Cancer Chemother Pharmacol (1992) 30: 126–130

Cancer Chemotherapy and Pharmacology

Phenotypic analysis of 1-B-D-arabinofuranosylcytosine deamination in patients treated with high doses and correlation with response*

W. Kreis, M. Lesser, D. R. Budman, Z. Arlin, L. DeAngelis, P. Baskind, E. J. Feldman, and S. Akerman

Departments of Medicine and Research, North Shore University Hospital and Cornell University Medical College, Manhasset, New York 11030, New York Medical College, Valhalla, New York 10595, and Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA

Received 9 September 1991/Accepted 8 January 1992

Summary. Two phenotypes for 1-B-D-arabinofuranosylcytosine (ara-C) deamination corresponding to a ratio of distribution for "slow" (ratio, ≤ 14) vs "fast" (ratio, >14) deaminators of 70%:30%, have been determined on the basis of studies on plasma ratios of 1-B-D-arabinofuranosyluracil/ara-C (ara-U/ara-C) in 56 subjects treated with high-dose ara-C (3 g/m^2 infused i.v. over 3 h). A positive correlation of age with the concentration of ara-U was observed. In a subgroup of 36 patients with leukemia, the ara-U/ara-C pattern was similar to that observed for all 56 subjects. In these leukemic patients, who were treated with combinations of ara-C plus other conventional agents, a tendency toward a positive response (complete response + partial response) was found for those showing low ara-U/ara-C ratios (slow deaminators). The phenotypic effect of deamination in acute leukemia needs to be evaluated prospectively.

Introduction

Following high-dose treatment with 1-B-D-arabinofuranosylcytosine (ara-C), only about 60%–80% of acute leukemia patients achieve a complete (CR) or partial (PR) response [1]. Although the mechanism of primary resistance in humans remains unclear, degradation of ara-C into inactive metabolites may be an important factor contributing to the lack of therapeutic activity. Rapid deamination of ara-C in patients is well established [3, 7, 11], with the liver having the highest levels of cytidine deaminase (CRD, E.C.3.5.4.5) [2, 10]. Normal and leukemic granulocytes also contain CRD [6, 9], and a correlation of high deaminase activity in blasts of leukemic patients with the response to treatment has been reported by Stuart and Burke [27].

Studies on the pharmacokinetics of ara-C have demonstrated large interindividual differences in its metabolism [7, 11, 28] (anabolic and catabolic), which might be explained by genetic polymorphism. Genetic factors responsible for the success or failure of drug treatment are well established [16]; these factors can be polygenic or monogenic. The deamination product of ara-C (1-B-D-arabinofuranosyluracil (ara-U), has been reported to enhance the cytotoxicity of ara-C in tissue cultures of L5178Y cells; high concentrations of ara-U (10^{-3} M) delayed cell progression through the S phase [30]. Large amounts of ara-U produced by treatment with high-dose ara-C (HiDAC) might contribute to the clinical toxicity, especially the CNS toxicity.

The present study on ara-C was initiated in our laboratory following the observation of large variations in ara-U/ara-C plasma levels shortly before the discontinuation of an HiDAC infusion. HiDAC (3 g/m^2 infused over 3 h) was chosen for this study because this dose and schedule is routinely used for the treatment of both acute and chronic myeloblastic leukemia (AML, CML). This dose may also saturate major sites of CRD. Plasma ara-C and ara-U levels have been evaluated at time points other than at the end of the infusion of HiDAC [7], but the most consistent values were obtained at 3 h, even following different treatment cycles. Single time points as used in our investigation have been applied extensively in many other studies on the genetics of drug metabolism [16]. A preliminary report on our findings has been published elsewhere [13].

Patients and methods

Patients. Individuals presenting with leukemia or primary CNS lymphoma were treated with a variety of HiDAC protocols. All patients signed a written informed consent form according to institutional IRB guidelines. Of the 58 subjects entered in the study, 56 were evaluable for ara-C and ara-U plasma levels. The evaluation of the response of leukemic patients to the treatment was complicated in that a heterogeneous group of individuals received combination therapy on a variety of

* This work was supported by the Don Monti Memorial Research Foundation

Offprint requests to: W. Kreis, Department of Medicine, North Shore University Hospital, 300 Community Drive, Manhasset, N. Y. 11030, USA

