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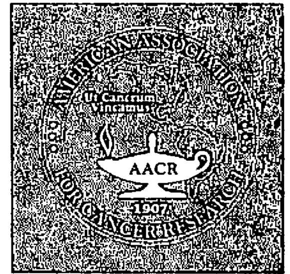

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408 Inhibition of Ras expression in lymphoma by isoform specific antisense oligonucleotides: effects on proliferation, survival and gene expression profiled by cDNA microarrays. Clarke, Paul, A.[1], Sumpter, K.[1,2], Te Poele, R.[1], Di Stefano, F.[1], Wooster, R.[3], Cunningham, D.[2], Monia, B.[4], and Workman, P[1]. *CRC Centre for Cancer Therapeutics[1], Dept. Molecular Carcinogenesis[3], Lymphoma Unit[2], Institute of Cancer Research and Royal Marsden Hospital, UK, and ISIS Pharmaceuticals, USA[4].*

Ras proteins play a central role in signal transduction. The specific function of individual Ras-isoforms is unclear, but evidence suggests that they may have distinct functions. Different isoforms are mutated in specific tumours for example Kirsten (Ki) - ras mutations in colorectal cancer and N-ras mutations in haematological malignancies. The use of recombinant knock-out mice has demonstrated that Ki-ras expression is essential for embryogenesis, while N-ras and Harvey (Ha) - ras knockout mice show few defects. Therapeutic strategies targeting the expression or post-translational modification of Ras are currently under development. However it is not entirely clear which particular isoform, if any, is critical to tumour cell proliferation or survival. In this study our aims were to characterise the contribution of each specific isoform to lymphoma cell proliferation, survival and gene expression by using an antisense strategy to specifically inhibit the expression of each isoform in non-Hodgkin's lymphoma (NHL) cell lines. Six different lymphoid cell lines were treated with antisense oligonucleotides specific for each individual Ras isoform. Western blotting confirmed that these antisense oligonucleotides specifically inhibited the expression of their target genes. Growth in media supplemented with 10% serum was not effected by any of the Ras antisense oligonucleotides. However, under restrictive growth conditions (0.1% serum) proliferation was significantly reduced following treatment with N-ras antisense. FACS analysis demonstrated there was no significant increase in cell death following treatment with any of the antisense molecules, nor was there a marked change in cell cycle distribution. Gene expression profiling using an in-house microarray with 5800 cDNAs detected changes specific for the inhibition of each Ras-isoform. In conclusion, N-ras appears to be required for lymphoma cell proliferation and would be a suitable therapeutic target in NHL.

409 Cerivastatin triggers apoptosis of AML cells with higher potency than lovastatin. Tan, M., Xia, Z., Wong, W. W.-L., Dimitroulakos, J., Minden, M., and Penn, L.Z. *Ontario Cancer Institute, University Health Network, Toronto, Canada.*

The vastatin family of drugs inhibits the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme that catalyzes the rate-limiting step in the mevalonate pathway. We have previously reported that lovastatin, a member of the vastatin family, induces apoptosis in human acute myeloid leukemic (AML) cells. We have recently shown that inhibiting protein geranylgeranylation downstream of mevalonate is critical for lovastatin-induced apoptosis. In the present study we evaluate the potency and mechanism of cerivastatin, a third generation member of the vastatin family. Unlike lovastatin, which requires activation from pro-drug to active form, cerivastatin is administered in active configuration. Our results show that cerivastatin is at least ten fold more potent than lovastatin at inducing apoptosis in AML cell-lines as well as in primary AML patient samples in vitro. Importantly, like lovastatin, cerivastatin is not cytotoxic to normal human bone marrow cells. Moreover, we show that inhibition of protein geranylgeranylation is essential for cerivastatin to trigger apoptosis. These results strongly suggest that the active form of the vastatin family induces apoptosis in AML cells by inhibiting HMG-CoA reductase. In addition, the apoptotic activity of the vastatin family occurs through a common mechanism that requires the abrogation of protein geranylgeranylation. Taken together, cerivastatin may be the drug of choice for inhibiting HMG-CoA reductase and triggering tumor cell apoptosis in the clinical management of AML.

