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differentiation of myeloid leukemia cell lines (NB4, HL-60 and U937). SS was identified in our recent studies as a potent inhibitor of PTPases. Herein, we present data demonstrating that SS (250 μg/ml, 6 days) induced 87% of NB4 cells to reduce nitroblue tetrazolium (NBT), in comparison to the 90% induced by ATRA (1 μM, 6 days). SS-induced NB4 cell differentiation was confirmed by increased CD11b expression and associated with growth arrest at S phase and increased cell death. Our results showed further that SS-induced NB4 differentiation was irreversible and required continuous drug exposure for optimal induction. Moreover, SS (400 μg/ml, 6 days) induced 60% and 55% of NBT-positive cells in HL-60 and U937 cell lines, which were augmented in the presence of GM-CSF (2 ng/ml) to levels (85% and 81%, respectively) comparable to those induced by ATRA. These results provide the first evidence of a differentiation induction activity of PTPase inhibitor SS in myeloid leukemia cell lines and ssuggest its potential therapeutic use in myeloid leukemia. Since SS induces differentiation via targeting PTPases, a mechanism distinct from that of ATRA, it may be particularly useful in AML cases unresponsive or developed resistance to ATRA.

#356 NAD(P)H:quinone oxidoreductase (NQO1)-dependent and -independent cytotoxicity of potent quinone cdc25 phosphatase inhibitors. Yusheng Han, Hongmei Shen, Brian Carr, John S. Lazo, and Su-Shu Pan. *University of Pittsburgh Cancer Institute, Pittsburgh, PA, and University of Pittsburgh, Pittsburgh, PA.*

A vitamin K analogue, compound 5 (Cpd5, a thioethanol naphthoquinone), inhibits oncogenic Cdc25 phosphatases, and arrests cell cycle progression at both G1 and G2/M. Recently, we evaluated >10,000 compounds in the NCI chemical repository for *in vitro* inhibition against recombinant human Cdc25B phosphatase and identified a quinone substructure in many of the active compounds. Bioreductive enzymes in cells, however, are known to reduce various quinones resulting in either detoxification or activation. Therefore, we used an isogenic set of human colon cancer cell lines to evaluate the effect of NQO1 on the cytotoxic activity of Cpd5 and the two most potent phosphatase inhibitors from the repository: NSC 95397 (a bis-thioethanol naphthoquinone) and NSC 663284 (a quinolinedione). The two cancer cell lines used were HCT116, which has intermediate NQO1 activity, and its mitomycin C-resistant sub-line HCT116-R30A (R30A), which has minimum NQO1 activity. Cell survival was measured by colony formation after 7 days drug exposure. Cell cycle arrest was evaluated by flow cytometry after 6 hr drug exposure. Cpd5 had an IC_{50} of 2.2$\pm$0.3 μM for HCT116 and 0.23$\pm$0.05 μM for R30A, i.e. a 10-fold difference. Inclusion of dicoumarol (10 μM), an inhibitor of NQO1, decreased the IC_{50} of Cpd5 for HCT116 to 0.24$\pm$0.04 μM, but had no effect on R30A. In contrast, HCT116 and R30A cells were equally inhibited by NSC 95375 with IC_{50}s of 1.4$\pm$0.3 μM and 1.3$\pm$0.2 μM, respectively. Similarly, HCT116 and R30A cells were equally inhibited by NSC 663284 with IC_{50}s of 2.4$\pm$0.3 μM and 2.6$\pm$0.5 μM, respectively. All three compounds blocked the two cell lines at the G2/M phase transition, consistent with cdc25 inhibition. Cpd5 at 2.5 μM arrested R30A cells at G2/M but 7.5 μM Cpd5 was needed to arrest HCT116 cells to a similar degree. NSC 95375 and 663284 arrested cell cycle progression at G2/M of HCT116 and R30A cells similarly, and did so in a concentration-dependent manner between 2.5 and 7.5 μM. Our data imply that NQO1 in HCT116 cells protected cells from the action of Cpd5, probably by the reduction of Cpd5 to a less active hydroxylquinone. In contrast, both NSC 95397 and 663284 displayed cytotoxicity that was independent of NQO1 levels. (Support: NCI CA61862 and CA78039)

