/GBF) is a water-soluble, semi-synthetic analog of epothilone B that has been evaluated in phase I trials with 2 dosing schedules.^{73,74} DLTs were neutropenia and hyponatremia, and diarrhea. Sensory neuropathy, neutropenia, and diarrhea were the most common adverse effects. No hypersensitivity reactions were observed.

Other Nonepothilone Antimicrotubule Agents

Other antimicrotubule compounds have been isolated from natural sources, including discodermolide from the marine sponge *Discodermia dissolute*, dolastatin from

Table 2. Efficacy of Single-Agent Ixabepilone in Phase II Trials

Study	Tumor Type	Pretreatment/Resistance	Evaluable Patients	Response Rate
Dreicer et al ⁴⁹	Bladder	Both taxane-pretreated and -naive	37	14% (8% pretreated)
Baselga et al ³⁶	Breast, early stage	Treatment-naive	96	19% (pCR)
Denduluri et al ³⁷	Breast, metastatic	Taxane-naive	23	57%
Low et al ³⁸	Breast, locally advanced or metastatic	Taxane-pretreated	37	22%
Roche et al ³⁹	Breast, metastatic	Anthracycline-pretreated	65	41.5%
Thomas et al ⁴⁰	Breast, metastatic	Taxane-resistant	49	12%
Perez et al ⁴¹	Breast, metastatic (29% “triple negative”)	Anthracycline-, taxane-, and capecitabine-resistant	113	18.6%
Eng et al ⁵⁵	Colorectal, advanced	Irinotecan/5-fluorouracil/leucovorin-refractory	25	0%
Ajani et al ⁵⁰	Gastric	Taxane-pretreated	45	4%
Chen et al ⁵¹	Gynecologic and breast	Almost all taxane-pretreated	21	14% (31% in breast)
Burtneess et al ⁵²	Head and neck, squamous cell, recurrent or metastatic	Mixed taxane-naive and -exposed (2 arms with different dosing)	75 (Arm A: 32; Arm B: 43)	A: 0%; B: 14.3% (of taxane-naive)
Singh et al ⁵³	Hepatobiliary (75% metastatic)	Chemotherapy-naive	48	8%
Pavlick et al ⁵⁶	Melanoma, stage IV	50% untreated, 50% pretreated	23	0%
Smith et al ⁴²	NHL, relapsed aggressive	All heavily pretreated	14	73% remission rate
O'Connor et al ⁴³	NHL, indolent and mantle cell	Resistant disease (≤4 prior treatments)	11	14%
Vansteenkiste et al ⁴⁴	NSCLC	Platinum-refractory	112 (Arm A: 52; Arm B: 60)	14% (A); 1% (B)
Whitehead et al ⁴⁵	Pancreatic, advanced	Chemotherapy-naive	56	9%
Hussain et al ⁴⁶	Prostate, metastatic	Hormone-refractory, chemotherapy-naive	42	15% (33% PSA)
Galsky et al ⁴⁷	Prostate, metastatic, with (-) or without (+) extramustine phosphate (EMP)	Progressive castrate, chemotherapy naive	48 (25 -EMP; 23 +EMP)	32% -EMP (48% PSA); 48% +EMP (68% PSA)
Fojo et al ⁴⁸	Renal	Prior systemic immune therapy (39%); prior nephrectomy (91%)	57	14%
Okuno et al ⁵⁴	Sarcoma, advanced or metastatic	Adjuvant chemotherapy (39%); chemotherapy-naive metastatic disease	31	6%

NHL=non-Hodgkin lymphoma; NSCLC=non-small cell lung cancer; pCR=pathologic complete response; PSA=prostate-specific antigen.

the sea hare *Dolabella auricularia*, halichondrin B from the marine sponge *Halicondrin okadae*, and hemiasterlin from the marine sponge *Hemiasterella minor*.^{21,75} All of these compounds have been synthesized and have synthetic or semi-synthetic analogs that have been evaluated in clinical studies. Sarcotidicytins A and B and eleutherobin, marine soft-coral-derived natural products, and laulimalide and isolaulimalide, marine-derived microtubule-stabilizing agents, have been less well studied clinically.

