Impact of the Putative Differentiating Agent Sodium Phenylbutyrate on Myelodysplastic Syndromes and Acute Myeloid Leukemia[1]

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ABSTRACT

Sodium phenylbutyrate (PB) is an aromatic fatty acid with cytostatic and differentiating activity against malignant myeloid cells (ID_{50}, 1–2 mM). Higher doses induce apoptosis. Patients with myelodysplasia ($n = 11$) and acute myeloid leukemia ($n = 16$) were treated with PB as a 7-day continuous infusion repeated every 28 days in a Phase I dose escalation study. The maximum tolerated dose was 375 mg/kg/day; higher doses led to dose-limiting reversible neurocortical toxicity. At the maximum tolerated dose, PB was extremely well tolerated, with no significant toxicities; median steady-state plasma concentration at this dose was 0.29 ± 0.16 mM. Although no patients achieved complete or partial remission, four patients achieved hematological improvement (neutrophils in three, platelet transfusion-independence in one). Other patients developed transient increases in neutrophils or platelets and decrements in circulating blasts. Monitoring of the percentage of clonal cells using centromere fluorescence *in situ* hybridization over the course of PB administration showed that hematopoiesis remained clonal. Hematological response was often associated with increases in both colony-forming units-granulocyte-macrophage and leukemic colony-forming units. PB administration was also associated with increases in fetal erythrocytes. These data document the safety of continuous infusion PB and provide preliminary evidence of clinical activity in patients with myeloid malignancies.

INTRODUCTION

Current therapies offer limited benefit to patients with MDS[3] and resistant subsets of AML. Supportive care remains a standard of care for the majority of patients with MDS. Subsets of AML can be identified in which available therapies have been mainly palliative: the elderly, AML arising from MDS, AML with poor risk cytogenetic abnormalities, and therapy-induced AML (1–4). In MDS and resistant AML, allogeneic bone marrow transplantation represents the only potentially curative therapy for these patients (5). Unfortunately, transplantation is not a feasible treatment modality for many patients.

Successful application of molecules that promote the terminal differentiation of malignant myeloid cells could have at least three potential roles in the treatment of myeloid neoplasms: terminal differentiation of a malignant clone to clonal extinction, as in retinoic acid remission-induction of acute promyelocytic leukemia (6), enforced clonal differentiation leading to functional but clonal hematopoiesis, and prolongation of remission duration in patients with AML or MDS with residual disease after chemotherapy through suppression of proliferation of the malignant clone.

We have recently shown that the aromatic fatty acid compound PB induces differentiation and inhibits the growth of primary leukemic cells (7, 8) *in vitro* at concentrations similar to those achieved in patients receiving this drug for nonmalignant disorders (9, 10). PB inhibits CFU-L production from bone marrow specimens from patients with MDS (8). In the ML-1 myeloid leukemia cell line, PB-induced differentiation is associated with induction of $p21^{WAF1/CIP1}$, hypophosphorylation of Rb protein, and arrest in the G_1 phase of the cell cycle (7). PB has been effectively used to induce fetal erythropoiesis in patients with sickle cell anemia and β-thalassemia (9–11).

Encouraged by the biological impact of PB on primary bone marrow samples from patients with MDS and AML studied *in vitro* at doses similar to those achieved in patients treated for inborn errors of metabolism and hemoglobinopathies, we

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[3] The abbreviations used are: MDS, myelodysplastic syndromes; AML, acute myeloid leukemia; PB, sodium phenylbutyrate; CFU-L, leukemia colony-forming unit; CFU-GM, colony-forming units-granulocyte-macrophage; CNS, central nervous system; MTD, maximum tolerated dose; FISH, fluorescence *in situ* hybridization; HI, hematological improvement; PAG, phenylacetylglutamine; PA, phenylacetylate; AUC, area under the curve; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; RAEB, refractory anemia with excess blasts; RAEB-t, refractory anemia with excess blasts in transformation.

undertook a pharmacokinetic and pharmacodynamic study of PB in patients with MDS and AML.

