# PROCEEDINGS

## EIGHTY-NINTH ANNUAL MEETING

*In Cooperation with the*
*Center for Continuing Education at*
*Tulane University Medical Center*

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cancer therapy has been the topic of intense study over the last five years. Many compounds discussed in the literature are potent FPT inhibitors but lack the n-cell potencies or have poor oral pharmacokinetics. We have developed novel rhalobenzocycloheptapyridine heterocycles that are potent, selective inhibitors of FPT and are antitumor agents in mice when given orally. These compounds ave good serum levels and half-lives when given orally to rodents and primates. Structural modifications in this series led to the discovery of SCH 66336, a highly potent (FPT $IC_{50}$ of 1.9 nM), orally active antitumor agent which is undergoing early clinical trials. A detailed structure activity relationship will be disclosed for the first time.

**#2177 Pharmacokinetics of a potent orally bioavailable inhibitor of farnesyl protein transferase in the mouse, rat and cynomolgus monkey.** Bryant, M.S., Liu, M., Wang, S., Nardo, C., Kumari, P., Chen, K.-J., Watkins, R., Korfmacher, W.A., Lin, C.-C., Taveras, A., Kelly, J., Remiszewski, S., Mallams, A.K., Alonso, A., Wolin, R., Alvarez, C., Cooper, A.B., Hollinger, F.P., Liu, Y.-T., Rane, D., Saksena, A.K., Snow, M.E., Vibulbhan, B., Ganguly, A.K and Nomeir, A.A. *Schering-Plough Research Institute, Kenilworth, NJ 07033*

The tricyclic farnesyl protein transferase inhibitor (SCH 66336) was subjected to pharmacokinetic evaluation in mice, rats and cynomolgus monkeys and found to possess desirable oral pharmacokinetic properties. In the athymic (nude) mouse model, a peak serum concentraton (Cmax) of 8.8 $\mu$M was achieved after an oral dose of 25 mg/kg and persisted at a concentration above its effective concentration for inhibiting soft agar growth of a number of tumor cell lines for 14 hr. The oral bioavailability was 76% and the half-life after intravenous (IV) administration was 1.4 hr. A dose-response study was conducted in the rat wherin plasma levels reached Cmax values of 3, 10 and 30 $\mu$M at oral doses of 10, 30 and 100 mg/kg, respectively. SCH 66336 was administerd orally in 4 different vehicles or by IV administration to the cynomolgus monkey at 10 mg/kg. Plasma levels were similar for all vehicles, achieving about 50% bioavailability and reached Cmax values of 1.3 - 2.5 $\mu$M. In human tumor xenograph efficacy studies in the nude mouse, concentrations of SCH 66336 in serum and tumor tissue samples were found to parallel closely. These studies indicate that the drug readily reaches the target tumor tissue after oral administration and thus accounts for its potent in vivo antitumor activity.

**#2178 Cellular actions of a farnesyltransferase inhibitor, RPR-115135, in a human isogenic colon cancer cell line system.** Russo, P., Reinhold, W., Yu, J., Ottoboni, C., Kohn, K.W., Riou, J.F., and O'Connor, P.M. *Laboratory of Molecular Pharmacology, Division of Basic Sciences, National Cancer Institute, Bethesda, MD 20892, Rhone-Poulenc Rorer S.A. 9403 Vitry sur Seine, France, Department of Experimental Oncology, National Institute for Research on Cancer, 16132 Genova, Italia.*

A non peptidomimetic farnesyltransferase inhibitor, RPR-115135, was studied in an isogenic cell line model system consisting of human colon cancer HCT116 cells which harbor a K-RAS mutation. HCT116 were transfected with a control pCMV vector or with a dominant-negative mutated p53 gene to disrupt p53 function. We found that relative to a control transfectant (CMV-2), there was a tendency for the mutated-p53 transfectant of HCT116 cells (mup53–2) to be more sensitive to RPR-115135 (<3-fold difference at the $IC_{50}$ value). Time-course experiments conducted over 6 days with a continual exposure to 10 $\mu$M RPR-115135 revealed that HCT116 cells were still able to grow for up to 24 h following drug administration but thereafter, clear growth inhibition was observed. Growth inhibition could not easily be accounted for on the basis of a specific cell cycle arrest phenotype, as assayed by flow cytometry. Rather there was a tendency for cells treated with RPR-115135 to accumulate a subdiploid population. In contrast addition of RPR-115135 to cells undergoing serum starvation enhanced the ability of HCT116 cells to arrest in $G_0/G_1$ phase and this arrest response appeared independent of p53 function. These latter results suggest that RPR-115135 might down-regulate cell cycle factors that would normally impede $G_0/G_1$ arrest. Studies are presently underway in HCT116 cells to investigate the effects of RPR-115135 on cell cycle and cell death regulatory molecules.

