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therapy is more rational than sequential therapy. Finally, the combination of PB + CRA and TX was evaluated in vivo. PC3 tumor growth was significantly delayed in animals treated with either TX or concomitantly with TX and PB + CRA, as compared to control. However, animals treated with all three agents demonstrated further growth delay than the TX alone arm ($P < 0.012$). These results suggest a rational therapeutic approach for combination of these agents given concomitantly, but not sequentially.

#1969 The Up-Regulation of GADD153 Expression in Human Epithelial Tumor Cells Treated with *N*-(4-Hydroxyphenyl)Retinamide (4HPR). Yuhe Xia, Nai-Sum Wong, Wang-Fun Fong, and Henk Tideman. *City University of Hong Kong, Hong Kong, China, and The University of Hong Kong, Hong Kong, China.*

Previous clinical trial studies have demonstrated a favorable therapeutic index for the semi-synthetic retinoid *N*-(4-hydroxyphenyl)retinamide (4HPR, fenretinide), but the underlying molecular mechanism of actions for this drug is largely unknown. This issue is addressed with the use of the CNE3 human epithelial tumor cell line established from a poorly differentiated nasopharyngeal carcinoma. Treatment of these cells with 4HPR (2.5-40 μM) initially resulted in dose-dependent cell-cycle arrest, with cells accumulating in the G_0/G_1 phase, and a corresponding reduction in the G_2/M phase. Continued application of 4HPR resulted in apoptosis that was evident by an apoptotic morphology (detected by fluorescence microscopy), the presence of internucleosomal cleavage of DNA, and the presence of a sub-G_1 DNA fraction. Using the cDNA-array technique, a series of cell cycle regulatory genes were found to have down-regulated in 4HPR-treated cells, of which CDC25B, CDK1 (cyclin-dependent kinase 1), and CCNB1 (cyclin B1) were 6.5, 10.3 and 14-fold reduced respectively when compared to the control. The stress-responsive gene GADD153 was the only gene that was up-regulated by 7-fold. This up-regulation of GADD153 in response to 4HPR treatment was confirmed by RT-PCR and Western analysis, and was found to occur in two other epithelial tumor cell lines established from the head and neck region. The up-regulation of GADD153 in CNE3 cells preceded apoptosis after application of 4HPR at clinically relevant doses. Treatment of CNE3 cells with the MAPK(mitogen-activated protein kinase) kinase inhibitor (PD98059) produced a similar up-regulation of GADD153 expression. By contrast, aurintricarboxylic acid (ATA), a MAPK-activator had no effect on GADD153 expression. Taken together, our findings indicate that treatment of cells with 4HPR first induces a stress-response, with an increase in the expression of GADD153 and this is followed by apoptosis. A reduction of the activity of the MEK/MAPK pathway in CNE3 cells may be responsible for the up-regulation of GADD153 and further study is required to examine if the same mechanism is adopted by 4HPR to up-regulate the expression of GADD153.

#1970 STI571 Decreases Glucose Derived Nucleic Acid Synthesis but Increases Direct Glucose Oxidation in K562 Myeloid Blast Cells. Laszlo G. Boros, Joan A. Boren, Silvia Marin, Marta Cascante, Shu Lim, Sara Bassilan, Ahmed Sayed, and Wain-Nang P. Lee. *Department of Biochemistry and Molecular Biology, Barcelona, Spain, and Harbor-UCLA Research and Education Institute, Torrance, CA.*

