Cancer Chemother Pharmacol (2009) 64:1029–1038
DOI 10.1007/s00280-009-0959-z

ORIGINAL ARTICLE

Bone marrow CFU-GM and human tumor xenograft efficacy of three tubulin binding agents

Leslie S. Kurtzberg · Stephanie D. Roth · Rebecca G. Bagley · Cecile Rouleau · Min Yao · Jennifer L. Crawford · Roy D. Krumbholz · Steven M. Schmid · Beverly A. Teicher

Received: 30 October 2008 / Accepted: 12 February 2009 / Published online: 10 March 2009

Abstract
Purpose The dynamic instability of microtubules in cells is one of the key targets of anticancer therapeutics. Microtubule-disrupting agents such as vinca alkaloids and microtubule-stabilizing agents such as taxanes are important antitumor agents. The bone marrow toxicity and human tumor xenograft activity of three tubulin-binding compounds, vincristine, paclitaxel, and tasidotin were compared.
Methods Mouse and human bone marrow were subjected to colony-forming (CFU-GM) assays over a 5-log concentration range in culture. In vivo, a range of tasidotin doses was compared with vincristine, paclitaxel, and docetaxel for efficacy in several human tumor xenografts.
Results The IC_{90} concentrations for vincristine and paclitaxel for mouse CFU-GM were 30 and 27 nM, and for human CFU-GM were 3 and 9 nM, giving mouse to human differentials of ten- and threefold. Tasidotin produced IC_{90}s of >300 nM in mouse and 65 nM in human CFU-GM, thus a >4.6-fold differential between species. In vivo, tasidotin resulted in a dose-dependent increase in tumor growth delay in the RL lymphoma, the RPMI 8226 multiple myeloma, and MX-1 breast carcinoma models. Vincristine and tasidotin were also very effective against these tumors. The PC-3 prostate carcinoma was very responsive to full-dose paclitaxel and docetaxel while tasidotin generated a dose dependent effect.
Conclusions Bringing together bone marrow toxicity data, pharmacokinetic parameters, and human tumor xenograft efficacy provides valuable information for the translation of preclinical findings to the clinic.

Keywords Tasidotin · Vincristine · Paclitaxel · Bone marrow CFU-GM · Tumor xenografts

L. S. Kurtzberg (✉) · R. G. Bagley · C. Rouleau · M. Yao · B. A. Teicher
Genzyme Corporation, 49 New York Avenue, Framingham, MA 01701, USA
e-mail: Leslie.Kurtzberg@Genzyme.com

S. D. Roth · J. L. Crawford · R. D. Krumbholz · S. M. Schmid
Genzyme Corporation, 4545 Horizon Hill Blvd, San Antonio, TX 78229, USA

Introduction

The cellular requirement for the dynamic flux of microtubules is one of the key targets of anticancer therapies. Although principally recognized in mitotic function for their role in separating the duplicate set of chromosomes during cell division, microtubules are an essential component of the cytoskeleton and are critical in many interphase functions including directional transport of proteins and organelles, maintenance of cell motility, cell shape and scaffolding, intracellular transport, secretion, neurotransmission and relay of signaling between cell surface receptors, and the nucleus [5, 49]. Microtubules are polymers composed of molecules of tubulin which are each dimers of tightly linked globular protein subunits called α- and β-tubulin. The biologic function of microtubules relies on the unique dynamics of tubulin polymerization [24, 25]. Microtubule assembly and disassembly are in dynamic equilibrium. Cells modify the rate of dynamic flux of microtubules to perform specific functions. During mitosis, the rates of both microtubule assembly and disassembly are increased so that chromosomes can readily capture microtubules that are actively forming mitotic spindles.

The cytotoxicity of the vinca alkaloid anticancer agents, vincristine, vinblastine, vinorelbine, and vinflunine, is caused by their interaction with tubulin and subsequent

disruption of microtubules [23, 40]. The stimulation of the vinca alkaloid-binding site on tubulin suppresses microtubule dynamics (switching between phases of slow growth and rapid shortening at microtubule ends) and microtubule treadmilling (initiating growth at the plus end and shortening at the minus end of the microtubules) causing cell cycle arrest at G2/M [28, 29, 39, 40, 46, 51]. Thus, these compounds cause a disruption of microtubules comprising the mitotic spindle apparatus, thereby inducing metaphase arrest in dividing cells. The vinca alkaloids also affect microtubules involved in chemotaxis and directional migration, intracellular transport and movement of organelles such as mitochondria and secretory granules especially in neural cells, secretory processes, membrane trafficking and transmission of receptor signals, and structural integrity in some cells. Platelets, in particular, are rich in tubulin and depend upon microtubules for structure. The relationships between the inhibitory effects of vinca alkaloid on cell proliferation, mitotic arrest, mitotic spindle disruption, and depolymerization of microtubules have been described in detail [3, 19, 54].

The dolastatins are a family of naturally occurring unique polypeptides containing unusual amino acids [45]. Tasidotin is a third-generation synthetic dolastatin depsipeptide analog with antitumor activity [6, 9, 10, 14, 27, 37]. In a manner similar to the vinca alkaloids, tasidotin suppresses microtubule spindle dynamics by reducing the rate of shortening, reducing the switching frequency from growth to shortening (catastrophe frequency), and reducing the time of microtubule growth [6, 47].

Microtubule-stabilizing agents including taxanes and epothilones, on the other hand, exert cytotoxicity by stabilizing microtubules [15, 20]. Taxanes, specifically paclitaxel, was discovered during a NCI screen of plant extracts for anticancer activity. It was later found that paclitaxel is produced by *Taxomyces andreanae*, a fungal endophyte endemic to the inner bark of the Pacific yew [52]. The site of paclitaxel binding on microtubules is different from the binding sites for vinca alkaloids and dolastatins. Paclitaxel binds to the N-terminal 31 amino acids of the β-tubulin subunit in tubulin oligomers or polymers rather than to tubulin dimers. Thus, taxanes shift the dynamic equilibrium between tubulin dimers and microtubules toward microtubule assembly and stabilize microtubules thus preventing depolymerization. The taxane-bound microtubules are very stable and the dynamic reorganization of the microtubule network is inhibited [48]. Taxanes induce microtubule bundling in cells and the formation of numerous abnormal mitotic asters. At low concentrations, taxanes kill cells by producing a sustained mitotic block at the metaphase-anaphase boundary which triggers apoptosis. The epothilones are also microtubule-stabilizing agents which induce microtubule bundling, formation of multipolar spindles, and mitotic arrest. Epothilones and taxanes do not bind to tubulin polymers in an identical manner [8, 18, 32, 38, 42, 53, 54].

Bone marrow is critically sensitive to many antineoplastic agents. [43]. It is important to understand the toxicity of agents to bone marrow, and to determine whether bone marrow progenitor cells will have greater or less sensitivity to the agent than human malignant cells. Bone marrow granulocyte–macrophage-colony forming unit (CFU-GM) assays that compare the sensitivity of bone marrow cells across species are useful in predicting the blood levels of an agent that might be achieved in patients using blood levels tested in preclinical efficacy and safety studies as a guide. Mouse bone marrow is less sensitive to many cytotoxic agents than is human bone marrow, allowing blood levels in preclinical efficacy testing that cannot be achieved in patients [11, 30, 34, 43]. An efficacious level of a compound with smaller or no differential in bone marrow progenitor sensitivity among species may have a better potential for reaching similar blood levels in patients as in mice. If bone marrow toxicity is dose-limiting, these compounds may be more likely to be successful in reaching therapeutic doses. Pessina et al. [43] suggested that through use of the ratio of mouse/human CFU-GM IC_{90} values and the mouse MTD, the human MTD of a compound could be predicted and thus the potential for achieving a therapeutic blood level in patients estimated.