MATERIALS AND METHODS

Patients. Patients were recruited from referrals to the Division of Hematological Malignancies at The Johns Hopkins Oncology Center. Previously treated or untreated patients age ≥18 years with any French-American-British classification subset of MDS were eligible if patients were transfusion-dependent, their granulocyte count was <1,000/μl, their platelet count was <50,000/μl, or excess blasts were present. The most recent chemotherapy must have been administered ≥1 month before enrollment.

Patients with relapsed AML were eligible. Untreated patients who were poor candidates medically for AML induction chemotherapy or who refused chemotherapy were also eligible. All patients with AML were required to have a WBC count <30,000/μl that had been stable for at least 2 weeks, and they had to be unlikely to require cytotoxic therapy during the duration of the trial.

Eligibility criteria included: Zubrod performance status ≤2, absence of active infections at the time of study entry, serum creatinine <2.0 mg/dl, total serum bilirubin <2.5 mg/dl, no clinical evidence of CNS or pulmonary leukostasis; no evidence of disseminated intravascular coagulation or CNS leukemia, and negative serum β-HCG. Hematopoietic growth factors must have been discontinued 3 weeks before protocol entry. All patients gave written informed consent approved by the Institutional Review Board.

Treatment. PB is manufactured by the Elan Pharmaceutical Research Corporation (Gainesville, GA) and was supplied through the Cancer Therapy Evaluation Program of the Division of Cancer Treatment and Diagnosis in the National Cancer Institute as 50-ml vials of a 40% viscous solution of PB in sterile water (400 mg/ml) containing 4.9 g of sodium. The total daily dose was diluted in 1 liter of sterile #590 dextrose in water, USP.

After registration, patients had prestudy blood testing and bone marrow aspiration (see below). Patients were hospitalized on the Hematological Malignancy unit of The Johns Hopkins Oncology Center or at the General Clinical Research Center of The Johns Hopkins School of Medicine. PB was administered as a 7-day continuous infusion through a peripheral or central venous catheter; stable patients were followed as outpatients. Patients were readmitted on day 29 to receive a second 7-day infusion of PB. Response was formally assessed on day 29 of the second treatment cycle. Monthly administration was continued in responding patients as long as response was maintained. Growth factor administration was prohibited while on study.

Patients were treated in cohorts of three patients at each dose level. Doses were escalated separately for each of these two diagnostic groups, and the MTD was determined separately. Planned doses were 125, 250, 375, 500, 750, and 1000 mg/kg/day. Patients who developed non-life-threatening dose-limiting toxicity had infusions stopped until resolution of the toxicity to grade I or less. The infusion was then restarted at the next lower dose level. AML patients whose WBC count exceeded 50,000/μl on 2 consecutive days, or who exhibited any clinical symptoms of hyperleukocytosis, were removed from the trial and were ineligible for additional treatment with PB.

Grade 3 nonhematological toxicity was considered dose limiting. MTD was the dose level where 0 of 6 or 1 of 6 patients experienced dose-limiting toxicity, with the next higher dose having at least 2 of 3 or 2 of 6 patients experiencing dose-limiting toxicity. If administration of PB at the MTD yielded plasma concentrations <6 mM, the originally targeted level based on early *in vitro* studies (8), a cohort of patients could be enrolled at a dose halfway between the MTD and the higher dose previously tested to identify the maximum sustainable plasma concentration.

