software (Biosoft, Inc.). PK studies were performed in mice injected IP with 1 mg/kg BTZ. Plasma and peritoneal fluid were sampled from 1 - 95 min and BTZ measured by HPLC/MS/MS. Results: Pretreatment of 2008 cells with BTZ blocked the DDP-induced down-regulation of CTR1 in a concentration dependent manner. Exposure to DDP alone reduced CTR1 levels 49% on western blot analysis, whereas BTZ pretreatment limited this decrease to 10%. This effect was confirmed by confocal microscopy and flow cytometry. The maintenance of CTR1 levels was functionally significant. BTZ pretreatment increased cellular Pt levels 2.4-fold (95% CI 1.8, 3.1-fold) over levels produced by exposure to DDP alone. Median effect analyses showed marked synergism with a CI of 0.37 at 50% cell kill. After IP injection peritoneal BTZ levels decreased in a mono-exponential manner; mean peritoneal C_{max} was 63.4 uM and the $t_{1/2}$ was 37.9 min. Peritoneal drug clearance was 2.2 mL/min. Cytotoxic quantities of BTZ were detectable in the peritoneal cavity even at 95 min. In contrast, BTZ was detectable in the plasma only sporadically and the mean C_{max} was 2.4 uM. The ratio of the exposure for the peritoneal cavity relative to plasma (AUC ratio) was 252. Conclusions: BTZ enhances cellular accumulation of DDP in ovarian cancer cells, an effect mediated in part by its ability to prevent DDP from down-regulating its influx transporter CTR1. BTZ and DDP also demonstrate marked synergy. The relative advantage of administering BTZ by the IP route was 252-fold, and the duration of exposure to detectable levels of drug was 6-fold greater in the peritoneal cavity than in plasma. These results suggest that IP BTZ administration is an attractive strategy for increasing tumor exposure, reducing systemic toxicity, and increasing Pt delivery to tumors confined to the peritoneal cavity.

#3261 NSCLCs harboring EGFR kinase domain mutations are sensitive to proteasome inhibition . Takeshi Shimamura,[1] Susumu Kobayashi,[2] Danan Li,[1] April M. Lowell,[1] Christa L. Borgman,[1] Kwok Kin Wong,[1] Balázs Halmos,[3] Daniel G. Tenen,[2] Geoffrey I. Shapiro[1]. *[1]Dana-Farber Cancer Inst., Boston, MA; [2]Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [3]University Hospitals of Cleveland and Case Western Reserve University, Cleaveland, OH.*

The ubiquitin-proteasome pathway regulates the intracellular concentration of key proteins including oncogenic kinases. Proteasome inhibition in NSCLC has been tested preclinically, but previous studies have not identified a subset most likely to benefit. Here we report that NSCLC cell lines harboring EGFR kinase domain mutations are routinely sensitive to proteasome inhibition. Activating mutations in the kinase domain of EGFR are most commonly exon 19 deletions and substitutions for L858 in exon 21, and confer sensitivity to the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. Over time, resistance universally emerges,associated with a second somatic T790M mutation in approximately 50% of cases. Currently, irreversible EGFR inhibitors are being tested in erlotinib-resistant NSCLCs, with limited efficacy reported in preliminary studies. We propose proteasome inhibition as a novel strategy for NSCLCs resistant to EGFR inhibitors. We have challenged two groups of NSCLC cell lines with bortezomib: one with EGFR mutation and the other with wild-type (WT) EGFR. Annexin V apoptosis assay revealed that IC50s for mutant EGFR NSCLC cells are routinely below 50nM, whereas IC50s for the majority of WT cell lines are above 50nM. Identical results were obtained with other proteasome inhibitors, including MG262 and MG132. In NCI-H1975 cell (EGFR L858R-T790M), 25nM bortezomib treatment caused caspase 3 induction at 16hrs while the induction was not in evident in NCI-H460 cell (EGFR-WT). The induction of apoptosis was partially reversed with a pan-caspase inhibitor Z-VAD-fmk. Proteasome inhibition caused an initial increase in the expression of EGFR, followed by depletion of the protein by 24 hours. Real-time qRT-PCR indicated that EGFR depletion does not occur at the transcription level. The treatment of H1975 or HCC827 (EGFR del) cells with 100nM bortezomib resulted in the induction of Grp78 and Hsp70 at 8 hrs, suggesting the initiation of ER stress. Phosphorylation of eIF2a was evident at 16hrs, which coincided with EGFR depletion, caspase 3 activation, and PARP cleavage. Pretreatment of HCC827 and H1975 cells with a low dose of cycloheximide reversed the effects of bortezomib. Bortezomib also caused tumor regression in an inducible bitransgenic murine model of adenocarcinoma driven by EGFR harboring compound L858R/T790M mutation. Moreover, HCC827 and H1975 cells were sensitive to Tunicamycin or 2-deoxyglucose, compounds that induce ER stress with a depletion of EGFR. In summary, proteasome inhibition is effective against NSCLCs with EGFR mutations. The mechanism connecting ER stress to apoptosis is under active investigation.