410 Blocking protein geranylgeranylation is essential for apoptosis of AML cells triggered by inhibitors of HMG-CoA reductase. Zhenlei Xia[1], Melissa M. Tan[1], Jim Dimitroulakos[1], Mark D. Minden[1,2], W. Wei-Lynn Wong[1,2], Linda Z. Penn[1,2]. *Department of Cellular and Molecular Biology, the Ontario Cancer Institute, University Health Network, Toronto[1] Department of Medical Biophysics, University of Toronto, Toronto[2].*

We have previously reported that lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, induces apoptosis in human acute myeloid leukemia (AML) cells. To understand the critical biochemical mechanism linking inhibition of the mevalonate pathway and apoptosis induced by lovastatin in AML cells, add-back experiments were employed in the present study. Apoptosis induced by lovastatin was completely prevented by mevalonate and geranylgeranyl pyrophosphate (GGPP), while only partially prevented by farnesyl pyrophosphate (FPP). Other products of the mevalonate pathway including cholesterol, squalene, lanosterol, desmosterol, dolichol, dolichol phosphate, ubiquinone, and isopentenyladenine had no effect. On the induction of apoptosis by lovastatin, our results suggest that the inhibition of geranylgeranylation of target proteins is the predominant mechanism of lovastatin-induced apoptosis in AML cells. Supporting evidence showed that the geranylgeranyl transferase inhibitor (GGTI-298) mimicked the effect of lovastatin, whereas the farnesyl transferase inhibitor (FTI-277) was much less effective at inducing apoptosis in AML cells. In addition, inhibition of Rap1A and Rab5 prenylation was observed with lovastatin and GGTI-298 treatment, but not with FTI-277. GGPP completely reverted the effect of lovastatin on protein prenylation, while FPP had no significant effect on these processes. We conclude that blockage

411 The use of expression profiling to identify genes regulated by the PI3K inhibitor LY294002. Robert te Poele, Karine Maillard, Richard Wooster and Paul Workman. *The Institute of Cancer Research, 15 Cotswold Road, Sutton, SM2 5NG, UK.*

The PI3K pathway plays an important role in regulating cellular proliferation and survival, and mutations in components of this pathway have been implicated in cancer. To determine which genes are regulated by the PI3K pathway, we have used DNA microarrays to establish expression profiles in tumour cells treated with the PI3K inhibitor LY294002 versus non-treated colon adenocarcinoma cells.

Our DNA expression arrays are based on cDNAs from the Unigene set of ESTs. PCR products from an initial set of 5808 cDNAs were gridded on a single double poly-L-Lysine coated glass microscope slide. Hybridisations were performed using Cy3- and Cy5-labelled first strand cDNA from treated and untreated cell line mRNA. After washing, the slides were scanned using an Axon Genepix 4000 scanner. Data were analysed with the Genepix software. Ratios from this process were normalised and used for interpretation and cluster analysis.

Concentrations of 30 and 100 μM of LY294002 were used in time course experiments, both concentrations inhibited phosphorylation of the Akt/PKB protein. The lower concentration mainly caused cell cycle arrest whereas at the higher concentration cell death was induced. Expression changes in genes involved in cell cycle regulation and apoptosis were observed. Cluster analysis grouped the cells treated with 30 μM and those treated with 100 μM on separate branches. A subset of differentially expressed genes in the cells treated with 30 or 100 μM of LY294002 could be identified. One of the genes was an apoptotic cysteine protease.

