

## Abstract# 1610

## Poster Board#/Session: 282-III

**LOW LEVEL OF DEOXYCYTIDINE KINASE EXPRESSION AND ACTIVITY IS COMMON IN SAMPLES FROM NEWLY DIAGNOSED ACUTE MYELOGENOUS LEUKEMIAS RESISTANT TO ARA-C.** D. Martincic\*, V. Kravtsov, M. Koury, J.A. Whitlock. *Departments of Pediatrics and Medicine, Vanderbilt University, Nashville, TN, USA.*

Deoxycytidine kinase (dCk) catalyzes the rate-limiting phosphorylation of deoxycytidine and its analogue ara-C. Phosphorylated ara-C is converted to its cytotoxic triphosphate metabolite that inhibits DNA synthesis. Loss of dCk activity has been associated with resistance to ara-C in leukemic cell lines and animal models. Limited studies in patients with acute myelogenous leukemias (AML) resistant to ara-C after standard or high-dose ara-C therapy have suggested that mutations in dCk coding region contribute to resistance to ara-C. We analyzed twelve samples from patients with newly diagnosed AML for resistance to ara-C and level of dCk expression and activity. In vitro resistance to ara-C of blasts from patients at diagnosis was analyzed using microculture kinetic (MiCK) assay. The results of MiCK assay were correlated to clinical outcome after induction therapy. dCk protein activity was measured using [<sup>3</sup>H] deoxycytidine as a substrate in the presence of excess thymidine. dCk mRNA level was assessed by semiquantitative polymerase chain reaction (PCR). In the MiCK assay, 9/12 samples were intrinsically resistant to ara-C while 3 patients showed varying levels of sensitivity. To correlate clinical response to induction therapy with in vitro resistance, samples were also analyzed for in vitro resistance to idarubicin or daunomycin. Correlation with clinical outcome showed that 7/9 in vitro ara-C resistant patients did not achieve complete remission (CR), 1 patient achieved CR and 1 patient underwent bone marrow transplant. In a group of patients sensitive to ara-C, 2 patients achieved CR and 1 achieved partial remission. PCR for dCk mRNA expression revealed a low level of mRNA expression in 8/9 patients resistant to ara-C and a high level of mRNA expression in one patient. dCk protein activity assay showed low level of dCk activity in 8/9 resistant samples. Two of three samples sensitive to ara-C also showed a low level of dCk activity. We conclude that decreased expression and activity of dCk is common in AML samples and may contribute to resistance to ara-C during induction therapy. High level of dCk expression and activity in some samples resistant to ara-C suggests involvement of mechanisms of resistance to ara-C other than low dCk expression and activity such as point mutations in dCk mRNA or increased activity of cytidine deaminase. Low level of dCk activity in patients in vitro sensitive to ara-C suggests another activity that may activate ara-C.

## Abstract# 1611

## Poster Board#/Session: 283-III

**DIPHTEA TOXIN FUSED TO GM-CSF OVERCOMES RESISTANCE TO APOPTOSIS MEDIATED BY BCL-2 AND IS SYNERGISTIC WITH ARA-C AGAINST HUMAN AML HL-60 CELLS.** K. Bhalla, C.N. Kim\*, R.J. Kreitman\*, P. Hall\*, T. Jia\*, M. Willingham\*, A. Frankel. *Emory University School of Medicine, Winship Cancer Center, Atlanta GA, and Wake Forest University School of Medicine, Comprehensive Cancer Center, Winston-Salem NC, USA.*

We have determined the cytotoxic effects of human GM-CSF fused to truncated diphtheria toxin (DT388-GMCSF) and/or Ara-C against HL-60 cells and their Bcl-2 overexpressing (6-10 fold) counterparts (HL-60/Bcl-2 cells) (Leukemia 10:17-31, 1996). Exposure of HL-60/Bcl-2 cells to DT388-GMCSF (0.7 nM for 48 hours) produced a 4- to 8-fold increase in the cytosolic accumulation of cytochrome c (cyt c), increase in DFF45 (inhibitor of caspase-activated DNase) and poly(ADP-ribose) polymerase (PARP) cleavage activity of caspase-3 and apoptosis (as determined by Annexin V staining and FACS analysis and morphologic evaluation). HL-60/Bcl-2, but not the control HL-60 cells were resistant to Ara-C-induced (0.2 to 10 μM) cyt c-mediated molecular cascade of apoptosis. Notably, Ara-C (200 nM for 24 hours) demonstrated dramatic synergy (combination index 0.07) with DT388-GMCSF (0.7 nM for 48 hours) against HL-60 cells. No changes in Ara-C incorporation into cellular DNA were seen. Synergy was not seen with two other protein synthesis inhibiting drugs - ricin and cycloheximide. GMCSF alone failed to modulate Ara-C sensitivity. As compared to either drug alone, co-treatment of HL-60 cells with Ara-C and DT388-GMCSF produced a significant increase in the cytosolic accumulation of cyt c, associated with a higher percentage of cells demonstrating loss of mitochondrial membrane potential and an increase in the reactive oxygen species. Augmented cytosolic accumulation of cyt c also resulted in greater induction of the PARP and DFF45 cleavage activities of caspase-3 as well as apoptosis of HL-60 cells. Marked enhancement of HL-60 and HL-60/Bcl-2 cell kill with the DT388-GMCSF/Ara-C combination was observed using thymidine incorporation for cell proliferation and colony formation. Neither DT388-GMCSF nor Ara-C treatment significantly altered Bcl-2, Bcl-xL, Bax, or Fas receptor (FasR) levels in HL-60 or HL-60/Bcl-2 cells. These findings suggest that co-treatment with DT388-GMCSF overcomes resistance to apoptosis mediated by Bcl-2 overexpression. This may lead to a lowered apoptotic threshold and clonogenic survival of human AML blasts due to Ara-C. These observations also suggest that clinical trials of the combination therapy may be warranted in patients with AML.