#357 Antitumor and anticarcinogenic action of Cpd 5: A new class of protein phosphatase inhibitor. Siddhartha Kar, Meifang Wang, Zhenggang Ren, Xiangbai Chen, and Brian I. Carr. *University of Pittsburgh, Pittsburgh, PA.*

Background: We have chemically synthesized a new class of inhibitors of dual specificity phosphatases (DSP), which play an important role in cell cycle and signal transduction. Cpd 5 or 2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone is one of the most potent. It inhibits DSPs (especially the Cdc25 family) in tissue culture cells and induces tyrosine phosphorylation of various DSP substrates, including Cdks and inhibits cell growth both *in vitro* and *in vivo* (JBC 270:28304, 1995; Proc. AACR 39:224, 1998). Purpose: In this study we evaluated (a) the antitumor and (b) the anticarcinogenic activity of Cpd 5 for the first time. Methods: (a) JM1 hepatomas were grown in 2 month old Fischer male rats by subcutaneous injection on the back or intra-portally in the liver. Rats were treated with Cpd 5 by intratumor, subcutaneous (nearby site), intramuscular (distant site), or intraperitoneal injection, either as a single high acute dose or chronically as several low doses. (b) Rats were injected intraperitoneally with a single dose of the carcinogen N-Nitrosodiethylamine (DEN). Immunostained liver sections for glutathione-S-transferase-pi (GST-pi) detected pre-neoplastic foci after 3 weeks. Cpd 5 was injected subcutaneously or intraperitoneally two weeks after DEN as a single high acute dose or chronically as several low doses. Results: (a) Cpd 5 had significant inhibitory effect on both intrahepatic (14% of control, $p<0.00000008$) and subcutaneous (33% of control, $p<0.00008$) tumor growth and also had significant inhibitory effect when injected intramuscularly at a site distant from the tumor (50% of control, $p<0.002$). There was no significant difference between the effects after acute or chronic injections. However, toxicity was much lower with chronic treatment. (b) The number of enzyme altered foci was also significantly reduced when rats were treated with acute (40% of control, $p<0.00002$) or chronic (50% of control, $p<0.02$) Cpd 5. Conclusions: Cpd 5 had significant inhibitory effect on growth of tumors and foci.

#358 Bosentan, a novel endothelin-A and -B receptor antagonist inhibits proliferation of malignant melanoma cells. Aleksandar Sekulic, Padma Suresh, Mark R. Pittelkow, and Svetomir N. Markovic. *Mayo Foundation, Rochester, MN.*

Here we tested a feasibility of endothelin (ET) receptor blockade with a dual endothelin-A and -B receptor (ETR-A and ETR-B) antagonist, Bosentan, as a novel therapeutic approach for malignant melanoma. ETs are 21aa peptides primarily produced by endothelial cells and implicated in a variety of physiological functions. Binding of ET to ETR-A on vascular structures potently stimulates angiogenesis and, thus, likely plays an important role in growth of multiple cancers. Activation of ETR-Bs, among other things, regulates melanocyte development and function. We first examined patterns of ETR subtype expression on six established melanoma cell lines using flow cytometry and immunocytochemistry. Following this sections of primary and metastatic melanoma tissues were evaluated for ETRA/ETRB expression by immunohistochemistry. To test functional effects Bosentan on melanoma cell proliferation, six melanoma cell lines were subjected to standard 3H-thymidine incorporation assays in presence or absence of various concentrations of Bosentan. All examined melanoma tissues (2 primary, 9 metastatic) express ETRB, albeit to different levels, whereas ETRA was expressed to low levels in only 3 metastatic tumors. In functional assays Bosentan inhibited proliferation of all examined cell lines with the IC50 ranging between 7 and 40 μg/ml. Our results suggest that malignant melanocytes express functional ETRs, and their treatment with Bosentan leads to significant growth inhibition. Concurrent inhibition of ETR-A and ETR-B *in vivo* by low toxicity, orally available inhibitor Bosentan might therefore prove useful as a novel mode of anti-melanoma therapy through simultaneous inhibition of cancer cell growth and process of angiogenesis.