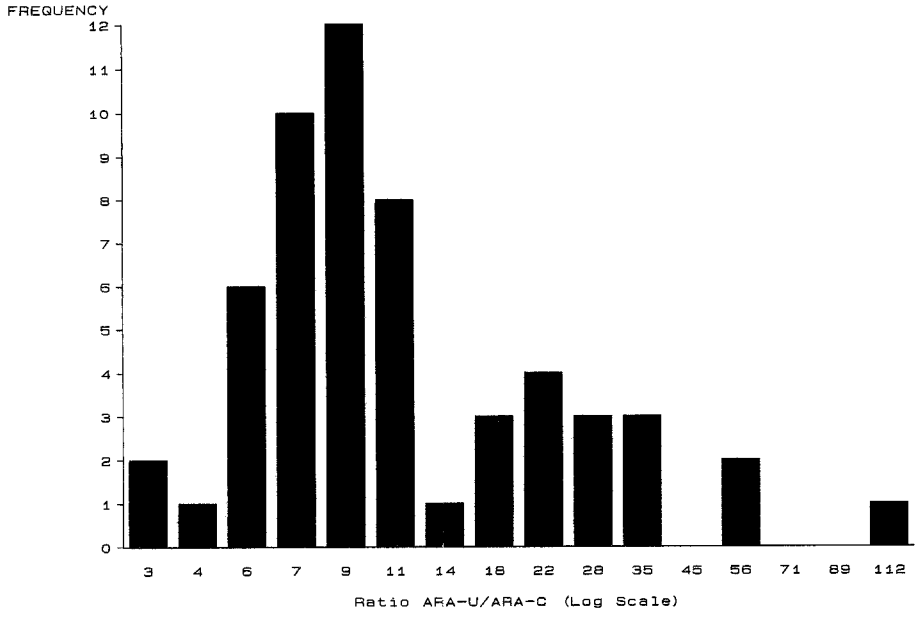

Fig. 1. Frequency histogram for the ara-U/ara-C ratio (log scale). Note: although the midpoints of class intervals have been rounded off, they do represent a log scale. *Abscissa*, Ara-U/ara-C ratio as evaluated shortly before the end of the infusion; *ordinate*, frequency of observation in the numbers of patients indicated

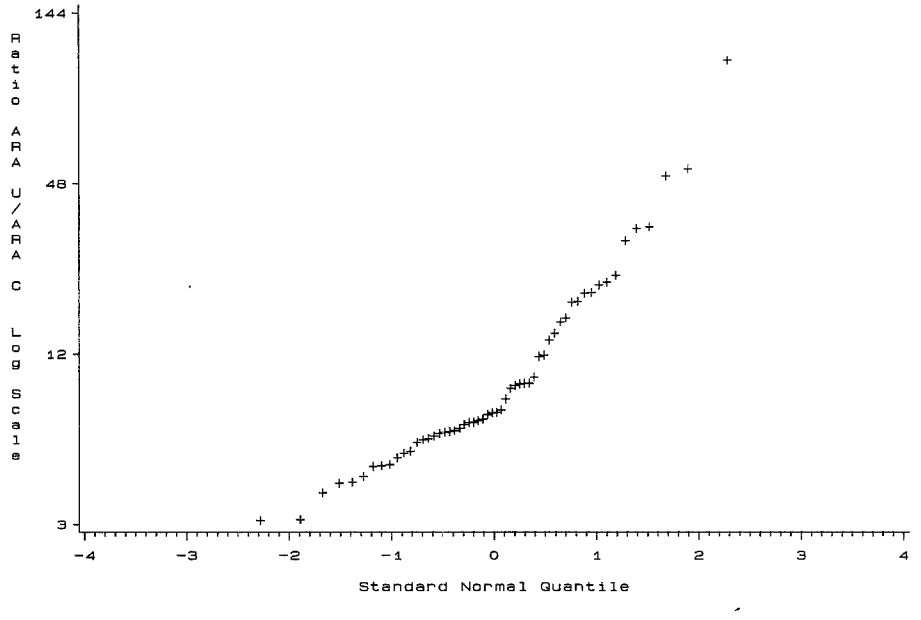

Fig. 2. Normal probability plot of the ara-U/ara-C ratio (log scale). *Abscissa*, rank for variable ratio; *ordinate*, ara-U/ara-C ratio

treatment protocols. All leukemic patients received infusions of 3 g/m^2 ara-C over 3 h as initial treatment. Just prior to the end of the first infusion, blood was drawn from a vein contralateral or peripheral to the infusion site into tubes containing 45 USP units sodium heparin and 0.5 mg tetrahydrouridine (THU). Plasma was separated by centrifugation at 3000 rpm for 10 min and frozen at –20° C until analysis.