Expression profiling using DNA microarrays can be used to identify PI3K inhibitor regulated genes and may help understand signal transduction pathways for proliferation and survival in tumour cells. *This work was supported by the ICR and the CRC.*

412 p53 cooperates in enforcing rapamycin-induced G1 arrest and protects from apoptosis. S. Huang, L.N.Liu, H. Hosoi, M.B.Dilling, T. Shikata, P.J. Houghton. *Dept. Molecular Pharmacology, St. Jude Children's Research Hospital, Memphis, TN 38105, U.S.A.*

The macrolide antibiotic rapamycin specifically inhibits the Ser/Thr kinase activity of mTOR/FRAP, a signaling molecule that links extracellular growth factor signals to protein translation. Rapamycin is a potent inhibitor of tumor cell growth, and an analog, CCI-779 is currently under Phase I investigation for treatment of cancer. In this work, the relationship between G1 checkpoint function and rapamycin-induced apoptosis was examined using human rhabdomyosarcoma cells, and mouse embryo fibroblasts (MEFs). Serum-starved tumor cells expressing mutated p53 alleles became apoptotic when exposed to rapamycin, and were protected by expression of a rapamycin resistant mutant mTOR. Replication defective adenovirus expressing either p53, or $p21^{cip1}$, but not control virus, induced G1 accumulation, up-regulation of $p21^{cip1}$, and complete protection of cells from rapamycin-induced apoptosis, but not against apoptosis induced by cycloheximide. Under serum-free conditions rapamycin induced apoptosis of MEFs with $p53^{-/-}$ or $p21^{cip1-/-}$ genotype, but not wild type or $p19^{ARF-/-}$ cells. Cycloheximide induced apoptosis independent of the genotype. Ad-p21 and Ad-p53 rescued cells from rapamycin-induced death, whereas expression of dominant negative p53 in wild type MEFs restored the ability of rapamycin to induce apoptosis. Under serum-containing conditions rapamycin suppressed levels of cyclin A, only in wild type, and $p19^{ARF-/-}$ MEFs. Rapamycin also inhibited DNA replication or S-phase progression significantly more in wild type, $p19^{ARF-/-}$, and $p21^{cip1-/-}$ MEFs compared to $p53^{-/-}$ cells. Suppression of G1 progression by p53 was therefore partly independent of $p21^{cip1}$. However, $p21^{cip1}$-mediated G1 block ultimately protected cells from apoptosis. The data suggest p53 cooperates in enforcing G1 cell cycle arrest leading to a cytostatic response to rapamycin. In contrast, in tumor cells having deficient p53 function the response to this agent may be apoptosis. These data suggest a mechanistic basis for selective tumor cytotoxicity for rapamycin and CCI-779. Supported by USPHS grant CA77776 and by ALSAC.

413 Phase I and pharmacological study of CCI-779, a cell cycle inhibitor. M Hidalgo, E Rowinsky, C Erlichman, R Drengler, B Marshall, A Adjei, L Hammond, E Galanis, T Edwards, J Burton, J Boni, A Tolcher, G Dukart, and J Buckner. *CTRC, San Antonio, TX; Mayo Clinic, Rochester, MN; Wyeth-Ayerst Research, Radnor, PA.*

CCI-779, an ester of rapamycin, binds to FKBP-12 intracellularly, forming a complex that inhibits the kinase activity of mammalian target of rapamycin (mTOR). This interferes with key signal transduction pathways, including those regulated by p70s6 kinase and PHAS-I protein, resulting in inefficient translation of proteins involved in cell cycle progression. CCI-779 inhibited tumor growth in preclinical models and is being developed for treatment of cancer. This study is evaluating the safety and pharmacokinetics (PK) of escalating doses of CCI-779 administered as a 30-minute IV infusion daily x 5 every 2 weeks in patients (pts) with solid neoplasms. As of May 2000, 63 patients had been entered. This abstract is based on 45 patients in the data base as of April 2000, treated with doses ranging from 0.75–19.1 mg/m²/d. A total of 214 courses were administered. Two episodes of DLT in the first cycle were observed: asymptomatic, grade 3 hypocalcemia (2.16 mg/m²/d) and grade 3 elevation in transaminases (19.1 mg/m²/d). In addition, grade 3 thrombocytopenia requiring dose reduction

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observed also. Other adverse events, generally mild-moderate in severity, occurred over a broad range of doses. Toxicities include asthenia, cutaneous toxicity, mucositis, and hypertriglyceridemia. In 17 pts receiving 0.75 to 3.12 mg/m²/d, CCI-779 exhibited little accumulation from cycle to cycle, preferential binding to RBCs, dose-related increase in AUC, and mean $t_{1/2}$ of 32.6 h. Preliminary evidence of antitumor activity has been observed, with 1 PR (non-small cell lung cancer) and minor responses in other tumor types. The safety profile and antitumor activity observed to date, associated with plasma concentrations at which biological activity was observed in vitro, are encouraging.