Study Parameters. All patients had the following studies obtained before starting treatment: complete blood count with differential WBC count, platelet and reticulocyte count, serum chemistry profile, prothrombin time, partial thromboplastin time, fibrin degradation products, fibrinogen, determination of fetal RBCs (F-cells) and reticulocytes (F-reticulocytes; see below), bone marrow aspirate, and biopsy. Marrow aspirate was processed for karyotyping, correlative studies (see below), and FISH (see below). Peripheral blood was collected monthly for FISH. Electrocardiograms and chest radiograph were also obtained. Blood tests were repeated weekly while patients were on the study; bone marrow was repeated monthly for the first two cycles, as were the studies of fetal hemoglobin. Correlative studies were repeated on bone marrow samples after the first infusion of PB (day 8) and before the second treatment cycle (day 29). Patients continuing to receive PB beyond the first two cycles had bone marrow aspirates repeated every 3 months while the patients were on the study.

Response Criteria (12)

MDS. Responses were graded according to Cancer and Leukemia Group B criteria (12). These criteria include a category of HI defined as a 50% or greater restoration of the deficit from normal in one or more peripheral blood cell lines, but insufficient to meet criteria for partial or complete remission; or a ≥50% decrease in packed red blood cell or platelet transfusion requirements. HI constituted a remission for the purpose of follow-up.

AML. Standard criteria were used to assess responses in the AML patient, with the addition of a category of HI, as described for MDS, without a significant change in bone marrow blast percentage.

Pharmacokinetic Studies

Two major objectives of this study were to describe the pharmacokinetics of PB in an attempt to achieve plasma concentrations in the 5–6 mM range, and to correlate the pharmacokinetic results with clinical and laboratory end points. Pharmacokinetic samples were drawn preinfusion, 1, 4, 12, 24, 48, 72, 96, 120, 144 h, and just before completion of the infusion. Postinfusion samples were drawn 10, 20, and 30 min, and 1, 2, 4, 8, and 24 h postinfusion. In addition, 24-h urine collections on days 1 and 7 were obtained and processed for determination of PAG, the biologically inert conjugate of PA, which is the direct metabolite of PB (13, 14).

Analytic Assay. Plasma PB, PA, and PAG concentrations were determined in all blood specimens by reverse-phase

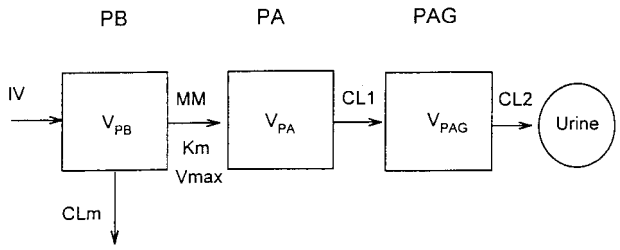

Fig. 1 Pharmacokinetic model describing the disposition of PB, PA, and PAG, illustrating the pharmacokinetic parameters. V_{PB}, V_{PA}, and V_{PAG} indicate volumes of distribution for PB, PA, and PAG, respectively. *CL1*, formation clearance of PA to PAG. *CL2*, clearance of PAG into urine. *Clm*, clearance of PB from central compartment.

high-performance liquid chromatography assay. Two hundred ml of plasma containing compound was transferred to a microcentrifuge tube, with the subsequent the addition of 50 ml of 10% perchloric acid (Sigma Chemical Co., St. Louis, MO) for protein extraction. The samples were vortexed and centrifuged (4°C; 8500 rpm; 10 min). One hundred fifty ml of supernatant was added to 5 ml of super-saturated potassium bicarbonate solution for neutralization and was centrifuged. All of the supernatant was transferred to an autosampler vial for analysis. The chromatographic apparatus consisted of a Thermoquest Liquid Chromatograph (Thermo Separation Products, Piscataway, NJ) with autosampler compartment AS3000, solvent delivery system, and a diode-array UV absorbance detector with a resolution of 2 nm. The absorbance wavelength was 208 nm (bandwidth, 10 nm). Mobile phase A consisted of 100% deionized water (Milli-QUV Plus; Millipore Corporation, Bedford, MA) with 0.005 M phosphoric acid (Sigma Chemical Co., St. Louis, MO) buffer. Mobile phase B consisted of 100% high-performance liquid chromatography grade acetonitrile (J. T. Baker, Phillipsburg, NJ) with 0.005 M phosphoric acid buffer. All mobile phases were run at a combined flow rate of 1 ml/min for a run time of 45 min using gradient profile. A Waters Nova-Pak C18 guard column was placed in line before the analytical column. The samples were injected onto a reverse-phase (Waters Bond-Pak C18, 3.9 × 300 mm, Millipore Corporation) column, which was maintained at 60°C. PA, PB, and PAG had retention times of approximately 18.2, 31.4, and 10.2 min, respectively. Chromatographic peak area was used for quantification by linear regression analysis. The lower limit of detection for the assay was 10 μM. Quality control samples were assayed at concentrations of 25, 500, and 2000 μM, and the inter-day coefficients of variation were <20%. The lower limit of quantification was 10 μM for PB, PA, and PAG. PAG concentrations were determined in the urine specimens and the amount of PAG excreted during the urine collection period was calculated as: concentration × urine volume.

