Relationship of Deoxycytidine Kinase and Cytoplasmic 5′-Nucleotidase to the Chemotherapeutic Efficacy of 2-Chlorodeoxyadenosine

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The agent 2-chlorodeoxyadenosine (2-CdA) has chemotherapeutic activity in hairy cell leukemia (HCL) and in refractory chronic lymphocytic leukemia (CLL). The cytotoxic activity of 2-CdA requires the intracellular accumulation of 2-CdA nucleotides. Deoxycytidine kinase (dCK) and cytoplasmic 5′-nucleotidase (5′-NT) are the principal enzymes that phosphorylate 2-CdA and dephosphorylate 2-CdA 5′-monophosphate, respectively. The net accumulation of 2-CdA nucleotides may therefore depend on both dCK and 5′-NT. The purpose of the present experiments was to determine if there is a relationship between pretreatment levels of dCK and 5′-NT in HCL and in CLL cells, and the clinical outcome of 2-CdA treatment. As measured by a direct immunoassay for dCK in 25 CLL patients, and by a 5′-NT activity assay in 23 patients, mean dCK levels were significantly higher in 2-CdA responders than in nonresponders ($P < .01$), whereas mean 5′-NT levels were significantly lower in 2-CdA responders than in nonresponders ($P < .05$). Mean dCK levels were higher in six HCL 2-CdA responders than in one nonresponder, whereas mean 5′-NT levels were lower in the 2-CdA responders than in the nonresponder. These results suggest that both dCK and 5′-NT are determinants of 2-CdA responsiveness.

THE AGENT 2-chlorodeoxyadenosine (2-CdA) has chemotherapeutic activity in refractory chronic lymphocytic leukemia (CLL)[1-4] and in hairy cell leukemia (HCL).[1,3,5] However, the biochemical basis for the clinical efficacy of 2-CdA has not been clearly established. The in vitro toxic effects of 2-CdA require the formation of 2-CdA nucleotides. 2-CdA is phosphorylated exclusively by deoxycytidine kinase (dCK), since mutant lymphoblastoid cell lines that lack dCK are impervious to 2-CdA toxicity.[6] In addition, cellular levels of cytoplasmic 5′-nucleotidase (5′-NT) profoundly influence 2-CdA sensitivity.[7] This enzyme can dephosphorylate a variety of purine nucleotides. Mutant lymphoblasts with elevated cytoplasmic 5′-NT levels have a 2-CdA–resistant phenotype.[7] Given a long enough period of exposure to 2-CdA, cells in which dCK levels greatly exceed 5′-NT levels should progressively accumulate 2-CdA nucleotides. In contrast, cells that have more 5′-NT than dCK should be relatively 2-CdA–resistant. Hence, one might predict that the levels of both dCK and 5′-NT may influence the sensitivity of a malignant cell to 2-CdA toxicity.

In the present experiments, we used a direct immunoassay for dCK and a cytoplasmic 5′-NT activity assay to study pretreatment cells from HCL and CLL patients who subsequently received 2-CdA, with varying outcomes. We found that 2-CdA responders had significantly higher dCK levels, but significantly lower 5′-NT levels, than 2-CdA nonresponders. Collectively, these results suggest that the chemotherapeutic activity of 2-CdA may depend on levels of both dCK and 5′-NT.

MATERIALS AND METHODS

Immunoassay of dCK. The immunoassay of dCK was performed as described.[8] Briefly, human dCK cDNA was amplified by polymerase chain reaction (PCR), and expressed as a fusion protein in the pMALcR1 vector (New England Biolabs, Beverly, MA). Antibodies against the purified recombinant dCK fusion protein recognized the 30.5-Kd dCK polypeptide in immunoblots. Antibody binding was quantitated by laser densitometry, compared with purified dCK. The results are expressed as nanograms of dCK per milligram of cellular protein, as determined by Bradford's method.[9] In crude extracts of leukemia cell lines, and in fresh tumor cells, there was a strong correlation ($r = .84$, $P < .001$) between dCK catalytic activities, as measured by radioassay,[10] and dCK protein levels, as measured by immunoassay. The dCK levels in separate samples from the same patient before treatment differed by only 5%, verifying the reproducibility of the assay.

