

tion. NAMI-A was able to down-regulate MAPK/ERK activity both in serum- and in phorbol ester-stimulated cells. Cell exposure to a caspase-3 inhibitor prevented the appearance of DNA laddering induced by NAMI-A or by a selective ERK inhibitor in both serum- and phorbol ester-stimulated cells. NAMI-A caused the increase of cells in G<sub>2</sub>-M depending on the duration of drug exposure; the effect was transient and completely reversed within 48 hrs after treatment. Correspondingly, cells of Lewis lung carcinoma, harvested from the primary tumour of mice *in vivo* treated with doses active on lung metastasis formation, grew normally following implantation into syngeneic mice but show the loss of capacity to further metastasis. These data suggest that NAMI-A selectively interacts with the fine mechanisms that regulate metastasis growth and progression.

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**502 Synergistic effect of E7070 combined with CPT-11 in human tumor xenograft models.** Yoichi Ozawa, Junko Kai, Kazutomi Kusano, Makoto Asada, and Kentaro Yoshimatsu, *Tsukuba Res Lab, Eisai Co. Ltd., Tsukuba, Japan.*

E7070, N-(3-chloro-7-indolyl)-1, 4-benzenedisulfonamide, shows inhibitory activity on phosphorylation of cdk2 and transcription of cyclin E in cancer cells resulting in disturbance of G1/S progression of the cell cycle and apoptosis. Phase I clinical trial of E7070 is ongoing in European countries.

To investigate combination effects of E7070 with other anticancer drugs, experimental combination studies were conducted using human tumor xenograft models. Seven anticancer drugs, ADM, CDDP, 5-FU, MMC, Taxol, CPT-11 and Gemcitabine, were examined as combined drugs for E7070 in human colorectal cancer HCT15 xenograft model. E7070 was administered daily for 5 days and 7 anticancer drugs were administered reported optimal dosing schedules, i.e. single dose for ADM, CDDP and MMC, every 4 days 3 times for 5-FU and CPT-11, daily for 5 days for Taxol and every 3 days for 4 times for Gemcitabine.

As the results show, the synergistic effect was observed in the combination between E7070 30 mg/kg and CPT-11 62.5 mg/kg, and between E7070 20 mg/kg/day and MMC 3.35 mg/kg. The combinations with other anticancer drugs were additive and there was no antagonistic combination. The synergistic effect of the combination of E7070 with CPT-11 was confirmed in other tumor xenograft model, SW620 human colorectal cancer, on 3 dosing schedules, simultaneous and sequential (E7070 dosing on day 1-5 and CPT-11 dosing on day 6, 10, 14, CPT-11 dosing on day 1, 5, 9 and E7070 dosing on day 10-14). In this model, the antitumor effect of the combination was apparently superior to those of monotherapy at MTDs in all schedules [i.e. T/C values on 20 days after first treatment; E7070 40 mg/kg/day (MTD) = 16.4%, CPT-11 100 mg/kg/day (MTD) = 15.0%, Combination (E7070 25 mg/kg - CPT-11 62.5 mg/kg) = 2.4%, E7070 25 mg/kg = 29.1%, CPT-11 62.5 mg/kg = 33.6%].

These results suggest that E7070 plus CPT-11 is a promising combination.

**503 Sequence dependence using combinations of Alimta™ (pemetrexed disodium, LY231514, MTA), gemcitabine, and oxaliplatin in human colorectal carcinoma cell lines.** R. Schultz<sup>1</sup>, M. Rothenberg<sup>2</sup>, M. Koury<sup>2</sup>, W. D. Hankins<sup>3</sup>, and V. Kravtsov<sup>2</sup>. <sup>1</sup>Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN; <sup>2</sup>Vanderbilt University Medical Center, Nashville TN; <sup>3</sup>John Hopkins, Baltimore, MD.