**#2179 p21WAF promoter is upregulated by the geranylgeranyltransferase-I inhibitor GGTI-298 through an Sp1-binding site.** Adnane, J., Qian, Y., Hamilton, A.D. and Sebti, S.M. *H. Lee Moffitt Cancer Center & Research Institute of the University of South Florida, Tampa, FL 33612 (JA, SMS) and Yale University, New Haven, CT 06511 (YQ, ADH).*

We have previously reported that the geranylgeranyltransferase-I inhibitor GGTI-298 arrests cells in G1 phase of the cell cycle and induces the accumulation of p21WAF protein. Here we show that GGTI-298 acts at the promoter level to induce p21WAF in various murine and human cell lines. The p53 consensus site of p21WAF promoter was not required for the up-regulation by the GGTI-298. To identify the region which is responsive to GGTI-298 we performed a functional analysis of p21WAF promoter. The results showed that a GC-rich region between sequences -93 and -62, containing a TGF$\beta$-responsive element and two Sp1-binding sites, was sufficient for the upregulation of p21WAF promoter by GGTI-298. Further analysis of the -93 to -62 region showed the sequences between -83 and -77, which represent an Sp1 consensus site, were essential for the upregulation by GGTI-298. In addition, Sp1 transcription activity was increased at least 3 fold. Taken together, these results show that GGTI-298 activates p21WAF transcription by upregulating Sp1-mediated transcription. This work was supported by U19-CA-67771.

**#2180 Apoptosis induction by novel anti-cancer compound, HMN-154 (HMN-176) through suppressing the expression of cell cycle controllers.** Tanaka, H., Ohshima, N., and Hidaka, H. *Nagoya University School of Medicine, Nagoya 466, Japan.*

Our novel anti-cancer compound HMN-154 (HMN-176) induced apoptosis of target cancer cells, following cell-cycle arrest at M-phase. HMN-154 induced apoptotic nuclear event (DNA fragmentation) from 12 hrs after treatment, and consequently, 80% of treated cells showed this apoptotic feature at 48 hrs. Recently, we developed "drug-western' method to isolate the genes for cellular drug binding factors. By this method, we found a component of transcriptional activator NF-Y complex to be one of the direct cellular target of HMN-154. We characterized HMN-154 binding motif in B-subunit of NF-Y, which is necessary for DNA-binding of total NF-Y complex. This binding resulted in the loss of accession to its target site. NF-Y has been known to play a critical role for cell cycle dependent expression of cdc2, cyclin A and cdc25C. HMN-154 suppressed the promoter activity of these genes and mRNA level was clearly decreased in HMN-154 treated cells. These results suggested that the anti-cancer action of HMN-154 was carried out by disrupting the expression cell-cycle controllers, which might be necessary for tumor cell growth. We will also discuss the oncogenic properties of NF-Y.

**#2181 3'-Deamino-3'-hydroxy-doxorubicin (WP159)-resistant cells exhibit collateral hypersensitivity to anti-mitotic drugs and serine/threonine protein phosphatase inhibitors.** Lothstein, L.* and Priebe, W. **Univ. of Tennessee Health Science Center, Memphis, TN 38163 and Univ. of Texas M.D. Anderson Cancer Center, Houston, TX 77030.*

WP159 is an uncharged anthracycline that circumvents P-glycoprotein (P-gp)-mediated multidrug resistance. However, WP159 inhibits topoisomerase II (topo II) and selects for resistant cells with reduced topo II activity. Murine J774.2 cells selected for 10-fold WP159 resistance (WP159/R) are cross-resistant to other topo II inhibitors but are two-fold more sensitive than parental cells to anti-mitotic drugs, including vinblastine and taxol. Doxorubicin-resistant cells are cross-resistant to anti-mitotic drugs, consistent with the overexpression of P-gp. WP159/R cells are also 2-fold more sensitive than parental cells to okadaic acid and cantharidin, protein phosphatase inhibitors with greater potency against phosphatase 2A (PP2A) than phosphatase 1 (PP1), but not to calyculin A, an inhibitor with greater potency against PP1. PP2A expression is not reduced in asynchronous WP159/R cells, nor do PP2A mRNA levels appear to change during cell cycle transit in synchronous cells. However, we propose that PP2A activity is lower during G2/M transit and may induce increased mitotic spindle instability, thus accounting for anti-mitotic drug hypersensitivity.