Of these agents, E7389 (Eribulin, Eisai), a simplified synthetic macrocyclic ketone analog of halichondrin B, is the farthest along in clinical development. It appears to work by a unique “end-poisoning” mechanism, whereby it inhibits microtubule growth, but not shortening, ultimately resulting in abnormal mitotic spindles that cannot pass the metaphase/anaphase checkpoint, leading to initiation of apoptosis.⁷⁶ Two different dosing schedules have been studied in phase I clinical trials.⁷⁷⁻⁷⁹ In both schedules, the DLT was neutropenia.

The results of several ongoing phase II studies have recently been reported in abstract form. In 103 patients with “heavily pretreated” (at least a prior anthracycline and taxane) advanced breast cancer, the overall objective response rate was approximately 11.5%.⁸⁰ Grade 3/4 neutropenia occurred in 61% and grade 3 neuropathy in only 5% of patients. In 103 patients with advanced non-small cell lung cancer who had been treated with platinum-based doublet chemotherapy (median 2 prior therapies, the majority of which were 2 cytotoxic regimens), E7389 showed an overall partial response rate of 9.6% (10.8% in taxane-pretreated patients) and 9.6 months median survival. The incidence of grade 3/4 neutropenia was 49% and grade 3 peripheral neuropathy was only 2%.⁸¹ In a phase II study in men with advanced and/or metastatic hormone-refractory prostate cancer stratified to no prior chemotherapy (except mitoxantrone or estramustine) and no more than one prior regimen containing a tubulin-binding agent (ie, a taxane), there was some evidence of single-agent activity for E7389 based on preliminary data.⁸² There were 2 of 21 prostate-specific antigen (PSA) responses (10%) in the taxane-pretreated group and 4/14 responses (29%) in the taxane-naïve group. This study is proceeding to stage 2 with further accrual.

Two phase III studies are underway with E7389, both in metastatic or locally advanced breast cancer. The first compares E7389 versus capecitabine and requires prior treatment with a taxane and an anthracycline in patients refractory to their most recent chemotherapy. The second compares E7389 versus physician choice of chemotherapy in patients previously treated with a taxane and anthracycline.

Targeting Nonmicrotubule Mitotic Proteins and Kinases

The intense focus on molecularly targeted agents, combined with a better understanding of the biochemical and molecular mediators of mitosis have spurred the discovery of new agents that target these mediators. The novel ways by which these agents interfere with mitosis, coupled with the specificity with which they target cells undergoing mitosis, create the potential to move beyond some of the difficulties encountered with microtubule-targeted agents and broaden the scope of cancer treatment. Because these drugs are microtubule-sparing, they may potentially avoid problems with neurotoxicity, whereas their specificity may result in better antitumor efficacy. They also serve as valuable tools to better understand cell division, as more mitotic players and their roles are uncovered. Inhibitors of the aurora kinases, polo-like kinase1 (PLK1), kinesin spindle protein (KSP), and centromeric protein E (CENPE) are in clinical development.

Aurora Kinase Inhibitors

The Aurora kinases in humans are a three-member family of serine/threonine kinases: Aurora A, Aurora B, and Aurora C. Aurora A is primarily centrosomal and localizes to the mitotic spindle. It functions in early mitosis when it is required for centrosome separation and mitotic spindle assembly.⁸³ Inhibition of Aurora A leads to severely defective spindle morphology, and ultimately to terminal mitotic arrest and apoptosis. Overexpression leads to tumorigenesis and elevated levels of expression have been found in many different tumor types.⁸⁴ Aurora kinase B is recruited to the centromere and spindle midbody during later stages of mitosis and is required for chromosome biorientation, the spindle assembly checkpoint, and cytokinesis.⁸⁵ Inhibition by small molecule inhibitors of Aurora kinase B, because it is required to induce the spindle checkpoint, abrogates the mitotic-spindle checkpoint, causing untimely mitotic exit without completion of cytokinesis, which leads to 4N DNA-containing cells that continue to progress through the cell cycle.⁸⁶⁻⁸⁸ With continued inhibition of Aurora B, cytokinesis never occurs through several rounds of the cell cycle, which leads to polyploidy and eventually apoptosis. Interestingly, when combined with other anti-mitotic agents, including Aurora A inhibitors, Aurora B inhibitors have a dominant phenotype.⁸⁴ By contrast, much less is known about the function of Aurora C, although recent studies have begun to illuminate its role.⁸³

VX-680

VX-680 (MK-0457, Vertex/Merck) was the first aurora kinase inhibitor to enter clinical trials. It inhibits Aurora A,