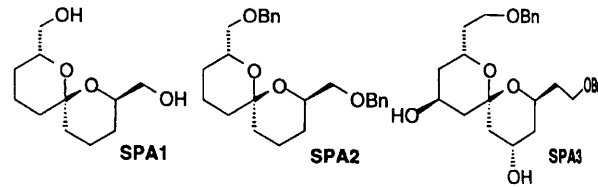
## Abstract# 1612

## Poster Board#/Session: 284-III

**DESIGN AND SYNTHESIS OF NOVEL FUNCTIONAL ANALOGS OF SPONGISTATIN AS ANTI-CANCER AGENTS.** C. Mao\*, H. Huang\*, S.T. Jan\*, C. Navara\*, R.K. Narla\*, F.M. Uckun. *Parker Hughes Cancer Center, Drug Discovery Program, Departments of Structural Biology, Chemistry and Experimental Oncology, Hughes Institute, St. Paul, MN, USA.*

Spongistatin 1 (SP) is a potent tubulin depolymerizing anti-cancer agent which has been isolated from an Eastern Indian Ocean sponge in the genus *Spongia*

(*J. Org. Chem.* 1993, 58, 1302). A three-dimensional computer model of tubulin was constructed based upon its recently resolved electron crystallographic structure (*Nature*, 1998, 391, 199) for the rational design of three functional SPA analogs (SPA) with potent inhibitory activity on tubulin polymerization. Our modeling studies predicted that the designed SPA, which all contain a spiroketal subunit corresponding to the A-B spiroketal fragment of SP, would bind to a specific site with dimensions approximately 8 Å wide × 18 Å long × 11 Å deep at the tubulin surface which is different from that of taxol, vinblastine, vincristine, and dolastatin. Remarkably, the SP binding site consisted of a cluster of 9 residues containing aromatic rings situated in close proximity, an event which is very rarely observed on the surface of proteins. The SP binding site is reminiscent of the rapamicin binding site observed in the crystal structure of FKBP12-rapamicin human FRAP complex which includes 12 aromatic residues in its binding site. The identified binding site is immediately adjacent to a GDP exchangeable site on the β subunit of heterodimeric tubulin, suggesting that SP can inhibit the displacement of the bound GDP. The convergent synthesis of these SPA was accomplished in a stereocontrolled fashion. All three SPA (SPA-1, SPA-2, and SPA-3) caused rapid tubulin depolymerization in turbidity assays, destroyed the microtubule organization, prevented mitotic spindle formation as determined by confocal laser scanning microscopy, and induced apoptotic death as confirmed by transmission electron microscopy, DNA gels, and TUNEL assays in NALM-6 (pre-B ALL) and MOLT-3 (T-ALL) cell lines as well as primary leukemic cells from 5 of 5 children with high risk acute lymphoblastic leukemia. Modeling studies of SPA-1, SPA-2, and SPA-3 docked into the target binding site of tubulin also revealed binding features which could be successfully exploited for the design of potentially more effective analogs of these novel anti-leukemic agents.