Measurement of 5′-NT activities. Cell extracts were prepared by sonication, and suspended in a buffer consisting of 20 mmol/L imidazole HCl, pH 7.0, 20 mmol/L $MgCl_2$, 0.1 mmol/L EGTA, and 0.1 mmol/L phenylmethylsulfonyl fluoride (buffer A). Adenosine monophosphate (AMP)-Sepharose (Sigma, St Louis, MO) affinity chromatography was used to separate 5′-NT from nonspecific phosphatases, according to the method of Spychala et al.[11,12] Two hundred microliters of extract in buffer A was applied to 0.2 mL AMP-Sepharose, which was washed with 1 mL of the same buffer. Then, cytoplasmic 5′-NT was eluted with 400 μL buffer A containing 0.5 mol/L NaCl. The 5′-NT activities were then determined using [^{14}C]-inosine monophosphate (IMP) (5 mmol/L, 2×10^6 cpm/mmol; Amersham, Arlington Heights, IL). Reaction mixtures contained 20 mmol/L magnesium chloride, 5 mmol/L dithiothreitol, 0.2 mg/mL bovine serum albumin, 5 mmol/L adenosine triphosphate (ATP), and 0.5 mol/L sodium chloride in 50 mmol/L Tris chloride (pH 7.5). After a 30-minute incubation at 37°C, the reactions were terminated by addition of 1 mL of cold 50 mmol/L acetic acid. Each sample was filtered through a 0.5-mL column of anion-exchange AG1-X2 (Bio-Rad, Richmond, CA; 100 to 200 mesh, chloride form) to retain unreacted nucleotides. The column was washed with 2 mL of 50-mmol/L acetic acid. The flow-through and wash fractions were collected directly in an scintillation vial for determination of their radioactivities. The reaction was linear with protein and with time up to 60 minutes.

Cell and tissue sources. CLL and HCL cells were isolated from heparinized peripheral blood by Histopaque (Sigma) centrifugation, and stored frozen in liquid nitrogen. We chose samples that contained

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at least 73% malignant cells, as determined by flow cytometry using monoclonal antibodies against CD5, CD11c, CD19, and κ and λ light chains, as described.[13] The CLL patients' characteristics are summarized in Tables 1 and 2.

Treatment protocol. The 2-CdA was administered by continuous intravenous infusion, according to our previously described protocol.[1,2] In CLL, "2-CdA responders" refers to patients who had partial remissions, according to the National Cancer Institute Working Group recommendations.[14] "Nonresponders" refers to patients who had progressive or static disease. HCL responders were as previously described.[1] The data for the various groups are presented as the means ± SD. The Mann-Whitney *U* test was used to compare the mean enzyme levels in the two patient groups. To assess the overall value of each test to predict 2-CdA responsiveness, a nonparametric modification of the receiver operating characteristic (ROC) curve was used, according to the method of DeLong et al.[15]

RESULTS

dCK levels in CLL. Immunoreactive dCK levels were determined in pretreatment CLL from 25 patients who received 2-CdA, including 11 responders and 14 nonresponders. The mean ± SD level of dCK in the 2-CdA responders was 1,988 ± 466 ng/mg cellular protein, compared with 1,131 ± 609 ng/mg protein in the nonresponders ($P < .01$). These data are shown in Fig 1, excluding two responders (patients 1 and 7) who had dCK levels of 1,869 and 1,853 ng/mg protein, respectively. Thus, the mean dCK level was 1.78 times higher in the 2-CdA responders than in the nonresponders. None of the 11 patients with dCK levels greater than or equal to 1,250 ng/mg protein responded to 2-CdA treatment, as indicated by the vertical division in Fig 1. The dCK level in two patients (one responder and one nonresponder) that were measured before and after 2-CdA treatment differed by less than 15%.