Pharmacokinetic Analysis. The data were analyzed by both compartmental and noncompartmental methods. For compartmental analysis, ADAPT II, version 4 (Biomedical Stimulation Resource, University of Southern California, Los Angeles, CA) was used. A model simultaneously describing the plasma concentrations of PB and the two metabolites (PA and PAG) was applied to the data (see Fig. 1; Ref. 15). Briefly, the model incorporates Michaelis-Menten nonlinear clearance of PB to PA, and elimination of PA by glutamination to PAG. Data were modeled as molar equivalents. The pharmacokinetic parameters were estimated using Bayesian estimation (the maximum *a posteriori* probability; 15). The plasma elimination half-life ($t_{1/2}$) was calculated as: $t_{1/2} = 0.693/Ke$. The AUC for each subject was calculated using the linear trapezoidal method from time zero to the last concentration time point obtained and was extrapolated to infinity by dividing the last concentration by Ke. The steady-state concentrations of PB, PA, and PAG were calculated as the mean of concentrations from 12 h to end of infusion.

Bone Marrow Correlative Studies

Clonogenic Assay. Clonogenic assays were performed as described previously using freshly isolated bone marrow mononuclear cells (16). CFU-L were scored on days 5 through 7 of culture; CFU-GM were scored on day 14.

CD34 Isolation. CD34+ cells were isolated from bone marrow mononuclear cells using immunomagnetic beads (Dynabeads; Dynal, Inc., Lake Success, NY) and removed from the beads using Detachabead (Dynal, Inc.). All isolation procedures were performed according to the manufacturer's instructions.

Flow Cytometric Determination of Differentiation. Two-color immunofluorescence was performed as previously described. (8, 17) The following antibodies were used to examine changes in the progenitor cell fraction and the mature myeloid compartment: CD34 (HPCA-2; Becton Dickinson, Mountain View, CA) and HLA-DR (CR3/43; Dako, Carpinteria, CA); and CD14 (Tuk4; Dako) and CD15 (C3D-1; Dako). Isolated CD34+ cells were stained for the following antigens: CD13 (Leu-M7; Becton Dickinson), CD33 (WM-54; Dako), HLA-DR, CD38 (Leu-17; Becton Dickinson), and glycophorin (GA-R2; PharMingen, San Diego, CA). C-kit expression was measured after the binding of phycoerythrin-labeled Steel Factor (SCF-PE; R and D Associates, Minneapolis, MN). Fluorochrome-labeled isotype-matched controls were purchased from Dako.

Cell Cycle Analysis and Determination of Apoptosis. Bone marrow mononuclear cells were incubated with 1 mM bromodeoxyuridine (Sigma Chemical Co.) for 2 h at 37°C before CD34 isolation. Cell cycle analysis of the isolated CD34+ cells was performed according to Dolbeare's technique (18) as described previously (19). Apoptosis was determined using the TUNEL technique as described previously (7) on isolated CD34+ cells (20–22). Although cell cycle analysis of bone marrow mononuclear cells obtained on bone marrow aspirates may be confounded by dilution with peripheral blood (23), by restricting the analysis to isolated CD34+ cells, peripheral blood dilution (with few, if any, CD34+ cells) should not impact this analysis significantly.

