were scattered within the nuclear region. Strikingly, BAL27862 abrogated both vinblastine- and colchicine-induced aster formation (assessed by IF) and disaggregated paclitaxel- and epothilone B-stabilized MTs (assessed by IB). MT destabilization occurred in isolated human peripheral blood mononucleocytes treated with BAL27862 *ex vivo*, suggesting a potential for a blood-based pharmacodynamic assay. Following a single 1 h pulse treatment, BAL27862 inhibited the formation of endothelial cell (HUVEC) tubular structures (maximal at 100 nM), while disrupting established tubules at 30 nM. Compared with its anti-proliferative activity against HUVECs, the theoretical 'therapeutic index' for vascular disruption activity (VDA) *in vitro* was 18−25; higher than observed for combretastatin A-4 (index: 6−7), an agent with known VDA.
Conclusions: BAL27862 is a new tubulin-interacting agent with an apparently novel mechanism of action. A broad antitumor activity, also in drug resistant tumor models, and potential vascular disruption activity strongly support further development of BAL27862 as a novel anticancer agent with a possibility for both i.v. and p.o. administration.

445 POSTER

Class III beta-tubulin overexpression in non-small cell lung, breast and prostate carcinoma xenografts confers innate or acquired resistance to taxanes and sensitivity to ixabepilone

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Background: Taxane type microtubule (MT) inhibitors are active agents but their therapeutic benefits are limited by multifactorial drug resistance. Substantial recent evidence suggests that suboptimal clinical response to taxanes in a variety of tumor types may be related to overexpression of class III beta-tubulin (TUBB3). Compared to other tubulin isoforms, TUBB3 has a lower affinity for paclitaxel (PTX) and is less susceptible to PTX-induced disruption of MT dynamics, the main mode of action of taxanes. Ixabepilone (IXA), an analog of epothilone B is the first of a new class of MT inhibitors designed to have reduced susceptibility to multiple mechanisms of drug resistance including MDR1, BCRP, MRP1 and tubulin mutation. In contrast to PTX, IXA is effective in disrupting the dynamicity of purified TUBB3 in vitro. We tested if IXA retains efficacy in a broad spectrum of TUBB3 overexpressing tumors, and whether TUBB3 overexpression can be induced during the development of acquired resistance to a taxane in vivo.
Methods: TUBB3 expression was determined by Western blot and immunohistochemistry with a TUBB3 specific antibody. Sensitivity to docetaxel (DTX), IXA and vinorelbine (VRB) was determined in mice administered each agent at its maximum tolerated dose (MTD). Sensitivity is defined as tumor response $\geqslant$1 log cell kill (LCK). Acquired resistance to DTX was developed over the course of 2 years (7 treatment courses) in the CWR22 prostate cancer xenograft by repeat cycles of treatment and re-transplantation of a regrown tumor at each relapse.
Results: All 5 tumors overexpressing TUBB3 were resistant to DTX and VRB, yielding activity ranging 0.2–0.9 and 0.1–0.3 LCK, respectively. IXA was active in all 5 tumors, yielding 1.6–4.2 LCK (Table 1) at its MTD. The parent CWR22 has equal sensitivity to DTX and IXA. Of clinical relevance, high TUBB3 staining was observed in breast cancer samples from taxane-resistant patients enrolled in a phase III clinical trials of IXA.
Conclusion: IXA exhibits reduced susceptibility to multiple drug resistance mechanisms and has robust activity in tumors overexpressing TUBB3. A randomized trial of IXA- versus PTX-containing regimens in NSCLC patients is planned.

Efficacy of IXA, DTX and VRB in 6 human tumors including 5 overexpressing TUBB3

Tumor	Histology	Activity (LCK)		
		IXA	DTX	VRB
H1155	NSCLC	4.2	0.2	0.1
DU4475	Breast	2.6	0.9	0.2
Pat-21	Breast	1.6	0.3	0.3
LX-1	NSCLC	2.6	0.5	0.1
CWR22/R	Prostate	1.6	0.7	ND
CWR22	Prostate	1.5	1.8	ND

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A phase I study of eribulin mesylate (E7389) in patients with refractory cancers