5′-NT activities in CLL. Cytoplasmic 5′-NT activities were determined in pretreatment CLL cells from 23 patients who received 2-CdA, including nine responders and 14 nonresponders. In patients 1 and 7, insufficient material was available for 5′-NT analyses. The mean ± SD level of 5′-NT in the 2-CdA responders was 5.04 ± 2.30 pmol/min/mg protein, compared with 12.0 ± 7.76 pmol/min/mg protein in the nonresponders ($P < .05$). Thus, 2-CdA nonresponders had mean 5′-NT levels that were 2.38-fold higher than 2-CdA responders. The individual data points are shown in Fig 1. None of the eight patients with 5′-NT activities greater than 10 pmol/mg/mg protein responded to 2-CdA treatment, as indicated by the horizontal division in Fig 1. In one CLL patient who responded to 2-CdA, the 5′-NT activities were measured before and after treatment. The posttreatment value was 24% higher.

dCK to 5′-NT ratios. Both dCK protein and cytoplasmic 5′-NT were determined in pretreatment CLL from 23 patients who received 2-CdA. The calculated mean ± SD of the dCK to 5′-NT ratio in the 2-CdA responders with CLL was 461 ± 173, compared with 177 ± 204 in the nonresponders ($P < .01$). For the assessment of the overall value of each test in predicting 2-CdA responsiveness, a nonparametric ROC curve was applied (Fig 2). For a test with no predictive value, the area under the ROC curve is expected to be 0.5 of the total area in the box. However, the areas under the ROC

Table 1. Characteristics of 2-CdA Responders

Patient No.	Sex	Age at Treatment	Rai Classification	WBC Count	% Lymphocytes	% CD19⁺ and CD5⁺	Previous Treatment
1*	M	77	III	258.4	97	99	CLB ADR/VCR/PRED CVP
2	F	61	IV	102.0	73	99	CLB CVP
3	F	71	III	78.1	93	99	CLB
4	M	65	III	326.0	98	99	CLB
5	M	55	III	62.2	89	76†	None
6	M	59	III	231.0	98	99	CLB
7*	M	67	IV	25.4	90	99	CLB/PRED
8	M	72	IV	32.6	99	99	CLB/PRED
9	M	48	II	76.3	90	99	CVP ADR/CTX/PRED CLB/PRED VCR/ADR/DEX
10	F	66	IV	76.1	98	99	CLB CVP CHOP CTX/PRED
11	F	76	III	215.2	94	99	CVP CLB

Abbreviations: ADR, Adriamycin (Adria Laboratories, Columbus, OH); CLB, chlorambucil; CTX, cyclophosphamide; VCR, vincristine; DEX, dexamethasone; PRED, prednisone; CHOP, cyclophosphamide, Adriamycin, vincristine, prednisone; CVP, cyclophosphamide, vincristine, prednisone; PROMACE, prednisone, vincristine, methotrexate, Adriamycin, cyclophosphamide, etoposide.

* Deoxycytidine kinase assay only due to insufficient sample for 5′-NT assay.

† Although 92% of WBC were CD19⁺, a substantial percent of the cells did not express CD5.

Table 2. Characteristics of 2-CdA Nonresponders

Patient No.	Sex	Age at Treatment	Rai Classification	WBC Count	% Lymphocytes	% CD19+ and CD5+	Previous Treatment
12	M	59	IV	142.0	96	99	CHOP (×2) CVP CLB
13	F	67	IV	108.8	99	99	CTX/PRED CVP CLB
14	M	58	III	697.0	99	99	CLB
15	M	60	IV	271.3	96	99	CLB/PRED CVP Fludarabine/PRED ADR/VCR
16	M	62	IV	201.0	96	99	CLB/PRED CTX/Methyl-PRED
17	F	59	IV	247.0	98	56*	CHOP (×3)
18	M	68	IV	62.4	85	99	CVP (×6) PROMACE
19	M	68	IV	78.5	95	99	CLB/PRED Fludarabine
20	M	59	II	105.8	93	97	None
21	M	51	IV	62.6	97	99	CLB/PRED Fludarabine
22	M	72	III	210.0	98	9	CTX/ADR/VCR/PRED CLB/PRED
23	M	55	II	41.9	87	99	CLB IFN-γ CHOP
24	M	72	III	87.4	97	99	CVP (×3) CLB/PRED (×2)
25	M	63	III	218.4	97	99	CLB

Abbreviations: See Table 1.