Drug assays. Analysis of ara-C was done by radioimmunoassay (RIA) according to Piall et al. [19]. Antibody for ara-C analysis was purchased from the University of Surrey (Guilford, England). Ara-U was assayed as previously described [7] by the addition of 6 µg deoxyuridine (dUR) + 2 ml 6% cold trichloroacetic acid (TCA) per ml plasma followed by mixing and centrifugation for 10 min at 3000 rpm. To the supernatant, 2 ml freshly prepared Freon-amine solution [1,1,2-trichloratrifluoroethane + trioctylamine, 5 : 1 (v/v); Aldrich Chemical Inc., Milwaukee, Wis.] was added twice for the extraction of TCA.

The supernatant was centrifuged and filtered through a 0.45-µm Millipore filter (Nikon Millipore Kogyo, Yonezawa, Japan). The separation of ara-C, ara-U, and dUR was performed on a Beckman C_{18} ultrasphere ODS 5-µm column (Beckman, San Remo, Calif.) by elution with ammonium phosphate (0.01 *M*, pH 5.1) containing 1% methanol. Absorption was measured at 265 nm, and calculations were based on the ratio of the AUC for dUR over that for ara-U as determined by UV detection at 265 nm. The sensitivity of the assay for ara-U was 0.25 µg/ml. Elution times were 10.06 min for ara-C, 17.92 min for ara-U, and 19.39 min for dUR. The ratio of ara-U/ara-C was derived by dividing the concentration of ara-U by that of ara-C as expressed in micrograms per milliliter, with no correction being done for the small differences in molecular weight (244.21 vs 243.22 kDa).

Statistical analysis. The analysis of the distribution of ratios of ara-U/ara-C (i. e., to identify groups of patients showing different rates of deamination) was carried out using histograms, probability plots [4], and the Shapiro-Wilk test for normality [25]. These analyses were applied to the base-10 logarithms of the ratios, as distributions of ratios are usually highly skewed and a log transformation often results in a nearly normal distribution (provided that the distribution is not a mixture distribution or multimodal to begin with). Possible correlations of plasma levels of

Table 1. Relationships between several clinical variables and plasma levels of ara-C and ara-U as well as the ara-U/ara-C ratio

Variables		Ara-U	Ara-C	Ara-U/ara-C ratio
Age[a]		$r = 0.37$ ($P < 0.005$)	$r = -0.11$ ($P < 0.44$)	$r = 0.15$ ($P < 0.28$)
BSA[a]		$r = -0.22$ ($P < 0.11$)	$r = 0.21$ ($P < 0.12$)	$r = -0.17$ ($P < 0.20$)
Sex[b]				
	F	105.2 µg/ml (17.5)	10.1 µg/ml (4.8)	2.94 µg/ml[c] (1.3)
	M	93.7 µg/ml (18.2) ($P < 0.021$)	10.3 µg/ml (6.1) ($P < 0.90$)	2.85 µg/ml (1.4) ($P < 0.73$)
Initial WBC		$r = 0.24$ ($P < 0.11$)	$r = -0.27$ ($P < 0.07$)	$r = 0.36$ ($P < 0.013$)

[a] Pearson correlation coefficient
[b] Mean value (SD)
[c] Geometric mean (SD)

ara-U and ara-C as well as the log ara-U/ara-C ratio with age and body surface area (BSA) were analyzed using scatter plots and the Pearson correlation coefficient. Student's *t*-test was used to compare these three variables in men vs women. Response rates for "slow" and "fast" deaminators were compared using the Fisher exact test for 2×2 tables. Summarized statistics are expressed as mean values ± SD.