## Abstract# 1613

## Poster Board#/Session: 285-III

**THE POST-INDUCTION RESIDUAL DISEASE IN CHILDHOOD ALL CORRELATES TO THE IN-VITRO PREDNISON RESISTANCE.** K. Schmiegelow\*, G.J.L. Kaspers\*, C. Nyvold\*, M.M.A. Rotter\*, J. Seyfang\*, R. Pieters\*, N. Knabe\*, H.O. Madsen\*, L.P. Ryder\* (Intr. by Christian Geisler). *Depts. Pediatric Hematology/Oncology, The University Hospital, Rigshospitalet, Copenhagen; Dept. Clinical Immunology, The University Hospital, Rigshospitalet, Copenhagen, Denmark; The University Hospital, Vrije Universiteit, Amsterdam, The Netherlands.*

The in-vitro resistance to the drugs applied for induction therapy in childhood ALL is related to the long-term outcome. Similarly, the early response to chemotherapy quantified by PCR-measurements of the day 29 residual disease reflects the risk of relapse. To explore the correlation between these two prognostic factors, we studied 33 children (8 females) 1.0-14.9 years of age (median: 7.2) with non-B cell ALL (pre-B/T/biphenotypic: 25/7/1) diagnosed at Rigshospitalet 1993-1997. Induction therapy consisted of vincristine (VCR)/prednisone (PRED)/doxorubicin (DOXO)/i.t.MTX. The in-vitro resistance to PRED, VCR, and DOXO was determined with the MTT-assay comparing the survival of leukemic cells incubated for 4 days at different drug concentrations with untreated control leukemic cells. LC50 values (lethal values to 50% of the cells) were determined for each drug. PCR was performed on bone-marrow (BM) samples at diagnosis (TCR-δ, TCR-γ, IgH) to identify the clone-specific Ig- and TCR-gene rearrangements. Subsequently, we generated a competitor for each leukemic clone (differing from the clonal immune gene rearrangement only by an inserted restriction site *XhoI*). After 4 weeks of induction therapy, quantification of the residual disease (RD) was performed on bone-marrow (BM) samples adding an increasing number of copies of the competitor to 100,000 copies of genomic DNA. Following PCR and digestion with the restriction enzyme *XhoI*, the number of malignant clone copies in the BM could be determined, being equal to the total competitor in the gel electrophoresis lane where the bands of competitor and genomic DNA had similar intensity. The median LC50 for PRED was 2.3 μg/ml (75% range: 0.05-323). The median RD was 0.02% (75%-range: 0.001-1.9%). The RD was significantly related to the LC50 for PRED ( $r_s = 0.43$ ,  $p = 0.01$ ), neither to the LC50 of DOXO nor VCR. Apart from girls with T-cell disease ( $n = 3$ ) the correlation between the RD and the LC50 for PRED was even more pronounced when the patients were subdivided by gender and phenotype (Male pre-B:  $n = 21$ ,  $r_s = 0.50$ ,  $p = 0.02$ ; male + T-cell:  $n = 4$ ,  $r_s = 0.95$ ,  $p = 0.05$ ; female + pre-B:  $n = 5$ ,  $r_s = 0.90$ ,  $p = 0.04$ ). Large, prospective studies are needed to determine whether in-vitro drug resistance and determination of postinduction RD should be combined to obtain the most reliable prediction of good- and bad-responding patients.

## MOLECULAR GEN

## MOLECULAR

## Abstract# 1614

**PARENTAL EXPRESSION OF THE ETIOLOGY OF INFANTILE SAINATI\***, E. Barisoni, S. Milan, Ospedale S. Maria, Clinica Pediatrica U. Ospedale Bambino Gesù, Rome, Italy.

Infant leukemia rearrangements is a rare disease such as exposure to a toxic agent in the mother during pregnancy. The polymorphisms in glutathione transferase (GST) in infantile leukemia disease in the infant are related to metabolites. We assayed CYP2E1, and CYP1B1 polymorphisms in patients diagnosed with leukemia. The PCR reactions were performed on the polymorphisms of the GSTM1 and GSTT1 polymorphisms in patients with leukemia. The frequency compared to 0.5 in the control population which was not significant. This was true for the frequency versus the expected 0.5 in the control families, 6 out of 10 patients with GSTM1 and GSTT1 genes. This frequency was significant (chi-square 7.65,  $p < 0.005$ , expected 0.19, observed 0.04). These preliminary results in conjunction with the clinical data on leukemia by modifying the etiology of the disease.

## Abstract# 1615

**TEL-AML1 FUSION GENE IN STANDARD RISK CHILDHOOD ALL WHO HAVE AN EARLY SINGLE DELAYED RESPONSE.** L.F. Odom, L. Stork, M. Bhat, *The University of Colorado*

Children with SR-ALL (WBC < 50,000), have a good response to chemotherapy resulting in the overall outcome. In our study, we included additional information on the Cancer Group (CCG) 1989-1991 (DDI). Results indicate that ~85% DDI was associated with relapse and hospitalizations. In our study, we identified at diagnosis. One genetic factor associated with prognosis is the presence of the *TEL-AML1* fusion gene, which was retrospectively used in a cohort of patients with SR-ALL. Fusion has been associated with relapse in patients who were diagnosed with SR-ALL. In our study, specimens were available for 44% (44%) had *TEL-AML1* fusion and 55% for *TEL-AML1* positive patients. In our study, negative patients who relapsed have remained in remission. These results are based on subsets of SR-ALL patients. These data are warranted. These data have an impact on intervention.