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Background: Eribulin mesylate (E7389), a structurally simplified, synthetic analogue of halichondrin B, shows anticancer activity against various types of tumors by inhibiting microtubule dynamics. A phase I study of eribulin was conducted to determine a recommended phase II dose and to assess safety and pharmacokinetics.
Methods: Patients with advanced solid cancers were enrolled. Eribulin mesylate was administered intravenously over 5 minutes on days 1 and 8 every 21 days. Cohorts of three patients were treated at 0.7, 1.0, 1.4 and 2.0 mg/m^2. Tumor measurements were performed at baseline and every 6 weeks. Pharmacokinetics were investigated on days 1 and 8 of the first cycle.
Results: A total of 15 patients with various cancers were treated (3, 3, 6 and 3 patients at 0.7, 1.0, 1.4 and 2.0 mg/m^2, respectively). The number of cycles ranged from 1 to 15, and 7 patients received >4 cycles. Dose-limiting toxicities were observed in 2 of 6 patients treated at 1.4 mg/m^2, and in all 3 patients at 2.0 mg/m^2. Of these patients, one patient treated at 1.4 mg/m^2 experienced grade 4 neutropenia for 5 days, and the other patient had febrile neutropenia and skipped day 8 administration. At 2.0 mg/m^2 one patient each developed either grade 4 neutropenia lasting 5 days or febrile neutropenia; neither patient received the day 8 administration. Administration on day 8 was also skipped in the third patient at 2.0 mg/m^2. Thus, administration on day 8 was omitted in 1 of 6 patients at 1.4 mg/m^2 and in all 3 patients at 2.0 mg/m^2. All omissions were because of grade 3 neutropenia on day 8. Other frequently observed non-hematological toxicities included fatigue, alopecia, nausea, anorexia, neuropathy, liver enzyme elevations, hyperglycemia, and increased CRP levels. However, these were generally mild, and grade 3 toxicities were fatigue (2 patients) and elevation of γ-glutamyltransferase (1 patient). No differences were observed between day 1 and day 8 in pharmacokinetic profiles. The systemic clearance on day 1 was 1.50–2.69 L/hr/m^2, and the volume of distribution was 93.4–106.8 L/m^2. Partial responses were achieved in 3 patients (two with non-small cell lung cancer and one with head & neck cancer) at 1.4 mg/m^2. Stable disease >12 weeks was observed in 3 patients (two with breast and one with cervical cancer).
Conclusions: The main toxicity of eribulin mesylate is neutropenia and easily managed. A dose of 1.4 mg/m^2 administered on days 1 and 8 every 3 weeks is recommended for phase II studies. Major responses observed warranted further clinical study.

447 POSTER

ARRY-520, a KSP inhibitor with potent in vitro and in vivo efficacy and pharmacodynamic activity in models of multiple myeloma

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Background: Kinesin spindle protein (KSP) plays a key role in spindle pole separation and production of the bipolar spindle. Inhibition of KSP causes cells to arrest at the prophase-metaphase transition with formation of monopolar spindles. Maintenance of this arrest leads to cell death. The KSP inhibitor ARRY-520 is currently in phase I testing for solid tumors and acute myeloid leukemia. We report here the characterization of the *in vitro* and *in vivo* activity of ARRY-520 in preclinical models of multiple myeloma.
Materials and Methods: The *in vitro* antiproliferative activity of ARRY-520 was determined using logarithmically growing cells. *In vivo* antitumor activity was determined using human multiple myeloma xenografts grown subcutaneously in SCID-beige mice. *In vivo* pharmacodynamic activity (accumulation of monopolar spindles and apoptotic cells) was evaluated by immunohistochemical analysis of tumor xenograft tissue harvested from mice after treatment with ARRY-520.
Results: ARRY-520 inhibited proliferation and induced mitotic arrest and apoptosis in the human multiple myeloma cell lines RPMI8226, JJN3 and H929, with EC_{50}s for proliferation of 1.5–2.5 nM. *In vivo*, treatment of mice bearing established subcutaneous tumors with the compound at 20 mg/kg IP, q4dx3 caused significant regression, including a 100%