Results

Of the 56 evaluable patients, 32 (57%) were men. The mean age of the total group was 49 ± 16 years (range, 18–82 years). Values for BSA averaged 1.84 ± 0.22 m^2 (range, 1.4–2.4 m^2). When the frequency histogram for the ara-U/ara-C ratio was plotted on a log scale (Fig. 1), two groups of ara-C deaminators could be discriminated. Based on visual inspection of the histogram, there appeared to be a group of slow deaminators whose ratios were ≤ 14.0 and a group of fast deaminators whose ratios were >14.0. According to this cutoff point, 40 patients (71%) were slow deaminators. A further indication of a possible mixture of distributions (i.e., two phenotypes) was the apparent break point in the normal probability plot for ratio (Fig. 2), which appeared to occur at a ratio of about 14 (on the vertical axis). The test for normality showed that the distribution deviated significantly from a normal distribution ($P < 0.001$). [Although a lack of normality does not prove a mixture of distributions, this result does at least rule out the possibility of a "nicely behaved" (unimodal) normal distribution]. We also examined the distribution of ara-U and ara-C in both their original and their logarithmic units and found no evidence of either bimodality or a mixture of distributions.

We examined the possible correlation of age, sex, BSA, and initial WBC with plasma ara-U and ara-C levels and the ara-U/ara-C ratio. Only three significant associations were observed (Table 1): age was positively correlated

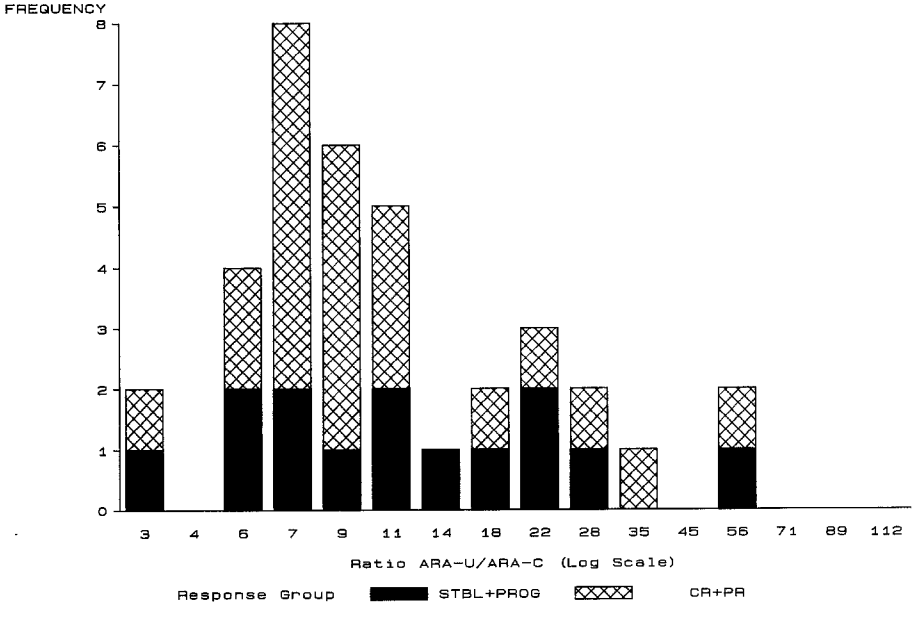

Fig. 3. Frequency histogram for the ara-U/ara-C ratio (log scale) according to the response category for patients with AML, ALL, and CML on induction therapy. *Abscissa*, Ratio of ara-U/ara-C; *ordinate*, frequency of observation in the numbers of patients indicated; *STBL + PROG*, stable + progressive disease