* Although 98% of WBC were CD19+, only 56% expressed CD5.

curves were 0.88, 0.83, and 0.87 of the total for dCK, 5'-NT, and dCK to 5'-NT ratio, respectively ($P < .05$ in each case). These data indicate that both dCK and 5'-NT levels are related to the outcome of 2-CdA treatment.

dCK levels and 5'-NT activities in HCL. Because most of the HCL patients had less than 70% malignant cells in the blood, and because very few failed to respond to 2-CdA, it was not possible to obtain representative samples of 2-CdA responders and nonresponders. The mean ± SD immunoreactive dCK values in six 2-CdA responders who had more than 85% HCL cells in the blood was 353 ± 96 ng/mg protein. The dCK level in the one measurable nonresponder was only 113 ng/mg protein. In comparison, 5'-NT activity in the six responders was 4.08 ± 2.20 pmol/min/mg of protein, compared with 29.67 pmol/min/mg of protein in the nonresponder.

DISCUSSION

These studies demonstrated a relationship between the response to 2-CdA chemotherapy in CLL and the pretreatment levels of dCK and cytoplasmic 5'-NT. The dCK levels were higher in 2-CdA responders than in nonresponders, whereas the reverse was true for cytoplasmic 5'-NT levels. These results suggest that both dCK and 5'-NT play a role in the in vivo activity of 2-CdA in CLL.

The dCK protein levels were as high in CLL cells as in rapidly proliferating leukemia cell lines. This finding is consistent with previous results showing that the activity of dCK is highest in lymphocytes and does not vary substantially during the cell growth cycle.[16] The exact function of the enzyme in normal and malignant lymphocytes with a low growth fraction is still not entirely clear. Based on the observation that dCK can phosphorylate deoxyadenosine and deoxyguanosine, as well as deoxycytidine, we have suggested that the enzyme may function in the salvage of deoxynucleosides necessary for DNA repair.[17]

The mean dCK levels in HCL were threefold to fivefold lower than in CLL, despite the fact that HCL is much more responsive than CLL to 2-CdA chemotherapy. Among 278 HCL patients treated in San Diego with 2-CdA, 85% had complete remissions after a single course of treatment,[3,5,18] and almost all the others had significant cytoreduction. In contrast, 44% of 90 advanced, refractory CLL patients responded clinically to CdA, and 4% had complete remissions.[18,19] These results indicate that between different lymphocyte tumor types, dCK levels alone do not predict sensitivity to 2-CdA. We have previously postulated that the effectiveness of 2-CdA in HCL may also depend on the destruction of the lymphoid microenvironment, which facilitates the survival of the malignant cells.[1] However, within

the HCL group, the mean dCK levels were higher in 2-CdA responders than in the nonresponder, as measured by both immunoassay and catalytic assay. Indeed, in the HCL patient who showed no clinical response to 2-CdA, the dCK level was extremely low. These results suggest that low dCK levels may be one mechanism for 2-CdA resistance in HCL.

In addition to 2-CdA, both fludarabine and cytarabine are established substrates for dCK. Leiby et al reported no significant relationship between dCK catalytic activity and response to fludarabine in patients with non-Hodgkin's disease.[20] Tattersall et al described low dCK activity in leukemic patients who did not respond to cytarabine.[21] However, there was considerable overlap in enzyme activity in the cytarabine-responsive and nonresponsive patients. Recently, Owens et al demonstrated that structural gene mutations in dCK may mediate resistance to cytarabine in cultured cells.[22] Occasional instances of dCK deficiency have also been demonstrated to occur in the leukemic cells of patients and experimental animals treated with cytarabine.[23] It is not yet clear whether this constitutes a common mechanism of clinical resistance to the drug. In the 25 CLL and seven HCL patients analyzed in the present study, we found a strong correlation between dCK catalytic activities and dCK protein levels. Furthermore, one 2-CdA nonresponder with CLL and one nonresponder with HCL had very low levels of the enzyme (Fig 1).