Cytogenetics and FISH. Bone marrow was prepared by standard metaphase cytogenetic preparation. FISH was done after serial samples if a cytogenetic abnormality appropriate for FISH analysis (gain or loss of whole chromosomes) was discovered on initial cytogenetic screen.

Peripheral blood was prepared for FISH without culture. Buffy coat was lysed (0.04 M KCl, 0.02 M HEPES, and 0.05 mM

Table 1 Patient characteristics

	MDS			AML		
	No.	Median	Range	No.	Median	Range
Patients	11			16		
Courses (Courses/Patient)	33			24		
		2	(2–10)		2	(1–2)
Age		69	(62–81)		70	(46–85)
PS		0	(0–2)		0	(0–2)
FAB						
RA	1					
RAEB	5					
RAEB-T	5					
WBC		2,400	(600–11,400)		2,300	(400–17,300)
ANC		528	(224–9,120)		408	(34–7,785)
HCT		24.4	(23.6–29.5)		26.8	(24.3–34.1)
Platelet		40	(9–112)		22	(9–111)
Clonal cytogenetics	5			8		
Previous therapy						
Yes	1			7		
No	10			9		

EGTA; for 20 min; 37°C), fixed, washed twice (fresh; 3:1, methanol:acetic acid), and stored at −20°C until air-dried slides were made for use in FISH. α-satellite probes specific for the centromere of a given chromosome were purchased from Oncor (Gaithersburg, MD) and Vysis (Downers Grove, IL). Probes were chosen on the basis of metaphase chromosome results for patients' pretreatment marrow; loss or gain of signal for that probe was confirmed on interphase cells from the same specimen. When more than one numerical abnormality was present, choosing trisomic over monosomic probes and probes with higher efficiency over those with lower efficiency were selected for monitoring. Five hundred nuclei were scored with the probe of interest; a normal control was included with each experiment. A cutoff of 5% for considering FISH results abnormal was determined by mean ± 2 SD of a series of normal controls.

Fetal Hemoglobin. Erythrocytes and reticulocytes containing fetal hemoglobin (F-cells and F-reticulocytes) were measured in peripheral blood as described previously (10).

RESULTS

Patients Treated. Patient characteristics are listed in Table 1. Eleven patients with MDS were treated and their median age was 69 years. Most had RAEB or RAEB-t, and seven had clonal cytogenetic abnormalities. Ten patients were previously untreated. Sixteen patients with AML were treated; 9 were previously untreated. Thirteen had clonal cytogenetic abnormalities. Of the previously treated patients, four had achieved remission and subsequently relapsed, whereas three were primarily refractory to chemotherapy.

Dose Escalation, Toxicities, and Determination of MTD. Although separate dose escalations were planned for the two diseases, dose-limiting toxicity was identical. The protocol was therefore amended so that determination of MTD could use data combined from the two patient populations. The toxicities for the combined patient populations for doses of 375 mg/kg/day and greater are detailed in Table 2. No serious toxicities were seen in patients receiving 125 to 375 mg/kg/day. At 500 mg/kg/day, neurocortical toxicity was seen in two of three patients with AML and one of two patients with MDS. Each developed lethargy, confusion, and slurred speech. Clinically, this resembled a metabolic global encephalopathy, although no asterixis or other focal neurological signs were seen in any of these patients. The onset of severe neurocortical toxicity occurred at days 3, 6, and 7, at which point PB was discontinued. Lesser degrees of CNS toxicity were observed in these patients beginning on days 2, 4, and 5. At these earlier time points, patients stated that they could fall asleep easily at any time, but were completely arousable with normal mini-mental examinations. In addition, blunted affect, anorexia, and subjective confusion were reported. Upon cessation of the infusion, neurocortical function completely normalized within 24 to 48 h. A third patient with AML treated at 500 mg/kg/day developed CNS toxicity during her fourth cycle of PB on day 6 of infusion. Toxicity was relatively mild, and she was able to complete the full 7-day course. Although by NCI common toxicity criteria, the CNS toxicity did not exceed grade II, these complications were clinically severe and were considered functionally dose limiting. Four AML patients were subsequently treated at 440 mg/kg/day. Two developed identical CNS toxicity, with onset of mild symptoms on days 2 and 3 and peak encephalopathy on days 5 and 7. Thus, 375 mg/kg/day was considered the MTD and the recommended dose for additional investigation in this patient population.