B, and C in vitro; FMS-related tyrosine kinase 3 (FLT3); and the BCR-ABL wild-type and T315I mutant (resistant to both imatinib and dasatinib).⁸⁴ In phase I studies, the DLT was neutropenia.⁸⁹ A phase II study commenced in patients with treatment-refractory chronic myelogenous leukemia or Philadelphia chromosome-positive acute lymphocytic leukemia (Ph+ALL) containing the T315I mutation. However, on November 20, 2007 the manufacturer suspended enrollment pending full analysis of efficacy and safety data after 1 patient had QTc interval prolongation.⁹⁰ A phase II study of VX-680 has been planned in colorectal and non-small cell lung cancers.⁸⁴

AZD1152

AZD1152 (AstraZeneca) is a selective inhibitor of Aurora B currently under evaluation in phase I studies with various dosing schedules. Neutropenia has been the main DLT reported.⁹¹ A phase I/II study is underway in relapsed acute myeloid leukemia (AML). In human acute leukemia cells in vitro and in vivo, AZD1152 has been found to synergistically enhance the antiproliferative activity of a microtubule depolymerization agent (vincristine) and a topoisomerase II inhibitor (daunorubicin).⁹²

Polo-Like Kinase (PLK) Inhibitors

There are 4 known members of this family of mitotic serine/threonine kinases in humans: PLK1, PLK2 (also known as Snk), PLK3 (Fnk or Prk) and PLK4 (Sak). PLK1 has been the most widely studied and is overexpressed in many tumor types.⁸³ Elevated PLK1 expression, histologic grade, and poor prognosis have been correlated in a variety of tumors.⁹³ Small molecule or small interfering RNA (siRNA) inhibition of PLK1 leads to G2/M arrest and apoptosis through inadequate generation of spindle poleward pulling forces and failure of cytokinesis.^{94,95} Furthermore, although PLK1 depletion is lethal to cancer cells, normal cells showed little to no cytotoxicity in response to depletion. Thus, PLK1 is an attractive target for antimitotic cancer therapies. The first reported small molecule inhibitor of PLK1 was the natural marine product scytonemin.⁹⁶ The compounds BI 2536 and ON01910.Na are currently in clinical development.

BI 2536

BI 2536 (Boehringer Ingelheim) is highly selective for PLK1. In phase I studies involving 104 patients, 2 different dosing schedules were evaluated.^{97,98} The main DLT on both schedules was neutropenia, with the addition of thrombocytopenia on one of the schedules. Other notable adverse events included fatigue, nausea, and vomiting. Phase II studies evaluating BI 2536 are ongoing in metastatic or relapsed non-small cell lung cancer and in small cell lung cancer as second line therapy.

ON 01910.Na

ON 01910.Na (Onconova) is an ATP noncompetitive inhibitor of PLK1 that interferes with the ability of PLK1 to bind substrates. It also has low nanomolar potency against ABL, FLT1 and PDGFR.⁸⁴ In phase I studies, 3 different dosing schedules are being evaluated and results have been reported in abstract form on 2 of these.^{99,100} Adverse events included mild-moderate anemia, leukopenia, elevated liver enzymes, gastrointestinal symptoms, and fatigue.

Kinesin Spindle Protein (KSP) Inhibitors

KSP (also known as kinesin-related motor protein Eg5) is a kinesin motor protein that drives centrosome separation and is required to establish the bipolar spindle. Furthermore, there is evidence that KSP expression is increased in tumor cells when compared with normal cells.¹⁰¹ Inhibition of KSP causes mitotic arrest with a monopolar spindle, with no effect on nonproliferating cells.⁸⁴ KSP is absent in terminally differentiated neurons. The first small molecule selective inhibitor of KSP identified was monastrol.¹⁰² More potent KSP inhibitors have since been identified, of which ispinesib (SB-715992) has advanced the farthest in clinical testing.

Ispinesib

Ispinesib (SB-715992, Cyokinetics) is a small molecule inhibitor of KSP adenosine triphosphate (ATP)ase that is noncompetitive with ATP and adenosine diphosphate and 40,000 times more selective for KSP than any other kinesins.¹⁰³ In phase I studies, 3 schedules have been evaluated.¹⁰⁴⁻¹⁰⁶ The main DLT was neutropenia. Other adverse events included leukopenia, anemia, and fatigue.