#3262 Schedule-dependent bortezomib modulation of gemcitabine pharmacokinetics and pharmacodynamics in non-small cell lung cancer and blood mononuclear cells. Elisa Giovannetti,[1] Cecilia Ceresa,[2] Adrie C. Laan,[1] Jens Voortman,[1] Richard Honeywell,[1] Giuseppe Giaccone,[3] Godefridus J. Peters[1]. *[1]VU University Medical Center, Amsterdam, The Netherlands; [2]Department of Neurosciences and Biomedical Technologies, University of Milano Bicocca, Milano, Italy; [3]Medical Oncology Branch, National Cancer Institute, Bethesda, MD.*

In a phase IB trial of bortezomib combination with gemcitabine/cisplatin in patients with primarily non-small cell lung cancer (NSCLC) (Voortman et al. 2007) a transient drop in the plasma levels of deoxycytidine was observed, which has never been observed in cisplatin/gemcitabine-treated patients. The present study was aimed to investigate the pharmacokinetic and pharmacodynamic interactions between gemcitabine and bortezomib in NSCLC and peripheral blood mononuclear cells (PBMCs). Accumulation of gemcitabine metabolites, including difluorodeoxycytidine triphosphate (dFdCTP), was studied by LC-MS/MS and HPLC in PBMCs from patients receiving bortezomib 1.0mg/m^2 plus gemcitabine 1000mg/m^2 and cisplatin 70mg/m^2. dFdCTP was also measured in PBMCs from volunteers and NSCLC cells (H460 and SW1573) exposed to simultaneous 4h or sequential treatments consisting of 4h-exposure to gemcitabine (50μM) followed by 2h bortezomib (100nM), or the reverse order. Cytotoxicity was studied by MTT, while deoxycytidine kinase (dCK) expression was assessed by PCR and western blot. Gemcitabine total phosphate levels in PBMCs from 9 bortezomib/gemcitabine/cisplatin-treated patients were significantly reduced after 90 min until 4 h from the start of gemcitabine infusion, compared to gemcitabine/cisplatin treated patients. Bortezomib/gemcitabine combinations also reduced dFdCTP in PBMCs *in vitro*. In contrast, dFdCTP continued to increase for 24 h after drug removal in SW1573, while in H460 cells dFdCTP levels were reduced after the removal of the simultaneous and the gemcitabine→bortezomib combination. However, dFdCTP significantly increased in both cell lines after bortezomib pre-incubation with respect to gemcitabine alone. In line with dFdCTP increase, all the drug combinations showed a synergistic interaction in SW1573 cells. In H460 cells only the bortezomib→gemcitabine combination significantly reduced cell growth with respect to single drugs, associated with dFdCTP increase. The simultaneous and the gemcitabine→bortezomib combination showed a lower cytotoxicity than expected, associated with dFdCTP decrease. Western blot revealed a major increase of dCK expression after bortezomib pre-incubation in both cell lines. Our data show that bortezomib affects gemcitabine pharmacokinetics and pharmacodynamics differently in PBMCs and NSCLC cells, suggesting that PBMCs are not an adequate surrogate tissue to evaluate the anticancer activity of bortezomib/gemcitabine combinations. However the bortezomib→gemcitabine/cisplatin schedule appeared a safe and active combination for the treatment of advanced NSCLC patients, and the bortezomib→gemcitabine combination showed a synergistic interaction in NSCLC cells, associated with increase of dFdCTP levels and dCK expression. Voortman J, et al., Clin Cancer Res 2007;13:3642-51

#3263 Combination therapy of 2-methoxyantimycin-A3 with bortezomib in mesothelioma. Lidong Zhang, Charles Rodarte, Xiaobo Cao, James Littlejohn, W. Roy Smythe. *Scott & White Memorial Hospital, Temple, TX.*

Malignant pleural mesothelioma (MPM) is a solid neoplasm that is highly unresponsive to conventional therapy. Therefore, there is urgent need for development of new strategies of therapy. Previously, we and others have demonstrated that MPM overexpresses anti-apoptotic cellular protein Bcl-xL. Furthermore, we have demonstrated that the functional inhibition of Bcl-xL by its specific inhibitor 2-methoxyantimycin-A3 induces apoptosis and increases chemosensitivity in MPM cell lines *in vitro* and *in vivo*. In this study, we investigated the combination effects of 2-methoxyantimycin-A3 with bortezomib, a specific proteasome pathway inhibitor, on MPM *in vitro*. Our results showed that the both MPM cell lines I-45 and REN, displayed synergistically enhanced cell viability reduction after treatment with a sub IC_{50} dose of bortezomib (12.5 nM) and 10, 20, or 40μg/mL of 2-methoxyantimycin-A3 for 48 hours. Combination treatment with bortezomib at 12.5 nM and 2-methoxyantimycin-A3 at 20 μg/mL showed a significant increase in sub-G1 population in REN cell lines (28.25%) compared with treatment of either drug alone (6.74-7.23%). However, the same combination treatment only resulted in a slight increase in sub-G1 population in I-45 cell line (7.65%) when compared with treatment of either drug alone (1.6-3.9%), indicating that apoptosis induction is the main reason for the combination effect in REN cell line, while cell growth inhibition is major contribution to the combination effect in I-45 cell lines. For mechanism study, Noxa, a pro-apoptotic member of Bcl-2 protein family, displayed 500-600% increases in the both MPM cell lines treated with combined drugs when compared with treatment without either drugs. However, knockdown of Noxa gene expression by its siRNA did not protect REN cells from the combined therapy-induced apoptosis. Overall, our results provide a potential combination therapy for patients with MPM.

#3264 Cumulative dose of Bortezomib induces chromosome abnormalities in mouse bone marrow cells *in vivo*. R. Ellen Friday,[1] Francesco Turturro,[1] John B. Mailhes[2]. *[1]Louisiana State Univ. Health Sciences Ctr. Feist-Weiller Cancer Center, Shreveport, LA; [2]Louisiana State Univ. Health Sciences Ctr., Shreveport, LA.*

Proteasome-mediated proteolysis occurs during the metaphase-anaphase transition of mitotic and meiotic cells. It is required for the fidelity of sister chromatid cohe-