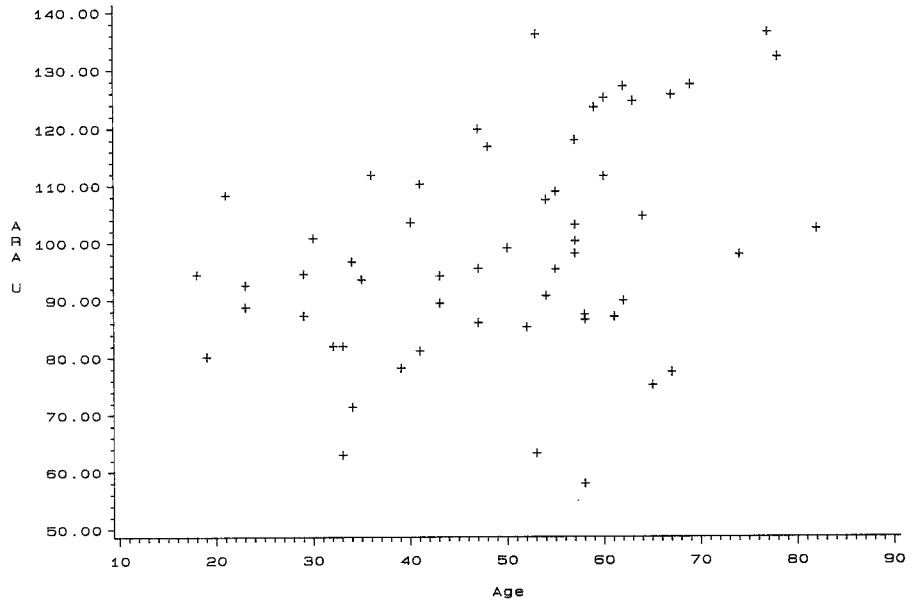

Fig. 4. ●

with plasma ara-U ($r = 0.37$, $P < 0.005$) (Fig. 4) but not with plasma ara-C or with ara-U/ara-C plasma levels [the variation in ara-U was substantially lower than that found for ara-C; coefficient of variation, 19% vs 55%, respectively); women had higher plasma ara-U levels (105.2 ± 17.5 μg/ml) than did men (93.7 ± 18.2, $P < 0.021$), but no significant difference in ara-C levels or in ara-U/ara-C ratios were observed; and a weak positive relationship between the initial WBC and the log-10 ara-U/ara-C ratio was found ($r = 0.37$, $P < 0.01$).

Analysis of response and deamination rates was restricted to 36 evaluable patients with leukemia [AML, CML, acute lymphocytic leukemia (ALL)]. No neurotoxicity was observed in this series of patients, who were treated with only one or two consecutive infusions of 3 g/m^2 ara-C. The histogram of the leukemic patients for the ara-U/ara-C ratio on a log scale (Fig. 3) was nearly identical to that shown for all patients in Fig. 1, with two groups of deaminators being apparent. According to a cut-off point of 14, 69% (25/36) of these subjects were slow deaminators. The response rate (CR + PR) was 68% among slow deaminators as compared with 45% among fast deaminators. However, this result was not statistically significant ($P = 0.27$) as judged by the sample size studied. Five patients with AML in the consolidation phase (all of whom achieved CRs), who were analyzed for the ara-U/ara-C ratio but were not included in the evaluation of response to treatment, were slow deaminators.

Discussion

A prospective study of deamination in a homogeneous group of leukemics using a standard regimen is needed. The lack of a significant correlation of ara-U/ara-C ratios with the response to treatment with HiDAC may have been attributable to the administration of combination therapy to the leukemic patients. Since the initial use of ara-C in the treatment of leukemia, extensive efforts have been devoted to determine predictive parameters for the response to ara-C treatment, such as kinase/deaminase (k/d) assays for target cells [6, 22], intracellular ara-C 5′-triphosphate (ara-CTP) accumulation and retention [20, 23], and the incorporation of ara-C into DNA [15]. None of these parameters has included the genetic aspects of ara-C metabolism.

Although Yang et al. [30] have reported in vitro enhancement of ara-C phosphorylation by ara-U and an increase in the number of L5178Y cells in the G1, S, or G2M phase, the ara-U concentrations used by these authors was 10^{-3} M; such levels of ara-U have not been found in vivo following the use of HiDAC. In contrast to this finding, Kessel and Shurin [12] observed an inhibition of the uptake of ara-C by ara-U, and Rustum et al. [24] concluded that ara-U could inhibit the phosphorylation of ara-C to its 5′di- and triphosphates [24].

Both the therapeutic and the toxic effects of various agents have been correlated with their metabolism, which has a genetic basis [16]. In an analogous manner, deamination of ara-C in humans can be classified into two phenotypes, a group of fast deaminators and a group of slow deaminators, on the basis of the ratio of serum levels of ara-U/ara-C. Our studies suggest that 30% of our study population were fast deaminators of ara-C and 70% were slow deaminators. As ara-U is also considered to produce some antileukemic effect, albeit inferior to that caused by ara-C [17], either phenotyping by the ratio of ara-U/ara-C or genotyping with probes may have prognostic significance for the treatment of leukemic patients with ara-C.