Using the new automated kinetics of response (KOR) assay which directly and continuously monitors drug-induced apoptosis, we examined the interactions of three drugs which are candidates for treatment of colorectal cancer. These studies were conducted to assist in the selection of the appropriate drug combinations or sequences for clinical development. We used three human colorectal carcinoma cell lines (HT29, COLO 320DM, and LoVo), which differ in their growth characteristics and relative expression of the tumor suppressor p53. Alimta and gemcitabine demonstrated minimal ability to induce apoptosis in any of these cell lines. In contrast, oxaliplatin induced dose-dependent apoptosis in two of the three cell lines. The highest apoptotic responses were observed with the sequence of alimta, gemcitabine or both administered prior to oxaliplatin in HT29 and LoVo colorectal carcinoma cells. Addition of alimta and gemcitabine after oxaliplatin had no potentiating effect. Alimta and gemcitabine have previously been shown to accumulate cells in the G<sub>1</sub>/S transition and early S phase, respectively. The present studies suggest potentiation in the therapeutic efficacy of oxaliplatin, and therein, might provide new treatment options for colorectal cancer patients. Further studies are needed to elucidate the mechanistic basis for potentiation of cytotoxicity with these drug combinations.

**504 Sequencing evaluation of ET-743 combinations with standard chemotherapy agents against a panel of human tumor cell lines.** R. Moore, M. Revilla, J. Jimeno, G. Faircloth, S. Weitman. *Institute for Drug Development, San Antonio, TX 78245, and PharmaMar, Madrid, Spain.*

ET-743, a potent alkaloid derived from the tunicate *Ecteinascidia turbinata*, was evaluated in combination with doxorubicin, taxol, SN-38, cisplatin, and gemcitabine against a panel of adult and pediatric tumor cell lines. These studies were designed to determine the type of drug-drug interaction between ET-743 and standard chemotherapy agents and the influence of sequence (24 hour pre-exposure to ET-743; 24 hour pre-exposure to standard agents; concurrent exposure) of exposure on antitumor activity. Multiple combinations of ET-743 with standard cytotoxic agents were used with a model-free design (Laska, et al. *Biometric* 50:834, 1994) to describe the type of drug-drug interaction. An additive pattern of drug-drug interaction was most typically observed, regardless of sequence of exposure between ET-743 and standard chemotherapy agents. An additive/synergistic drug-drug interaction was observed when ET-743 was combined with SN-38 (lung and colon tumor cell lines), cisplatin (osteosarcoma), and gemcitabine (breast). Evidence of antagonism was noted with taxol (lung tumor cell lines) and doxorubicin (rhabdomyosarcoma). These studies suggest that ET-743, which is in phase II clinical trials, could be combined with several cytotoxic agents against a broad-range of tumor types.

**505 Combination chemotherapy of pancreatic cancer with gemcitabine and perillyl alcohol.** P. L. Crowell and D. A. Wiseman. *Indiana University Purdue University Indianapolis, Indianapolis, IN 46202 U.S.A.*

Despite advances in pancreatic cancer chemotherapy, the disease has a >95% mortality rate. We have tested the potential of combining the pancreatic cancer chemotherapy drug Gemcitabine (Gem) with perillyl alcohol (POH), a novel therapeutic agent in Phase II trials, in the treatment of pancreatic carcinoma cells. MIA PaCa-2 human pancreatic carcinoma cells were exposed for 24-48 h to 0-500 μM POH and 0-100 nM GEM. The combination of POH and GEM showed a significant (p<0.05) inhibitory effect versus control and single-agent groups, and all GEM + POH combinations at the 24 h timepoint were synergistic. Furthermore, POH potentiated GEM-induced S phase arrest in that the % of cells in S phase was 7.0, 9.2, 25.6, and 50.3% for control, 300 μM POH, 20 nM GEM, and 300 μM POH + 20 nM GEM groups, respectively. In summary, the combination of POH and GEM was more beneficial than single agent therapy, suggesting that POH may be useful in combination with GEM in the therapy of human pancreatic cancer.