The current report compares the bone marrow CFU-GM and tumor cell CFU activity of three tubulin binding agents, vincristine, tasidotin, and paclitaxel. A smaller difference in bone marrow CFU-GM cytotoxicity between mouse and human addresses the practical issue of achieving doses in the clinic at a level comparable to those first tested in preclinical studies. Antitumor activity of the compounds in several xenograft models was assessed using the endpoints of tumor growth delay and body weight change.

Materials and methods

Materials

Tasidotin hydrochloride (*N*, *N*-dimethyl-L-valyl-L-valyl-*N*-methyl-L-valyl-L-prolyl-L-proline-tert-butylamide hydrochloride) was available in-house as a stable white solid. Aqueous solutions remain stable with maximum stability of aqueous solutions occurring at pH 5.0 in a phosphate buffered solution. Tasidotin was prepared as a 10-mM stock solution in PBS. Paclitaxel (#T1912) and vincristine (#V8879) were purchased from Sigma Chemicals (St. Louis, MO). Paclitaxel was prepared as a 10-mM stock solution in DMSO (Sigma, #D5879). Vincristine was prepared as a 10-mM stock solution in distilled water. For cell culture,

aliquots (10 µl) were made in sterile, capped polypropylene tubes and stored frozen at −20°C until use.

For colony-forming assays, a single aliquot of each compound was thawed the day of the assay, and a working stock of 10 µM was made from 5 µl of the 10 mM drug into 5 ml RPMI1640/5% FBS. Dilutions of 20× the desired final concentration were prepared from this working stock solution in RPMI1640/5% FBS.

For xenograft studies, vincristine, docetaxel, and paclitaxel were purchased from Sigma chemicals. Tasidotin was prepared in normal saline. Compounds were prepared freshly for injection into mice. Compounds were administered in volumes of 0.1 ml/10 g body weight. Vincristine was prepared in normal saline. Docetaxel was reconstituted in 13% ethanol for a 10 mg/ml stock solution and then further diluted with normal saline. Paclitaxel was reconstituted at 6 mg/ml in 1:1 Cremophor EL:ethanol and then further diluted with normal saline.

Mouse bone marrow

Male Balb/c mice were purchased from Charles River Laboratories (Wilmington, MA) at 6–8 weeks of age and used for studies at 7–10 weeks of age. The mice were housed four to eight per cage, had free access to tap water, were fed a normal diet and observed daily. The procedures were carried out according to a protocol approved by the Institutional Animal Care and Use Committee in accordance with the Federal Animal Welfare Act (9 CFR, 1992) and were conducted in an AAALAC accredited facility.

For bone marrow collection, naïve mice were killed by asphyxiation through inhalation of carbon dioxide. To ensure a sterility of the bone marrow, the killed donor mouse was placed in a biological laminar flow hood and the whole body was sprayed with a 70% ethanol solution. A skin incision was made throughout the whole hind limb from mid-point of inguinal ligament to the medial side of the foot. The muscle and other connective tissues were removed and both tibias and the femurs were extracted. The bone medullary cavity was accessed by removing the epiphysis of the tibia or the femur located at the end of long bones. A blunt syringe needle (27G) was inserted into the medullary cavity and the cavity was flushed with 2 ml of sterile RPMI1640/5% FBS until the color of bone shaft became pale indicating removal of the bone marrow. The resulting cellular suspension was collected into a 50-ml conical-bottom tube and kept on wet ice. Approximately 25 million cells were obtained from each mouse [31].

Bone marrow CFU-GM assay

For mouse CFU-GM assays, freshly isolated mouse bone marrow cells were cultured in MethoCult (#M3534) containing the cytokines rm SCF, rm IL-3, and rh IL-6 to assist growth (StemCell Technologies, Vancouver, BC).

For human CFU-GM assays, viable human bone marrow cells (purchased as frozen vials from All Cells, LLC via StemCell Technologies, #ABM009) were prepared with a DNase treatment (#07900, StemCell Technologies), washed in a protocol described by the vendor, and cultured. The culture medium used was MethoCult GF (#H4534), containing the cytokines hSCF, hGM-CSF, hIL-3 (StemCell Technologies).

Both the human and mouse MethoCult mediums were received frozen in 100 ml volumes. Upon receipt, bottles were thawed overnight at 4°C, and divided into 3 ml aliquots in sterile 15 ml tubes The MethoCult mediums solidify at 37°C, and were not warmed to greater than room temperature until the addition of cells and compounds immediately prior to transfer to 37°C. The MethoCult medium aliquots were refrozen at −20°C until thawing at 4°C overnight before the assay.

For experiments, 150 µl of 20× concentration of the compounds, along with 150 µl of a suspension of mouse or human bone marrow cells, were added to the 3-ml tube of thawed MethoCult medium and vigorously mixed by vortex. Final cell concentrations were 4×10^4 cells/plate for mouse cells, 8×10^4 cells/plate for human cells. Tubes were then allowed to sit undisturbed for 10–15 min until the air bubbles dissipated. A blunt needle attached to a 3-ml syringe was used to collect 2.5 ml of the cell suspension, the air was voided from the syringe, and 1 ml of cell suspension was placed in the center of duplicate treatment-labeled 100 cm^2 culture dishes. Dishes were gently rotated to allow even coverage of the bottom surface of each dish and then placed in a 37°C incubator for colony formation for 13 days for mouse bone marrow and 15 days for human bone marrow. Colonies were defined as clusters containing 30 or more cells.

Human and mouse bone marrow progenitor cells were continuously exposed to a concentration range of vincristine, paclitaxel, or tasidotin in three independent CFU-GM colony formation experiments. The compound concentrations were made in half-log intervals covering 5 logs (10 concentrations). The IC_{50} and IC_{90} values and the 95% confidence interval for each compound for human and mouse bone marrow were determined by non-linear regression analysis [31].

Tumor Lines

The RL lymphoma is a mature, EBV-negative, CD20+ B cell line from a diffuse large-cell lymphoma and carries a chromosomal marker *t*(14, 18)(q32;q21) [2]. The RPMI 8226 line is a B lymphocyte plasmacytoma, a cell line established from a 61-year-old male in the mid-1960s [35].

The cell line is most often described as multiple myeloma. The NCI-H460 large cell lung carcinoma was derived in 1982 from the pleural effusion fluid of a male patient with large cell lung cancer [1]. The human PC-3 prostate carcinoma line was developed from a bone metastasis of a grade IV prostate adenocarcinoma from a 62-year-old patient [26]. The PC-3 line is androgen independent, exhibits low acid phosphatase, and testosterone-5-alpha reductase activities. The MX-1 human mammary xenograft tumor was developed by Dr. A. A. Ovejera [41]. The original human xenograft was established from a 29-year-old female patient with no previous therapy and the tumor was diagnosed histologically as poorly differentiated infiltrating duct cell carcinoma.