Genetic polymorphism for CRD has been established by Teng et al. [29] in human granulocytes, whereby three different electrophoretic patterns, possibly representing isozymes, were observed [29]. Three common phenotypes of CRD in human white cells have also been discussed by Nelson et al. [18]. Hepatic deamination has not been eval-

uated. The liver is believed to be the major tissue responsible for deamination [2, 10].

A therapeutic approach to polymorphism of deamination would be to inhibit the degradation of ara-C. Inhibition of ara-C degradation can be accomplished using either ara-C derivatives that are less susceptible to deamination or CRD inhibitors such as tetrahydrouridine (THU) in combination with the parent drug. THU has been shown to increase levels of intracellular ara-CTP in vitro [5, 8]. THU has prevented the deamination of ara-C in humans as demonstrated by the observation of high plasma ara-C values at 3 h (before the end of treatment) in leukemic patients who were treated with 200 mg/m^2 ara-C co-infused with 350 mg/m^2 THU over 3 h; mean values found for ara-C at the time of discontinuation of the infusion at 3 h (Cp3h values) were 3.31 ± 1.9 μg/ml [14]. These Cp3h values are normally achieved using HiDAC regimens only [26] and are considered to be sufficient for the saturation of the kinases [5, 20, 21]. Whether THU is capable of inhibiting all or part of the deamination observed in the 30% of our patients who were fast deaminators and of producing a further therapeutic benefit in all or part of the slow deaminators remains to be established. Genetic polymorphism may be evident in humans, as deamination was not inhibited by THU in one of our patients [14].