414 CCI-779, an ester analogue of rapamycin that interacts with PTEN/PI3 kinase pathways: A phase I study utilizing a weekly intravenous schedule. E. Raymond, J. Alexandre, H. Depenbrock, N. Ady Vago, S. Faivre, A. Lahr-Randak, E. Materman, J. Boni, S. Abbas, E. Angevin, B. Escudier, J.P. Armand. *Institut Gustave Roussy, Villejuif, France; Onkologische Tagesklinik & Wyeth Ayerst Research / Genetics Institute, Munich, Germany.*

Background. CCI-779 inhibits mTOR, thus the phosphorylation of eIF4E-BP1 and $p70^{S6}$ kinases, prevents eIF4E to initiate protein synthesis and the phosphorylation of the ribosomal protein S6 required for the translation of mRNAs. **Patients and Methods.** CCI-779 was given as a weekly 30-min infusion in patients (pts) with advanced tumors using the modified CRM. **Results.** 18pts (M/F: 12/6) received: 7.5 (1pt), 15 (2pts), 22.5 (1pt), 34 (3pts), 45 (4pts), 60 (1pt), 80 (1pt), 110 (1pt), 165 (1pt) and 220 mg/m²/week (3pts). DLT was observed in only 1pt; MTD has not been reached. No prolonged immunosuppression has been induced. Grade (Gr) 1-2 skin toxicity was observed: dryness with mild itching (6pts), eczema-like lesions (2pts), sub-acute urticaria (2pts), and aseptic folliculitis (11pts). Gr1-2 and Gr-3 mucositis/stomatitis were observed in 10pts and 1pt, respectively. All pts receiving ≥ 8 doses experienced Gr-1 nail changes. Thrombocytopenia was observed in 9pts; 2pts with G-3 at 34 and 45 mg/m²/week. Leukopenia was reported in 4pts and anemia in 7pts. Asymptomatic increases of triglyceride and cholesterol levels were observed in 9pts and 5pts, respectively. A reversible decrease in testosterone concentrations with increased levels of LH/FSH were observed in 5/9 men receiving ≥4 doses at dosages ≥15mg/m²/week. Pharmacokinetic analysis from 12pts (doses: 7.5–60 mg/m²/week) indicates that CCI-779 C_{max} increased linearly but AUC increased sub-proportionately. Clearance and volume of distribution at steady state increased with increasing dose. Mean half-life was about 20hrs. Of the 16pts evaluable for anti-tumor activity, 3pts had a partial response (renal cell carcinoma with lung metastases; neuro-endocrine tumor with hepatic metastases and breast cancer with liver, lymph node and peri-orbital metastases). **Conclusion.** Current data show that CCI-779 has promising activity and mild-moderate toxicity over a broad range of doses.

415 Effect of the proteasome inhibitor PS-341 on cell cycle progression and bcl-2: A potentially unique mechanism of action. R. Perez-Soler†, YH Ling†, B Ng†, J Adams*, P Elliott*, L Liebes†. *Kaplan Cancer Center, †New York University School of Medicine, New York, NY, and *Millennium Pharmaceuticals, Cambridge, MA.*