Chronic myeloid leukemia cells contain a constitutively active Bcr-Abl tyrosine kinase, the target protein of STI571 phenylaminopyrimidine class protein kinase inhibitor. Here we provide evidence for metabolic phenotypic changes in cultured K562 human myeloid blast cells after treatment with increasing doses of STI571. We used [1,2-13C2]glucose as the single tracer and biological mass spectrometry in order to delineate glucose derived specific synthesis of RNA ribose, lactate, glutamate and palmitate. The synthesis pathways of these molecules from glucose determine the rate of cell proliferation, differentiation and apoptosis in leukemia cells, which are highly dependent on glucose carbons for their growth. Proliferation of K562 cells showed a 57%, 74% and a 99.5% decrease while glucose derived synthesis of RNA also decreased by 7.8%, 34.1% and 41.5% in response to 0.4, 4 and 40 mg/ml STI571, respectively. On the other hand, glucose consumption, lactate production, 13CO2 release and direct glucose oxidation through G6PD showed a remarked dose-dependent increase in the same cells. These metabolic changes were absent or less prominent in Bcr-Abl negative MIA pancreatic adenocarcinoma cells. The leukemia cell growth controlling characteristics of STI571 involve the regulation of anabolic glucose use for de novo nucleic acid synthesis through the non-oxidative steps of the pentose cycle. The excess glucose not used for nucleic acid synthesis is re-routed toward direct oxidation through the oxidative steps of the pentose cycle and released as lactate into the cell culture medium. Therefore, the Bcr-Abl tyrosine kinase construct is likely associated with the phosphorilation or transcriptional regulation of metabolic enzymes that are involved in glucose carbon redistribution among cell proliferation-related macromolecules (RNA, DNA) and direct oxidative glucose degradation. The profound metabolic changes observed in response to STI-571 treatment indicate that leukemia cell growth is oppositely influenced by increased glycolysis and the anabolic glucose utilizing reactions of the non-oxidative branch of the pentose cycle.

#1971 The Immunosuppressant Rapamycin Inhibits Growth of Human Hepatoma Cells Alone or Combined with Tacrolimus, while Tacrolimus Alone Accelerates Cell Growth. Guido Schumacher, Marijke Oidtmann, Anne Rüggeberg, Andrea R. Mueller, Jan M. Langrehr, Marcus Bahra, Herwig Gerlach, Klaus P. Platz, and Peter Neuhaus. *Charité Campus Virchow Klinikum, Humboldt Universtiy, Berlin, Germany.*

After human organ transplantation the incidence of a malignant tumors is up to 10% in all patients using standardized immunosuppressive regimens containing tacrolimus. Recurrence of hepatoma after liver transplantation occurs in over 50% in patients with high tumor stage and angioinvasion. The immunosuppressive drug Rapamycin has been shown to inhibit the cell cycle in various cell systems. We examined the effect on growth of the human hepatoma cell lines SK-Hep1 (wt-p53), Hep3B (mut-p53), and PLC/PRF/5 (mut-p53) using different concentrations of rapamycin, tacrolimus, and a combination of both. There was a dose dependent growth inhibition of all three cancer cell lines after treatment with rapamycin after 3 and 5 days with similar sensitivity, regardless of the p53 mutation status. Inhibition ranged from 16% at 1ng/ml in Hep3B cells to 74% at 100ng/ml in SK-Hep1 and Hep3B cells after 5 days. Treatment with tacrolimus showed a growth stimulating effect on all tumor cell lines. Cell proliferation was increased with a range of 12% after 5ng/ml to 25% after 1000ng/ml after three days in Hep3B cells. The combination of rapamycin and tacrolimus at equal doses resulted in growth inhibition of all cancer cells similar to treatment with rapamycin alone. In this combined treatment, tacrolimus had no impact on growth stimulation. Phase contrast microscopy revealed that there is no change in cell morphology. FACS analysis confirmed that there was no induction of apoptosis. An increase of the cell fraction in S-phase of up to 50% and G2-phase of up to 20% was observed in rapamycin treated cells. No changes were seen in tacrolimus treated cells. We conclude that rapamycin has strong inhibition of hepatoma cell growth in contrast to tacrolimus, which stimulates hepatoma cell growth. Immunosuppression after liver transplantation containing rapamycin rather than tacrolimus may reduce the incidence of tumor recurrence after liver transplantation.