References

1. Bolwell BJ, Cassileth PA, Gale RP (1988) High dose cytarabine: a review. Leukemia 2: 253–260
2. Camiener GW, Smith CG (1965) Studies of the enzymatic deamination of cytidine arabinoside I. Biochem Pharmacol 14: 1405–1416
3. Capizzi RL, Yang JL, Cheng E, Bjornsson T, Sahasrabudhe D, Tan RS, Cheng YC (1983) Alteration of the pharmacokinetics of high dose ara-C by its metabolite, high ara-U, in patients with acute leukemia. J Clin Oncol 1: 763–771
4. Chambers JM, Cleveland WS, Kleiner B, Tukey PA (1983) Graphical methods for data analysis. Wadsworth International Group, Belmont, California
5. Chou TC, Arlin Z, Clarkson BC, Philips FS (1977) Metabolism of 1-B-D-arabinofuranosylcytosine in human leukemic cells. Cancer Res 37: 3561–3570
6. Coleman CN, Johns DG, Chabner BA (1975) Studies on mechanisms of resistance to cytosine arabinoside: problems in the determination of related enzyme activities in leukemic cells. Ann NY Acad Sci 255: 247–251
7. DeAngelis LM, Kreis W, Chan K, Dantis E, Akerman S (1992) Pharmacokinetics of ara-C and ara-U in plasma and CSF after high-dose administration. Cancer Chemother Pharmacol 29: 173–177[a]
8. Furner RL, Mellett LB, Herren TC (1975) Influence of tetrahydrouridine on the phosphorylation of 1-B-D-arabinofuranosylcytosine (ara-C) by enzymes from solid tumors in vitro. J Pharmacol Exp Ther 194: 103–110
9. Harris AL, Graham-Smith DG (1982) The relationship of ara-C metabolism in vitro to therapeutic response in acute myeloid leukemia. Cancer Chemother Pharmacol 9: 30–35
10. Ho DHW (1973) Distribution of kinase and deaminase of 1-B-D-arabinofuranosylcytosine in tissue of man and mouse. Cancer Res 33: 2816–2820
11. Ho DHW, Frei E (1971) Clinical Pharmacology of 1-B-D-arabinofuranosylcytosine (ara-C). Clin Pharmacol Ther 12: 944–954
12. Kessel D, Shurin S (1968) Transport of two non-metabolized nucleosides, deoxycytidine and cytosine arabinoside, in a subline of the L1210 murine leukemia. Biochim Biophys Acta 163: 179–187
13. Kreis W, Arlin Z, Budman D, Allen S, Schulman P, DeAngelis L, Lesser M, Baskind P, Feldman EJ, Akerman S (1991) Phenotypic analysis of deamination of ara-C in patients treated with high dose ara-C (HiDAC). Proc Am Assoc Cancer Res 32: 176
14. Kreis W, Budman DR, Chan K, Allen SL, Schulman P, et al (1991) Therapy of refractory/relapsed acute leukemia with cytosine arabinoside plus tetrahydrouridine (an inhibitor of cytidine deaminase) – a pilot study. Leukemia 5: 991–998
15. Major P, Egan EM, Beardsley GP, Minden MD, Kufe DW (1981) Lethality of human myeloblasts correlates with the incorporation of ara-C into DNA. Proc Natl Acad Sci USA 78: 3235–3239
16. Meyer UA, Zanger UM, Grant D, Blum M (1990) Genetic polymorphisms of drug metabolism. Advances in drug research, 19: 197–241
17. Muller WEG, Zahn RK (1979) Metabolism of 1-B-D-arabinofuranosyluracil in mouse L5178Y cells. Cancer Res 39: 1102–1107
18. Nelson RL, Povey S, Hopkinson DA, Harris H (1977) Detection after electrophoresis of enzymes involved in ammonia metabolism using L-gentamate dehydrogenase as a linking enzyme. Biochem Genet 15: 1023–1035
19. Piall EM, Aherne GW, Marks VM (1979) A radioimmunoassay for cytosine arabinoside. Br J Cancer 40: 548–556
20. Plunkett W, Liliemark JO, Adams TM, Nowak B, Estey E, Kantarjian H, Keating MJ (1987) Saturation of 1-B-D-arabinofuranosylcytosine 5′-triphosphate accumulation in leukemia cells during high dose 1-B-D-arabinofuranosylcytosine therapy. Cancer Res 47: 3005–3011
21. Prooijen HC van, Dekker AW, Punt K (1984) The use of intermediate dose cytosine arabinoside (ID ara-C) in the treatment of acute non-lymphocytic leukemia in relapse. Br J Haematol 57: 291–299
22. Riva CH, Barra Y, Cano JP, Tubiana N, Lejeune C, Merlin M, deSousa G, Carcassonne Y, Kreis W, Lovecchio J, Rottach C, Rustum YM (1990) Correlation between deoxycytidine kinase and deaminase activities and response to therapy with ara-C. J Cell Pharmacol 1: 79–95
23. Rustum YM, Preisler HD (1979) Correlation between leukemic cell retention of ara CTP and response to therapy. Cancer Res 39: 42–49
24. Rustum YM, Slocum HK, Wang G, Bakshi D, Kelly E, Buscaglia D, Wrozosek C, Early AP, Preisler H (1982) Relationship between plasma ara-C and intracellular ara CTP pools under conditions of continuous infusion and high dose ara-C treatment. Med Pediatr Oncol [Suppl] 1: 33–43
25. SAS Procedures Guide for Personal Computers, Version 6. SAS Institute, Cary, North Carolina, pp 350–351
26. Slevin ML, Piall EM, Aherne GW, Harvey VJ, Johnston A, Lister TA (1983) Effect of dose and schedule on pharmacokinetics of high dose cytosine arabinoside in plasma and cerebrospinal fluid. J Clin Oncol 1: 546–551
27. Stuart DC, Burke PJ (1971) Cytidine deaminase and development of resistance to arabinosylcytosine. Nature New Biol 233: 109–110
28. Talley RW, O'Bryan RM, Tucker WG, Loo RV (1967) Clinical pharmacology and human antitumor activity of cytosine arabinoside. Cancer 20: 809–816
29. Teng YS, Anderson J, Giblett E (1975) Cytidine deaminase: a new genetic polymorphism demonstrated in human granulocytes. Am J Hum Genet 27: 492–497
30. Yang JL, Cheng EH, Capizzi RL, Cheng YC, Kute T (1985) Effect of uracil arabinoside on metabolism and cytotoxicity of cytosine arabinoside in L5178Y murine leukemia. J Clin Invest 75: 141–146

[a] *Note added in proof:* Subsequent to the acceptance of this manuscript, we used a more sophisticated analytic method to demonstrate the existence of two phenotypes. The E-M algorithm was used to determine whether the ratios arose from the mixture of two normal distributions [Atkins M and Wilson GT (1980) Mixture models, outliers and the E-M algorithm. Technometrics 22: 325–331]. The analysis showed the existence of two populations of patients, one with a mean ratio of 8.02, and the other with a mean of 32.6 (see Fig. 1). This result was statistically significant, $P<0.001$.