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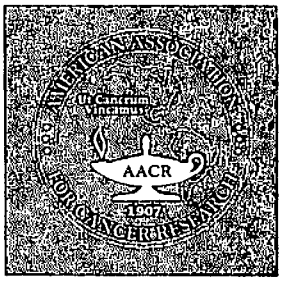

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EORTC
European Organization for Research and Treatment of Cancer

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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/m^2/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/m^2/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/m^2/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/m^2/week. Pharmacokinetic analysis from 12pts (doses: 7.5–60 mg/m^2/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-Solert, YH Linqt, B Ngt, J Adams*, P Elliott*, L Liebest. *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 (0.1 μM 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 most 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/m^2 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/m^2, 0.8mg/m^2, 1mg/m^2, 1.2mg/m^2 and 1.45mg/m^2. 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/m^2 experienced self-limiting G3 diarrhea. No objective responses have been documented. One pt with melanoma treated at 1mg/m^2 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/m^2, 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.