#1972 Antitumor Activity of RAD001, an Orally Active Rapamycin Derivative. Iwan Beuvink, Terence O'Reilly, Sabine Zumstein, Frederic Zilberman, Richard Sedrani, Sara Kozma, George Thomas, and Heidi A. Lane. *Friedrich Miescher Institute, Basel, Switzerland, and Novartis Pharma AG, Basel, Switzerland.*

RAD001 is a hydroxy-ethyl ether derivative of rapamycin that is orally bioavailable. RAD001 has demonstrated in vitro anti-proliferative activity against a number of human tumor cell lines. Concentration response curves, with IC10s, IC50s and IC90s differing by several orders of magnitude, suggest potent inhibition of cell proliferation but less potency in killing tumor cells. IC50 values ranged from fM to μM, indicating that although some tumor cell lines are very sensitive to RAD001 treatment others are intrinsically more resistant. Despite these differences in growth response, down-regulation of p70S6 kinase activity and reductions in 4E-BP1 phosphorylation (downstream effectors of the mTOR "Target Of Rapamycin" kinase pathway) were observed in both sensitive (A549; IC50: 6fM) and resistant (HCT-116; IC50: 6μM) cell lines. Washout experiments further demonstrated a dramatic, sustained down-regulation of mTOR targets for up to 2-3 days after a single 30 min RAD001 pulse-treatment. These data indicate that RAD001 has a similar mechanism of prolonged action as reported for rapamycin; however, resistant lines do exist which are able to compensate for RAD001-induced loss of mTOR function. In vivo, RAD001 was orally active, inhibiting the growth of human tumor xenografts in nude mice at doses ranging from 0.5-5.0mg/kg/d. At these doses RAD001 was well tolerated.

#1973 Optimized Synergistic Effect When Hyperthermia Precedes 2', 2' Difluorodeoxycytidine (dFdC, Gemcitabine) By 24 Hours. Roger A. Vertrees, Joseph B. Zwischenberger, and Paul J. Boor. *The University of Texas Medical Branch, Galveston, TX.*

Hyperthermia has been shown to increase cytotoxicity of various antineoplastic agents. We investigated the metabolism, cytotoxicity and sensitizing properties of 2', 2' difluorodeoxycytidine (dFdC, Gemcitabine) and hyperthermia (HT), alone and in three distinct temporal associations on human lung cancer cell lines *in vitro*. A malignant human lung cancer cell line (BZR-T33) was treated with various concentrations (0.1, 0.5, 1.0, 5.0, and 10.0 μM) of dFdC for 30, 60, 120 or 180 min to determine dFdC-LD33 (dose necessary to kill 33% of cells). Another aliquot of the same cells were subjected to various hyperthermic temperatures (40, 41, 42, 43, 43.5, 44°C) for either 60, 120 or 180 min to determine hyperthermia-LD33. LD33, dFdC was 0.15 μM for 3 h, and for hyperthermia was 43°C for 3 h. Cells were divided into six groups dependent on treatment received: no treatment is control (C), hyperthermia LD33 (H), Gemcitabine LD33 (G), hyperthermia LD33 then 24 h later Gemcitabine LD33 (H/G), Gemcitabine LD33 then 24 h later hyperthermia LD33 (G/H), and simultaneous hyperthermia LD33 and Gemcitabine LD33 (G&H). Cell survival was determined by 7-day growth curves and clonogenic assay, cell cycle perturbation was assayed by flow cytometry. Results indicate that when compared to controls, 7-day growth curves show significant reductions in number of surviving cells for all treatments: 0.5-log ($p<0.05$) reduction for heat only, 2-log ($p<0.05$) reduction for dFdC only, and a 3.5-log ($p<0.05$) reduction for combined modality. Compared to controls, clonogenic results show significant reductions in surviving fractions and colony size: control 72% and 16.5 mm^2, heat only 29% and 10.1 mm^2 ($p<0.05$), dFdC only 39% and 9.2 mm^2 ($p<0.05$) and for combined modality 12% and 2.7 mm^2 ($p<0.05$). Results indicate that 24 h after exposure, control cells had 43% of cells in G_0/G_1, 24% in G_2/M and 32% in S-phase. DFdC-treated cells had 72% of cells accumulated in G_0/G_1, 3.7% in G_2/M and 24% in S-phase. Heat-treated cells had 43% in G_0/G_1, 37% in G_2/M, and 20% in S-phase. The combined modality of heat followed 24 h later by dFdC resulted in 7% of cells in G_0/G_1, 33% of cells G_2/M and 60% in S-phase. We