In lymphocytes maintained in tissue culture, the net accumulation of 2-CdA phosphates depends on the balance between phosphorylation of the nucleoside by dCK, and the dephosphorylation of its nucleotide product by cytoplasmic 5'-NT.[24] In an extensive survey of human malignant cell lines, the dCK to 5'-NT ratio correlated more strongly with sensitivity to 2-CdA than did dCK levels alone.[7] Whether this is true for sensitivity to fludarabine or cytarabine is entirely unknown, insofar as the nucleotide derivatives of 2-CdA, fludarabine, and cytarabine are metabolized differently.

None of the CLL patients with dCK levels less than or equal to 1,250 ng/mg protein, or with 5'-NT activities greater

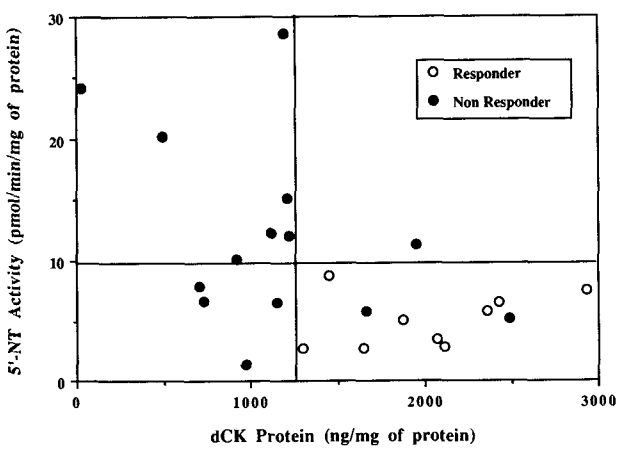

Fig 1. dCK protein levels in CLL extracts were determined by quantitative immunoblotting, using purified recombinant dCK as an antigen standard. Cytoplasmic 5'-NT activities were assessed radiochemically after removal of nonspecific phosphatases by AMP-Sepharose chromatography.

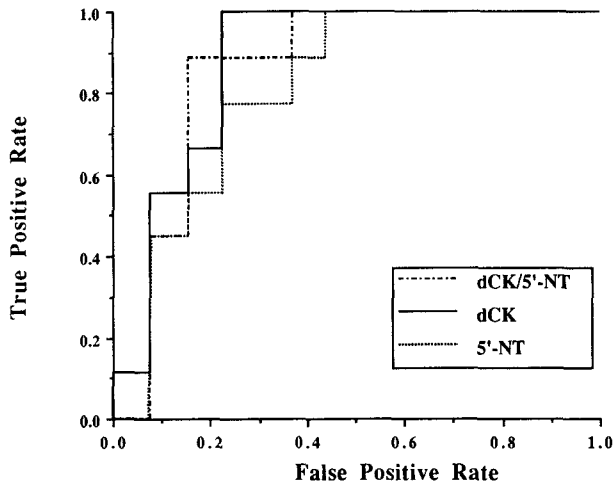

Fig 2. ROC curves for dCK, 5'-NT, and dCK to 5'-NT ratio.