Human tumor xenografts

The efficacy of vincristine, paclitaxel, docetaxel, and tasidotin was compared in human tumor xenograft studies. All procedures were carried out according to a protocol approved by the Institutional Animal Care and Use Committee in accordance with the Federal Animal Welfare Act (9 CFR, 1992) and were conducted in an AAALAC accredited facility. Prior to efficacy testing, the maximum tolerated dose (MTD) of each compound was determined by the method described by Corbett et al. [7]. SCID mice from Frederick Cancer Research and Development Center (Frederick, MD) or nu/nu mice from Harlan Sprague Dawley (Indianapolis, IN) were implanted subcutaneously with a 4-mg tumor fragment, and treatments were initiated when tumors reached 100–200 mm^3. At the time the tumor volumes reached 100–200 mm^3, the animals were pair-matched into treatment ($n = 10$) or control groups ($n = 10$).

For experiments with the human RL lymphoma, RPMI 8226 multiple myeloma, MX-1 breast carcinoma, PC-3 prostate carcinoma or NCI-H460 non-small cell lung carcinoma xenograft, vincristine (1 or 0.5 mg/kg) was administered by intravenous injection into a tail vein on alternate days for five injections, docetaxel (20 or 12 mg/kg) was administered by intravenous injection into a tail vein on alternate days for three injections, and paclitaxel (20 or 10 mg/kg) was administered by intravenous injection into a tail vein on alternate days for three injections. Tasidotin (120, 100, 80 or 40 mg/kg) was administered by intraperitoneal injection daily for 5 days.

Tumor volumes were calculated using the formula $(w^2 \times l)/2$ where 'w' is the width of the tumor and 'l' is the length of the tumor. Individual mice were weighed and tumor measurements taken by calipers twice weekly. The data are presented as mean tumor volume ± SEM. Mice were euthanized when the tumors reached a predetermined volume. Tumor growth delay was obtained by determining the difference between treatment and control group mean tumor growth in days at a predetermined tumor volume.

Results

The compounds vincristine, paclitaxel, docetaxel, and tasidotin are potent cytotoxic agents for both mouse, and human bone marrow CFU-GM (Fig. 1). Exposure to the compounds produced killing of cells in an exponential manner. The concentration response curves are shown in Fig. 2. For all compounds tested, concentrations killing 50 and 90% of the cells were achieved or nearly achieved. Human and mouse bone marrow CFU-GM IC_{50} concentrations were two- to threefold lower than the corresponding IC_{90} concentrations; thus the concentration response curves for these compounds are very steep, consistent with strong binding to a critical target in the cells (Table 1). Human bone marrow CFU-GM was more sensitive to the cytotoxicity of the compounds than was mouse bone marrow CFU-GM.

Vincristine was a more potent cytotoxic agent than paclitaxel or tasidotin toward human bone marrow CFU-GM and had the largest differential between cytotoxicity toward mouse bone marrow CFU-GM and human bone marrow CFU-GM. The ratios of the mouse and human bone marrow CFU-GM at the IC_{50} and IC_{90} concentrations were calculated (Table 1). For vincristine and tasidotin, the ratios were greater than or equal to 10 and for paclitaxel the ratios were two- and threefold.

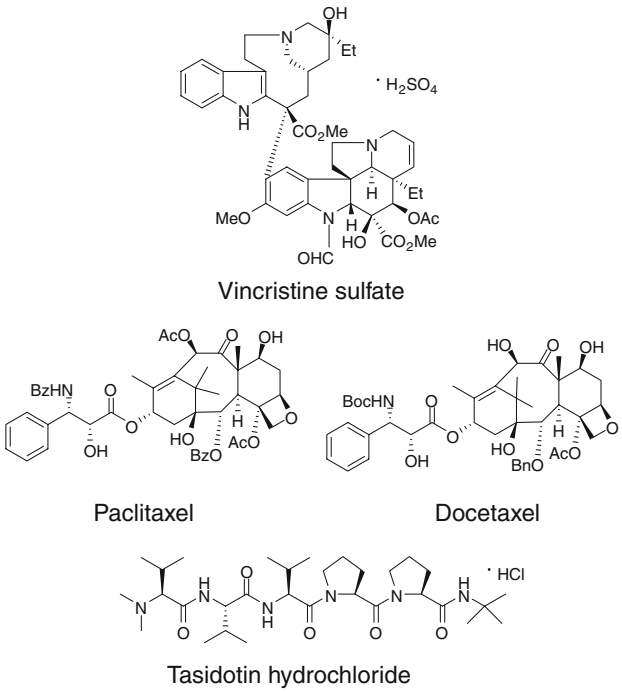

Fig. 1 Chemical structures of vincristine sulfate, paclitaxel, docetaxel and tasidotin hydrochloride are shown

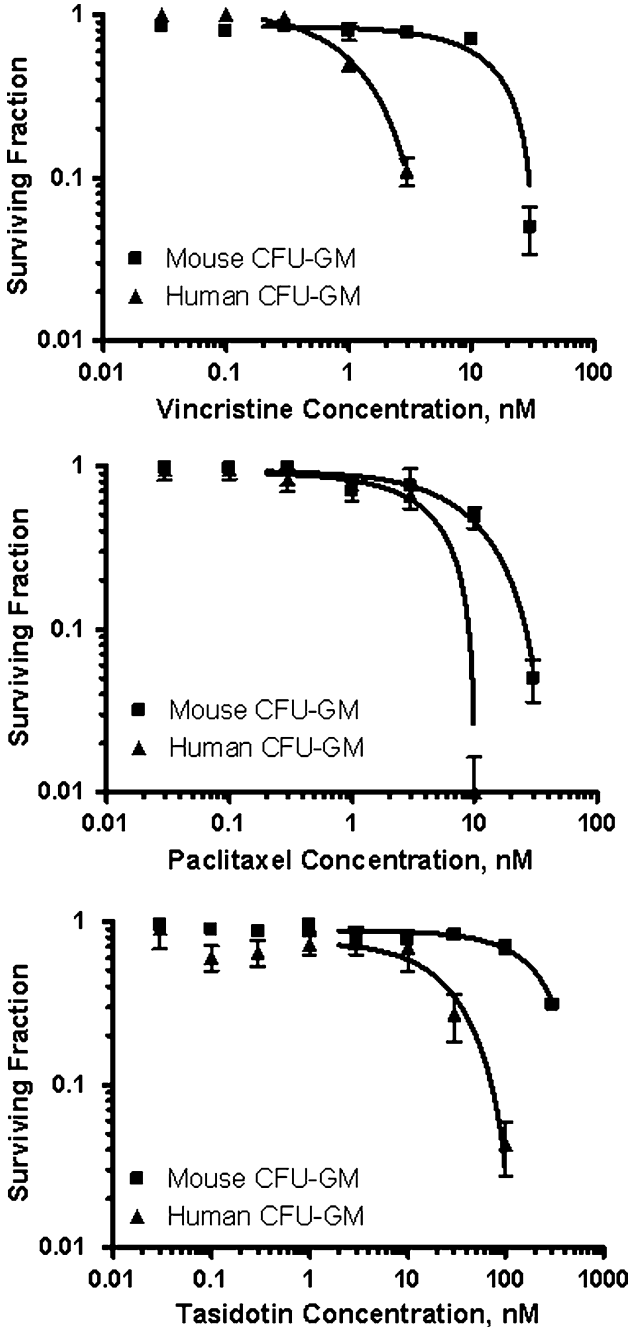

Fig. 2 Concentration response curves for each of three tubulin binders in mouse and human bone marrow CFU-GM. The data are the mean of two independent experiments; *bars* are SD