Serum ammonia was measured at the time of severe CNS changes in the three patients treated at 500 mg/kg/day and one of two patients treated at 440 mg/kg/day. Ammonia was significantly elevated in three patients at the time of toxicity (75, 150, and 210 μmol/liter), and was mildly elevated in the patient who developed CNS toxicity during cycle 4 (59 μmol/l). Four of six patients with CNS toxicity treated at 440 or 500 mg/kg/day received subsequent cycles of PB at 375 mg/kg/day with no evidence of CNS toxicity. Of the other two patients who experienced CNS toxicity, one was taken off study because of leucocytosis (see below), and one declined additional therapy

Table 2 Toxicity

	Dose Level 375					Dose Level 440					Dose Level 500				
NCI toxicity grade	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Nausea/Vomiting	9	1						4			2	3			
Mucositis	10					4					5				
Diarrhea	10					4					5				
Liver															
Billirubin	10					4					5				
Tranaminases	10					4					5				
Renal	10					4					5				
Skin	7		3			4					4		1		
CNS															
Headache	6	4				2	2				4	1			
Cortical	10					3			1		2	3			
Cardiac function	10										5				
Hypocalcemia	6	2	2			2		2			2	2		1	
Fever	9		1			3		1			4			1	
Hemorrhage	10					3			1		5				
Alopecia	10					4					5				
Other															
Hyperuricemia	8					4					5				
Thrombophlebitis	2					3									
Interstitial lung disease	1														
Abdominal pain	1					1					1				
Hiccup						1									
Auditory acuity											1				

with PB because of declining performance status after recovery from CNS toxicity.

PB was well tolerated otherwise. Hyperuricemia was regularly induced at all doses of 375 and greater: 4 of 4 patients treated at 440, 4 of 5 treated at 500, and 8 of 10 treated at 375 mg/kg/day developed hyperuricemia. The median onset of increased uric acid was day 3; the median date of peak uric acid was day 6. Mean day-7 urate levels were significantly increased for the cohorts treated at 440 and 500 mg/kg/day. Changes in uric acid were not associated with changes in other laboratory parameters that would suggest tumor lysis syndrome. A retrospective analysis of the fractional excretion of urate (FE_{UA}) was performed on archived urine samples. The mean FE_{UA} was decreased in all dose cohorts, although these differences were only significant when data were pooled across dose cohorts. Despite comparable decline in FE_{UA} at all dose levels, mean uric acid levels were significantly increased only in the higher dose cohorts. Among patients treated with doses of PB from 375 to 500 mg/kg/day, mean FE_{UA} declined from 15 $\pm$ 3 on day 1 to 9 $\pm$ 2 on day 7 ($P < 0.01$). This was associated with an increase in mean serum uric acid from 5.9 $\pm$ 0.39 to 8.9 $\pm$ 0.9 ($P < 0.01$). The mean serum day-7 uric acid among the higher dose cohorts was 11.0 $\pm$ 0.8 (440 mg/kg/day) and 10.8 $\pm$ 1.1 (500 mg/kg/day).