**#2182 Isolation of polyoxypeptin, a novel cyclic depsipeptide, from *Streptomyces* as an apoptosis inducer in human pancreatic carcinoma cells.** Umezawa, K., Nakazawa, K., Uemura, T., Hashizume, H., Masamoto, S., Ikeda, Y., Kondo, S., Naganawa, H., Kinoshita, N., Hamada, M., and Takeuchi, T. *Keio University, Yokohama 223, Institute of Microbial Chemistry, Tokyo 141, Japan.*

Microbial culture broths were screened for apoptosis-inducing agents that are effective in human pancreatic carcinoma AsPC-1 cells, since induction of apoptosis may be involved in the clinical effect of anticancer agents. Apoptosis was assayed by characteristic morphological changes observed in 24 hours. Polyoxypeptin, a novel cyclic depsipeptide, was isolated via the bioassay-guided fractionation of the culture broth of *Streptomyces* sp. Polyoxypeptin was extracted with ethyl acetate, purified on silica gel, and recrystallized from acetonitrile or ethyl acetate. Structural determination by 2-D NMR and X-ray crystallographic analysis revealed the structure of polyoxypeptin to be a novel cyclic hexadepsipeptide containing five hydroxylated amino acids, including the unusual and hitherto unreported amino acid 3-hydroxy-3-methylproline. Polyoxypeptin decreased the viability of AsPC-1 cells within 24 hours with an $IC_{50}$ of 80 ng/mL, though adriamycin, cisplatin, and vinblastine did not. It also induced nuclear fragmentation and internucleosomal DNA fragmentation in AsPC-1 cells.

**#2183 Ukrain, a semisynthetic alkaloid of Chelidonium majus, is selectively toxic to malignant cells by causing a metaphase block which results in apoptosis.** Panzer, A., Seegers, J.C. *Department of Physiology, University of Pretoria, PO Box 2034, Pretoria 0001, South Africa.*

Ukrain ($C_{66}$ $H_{75}$ $N_6$ $O_{18}$ PS.6HCl.) is a semisynthetic compound consisting of the chelidonine alkaloid of Chelidonium majus combined to the thiophosphoric acid triaziride (Austrian Patent No. 354644, Vienna, 1980). Previously, the National Cancer Institute (Maryland, USA) demonstrated Ukrain to have cytolytic activity against all 60 human cancer cell lines tested (NSC 631570). In this study, the effect of Ukrain was evaluated on two malignant cell lines (human cervical carcinoma, HeLa, and human oesophageal carcinoma, WHCO5, originally isolated from a biopsy specimen of squamous oesophageal carcinoma) and compared to normal equine lung cells (derived from embrional tissue). Spectrophotometrical analysis of DNA content showed the growth of the equine lung cells to be unaffected by 48 hours exposure to 50$\mu$g/ml Ukrain ($p=0.167$), while the

growth of the HeLa and WHCO5 cells was inhibited to 52.8% and 49.1% of control levels respectively ($p<0.0001$). Flow cytometry studies showed a dose-dependent increase in G2M cells in both of the malignant cell lines, while the equine lung cells did not differ from controls. Morphological studies of the HeLa and WHCO5 cells exposed to Ukrain, revealed abnormal mitotic spindles and apoptotic cells at concentrations where the equine lung cells remained unaffected. It is concluded that Ukrain is selectively toxic to malignant cells by causing a metaphase block which is characterised by abnormal chromosomal distribution, and results in the formation of micronuclei and in apoptosis.

**#2184 Silymarin decreases prostate specific antigen (PSA) expression concomitant with inhibition of cell growth, induction of neuroendocrine differentiation, and perturbations in cell cycle in human prostate carcinoma cells LNCaP.** Agarwal, R., and Zi, X. *Department of Dermatology, Case Western Reserve University, Cleveland, OH 44106.*

Prostate Cancer (PCA) is the most common non-skin cancer and second leading cause of cancer deaths in US' males. One approach to control PCA is chemo-intervention. Recently, we showed that a flavonoid antioxidant silymarin exerts strong protective effects against tumorigenicity in different models of epithelial origin. Since, reduction of serum PSA levels has been proposed as an end point for hormone-refractory human PCA intervention, we examined whether silymarin alters PSA expression in androgen-dependent human PCA cells LNCaP, and that this effect has biological relevance. Silymarin treatment resulted in a significant decrease in both fetal bovine serum- and dihydrotestosterone-mediated intracellular and secreted PSA levels concomitant with a significant inhibition of cell growth, an induction of neuroendocrine differentiation, and a G1 arrest. In additional studies, silymarin treatment resulted in a significant induction of cyclin dependent kinase inhibitors (CDKIs) Cip1/p21 and Kip1/p27 together with a decrease in CDK4 but no change in CDK2, CDK6, cyclin D1 and E. Cell treated with silymarin also showed an increased binding of CDKIs with CDKs together with a marked decrease in kinase activity of CDKs and associated cyclins. These results suggest that silymarin may be a useful agent for the intervention of human prostate cancer, and that decrease in PSA levels possibly leading to alterations in growth promoting cell cycle events could be the molecular mechanism of silymarin's effect.