Table 1 Mouse and human bone marrow CFU-GM IC_{50} and IC_{90} values for three tubulin binders in nanomolar concentrations are listed

	Vincristine	Paclitaxel	Tasidotin
Mouse CFU-GM			
IC_{50} (nM)	12	11	220
IC_{90} (nM)	30	27	>300
Human CFU-GM			
IC_{50} (nM)	1.2	4.7	19
IC_{90} (nM)	3	9	65
Ratio mouse: human (IC_{50}, IC_{90})	Tenfold, tenfold	2.3-fold, threefold	11.6-fold, >4.6-fold

The values were derived from curves in Fig. 2. Ratios of mouse bone marrow CFU-GM to human bone marrow IC_{50} and IC_{90} are given

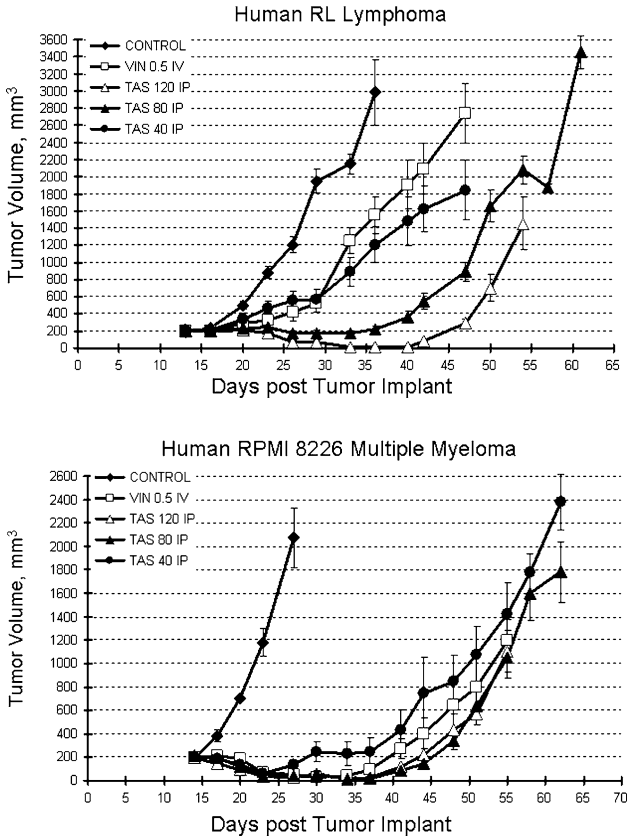

Fig. 3 Growth delay of subcutaneously implanted human RL lymphoma and RPMI 8226 multiple myeloma tumors after treatment with vincristine (0.5 mg/kg) intravenously on alternate days for five injections or tasidotin (120, 80 or 40 mg/kg) intraperitoneally daily for 5 days for 2 weeks. The *points* are mean for ten mice; *bars* are SEM

In vivo, a range of tasidotin doses (120, 80 and 40 mg/kg) were tested for efficacy in comparison with vincristine (0.5 mg/kg) in subcutaneously implanted human RL lymphoma (Fig. 3). Administration of vincristine at a dose of 0.5 mg/kg was well tolerated with no body weight loss. The vincristine treatment was minimally effective against RL lymphoma with a median tumor growth delay of 7 days (Table 2). Tumor growth delays produced by the dose range for tasidotin showed tumor response in a dose-dependent manner resulting in 27.5, 22.5, and 11 days for doses of 120, 80 and 40 mg/kg, respectively. Treatment with tasidotin was well tolerated with a maximum body weight loss of 4% at the two highest doses tested and no weight loss at the remaining doses (Table 2). A similar study was performed with the RPMI 8226 multiple myeloma (Fig. 3). Tasidotin was highly efficacious at each of the doses tested in the

Table 2 Mean percent maximal body weight loss for the treatment groups in in vivo experiments

Treatment	Body weight loss
Human RL lymphoma	
Vin 0.5 IV	None
Tas 120 IP	4.05%
Tas 80 IP	4.05%
Tas 40 IP	None
Human RPMI 8226 multiple myeloma	
Vin 0.5 IV	None
Tas 120 IP	4.0%
Tas 80 IP	None
Tas 40 IP	4.0%
Human MX-1 breast carcinoma	
Doce 12 IV	7.7%
Vincris 1 IV	23.9%
Tas 100 IP	None
Tas 50 IP	None
Pac 10 IV	4.0%
Human PC-3 prostate carcinoma	
Doce 20 IV	24.5%
Pac 20 IV	1.0%
Doce 12 IV	8.8%
Tas 100 IP	2.4%
Tas 50 IP	None
Tas 100/Doce 12	20.7%
Tas 50/Doce 12	14.3%
Human NCI-H460 MSCLC	
Doce 20 IC	24.5%
Pac 20 IC	10.1%
Doce 12 IC	17.4%
Tas 100 IP	4.2%
Tas 50 IP	2.8%
Tas 100/Doce 12	18.8%
Tas 50/Doce 12	13.8%

RPMI 8226 multiple myeloma with tumor growth delays of 33, 33, and 28 days for doses of 120, 80, and 40 mg/kg, respectively. Vincristine (0.5 mg/kg) was also a very effective therapy in this model producing a tumor growth delay of 31 days. Treatment with tasidotin was well tolerated with a maximum body weight loss of 4% at doses of 120 and 40 mg/kg and no weight loss at a dose of 80 mg/kg. Administration of vincristine was also well tolerated, with no observable body weight loss at the dose tested (Table 2).

In the human MX-1 breast carcinoma xenograft, docetaxel (12 mg/kg), and vincristine (1 mg/kg) administration each resulted in tumor growth delays of ≥50 days (Fig. 4). The marked effect seen in the docetaxel was not unexpected