#359 *In vivo* activity of RAD001, an orally active rapamycin derivative, in experimental tumor models. Terence O'Reilly, Juliane Vaxelaire, Melanie Muller, Heinz-Herbert Fiebig, Marc Hattenberger, and Heidi A. Lane. *Business Unit Oncology, Novartis Pharma AG, Basel, Switzerland, and Oncotest gmbH, Freiberg, Germany.*

RAD001 is a hydroxyethyl ether derivative of rapamycin that is orally bioavailable. RAD001 has demonstrated *in vitro* anti-proliferative activity against a panel of human tumor lines. For *in vivo* testing, tumor-bearing nude mice were administered RAD001 in a variety of doses and schedules. Tumors were established by transplantation of fragments generated from injection of cells, or by transplantation of fragments from stabilized tumors originating from surgically removed human tumors. When administered once daily p.o., at doses ranging from 0.5-5.0 mg/kg/day, RAD001 was a potent inhibitor of tumor growth in 10 different xenograft models of human tumors (including pancreatic, colon, epidermoid, lung and melanoma). In general, RAD001 was well tolerated and better tolerated in mouse xenograft models than standard cytotoxic agents (i.e. doxorubicin and 5-fluorouracil), while possessing similar antitumor activity. Only one instance of *in vivo* resistance has been observed (MAXF 401 mammary xenograft model), otherwise the activity of RAD001 was generally inhibition of tumor growth (persistent regressions in one tumor line, T/C values of 9 to 45 % in 8 tumor lines). Xenograft models sensitive to RAD001 treatment included tumors exhibiting comparative resistance *in vitro* (KB-31 and HCT116). Persistent tumor regressions (41 %) were observed in a line displaying sensitivity to RAD001 *in vitro* (A549). Pharmacokinetic analyses, following a 5 mg/kg administration, indicated rapid uptake into plasma (Cmax 2513 ng/ml; Tmax 1 h), but the time to Cmax was delayed in tumors (Cmax 102 ng/g; Tmax 2 h). Elimination from the tumor (t1/2, 16 hr) was apparently slower than for plasma (t1/2, 7.5 hr). RAD001 levels were above the IC50 of A549 cells for a 72 h period. Interestingly, tumor RAD001 levels, following a single 5 mg/kg administration, never exceeded the *in vitro* antiproliferative IC50 for either KB-31 or HCT116 cells; despite the sensitivity of these lines *in vivo*. From these observations, and given the extreme sensitivity of endothelial cells to RAD001, it is plausable that RAD001 may not only act on tumor cells but may also affect angiogenesis. Taken together, these data support the application of RAD001 as an antitumor agent.

#360 Discovery of anticancer agents from sponge-associated fungi. Frederick A. Valeriote, Karen Tenney, Charles Grieshaber, Halina Pietraszkiewicz, Akiko Amagata, Taro Amagata, Jeff Gautschi, Joseph Media, Joseph Stayanoff, Richard Wiegand, and Phil Crews. *Henry Ford Health System, Detroit, MI, and University of California Santa Cruz, Santa Cruz, CA.*

We have evaluated 1,112 extracts (from 660 sponge-associated fungi) for assessment of potential anticancer activity. Both broth and mycelia extracts were assayed in most cases. Each sample was assayed *in vitro* against up to 8 cell types (murine and human) in a disk diffusion/ clonogenic assay. From these results, the samples were assigned into one of 4 categories: Inactive (79% of the extracts), Equally active across cell types (16% of the extracts), Equally active and potent (9 extracts or 1%), and Solid tumor selective (42 extracts or 3.8%). The equally active and potent category is studied further since solid tumor selective compounds might exist in the extract but be concealed by one or more potent, cytotoxic compounds. Further, a novel, potent compound could form the basis for analog synthesis in an attempt to develop an active anticancer agent. One