**#2185 The anti-tumor efficacy of SU101 in human tumor models is exerted by the parent compound and not the metabolite.** Shawver, L.K., Sutton, B., West, K.A., Cropp, G.F., Strawn, L.M., Powell, T.J. *SUGEN, Inc., Redwood City, CA 94063.*

SU101, a signal transduction inhibitor that is currently in clinical trials, appears to inhibit tumor cell growth via two distinct mechanisms: 1) the parent compound inhibits PDGF-R signal transduction, and 2) its major metabolite (SU0020) inhibits pyrimidine biosynthesis. *In vivo* efficacy of SU101 was examined in tumor xenograft models using rat glioma (C6) and human lung tumor (Calu-6) cells. In both tumor models, parenteral (IP) administration of SU101 significantly inhibited tumor growth; in contrast, oral administration of SU101 inhibited the growth of rat C6 tumors, but not human Calu-6 tumors. In the C6 model, the efficacy of SU101 administered orally was completely reversed by coadministration of uridine, while efficacy following IP administration was not affected. Thus, SU101 accounted for the anti-tumor efficacy following IP administration, while SU0020 accounted for the efficacy following oral administration. Parenteral administration of SU101 to mice bearing C6 tumors resulted in detectable levels of SU101 in both the plasma and the tumor, while oral administration resulted in detection of only SU0020 in the plasma. We conclude that oral administration of SU101 resulted in its complete conversion to SU0020, and prevented efficacy in the human tumor model but not the rat tumor model. These results suggest that parenteral administration of SU101 is required to achieve efficacy in the treatment of human cancers.

**#2186 KF25706 (UCS1006-S15): A novel derivative of radicicol inhibiting multiple signal transduction pathways with in vivo antitumor activity in breast carcinoma xenograft models.** Shiro Akinaga[1], Shiro Soga[1], Yukimasa Shiotsu[1], Tsutomu Agatsuma[1], Chikara Murakata[1], Theodor Schulte[2], Len Neckers[2] and Tatsuya Tamaoki[1]. *Pharmaceutical Research Institute Kyowa Hakko Kogyo Co., Ltd., Japan[1] and Clinical Pharmacology Branch, Division of Clinical Science, National Cancer Institute, Bethesda, MD 20892[2].*

KF25706 (UCS1006-S15) is a novel derivative of radicicol, an inhibitor of multiple signal transduction pathways. KF25706 inhibited tyrosine kinase activity in v-src transformed rat fibroblast cells and Erk phosphorylation in K-ras transformed rat epithelial cells, with IC50 values of 120 and 100nM, respectively, after 48 h exposure. KF25706 also inhibited the growth of both cell lines with IC50's of 22 and 87nM, respectively, after 72 h exposure. KF25706 also inhibited the growth of human breast cell lines with IC50's ranging from 15nM to 150nM, and rapidly depleted the oncogenic tyrosine kinase p185 erbB-2 from SK-BR-3 cells. The multiple activities of KF25706 may stem from the drug's interaction with the hsp90 chaperone family, which was assessed by the drug's ability to compete for chaperone binding with the known hsp90-binding agent, geldanamycin. More importantly, KF25706 showed significant in vivo growth-inhibitory activity against ER (-) human breast carcinoma Mx-1 cell xenografts, following a regimen of 5 consecutive daily s.c. and/or i.v. injections at a dose of 100mg/kg. KF25706 was also shown to possess antitumor activity against ER (+) human breast carcinoma MCF-7 cells xenografts following the same treatment schedule. The compound exhibited no liver or no renal toxicity, as assessed by serum GPTase and level, and very mild myelotoxicity, as determined by peripheral blood cell ing. These results indicated that KF25706 with its novel mechanism(s) of is a candidate drug for further preclinical study.