complete response rate for RPMI8226 tumors and a 50% complete response rate for H929 tumors, with some complete responses lasting for several weeks after the final dose of compound. Regressions of 70−90% of tumor volume were observed after treatment of mice bearing JJN3 tumors as large as 2.5 cm^3. 100% of RPMI8226 tumors had significant regression after a single dose of the compound. ARRY-520 was also active against tumors that had progressed on conventional therapy: RPMI8226 and JJN3 tumors that had progressed during treatment with revlimid or bortezomib regressed following treatment with ARRY-520, including regression of 85–95% of tumor volume for JJN3 tumors that had progressed in mice treated with revlimid. *In vivo* activity of ARRY-520 correlated with accumulation of monopolar spindles and apoptotic cells in xenografts taken from treated mice.
Conclusions: ARRY-520 exhibited potent *in vitro* and *in vivo* activity in preclinical models of multiple myeloma. *In vivo* antitumor activity correlated with the pharmacodynamic activity of the compound. A study of ARRY-520 in resistant / refractory multiple myeloma is planned.

448 POSTER

NKTR-105, a novel PEGylated-docetaxel, demonstrates superior anti-tumor activity compared to docetaxel in human non-small cell lung and colon cancer xenografts

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Background: NKTR-105 is a novel polyethylene glycol (PEG) conjugate of docetaxel, a widely used antineoplastic agent approved for the treatment of breast, NSCLC, prostate, gastric and head and neck cancers. NKTR-105 was created using Nektar's innovative small molecule PEGylation technology and prior experience with NKTR-102 (PEG-irinotecan) in nonclinical studies demonstrates that adding a PEG moiety to anti-tumor drugs leads to an increase in exposure which is associated with improved anti-tumor activity. The purpose of these studies was to evaluate the anti-tumor activity of NKTR-105 in mouse xenograft models of human non-small cell lung (H460) and colon (LS174T, LoVo) tumors exhibiting degrees of docetaxel sensitivity.
Materials and Methods: H460, LS174T and LoVo tumors were established in female, athymic nude mice. Groups of 10 mice received NKTR-105 or docetaxel administered every 7 days x 3 at several doses up to maximum tolerated dose (MTD). Doses of NKTR-105 are expressed as docetaxel-equivalent doses. Control groups received no treatment after tumor implant. Animals were weighed and tumor volumes measured twice weekly after the first drug injection. Activity was assessed by tumor growth delay (TGD) and number of regressions.
Results: NKTR-105 treatment resulted in a significantly greater TGD than docetaxel ($p < 0.05$) in H460 tumor bearing mice at MTD (33 mg/kg NKTR-105, 25 mg/kg docetaxel) with % TGD of 122% and 48%. TGD with NKTR-105 treatment in LS174T tumor bearing mice was dose related, with three partial regressions at MTD; no partial regressions were observed with docetaxel. In this model, treatment with NKTR-105 produced a significantly greater ($p < 0.0001$) TGD than docetaxel; 266% vs. 166% at MTD (40 mg/kg NKTR-105, 30 mg/kg docetaxel). Greater TGD was also observed in LoVo tumor bearing mice at the MTD of 30 mg/kg: 128% for NKTR-105 vs. 64% for docetaxel. Body weight changes for NKTR-105 treatment were similar to those for docetaxel at respective MTDs in all xenograft models.
Conclusions: NKTR-105 demonstrates significantly greater anti-tumor activity than docetaxel in H460 and LS174T mouse xenograft models. At MTD, the % TGD for NKTR-105 was 2.5-, 2-, and 1.6-fold greater than docetaxel in H460, LoVo, and LS174T xenograft models, respectively. Tumor regressions were seen in LS174T with NKTR-105 but not with docetaxel. NKTR-105 is a promising oncolytic drug candidate that warrants further study in clinical trials.

449 POSTER

In vivo evaluation of ALB 109564, a novel tubulin inhibitor with improved efficacy over existing members of the Vinca alkaloid class