PS-341 is a proteasome inhibitor currently in Phase I clinical evaluation. We studied the effects of PS-341 on cell cycle progression and related events in human NSCLC H460 (p53 wild type) and H358 (p53 null) cells. Exposure to 0.1 μM PS-341 for 6 h resulted in a marked accumulation of cells at G2/M. This blockade was associated with a 6–10 fold time-dependent accumulation of cyclins A and B and a 10-fold elevation of cyclins A and B kinase activities as assessed by ^{32}P-γ-ATP incorporation into histone-H1. In addition, bcl-2 phosphorylation, a marker of mitotic arrest, was detected as early as 3 h after exposure to PS341. More importantly, a 25 kDa bcl-2 degradation product was detected as early as 12 h after exposure to PS341. This degradation product appeared specific for proteasome inhibition since it was observed with the proteasome inhibitors MG132 and PSI but not with the chemotherapeutic agents paclitaxel, vinblastine, camptothecin, etoposide, and cisplatin, or the PKC inhibitor staurosporine. In addition, it was not caspase-dependent since it was observed in the presence of caspase inhibitors and appeared to localize in the triton X-100 insoluble cellular fraction. In view of the ability of PS341 to induce arrest at G2/M we then studied *in vitro* cytotoxicity of the combination of PS341 and the antitubulin agent docetaxel against H460 and H358 cells. Cells were treated concomitantly with PS341 (0.1μM or 0.5μM) and docetaxel (0.1 to 4μM) for 48 h. An additive cytotoxic effect was observed with the combination 0.5μM PS341 and 0.5 and 1μM docetaxel. In conclusion, our results indicate that PS341 induces unique changes in bcl-2 that appear to be specific for proteasome inhibition. The functional consequences of these bcl-2 changes and their potential relationship with the demonstrated ability of this agent to retain its cytotoxicity against bcl-2 transfected cells is being investigated.

416 A phase I pharmacodynamic study of the proteasome inhibitor PS-341. J. P. Thomas, A. Adjei, C. Ehrlichman, P. Geiger, A. Haas, R. Arzoomanian, D. Alberti, R. Marnocha, K. Binger, J. Volkman, C. Feierabend, K. Tutsch, J. Adams, P. Eliot and G. Wilding. *University of Wisconsin Comprehensive Cancer Center, Madison, WI, Mayo Clinic, Rochester, MN and Millenium Pharmaceuticals, Cambridge, MA.*

The ubiquitin-proteasome pathway is the principal enzymatic degradation pathway for many intracellular proteins, including those involved in cell cycle regulation, diverse metabolic processes including stabilization of cell cycle regulatory proteins and inhibition of NF-κB activation. PS-341 has broad activity including MDR and Bcl-2 overexpressing cancer cell lines. *In vivo* PS-341 inhibits the growth of a number of tumors including the HT-29, NCI-H23 and PC-3 models. Toxicity was seen in preclinical models when the proteasome was inhibited by greater than 80%. We are conducting a phase I trial of PS-341 in patients with advanced refractory cancers. PS-341 is administered intravenously twice weekly for 4 weeks followed by a two week break. Dose levels of 0.5, 0.9, 1.25 and 1.50 mg/m² have been explored. A total of 9 patients have been treated at the UW. Toxicities seen have included rash, fatigue and thrombocytopenia. A MTD has not yet been reached. Proteasome inhibition by PS-341 has been monitored by measuring 20S proteasome activity in whole blood samples using a flourogenic peptide substrate. 20S proteasome inhibition in this study measured 1 hour after PS-341 administration correlates highly with PS-341 dose. We are achieving levels of proteasome inhibition (> 60%) associated with anti-tumor activity in the preclinical models. We have also examined patient peripheral blood mononuclear cells to determine whether levels of proteasome inhibition achieved in this study may be associated with accumulation of ubiquitinated proteins. Cell lysates were analyzed by Western blot for ubiquitin protein conjugates. Up to a 3 fold increase in ubiquitinated proteins were seen in some patients, peaking at 5 hours after PS-341 administration.

417 Proteasome inhibition by PS-341: A phase I study. A Hamilton[1], JP Eder[2], A Pavlick[1], JW Clark[3], A Chachoua[1], DP Ryan[3], K Farrell[1], H Wasserstrom[1], L Liebes[1], J Wright[4], P Elliott[5], J Adams[5] and F Muggia[1]. [1]*NYU Sch. of Med.*, [2]*Dana Farber Can. Inst.*, [3]*Mass. General Hosp.*, [4]*CTEP NCI*,[5]*Millennium Pharm.*