than or equal to 10 pmol/min/mg protein responded to 2-CdA. However, there were some patients with high dCK and low 5'-NT levels who nonetheless did not respond to 2-CdA therapy. Furthermore, the mean dCK to 5'-NT ratio in HCL was lower than in CLL. Thus, it is unlikely that chlorodeoxy (Cld)ATP levels in leukemia cells correlate linearly with response. Other factors that may influence the toxicity of 2-CdA are the size of the dCTP pool, the pace of deoxycytidine excretion,[23,24] cell volume changes, the activities of DNA polymerase-α and -β,[17] and the ratio of CldATP to dATP.[25,26] In tissue culture, any metabolic change that enhances the rate of endogenous deoxycytidine production can impair the functional activity of dCK, since deoxycytidine is the preferred substrate for the enzyme.[27] Such changes may potentially include increases in the activities of ribonucleotide reductase and CTP synthetase, as well as cytoplasmic 5'-NT.[24,28,29] In the future, it will be interesting to determine if the levels of these enzymes, in addition to dCK and 5'-NT, are related to the chemotherapeutic efficacy of 2-CdA.

The immunoassay for dCK makes it simple to measure enzyme levels in small tumor biopsy specimens. We are currently working on the development of a cytoplasmic 5'-NT immunoassay so that we can directory measure the enzyme in crude tissue extracts. Using the two immunoassays, it should be possible to assess the variability of dCK and 5'-NT in many different tumor cell types. Such evaluations could be used to identify tumors that may potentially respond to 2-CdA treatment. Based on the data presented here, one would predict that pharmacologic inhibition of cytoplasmic 5'-NT should potentiate the efficacy of 2-CdA in HCL and CLL cells with high dCK levels, but equally high cytoplasmic 5'-NT activities. To date, no potent inhibitors of cytoplasmic 5'-NT have been reported. More detailed studies of the human enzyme, as well as the synthesis of potential cytoplasmic 5'-NT inhibitors, appear to be warranted.

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REFERENCES

1. Piro LD, Carrera CJ, Carson DA, Beutler E: Lasting remissions in hairy-cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. N Engl J Med 322:1117, 1990

2. Piro LD, Carrera CJ, Beutler E, Carson DA: 2-Chlorodeoxyadenosine: An effective new agent for the treatment of chronic lymphocytic leukemia. Blood 72:1069, 1988

3. Piro LD: 2-Chlorodeoxyadenosine treatment of lymphoid malignancies. Blood 79:843, 1992

4. Cheson BD: The purine analogs—A therapeutic beauty contest. J Clin Oncol 10:352, 1992 (editorial; comment)

5. Saven A, Piro LD: Treatment of hairy cell leukemia. Blood 79: 1111, 1992

6. Carson DA, Wasson DB, Kaye J, Ullman B, Martin DW, Robins RK, Montogomery JA: Deoxycytidine kinase-mediated toxicity of deoxyadenosine analogs toward malignant human lymphoblasts in vitro and toward murine L1210 leukemia in vivo. Proc Natl Acad Sci USA 77:6865, 1980

7. Carson DA, Wasson DB, Taetle R, Yu A: Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. Blood 62:737, 1983

8. Kawasaki H, Carrera CJ, Carson DA: Quantitative immunoassay of human deoxycytidine kinase in malignant cells. Anal Biochem (in press)

9. Bradford M: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248, 1976

10. Durham JP, Ives DH: Deoxycytidine kinase. I. Distribution in normal and neoplastic tissues and interrelationships of deoxycytidine and 1-beta-D-arabinofuranosylcytosine phosphorylation. Mol Pharmacol 5:358, 1969

11. Carson DA, Carrera CJ, Wasson DB, Iizasa T: Deoxyadenosine-resistant human T lymphoblasts with elevated 5'-nucleotidase activity. Biochim Biophys Acta 1091:22, 1991

12. Spychala J, Madrid-Marina V, Fox IH: High Km soluble 5'-nucleotidase from human placenta. Properties and allosteric regulation by IMP and ATP. J Biol Chem 263:18759, 1988

13. Kipps TJ, Meisenholder G, Robbins B: New developments in flow cytometric analyses of lymphocyte markers. Lab Immunol 12: 237, 1992

14. Cheson BD, Bennett JM, Rai KR, Grever MR, Kay NE, Schiffer CA, Oken MM, Keating MJ, Boldt DH, Kempin SJ: Guidelines for clinical protocols for chronic lymphocytic leukemia: Recommendations of the National Cancer Institute-sponsored working group. Am J Hematol 29:152, 1988