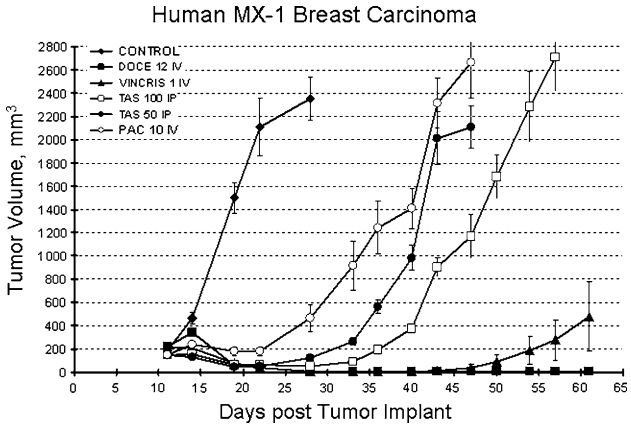

Fig. 4 Growth delay of subcutaneously implanted human MX-1 breast carcinoma tumors after treatment with vincristine (1 mg/kg) intravenously on alternate days for five injections, paclitaxel (10 mg/kg) intravenously once daily for five injections, or tasidotin (100 or 50 mg/kg) intraperitoneally daily for 5 days. The *points* are mean for ten mice; *bars* are SEM

in this model, as docetaxel is an FDA-approved treatment for breast cancer as a single agent. Paclitaxel (10 mg/kg) was less effective resulting in a tumor growth delay of 13.5 days in this tumor. Tasidotin had a dose-dependent effect with 26.5 and 21 days of tumor growth delay at doses of 100 and 50 mg/kg, respectively (Fig. 4). A dose of 12 mg/kg docetaxel produced a body weight loss of 7.7%, while a dose of 1 mg/kg vincristine produced severe body weight loss, reaching a maximum of 23.9%. Paclitaxel at 10 mg/kg resulted in a maximal body weight loss of 4.0%, and there were no body weight loss produced by tasidotin treatment at 100 or 50 mg/kg (Table 2).

The human PC-3 prostate carcinoma xenograft was also responsive to the tubulin binding agents (Fig. 5). In this model, docetaxel (20 mg/kg) at full dose produced a tumor growth delay >70 days while a dose of 12 mg/kg of docetaxel produced 28.5 days of tumor growth delay. A full dose of paclitaxel (20 mg/kg) resulted in 46.5 days of tumor growth delay. Tasidotin showed a dose-dependent effect on tumor growth delay resulting in 23 days and 17 days of tumor growth delay with doses of 100 and 50 mg/kg of tasidotin, respectively. Combination regimens including tasidotin and docetaxel were also tested. The combination of tasidotin (100 mg/kg) along with docetaxel (12 mg/kg) resulted in 41 days of tumor growth delay which was greater than the tumor response from docetaxel (12 mg/kg) alone while the combination of tasidotin (50 mg/kg) with docetaxel (12 mg/kg) resulted in 26 days of tumor growth delay which was not different from docetaxel (12 mg/kg) alone. Treatment with tasidotin was well tolerated with no body weight loss at the 50 mg/kg dose and 2.4% body weight loss at the 100 mg/kg dose. A maximal body weight loss of 8.8% occurred with docetaxel at 12 mg/kg. The group treated with a combination of

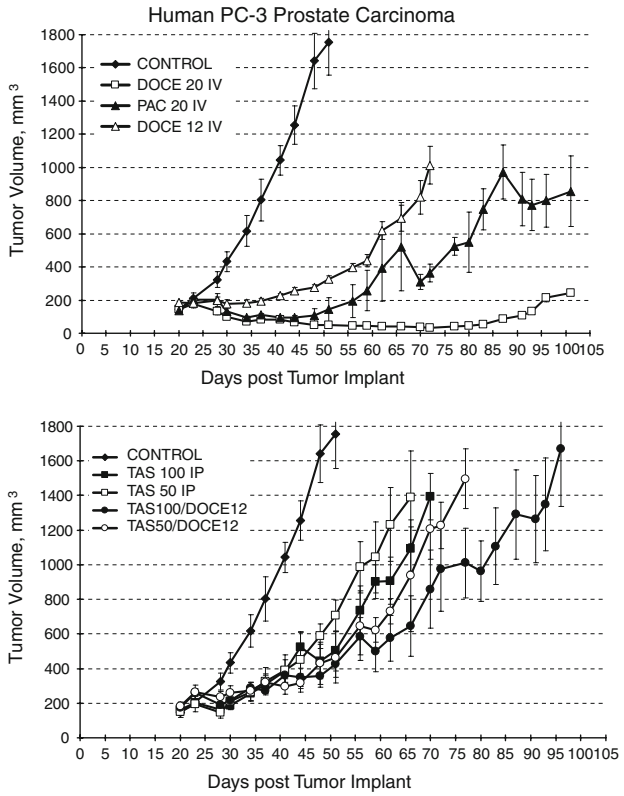

Fig. 5 Growth delay of subcutaneously implanted human PC-3 prostate carcinoma tumors after treatment with docetaxel (20 or 12 mg/kg) intravenously on alternate days for three injections, paclitaxel (20 mg/kg) intravenously alternate days for three injections, tasidotin (100 or 50 mg/kg) intraperitoneally daily for 5 days, or the combination of tasidotin (100 or 50 mg/kg) intraperitoneally daily for 5 days along with docetaxel (12 mg/kg) intravenously on alternate days for three injections. The *points* are mean for ten mice; *bars* are SEM

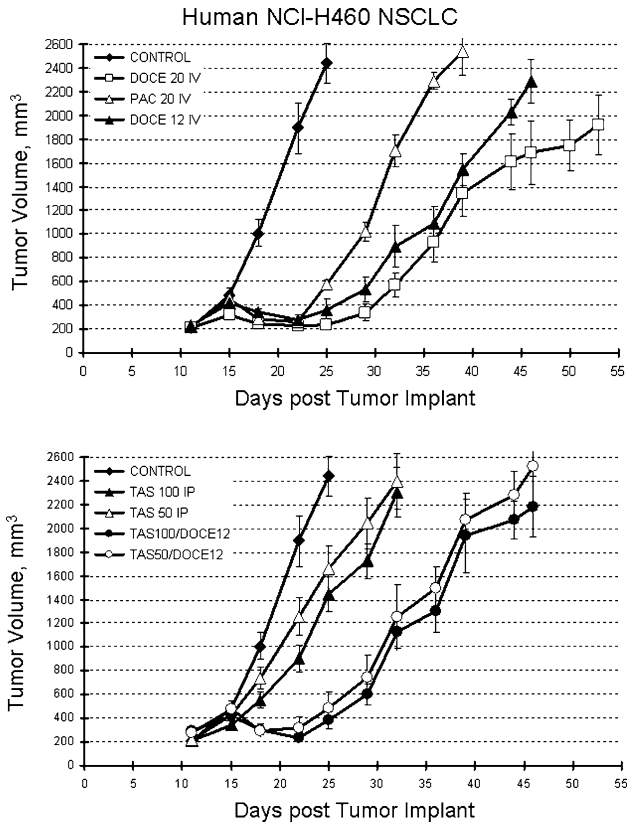

Fig. 6 Growth of subcutaneously implanted human NCI-H460 non-small cell lung carcinoma tumors after treatment with docetaxel (20 or 12 mg/kg) intravenously on alternate days for three injections, paclitaxel (20 mg/kg) intravenously alternate days for three injections, tasidotin (100 or 50 mg/kg) intraperitoneally daily for 5 days, or the combination of tasidotin (100 or 50 mg/kg) intraperitoneally daily for 5 days along with docetaxel (12 mg/kg) intravenously on alternate days for three injections. The *points* are mean for ten mice; *bars* are SEM

tasidotin and docetaxel had a maximal body weight loss of 14.3% for 50 mg/kg tasidotin plus 12 mg/kg docetaxel, and 20.7% for 100 mg/kg tasidotin plus 12 mg/kg docetaxel. The higher dose of docetaxel, 20 mg/kg, caused a body weight loss of 24.5%. The paclitaxel dose of 20 mg/kg was well tolerated, with a body weight loss of 1.0% (Table 2).