The proteasome is a multimeric protease complex that regulates cellular proteins by degrading ubiquinated proteins. Proteasome inhibition results in increased levels of a variety of key cellular proteins that may contribute to anti-tumor activity; IκB inhibits nuclear factor κB (NF-κB) mediated transcription, p53 inhibits apoptosis, and p21 inhibits cyclin-dependent kinase (CDK) activity. PS-341 is a dipeptide boronic acid derivative that inhibits the proteasome by stabilization of its active site. Animal models predicted dose limiting gastrointestinal toxicity at ≥80% proteasome inhibition (PI). PS-341 was administered as an IV bolus on D1&4 of a 2-week cycle. Five dose levels have been studied to date: 0.25mg/m², 0.8mg/m², 1mg/m², 1.2mg/m² and 1.45mg/m². 19 pts have been treated: 11M / 8F. Age: median 57, range 25–78. Primary tumors: colorectal (3), renal (3), NSCLC (3), melanoma (2), ST sarcoma (2), osteosarcoma (1), lymphoma (1), prostate (1), endometrial (1), esophagus (1), hepatoma (1). Prior therapies: chemotherapy (17), radiotherapy (13). Toxicities have been mild and non-specific. 1/6 pts treated at 1.2mg/m² experienced self-limiting G3 diarrhea. No objective responses have been documented. One pt with melanoma treated at 1mg/m² maintained a PR in lung with SD in skin for 6 months. PI was measured at 1, 4 and 24hrs after dosing. At all dose levels, peak PI was seen at 1hr, and recovery to approximately 50% of peak PI was seen at 24h. Peak mean PI were 21%, 54%, 48% and 59% at dose levels 1, 2, 3 and 4 respectively. Tumor PI at 24h in one pt was 87% and averaged 54% in 2 biopsies at 2–3 h in another pt. Accrual is ongoing at 1.9mg/m², and phase II studies are planned. Supported by U01 CA76642, M01 RR00096 and the Lynne Cohen Foundation (NY), and U01 62490 (Boston).

418 Pharmacodynamic evaluation of the protein kinase C (PKC) inhibitor CGP41251 (PKC412) in patients with metastatic melanoma. M. Millward[1], C. House[1], L. Webster[1], B. Linahan[1], I. Olver[2], G. Toner[1], J. Zalcberg[1], D. Bowtell[1]. [1]*Peter MacCallum Cancer Institute, Melbourne*, [2]*Royal Adelaide Hospital, Australia.*

PKC412 selectively inhibits PKC (IC_{50} <1μM) and has preclinical activity as a cytostatic and modulator of MDR. The recommended Phase II dose is 75mg tds which produces potentially active trough plasma levels (10μmol/l), and suppresses cytokine release and lymphocyte ERK2 levels (Thavasu 1999). Patients (pts) with measurable metastatic melanoma and ≥ 2 superficial lesions received 75mg tds; tumor biopsies and plasma were collected prior to and after 28 days treatment. Intra-tumoral total PKC activity was measured in cytosolic and particulate fractions using protamine sulphate as the substrate. Initial experiments showed addition of 10μM PKC412 to melanoma biopsies inhibited phosphorylation. Ability of plasma to modulate *ex vivo* intracellular daunorubicin accumulation in MDR cells was measured with activity of 20μg/ml valspodar (PSC833) defining 100% reversal. Compared to the pretreatment biopsy, cytosolic PKC activity was reduced by 7% to 91% in 7/9 pts. Particulate PKC activity was reduced by 11% to 79% in 4/9 pts. Only 1 pt had >50% inhibition in both fractions. Tumor PKC isoform profile in 1 pt resistant to PKC412 (unchanged cytosolic activity and >200% increase particulate activity) showed an abundance of PKCξ, an isoform refractory to inhibition by PKC412 (IC_{50} >1000μM). Addition of 20μg/ml PKC412 to pretreatment plasma produced 14%–64% (mean 40%) reversal of MDR. Plasma taken following 28 days PKC412 showed <10% reversal in 8/8 patients. All patients had progressive disease. This Phase IIA trial did not demonstrate consistent target inhibition or pharmacodynamic efficacy of PKC412 in melanoma patients.

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