## CLINICAL RESEARCH 7: Phase I Clinical Trials

**#2187 Phase I/II and pharmacology study of paclitaxel (P) plus or [1-(2-tetrahydrofuryl)-5-FU + uracil] and leucovorin (LV) in the secor treatment of patients (PTS) with metastatic breast cancer (MBC).** Kl U., Ehricke S., Hilger R., Borquez D., Oberhoff C., Chazard M., Ben Harstrick A. and Seeber S. *Dept. of Internal Medicine (Cancer Research) German Cancer Center, University of Essen, Germany.*

5-FU is the classic example of a cycle specific S-phase dependent druc short half life. Therefore conventional bolus injection may not be the mos tive schedule. Recent phase II study results demonstrate high efficacy a toxicity for a weekly schedule of 24-hour infusional 5-FU/LV (Wilke, Ann. 7:55–58,1996) as well as for the continuous infusion of 5-FU (Rezazzor Oncol. 7:807–13,1996) in intensively pretreated pts with MBC. UFT may al administration of long term low dose oral 5-FU with the same pharmacc profile as a continuous infusion (Tashiro, Jpn. J. Clin. Oncol. 24:212–217 Within this ongoing phase I/II study UFT, which is composed of 1-(2-tetra furyl)-5-FU (ftorafur) and uracil in a molar ratio of 1:4, was administered ora LV and in combination with P. So far 17 pts were treated as a part of an c phase I/II protocol in order to determine the safety, activity and pharmacol of this combination. After standard premedication pts received a fixed do 175 mg/m$^2$ 3h i.v. on day (d)1 at all dose levels (dl). UFT was administere in combination with 90 mg/d of LV in three divided doses for 14 d. UFT d were: dl1 300mg/d, dl2 400mg/d, dl3 500mg/d, dl4 600mg/d. The cycle repeated every 21 d. So far within Phase I 17 pts entered the trial: 6 pts dl dl2, 3 pts dl3, 3 pts dl4. All encluded pts have had prior CTX for MBC. T toxicity was neutropenia 68% WHO grade II/III. No febrile neutropeni arthralgia and myalgia WHO grade I and II were common but not dose lim pts had alopecia grade III. Even at dl 4 we did not see any dose gastrointestinal toxicity. 10 pts are evaluable for response: PR 4, SD 4, P will be followed within the ongoing phase II. Furthermore we will determi plasma levels in 6 more pts planned to be treated at dl 4 giving P alone w first cycle and in combination with UFT/LV during the second cycle in show pharmacokinetik interactions of the drug combination. To concl combination of P and oral UFT/LV seems to be a safe, convenient and regimen for pts with pretreated MBC.

**#2188 Dose-finding study of Docetaxel (Taxotere®) and Vinorelbi velbine®) D1 and D8 as 1st-line chemotherapy for metastatic breast (MBC).** Bonneterre, J., Cuvier, C., Bonneterre, M-E., Marty, M., Soe Assadourian, S. *Centre Oscar Lambret, Lille. Hôpital Saint Louis, Paris toires Rhône-Poulenc Rorer, Montrouge, France.*

Taxotere® (TXT) has shown very high activity as a single-agent chem for MBC. Navelbine® (NVB) has also shown significant activity in this Thus, we decided to perform dose-finding study combining NVB and T 1st-line chemotherapy for MBC. Eligible patients (pts) had histological MBC, measurable or evaluable disease, no previous chemotherapy for m disease (neoadjuvant and/or adjuvant chemotherapy allowed if ≥ 12 before acccrual), PS ≤ 2, age ≤ 75, normal hematological, hepatic functions and signed informed consent. NVB 20 mg/m$^2$ was administer D8 and TXT at D8 in escalated doses.

| TXT Dose Level | Nb Pts | Eval. cycles | Grade 4 neutrop. | Febrile neutrop. |
|---|---|---|---|---|
| 60 mg/m$^2$ | 6 | 19 | 52% | 5% |
| 75 mg/m$^2$ | 9 | 45 | 37% | 15% |

** : febrile neutropenias at 1st cycle requiring antibiotics and/or hospit*

Although the MTD was not formaly reached (3/9 pts presented a DL cycle, 1 patient died after a septic shock after the 2nd cycle), we decide evaluate the safety/efficacy profile of this 2-drug combination in accrual at dose level 75 mg/m$^2$ of TXT.

**#2189 Phase I study of a weekly schedule of CPT-11, follin and 5-FU in advanced colorectal cancer.** Vanhoefer, U., Harstrick, C.H., Achterrath, W., Wilke, H., and Seeber, S. *University Hospital, Robert Rössle Clinic, 13125 Berlin; Rhône-Poulenc Rorer, 50829 Co many.*

A weekly schedule of CPT-11, FA and 5-FU was evaluated in patie metastatic colorectal cancer as first line chemotherapy. Treatment weekly therapy × 4 followed by one week rest with fixed doses of C mg/sqm, 90 min inf.) and FA (500 mg/sqm, 2 h inf.). Doses of 5-FU w