The human NCI-H460 non-small cell lung carcinoma was less responsive to the tubulin binders than were the other human tumor xenografts tested. A full dose regimen of docetaxel (20 mg/kg) produced 23 days of tumor growth delay in the NCI-H460 lung while the lower dose regimen of 12 mg/kg resulted in 18.5 days of tumor growth delay in this tumor (Fig. 6). The NCI-H460 non-small cell lung carcinoma was also less responsive to tasidotin with 7 and 4 days of tumor growth delay resulting from administration of 100 and 50 mg/kg of tasidotin, respectively. Combination regimens were also tested in the NCI-H460 tumor model. The combinations of tasidotin (100 or 50 mg/kg) along with docetaxel (12 mg/kg) resulted in tumor responses that were not different from docetaxel (12 mg/kg) alone. Tasidotin was well tolerated in this model, with a maximal body weight loss of 4.2% at the 100-mg/kg dose and 2.8% at the 50-mg/kg dose. Severe body weight loss was exhibited in the mice receiving docetaxel, with a maximal body weight loss of 24.5% at the 20-mg/kg dose and 17.4% at the 12-mg/kg dose. Tasidotin did not increase the body weight loss when used in combination with docetaxel. A maximal body weight loss of 10.1% occurred with paclitaxel treatment at 20 mg/kg (Table 2).

Discussion

Bone marrow granulocyte-macrophage-colony forming unit (CFU-GM) assays comparing the sensitivity of bone marrows across species have been useful in predicting the blood levels of agents that might be tolerated in patients compared with blood levels in preclinical efficacy and safety species [43]. For many cytotoxic agents, the bone

marrow of mice is less sensitive than is human bone marrow, thus allowing blood levels to be achieved in preclinical efficacy testing that cannot be reached in patients. The toxicity of three tubulin binding agents was examined for murine and human bone marrow progenitor cells (Table 1) [11, 30, 34, 43]. The differential sensitivity between the cells from these two species to the tubulin binders may explain, in part, the difference in effects of these compounds in patients compared to preclinical studies. The corollary is that compounds with smaller or no differential in bone marrow progenitor sensitivity amongst species may have a better potential for reaching similar blood levels in patients as in mice. If bone marrow toxicity is dose-limiting in patients, compounds with smaller differences in bone marrow toxicity would more likely be successful in reaching therapeutic doses. Paclitaxel has the smallest difference in the IC_{90} value between human and mouse bone marrow and has been a very useful clinical antitumor agent for the treatment of many tumor types [13, 33, 55]. Pessina et al. [43] reported paclitaxel IC_{50} and IC_{90} values in the mouse of 0.004 and 0.007 μg/ml which compare very well with the values from the current study of 0.009 and 0.023 μg/ml, respectively. Similarly, Pessina et al. [43] reported paclitaxel IC_{50} and IC_{90} values for human bone marrow CFU-GM of 0.0039 and 0.00061 μg/ml which compare favorably with the values from the current study of 0.0039 and 0.0076 μg/ml. Pessina et al. [43] went further to suggest that through use of the ratio between mouse and human CFU-GM IC_{90} values as well as the maximum tolerated dose of the compound in mice, the maximum tolerated dose of the compound in patients could be predicted and a therapeutic blood level in patients estimated.

Each of the tubulin-binding agents studied has been used in the clinic; thus, the normal tissue adverse reactions upon administration of each of these agents to patients are known. The most frequent serious adverse event upon treatment with vincristine is the development of neurologic side effects. First, sensory impairment and parasthesia may occur. As treatment continues, neurotic pain and motor difficulties develop. Some neuromuscular complications may persist for prolonged periods post therapy in some patients [36, 57, 58]. Serious bone marrow depletion is usually not a major dose-limiting toxicity with vincristine treatment. However, leukopenia, anemia, and thrombocytopenia have been reported. Peripheral neuropathy may develop in patients receiving escalating doses of vincristine. On the other hand, 90% of patients treated with paclitaxel also develop some hematologic toxicity including neutropenia, leucopenia, and anemia [13, 33, 55]. Serious neutropenia (ANC < 500/mm3; grade 4) occurs in about 52% of patients and anemia in about 78% of patients receiving paclitaxel Symptoms of peripheral neuropathy are seen in 60% of patients as well as myalgia and arthralgia. About 52% of patients treated with paclitaxel also experience gastrointestinal distress [4, 12]. Docetaxel administration produces neutropenia in essentially all patients treated and grade 4 neutropenia occurs in about 80% of patients. Virtually all patients treated with docetaxel also experience leucopenia and anemia caused by the drug. About 50% of patients treated with docetaxel experience fluid retention. Severe neurosensory symptoms (paresthesia, dysesthesia, pain) were observed in about 5% patients treated with docetaxel. The major dose limiting toxicities associated with administration of tasidotin to patients are hematologic, especially neutropenia and thrombocytopenia [37]. Severe neutropenia occurred in 44% of patients receiving tasidotin. Dose-related non-hematologic toxicities were mainly gastrointestinal including nausea, vomiting, and diarrhea; fatigue also occurred frequently [37]. The principal toxicity of tasidotin is neutropenia that is similar to other clinical agents which interfere with microtubule dynamics [9, 10, 27, 37]. Interestingly, neurotoxicity, which occurs often with the taxanes, vinca alkaloids, and epothilones, was not observed in the early clinical trials of tasidotin [27, 32].

Several pharmacokinetic parameters for the tubulin inhibitors in patients and mice are compared in Table 3 [6, 17, 21, 22, 37, 50, 56]. Overall, the concentration needed to produce the same level of tasidotin-induced bone marrow CFU-GM killing in mouse bone marrow compared with human bone marrow is greater than eightfold. The mouse dose of 120 mg/kg (360 mg/m^2) is 7.7-fold higher than the recommended Phase II dose for tasidotin in patients of 46.8 mg/m^2 in very good agreement with the CFU-GM findings. At these doses, the C_{max} and AUC for tasidotin in mice is about 20-fold greater than the C_{max} and AUC in humans (Table 3) [6, 37]. The concentration of paclitaxel required to produce the same killing of mouse and human bone marrow CFU-GM differs by two- to threefold and is the most similar among the group of agents tested. The single mouse dose of paclitaxel is 20 mg/kg. This dose and the doses of the comparator compounds were determined in prior MTD experiments in the mouse [7]. By linear extrapolation of the mouse paclitaxel AUC data on Table 3, the AUC for a 20-mg/kg dose of paclitaxel in the mouse would be 13,070 ng × h/ml, which is within the range of the human AUC for 175 mg/m^2 of paclitaxel administered as a 3-h infusion [21, 22]. The MTD for a single dose of vincristine in mice is 4–5 mg/kg. By linear extrapolation from the vincristine AUC data on Table 3, 4–5 mg/kg of vincristine would produce an AUC of 3,360–4,200 ng × h/ml, which is seven- to ninefold greater than the AUC for patients treated with 2 mg/m^2 of vincristine. [17, 50, 56]. As with many anticancer agents, the resilience of the mouse to tasidotin and vincristine allows much greater exposure of