In Phase II studies (Table 3), ispinesib has shown activity in patients with metastatic breast cancer who progressed or relapsed after treatment with an anthracycline and taxane.¹⁰⁷ Unfortunately, there has been no activity seen in colorectal,¹⁰⁸ hepatocellular,¹⁰⁹ head and neck,¹¹⁰ ovarian,¹¹¹ or renal cell cancers,¹¹² or in melanoma.¹¹³ Studies in non-small cell lung and hormone-refractory prostate cancers have been conducted, but results have not yet been reported. Phase I studies in patients with hematologic malignancies are currently accruing. Ispinesib was generally well tolerated with mild hematologic and few other toxicities.

Other Mitotic Kinesin Inhibitors

SB-743921 (GlaxoSmithKline/Cytokinetics) is a KSP inhibitor more potent than ispinesib.¹¹⁴ The main DLT in phase I study was neutropenia, of which the onset and duration were predictable.¹¹⁵ Phase I/II studies are underway in NHL.

Table 3. Efficacy of Ispinesib in Phase II Trials

Study	Tumor Type	Pretreatment/Resistance	Evaluable patients	Clinical Results
Miller et al ¹⁰⁷	Breast, locally advanced or metastatic	Taxane- and anthracycline-pretreated	33	ORR, 9%, SD, 12%
El-Khoueiry et al ¹⁰⁸	Colorectal, metastatic	Heavily pretreated	53	ORR, 0%, SD, 9%
Knox et al ¹⁰⁹	Hepatocellular, advanced	Chemotherapy-naive	15	ORR, 0%, SD, 46%
Tang et al ¹¹⁰	Head and Neck (squamous), recurrent or metastatic	One prior line of chemotherapy	20	ORR, 0%, SD, 25% (>2 cycles)
Lee et al ¹¹³	Melanoma, metastatic or recurrent	No prior chemotherapy; adjuvant immunotherapy allowed	17	ORR, 0%, SD, 35%
Shahin et al ¹¹¹	Ovarian	Platinum/taxane refractory or resistant	22	ORR, 5%, SD, 26%
Beekman et al ¹¹²	Renal Cell	Prior immunotherapy (53%); prior sunitinib, sorafenib, or bevacizumab (79%)	15	ORR, 0%, SD, 47%

ORR=objective response rate; SD=stable disease.

GSK-923295 (GlaxoSmithKline/Cytokinetics) is a CENPE inhibitor. CENPE is a component of the mitotic checkpoint that catalyzes congression of chromosomes at the spindle equator before biorientation.⁶ A phase I study in patients with advanced solid tumors is ongoing.

Conclusion

The development of newer agents for cancer therapy has undergone a dramatic paradigm shift. Much more emphasis is being placed on therapies that focus on specific molecular targets produced in tumor cells, as opposed to nonspecific cytotoxic chemotherapies that affect all cells undergoing division. Part of this paradigm shift is due to a better understanding of tumor biology and the consideration of cancer as a chronic disease. Thus, minimizing toxicity with tumor-specific targets is of great importance. The only exception is the ongoing development of agents that target mitotic tubulin and microtubules, which are relatively less selective for cancer cells, and those that target related mitotic kinases and kinesins, which appear to be more selective for cancer cells.

The FDA approval of ixabepilone in the era of targeted therapy is an exciting development. Its success lies in the ability to overcome the resistance that hampers the taxanes, while maintaining a similar, broad antitumor efficacy. However, the problems of neurotoxicity and cumbersome formulation remain. Next-generation ephthalones

and anti-microtubule agents show promise in overcoming these problems, yet the only approved antimetabolic agents remain those that have tubulin as their target.

The mitotic kinase and kinesin inhibitors represent a chance to improve on antitubulin agents. In clinical studies of these targeted antimetabolites, neurotoxicity has not been observed to any significant degree and neutropenia has been the main toxicity. Unfortunately, early forays in their use, although showing an improved side effect profile, have been somewhat disappointing in terms of efficacy. We remain hopeful, however, that newer agents may improve the therapeutic window of this class of drugs.

As summarized in this review, a deeper understanding of cell biology has resulted in a vast array of agents targeting not just mitotic tubulin, but Aurora kinase, PLK, KSP and CENPE, with promising preclinical and early clinical results. The development of each of these agents shares the common, rational goal of improving oncologic care. Through further studies, we remain optimistic that these novel antimetabolic agents will continue to lengthen the survival of cancer patients while improving upon toxicities in the years ahead.

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(Saif and Shah, continued from page 53)

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