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Purpose: ALB 109564 is a novel semi-synthetic Vinca alkaloid. The purpose of the studies presented here is to compare the activity of ALB 109564 to vinorelbine in human tumor xenograft studies and to support the selection of ALB 109564 as a candidate for development.
Methods: A panel of five xenografts (H460, Colo205, PC3, H69, and MX-1) was used to explore the breadth of activity for ALB 109564. Vinorelbine was included in these studies for comparative purposes. Both vinorelbine and ALB 109564 were dosed ip using a q4 d $\times$4 schedule. Athymic nude mice (n = 10) were implanted sc with the respective tumor cells. Following establishment of measurable tumors mice received equivalent therapeutic doses, based on their maximum tolerated doses (MTDs), of vinorelbine and ALB 109564. Paclitaxel was included as a positive reference standard administered at 30 mg/kg, iv, q2 d $\times$5 and produced a significant tumor growth delay (TGD) in each of the tumor models.
Results: ALB 109564 demonstrated antitumor activity superior to vinorelbine when dosed at respective MTDs. Statistically significant TGDs were observed with ALB 109564 in four of the five xenografts studied while vinorelbine did not significantly delay tumor growth in any of the models.
The activity of ALB 109564 in the PC3 prostate carcinoma xenograft was confirmed in an experiment where the compound was evaluated on three different schedules with iv administration. When given iv, ALB 109564 is tolerated at considerably higher dose levels than when administered to mice ip. While the MTD was 6 mg/kg ip on a q4 d $\times$ 3 regimen, the MTD for iv ALB 109564 on a q4 d $\times$ 4 regimen proved to be 14.3 mg/kg. This regimen was compared to a qwk $\times$ 4 iv regimen and a qd $\times$ 3 qwk $\times$ 4 intensive iv regimen. The MTD of ALB 109564 on each of these three regimens produced highly significant TGDs of >40 days. Regressions of PC3 tumors were observed with all three iv treatment regimens.
Conclusions: ALB 109564 (12′-methylthiovinblastine dihydrochloride) is a tubulin inhibitor with a mechanism of action comparable to that of vincristine, vinblastine, and vinorelbine. Preclinical evaluation of ALB 109564 has demonstrated *in vivo* oncolytic activity against several human tumor cell types in xenograft models greater than that seen with vinorelbine when dosed ip and improved activity when dosed iv. These results support the clinical development of ALB 109564.

450 POSTER

NPI-2358, a novel tumor vascular disrupting agent potentiates the anti-tumor activity of docetaxel in the non small cell lung cancer model MV522

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Background: NPI-2358 is a diketopiperazine derivative of the marine Aspergillus sp. isolated compound halimide and represents a structurally novel tumor vascular disrupting agent (VDA). NPI-2358 acts at the colchicine binding site of β-tubulin, preventing tubulin polymerization and resulting in a mitotic block in rapidly proliferating cells and a selective disruption of tumor vascular endothelial cell shape and cohesion. In addition to its VDA activity, NPI-2358 is cytotoxic to various human tumor cell lines in vitro, with an IC50 of 10−20 nM. In vivo, as a single agent NPI-2358 selectively and markedly disrupts tumor blood flow for at least 24 hr post administration, resulting in tumor necrosis in both mouse (N202 breast tumor) and rat (P22 sarcoma) models. NPI-2358 also exhibits single-agent antitumor activity in various orthotopic xenograft models and potentiates the activity of chemotherapeutic agents in colon, breast and prostate subcutaneous cancer models.
Materials and Methods: Here we report on the evaluation of NPI-2358 in combination with docetaxel in the human MV522 non small cell lung cancer subcutaneous xenograft model in mice. The sequence and timing of docetaxel and NPI-2358 administration were investigated against small (100 mm3) as well as larger tumors (1700 mm3).
Results: Docetaxel administered at 15 mg/kg (IV) on days 1, 3 and 5 in combination with NPI-2358 at 3.75 mg/kg (IP) on days 1, 3, 5, 8, and 11 resulted in significant tumor size reduction, with an overall response rate of 75% and two complete tumor regressions, whereas the docetaxel group did not display partial or complete tumor regressions. In addition to being effective against small tumors NPI-2358 also significantly potentiated the effects of docetaxel against large tumors. An interesting observation made during these studies was that the addition of NPI-2358 to the docetaxel treatment regimen apparently alleviated the severe weight loss observed in the docetaxel treated mice.
Preliminary results on the sequence and timing of docetaxel and NPI-2358 administration in this MV522 xenograft model suggest that NPI-2358 at 3.75 mg/kg administered 1 hour after docetaxel at 15 mg/kg is an optimal dosing schedule to enhance docetaxel anti-tumor activity. NPI-2358 has been evaluated as a single agent in a Phase 1 clinical trial and a combination trial with docetaxel has been initiated in patients with non small cell lung cancer.