Table 3 Human and mouse pharmacokinetic parameters for the tubulin binding agents are listed

Species	Tasidotin dose	C_{max} (ng/ml)	AUC $_{(0-\infty)}$(ng × h/ml)	CL	V_{dss}
Human	46.8 mg/m^2 (1.23 mg/kg)	3,619 (1,565–5,459)	2,797 (1,370–4,049)	16.7 (11.6–34.2) L/h/m^2	9.9 (5.3–22.7) L/m^2
Mouse	120 mg/kg (360 mg/m^2)	72,766 (62,631–82,901)	45,217 (41,483–48,950)	44.5 (41–48) ml/min/kg	1,959 (1,708–2,209) ml/kg
	Paclitaxel dose				
Human	175 mg/m^2 (5 mg/kg) 3 h infusion	3,650 (2,393–5,060)	15,007 (9,552–24,634)	12.7(9.7–16.4) L/h/m^2	99 (45–199) L/m^2
Mouse	4 mg/kg (60 mg/m^2)	1,767	2,614		
	Vincristine dose				
Human	2 mg/m^2 (0.053 mg/kg)	277	464 (273–562)	5 (3.9–7.9) L/h/m^2	124 (80–290) L/m^2
Mouse	2 mg/kg (6 mg/m2)	140	1,680	80 (69–91) ml/min/kg	19,193 (15,168–23,218) ml/kg

Tasidotin data are from Bonate et al. [6] Mita et al. [37]. Paclitaxel data are from Huizing et al. [22] and Hosten et al. [21]. Vincristine data are from Sethi and Kimball [50], Webb et al. [56] and Guo et al. [17]

implanted human tumors to the drug molecule in the murine host than in the human patient.

All of the tubulin-binding agents were active and effective anticancer drugs in the human tumor xenografts examined. Vincristine was a very effective anticancer agent in the RPMI 8226 human multiple myeloma and the MX-1 human breast carcinoma models. The taxanes, paclitaxel, and docetaxel especially when administered at full dose, were very effective anticancer agents for treatment of the solid tumors, MX-1 breast carcinoma, PC-3 prostate carcinoma, and NCI-H460 non-small cell lung carcinoma. Like vincristine, tasidotin was most effective in the RPMI 8226 multiple myeloma and MX-1 breast carcinoma models. The NCI-H460 non-small cell lung carcinoma was not responsive to tasidotin. Interestingly, co-administration of tasidotin and docetaxel to mice bearing the PC-3 prostate carcinoma or NCI-H460 non-small cell lung carcinoma did not increase the tumor response compared with the response to the taxane alone. In the xenograft experiments, administration of tasidotin resulted in lower body weight losses than did docetaxel.

Because of the mouse's greater MTD compared to humans, the compound doses found to be efficacious in the mouse may be difficult to achieve in the human. The ratio of human and mouse bone marrow CFU-GM is a useful parameter along with preclinical mouse safety and efficacy results in the translation to the clinic. Pessina et al. [43] has validated a model utilizing the ratio between mouse and human CFU-GM IC_{90} values as well as the maximum tolerated dose of the compound in mice to accurately predict the maximum tolerated dose of the compound in patients and estimate therapeutic blood levels. The mouse tends to be more tolerant to xenobiotics than are humans; however, through the application of translational techniques, more accurate interpretation of preclinical tumor models may be achieved in the process of the development of new drugs [16, 44].

Acknowledgments We thank Dr. Dennis Lovett and Dr. William E. Bauta for the rendering of the chemical structures in this manuscript.

References

1. Banks-Schlegel SP, Gazdar AF, Harris CC (1985) Intermediate filament and cross-linked envelope expression in human lung tumor cell lines. Cancer Res 45:1187–1197
2. Beckwith M, Longo DL, O'Connell CD et al (1990) Phorbol ester-induced cell cycle-specific growth inhibition of human B-lymphoma cell lines. J Natl Cancer Inst 82:501–509
3. Bennouna J, Delord JP, Campone M et al (2008) Vinflunine: a new microtubule inhibitor agent. Clin Cancer Res 14:1625–1632
4. Bhagra A, Rao RD (2007) Chemotherapy-induced neuropathy. Curr Oncol Rep 9:290–299
5. Bhat KMR, Setaluri V (2007) Microtubule-associated proteins as targets in cancer chemotherapy. Clin Cancer Res 13:2849–2854
6. Bonate PL, Beyerlein D, Crawford J et al (2007) Pharmacokinetics in mice with xenografted tumors after intravenous administration of tasidotin (ILX651) or its carboxylate metabolite. AAPS J 9:E378–E387
7. Corbett TH, Polin L, Roberts BJ (2002) Transplantable syngeneic rodent tumors. In: Teicher BA et al (eds) Tumor models in cancer research. Humana Press, Totowa, pp 41–71
8. Cortes J, Baselga J (2007) Targeting the microtubules in breast cancer beyond taxanes: the epothilones. Oncologist 12:271–280
9. Cunningham C, Appleman LJ, Kirvan-Visovatti M et al (2005) Phase I and pharmacokinetic study of the dolastatin-15 analog tasidotin (ILX651) administered intravenously on days 1, 3 and 5 every 3 weeks in patients with advanced solid tumors. Clin Cancer Res 11:7825–7833
10. Ebbinghaus S, Rubin E, Hersh E et al (2005) A phase I study of the dolastatin-15 analog tasidotin (ILX651) administered intravenously daily for 5 consecutive days every 3 weeks in patients with advanced solid tumors. Clin Cancer Res 11:7807–7816
11. Erickson-Miller C (1997) Differential toxicity of camptothecin, topotecan and 9-aminocamptothecin to human, canine, and murine myeloid progenitors (CFU-GM) in vitro. Cancer Chemother Pharmacol 39:467–472
12. Ferguson T, Wilcken N, Vagg R et al (2007) Taxanes for adjuvant treatment of early breast cancer. Cochrane Database Syst Rev 4:CD004421
13. Ferlini C, Gallo D, Scambia G (2008) New taxanes in development. Exp Opin Invest Drugs 17:335–347

