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

F. Lee[1], K. Covello[1], M. Kimler[1], R. Lenhart[1], Y. She[2], S. Platero[2], R. Kramer[1]. *[1]Bristol-Myers Squibb Company, Oncology Drug Discovery, Princeton, NJ, USA; [2]Bristol-Myers Squibb Company, Molecular Pathology, Princeton, NJ, USA*

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

446 POSTER

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

H. Minami[1], T. Mukohara[2], S. Nagai[3], H. Mukai[3], M. Namiki[4]. *[1]Kobe University Graduate School of Medicine, Medicine, Kobe, Japan; [2]Kobe University Hospital, Medicine, Kobe, Japan; [3]National Cancer Center Hospital East, Medicine, Kashiwa, Japan; [4]Eisai Co. Ltd, New Product Development, Tokyo, Japan*

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

R. Woessner[1], B. Tunquist[2], E. Chlipala[3], M. Humphries[1], D. Trawick[4], A. Cox[1], P. Lee[1], J. Winkler[5], D. Walker[2]. *[1]Array BioPharma, Pharmacology, Boulder, CO, USA; [2]Array BioPharma, Translational Oncology, Boulder, CO, USA; [3]Premier Laboratory, Boulder, CO, USA; [4]Array BioPharma, Drug Metabolism, Boulder, CO, USA; [5]Array BioPharma, Discovery and Translational Biology, Boulder, CO, USA*

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%