15. DeLong ER, DeLong DM, Clarke-Pearson DL: Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach. Biometrics 44:837, 1988

16. Arner ES, Flygar M, Bohman C, Wallstrom B, Eriksson S: Deoxycytidine kinase is constitutively expressed in human lymphocytes: Consequences for compartmentation effects, unscheduled DNA synthesis, and viral replication in resting cells. Exp Cell Res 178:335, 1988

17. Terai C, Wasson DB, Carrera CJ, Carson DA: Dependence of cell survival on DNA repair in human mononuclear phagocytes. J Immunol 147:4302, 1991

18. Beutler E, Piro L, Saven A, Kay AC, Mcmillan R, Longmire R, Carrera CJ, Morin P, Carson DA: 2-Chlorodeoxyadenosine(2-CdA): A potent chemotherapeutic and immunosuppressive nucleoside. Leuk Lymph 5:1, 1991

19. Saven A, Carrera CJ, Carson DA, Beutler E, Piro LD: 2-Chlorodeoxyadenosine treatment of refractory chronic lymphocytic leukemia. Leuk Lymph 5:133, 1991 (suppl)

20. Leiby JM, Snider KM, Kraut EH, Metz EN, Malspeis L, Grever MR: Phase II trial of 9-beta-D-arabinofuranosyl-2-fluoroadenine 5'-monophosphate in non-Hodgkin's lymphoma: Prospective comparison of response with deoxycytidine kinase activity. Cancer Res 47: 2719, 1987

21. Tattersall MH, Ganeshaguru K, Hoffbrand AV: Mechanisms of resistance of human acute leukaemia cells to cytosine arabinoside. Br J Haematol 27:39, 1974

22. Owens JK, Shewach DS, Ullman B, Mitchell BS: Resistance to 1-beta-D-arabinofuranosylcytosine in human T-lymphoblasts mediated by mutations within the deoxycytidine kinase gene. Cancer Res 52:2389, 1992

23. Kees UR, Ford J, Dawson VM, Piall E, Aherne GW: Development of resistance to 1-beta-D-arabinofuranosylcytosine after high-dose treatment in childhood lymphoblastic leukemia: Analysis of resistance mechanism in established cell lines. Cancer Res 49:3015, 1989

24. Carson DA, Kaye J, Wasson DB: The potential importance of soluble deoxynucleotidase activity in mediating deoxyadenosine toxicity in human lymphoblasts. J Immunol 126:348, 1981

25. Santana VM, Mirro J, Jr., Harwood FC, Cherrie J, Schell M, Kalwinsky D, Blakley RL: A phase I clinical trial of 2-chlorodeoxyadenosine in pediatric patients with acute leukemia. J Clin Oncol 9:416, 1991

26. Avery TL, Rehg JE, Lumm WC, Harwood FC, Santana VM, Blakley RL: Biochemical pharmacology of 2-chlorodeoxyadenosine in malignant human hematopoietic cell lines and therapeutic effects of 2-bromodeoxyadenosine in drug combinations in mice. Cancer Res 49:4972, 1989

27. Hoglund L, Pontis E, Reichard P: Effects of deoxycytidine and thymidine kinase deficiency on substrate cycles between deoxyribonucleosides and their 5'-phosphates. Cancer Res 48:3681, 1988

28. Kubota M, Carrera CJ, Wasson DB, Carson DA: Deoxynucleoside overproduction in deoxyadenosine-resistant, adenosine deaminase-deficient human histiocytic lymphoma cells. Biochim Biophys Acta 804:37, 1984

29. Chiba P, Tihan T, Szekeres T, Salamon J, Kraupp M, Eher R, Koller U, Knapp W: Concordant changes of pyrimidine metabolism in blasts of two cases of acute myeloid leukemia after repeated treatment with ara-C in vivo. Leukemia 4:761, 1990