14. Garg V, Zhang W, Gidwani P et al (2007) Preclinical analysis of tasidotin HCl in Ewing's sarcoma, rhabdomyosarcoma, synovial sarcoma and osteosarcoma. Clin Cancer Res 13:5446–5454
15. Goodin S, Kane MP, Rubin EH (2004) Epothilones: mechanism of action and biologic activity. J Clin Oncol 22:2015–2025
16. Graham C, Tucker C, Creech J et al (2006) Evaluation of the antitumor efficacy, pharmacokinetics and pharmacodynamics of the histone deacetylase inhibitor depsipeptide in childhood cancer models in vivo. Clin Cancer Res 12:223–234
17. Guo P, Wang X, Zhou F, Gallo JM (2004) Determination of vincristine in mouse plasma and brain tissues by liquid chromatography–electrospray mass spectrometry. J Chromatogr B 809:273–278
18. Henderson IC, Bhatia V (2007) Nab-paclitaxel for breast cancer: a new formulation with an improved safety profile and greater efficacy. Exp Rev Anticancer Ther 7:919–943
19. Hinnen P, Eskens FA (2007) Vascular disrupting agents in clinical development. Brit J Cancer 96:1159–1165
20. Horwitz SB, Cohen D, Rao S et al (1993) Taxol: mechanisms of action and resistance. J Natl Cancer Inst Monogr 15:55–61
21. Hosten B, Abbara C, Petit B et al (2008) Effect of interleukin-2 pretreatment on paclitaxel absorption and tissue disposition after oral and intravenous administration in mice. Drug Metab Dispos 36:1729–1735
22. Huizing MT, Keung ACF, Rosing H et al (1993) Pharmacokinetics of paclitaxel and metabolites in a randomized comparative study in platinum-pretreated ovarian cancer patients. J Clin Oncol 11:2127–2135
23. Johnson IS, Armstrong JG, Gorman M et al (1967) The vinca alkaloids: a new class of oncolytic agents. Cancer Res 23:1390–1427
24. Jordan MA, Thrower D, Wilson L (1991) Mechanism of inhibition of cell proliferation by Vinca alkaloids. Cancer Res 51:2212–2222
25. Jordan MA, Wilson L (2004) Microtubules as a target for anticancer drugs. Nat Rev Cancer 4:253–265
26. Kaighn ME, Narayan KS, Ohnuki Y et al (1979) Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Investig Urol 17:16–23
27. Kaur KK, Verschraegen C (2005) Tasidotin HCl. Curr Opin Invest Drugs 6:631–638
28. Kruczynski A, Barret JM, Etievant C et al (1998) Antimitotic and tubulin-interacting properties of vinflunine, a novel fluorinated vinca alkaloid. Biochem Pharmacol 55:635–648
29. Kruczynski A, Etievant C, Perrin D et al (2002) Characterization of cell death induced by vinflunine, the most recent Vinca alkaloid in clinical development. Br J Cancer 86:143–150
30. Kummar S, Gutierrez M, Doroshow JH et al (2006) Drug development in oncology: classical cytotoxics and molecularly targeted agents. Brit J Clin Pharmacol 62:15–26
31. Kurtzberg LS, Battle T, Rouleau C et al (2008) Bone marrow and tumor cell CFU and human tumor xenograft efficacy of non-camptothecin and camptothecin topoisomerase I inhibitors. Mol Cancer Ther 7(10):3212–3222
32. Lee JJ, Swain SM (2008) The epothilones: translating from the laboratory to the clinic. Clin Cancer Res 14:1618–1624
33. Markman M (2008) Taxanes in the management of gynecologic malignancies. Exp Rev Anticancer Ther 8:219–226
34. Masubuchi N (2004) A predictive model of human myelotoxicity using five camptothecin derivatives and the in vitro colony-forming unit granulocyte/macrophage assay. Clin Cancer Res 10:6722–6731
35. Matsuoka Y, Moore GE, Yagi Y et al (1967) Production of free light chains of immunoglobulin by a haematopoietic cell line derived from a patient with multiple myeloma. Proc Soc Exp Biol Med 125:1246–1250
36. Mihelic R, Kaufman J, Lonial S (2007) Maintenance therapy in lymphoma. Clin Lymphoma Myeloma 7:507–513
37. Mita AC, Hammond LA, Bonate PL et al (2006) Phase I and pharmacokinetic study of tasidotin hydrochloride (ILX651), a third generation dolastatin-15 analog, administered weekly for 3 weeks every 28 days in patients with advanced solid tumors. Clin Cancer Res 12:5207–5215
38. Nettles JH, Li H, Cornett B (2004) The binding mode of epothilone A on alpha, beta-tubulin by electron crystallography. Science 305:866–869
39. Ngan VK, Bellman K, Hill BT et al (2001) Mechanism of mitotic block and inhibition of cell proliferation by the semisynthetic Vinca alkaloids vinorelbine and its newer derivative vinflunine. Mol Pharmacol 60:225–232
40. Ngan VK, Bellman K, Panda D et al (2000) Novel actions of the antitumor drugs vinflunine and vinorelbine on microtubules. Cancer Res 60:5045–5051
41. Ovejera AA, Houchens DP (1981) Human tumor xenografts in athymic nude mice as a preclinical screen for anticancer agents. Sem Oncol 8:386–393
42. Perez EA (2007) Novel enhanced delivery taxanes: an update. Semin Oncol 34:Suppl 1–5
43. Pessina A (2003) Application of the CFU-GM assay to predict acute drug-induced neutropenia: an international blind trial to validate a prediction model for the maximum tolerated dose (MTD) of myelosuppressive xenobiotics. Toxicol Sci 75:355–367
44. Peterson JK, Houghton PJ (2004) Integrating pharmacology and in vivo cancer models in preclinical and clinical drug development. Eur J Cancer 40:837–844
45. Poncet J (1999) The dolastatins, a family of promising antineoplastic agents. Curr Pharm Des 5:139–162
46. Pourroy B, Carre M, Honore S et al (2004) Low concentrations of vinflunine induce apoptosis in human SK-N-SH neuroblastoma cells through a post mitotic G1 arrest and a mitochondrial pathway. Mol Pharmacol 66:580–591
47. Ray A, Okouneva T, Manna T (2007) Mechanism of action of the microtubule-targeted antimitotic depsipeptide tasidotin (formerly ILX651) and its major metabolite tasidotin C-carboxylate. Cancer Res 67:3767–3776
48. Ringel I, Horwitz SB (1991) Studies with RP56967 (Taxotere): a semisynthetic analog of taxol. J Natl Cancer Inst 83:288–291
49. Rowinsky EK, Donehower RC (1996) Antimicrotubule agents. In: Chabner BA, Longo DL (eds) Cancer chemotherapy and biotherapy. Lippincott-Raven, New York, pp 263–296
50. Sethi VS, Kimball JC (1981) Pharmacokinetics of vincristine sulfate in children. Cancer Chemother Pharmacol 6:111–115
51. Simoens C, Vermorken JB, Korst AEC et al (2006) Cell cycle effects of vinflunine, the most promising Vinca alkaloid and its interaction with radiation in vitro. Cancer Chemother Pharmacol 58:210–218
52. Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260:214–218
53. Stinchcombe TE (2007) Nanoparticle albumin-bound paclitaxel: a novel cremophor-EL-free formulation of paclitaxel. Nanomedicine 2:415–423
54. Teicher BA (2008) Newer cytotoxic agents: attacking cancer broadly. Clin Cancer Res 14:1610–1617
55. Tusquets I, Estevez LG, Alvarez I et al (2007) The use of taxanes in the neoadjuvant treatment of breast cancer: a review of randomized phase II/III trials. Clin Breast Cancer 7:764–774
56. Webb MS, Logan P, Kanter PM et al (1998) Preclinical pharmacology, toxicology and efficacy of sphingomyelin/cholesterol liposomal vincristine for therapeutic treatment of cancer. Cancer Chemother Pharmacol 42:461–470
57. Zelnak AB (2007) Clinical pharmacology and use of microtubule targeting agents in cancer therapy. Methods Mol Med 137:209–234
58. Zwick C, Gleissner B, Pfreundschuh M (2007) Aspects of chemotherapy schedules in young and elderly patients with aggressive lymphoma. Clin Lymphoma Myeloma 8(suppl 2):S43–S49