Reductions in serum calcium were observed regularly during PB infusions: 3 of 6 patients treated at 125, 1 of 6 at 250, 5 of 11 treated at 375, 4 of 4 treated at 440, and 3 of 5 patients treated at 500 developed asymptomatic reversible decrements in serum calcium. Among patients whose calcium declined significantly, mean pre- and posttreatment values were 8.4 $\pm$ 0.1 and 7.6 $\pm$ 0.1, respectively ($P < 0.001$). The lowest posttreatment calcium value was 6.8 (pretreatment value of 7.7 and a dose level of 500 mg/kg/day).

Two patients with MDS developed symptomatic interstitial lung disease; both patients underwent open-lung biopsy revealing usual interstitial pneumonitis. The first patient, a man 71 years of age with RAEB, was treated at 125 mg/kg/day and achieved HI in neutrophil counts, receiving 10 cycles of PB before declining additional therapy because of the frequent hospitalizations. He had a preexisting history of obstructive pulmonary disease, and he developed progressive pulmonary dysfunction 5 months after discontinuing PB therapy. The second patient was a man 74 years of age with RAEB. He received one cycle of PB at 250 mg/kg/day. He was not retreated because of the occurrence of neutropenic fevers and comorbid problems. After resolution of these problems 6 months later, he received two cycles at 375 mg/kg/day, achieving HI (neutrophils). He developed progressive interstitial lung disease, leading to open lung biopsy. Review of pretreatment chest radiographs showed that the patient had underlying interstitial changes before entering the study.

Grade I nausea without emesis was seen intermittently in patients receiving 375 mg/kg/day or greater; nausea was completely relieved by granisetron. Grade I headaches were reported frequently at all dose levels and were completely relieved by acetaminophen. One patient with MDS and a history of superficial venous thromboses was found to have a deep venous thrombosis while receiving PB (375 mg/kg/day); however, his symptoms predated the onset of the infusion. Six patients developed thrombophlebitis at the site of i.v. catheters. Fever was not common in this group of granulocytopenic patients. One patient with untreated AML and history of congestive heart failure received excessive hydration during her PB infusion and developed pulmonary edema, requiring interruption of the infusion for diuresis. The patient was able to complete the infusion and receive a second cycle without recurrence (250 mg/kg/day).

One patient (500 mg/kg/day) reported decreased hearing during PB infusion. Audiometry was not performed; however, hearing subjectively returned to baseline after discontinuation of PB infusion.

Pharmacokinetic Analysis. The previously described model (Fig. 1) fit the data well. The mean coefficients of determination (r^2) for PB, PA, and PAG were 0.88 ± 0.07, 0.80 ± 0.09, and 0.91 ± 0.06, respectively. Pharmacokinetic parameters derived from the model are shown in Table 3 and are similar to those previously reported (15). Plasma PB disposition curves were characterized by a rise to an apparent plateau achieved by 4–6 h into the infusion. AUC and steady-state concentrations of PB increased in a linear fashion without changes in half-life from 125 to 440 mg/kg/day (Table 4; Fig. 2). At a dose of 500 mg/kg/day, there seemed to be nonlinear elimination for PB. At the MTD, the mean (± SD) PB steady-state plasma concentration was 0.287 ± 160 nM. PB exhibited saturable metabolism to PA (mean K_m = 0.2 ± 0.057 mM and V_{max} = 7.1 ± 4.3 mM/h). The mean volume of distribution for PB compartment was 14.0 ± 7.7 liters and for the PAG compartment was 9.6 ± 3.7 liters. PA appeared to exhibit nonlinear elimination in the current data set, clearance decreasing with dose (Table 3). AUC and steady-state concentrations increased in a nonlinear fashion with all doses of PB used, with a gradual prolongation of half-life.

Elevated PA concentrations were related to the occurrence of dose-limiting CNS toxicity; the median PA steady-state concentration was 3.3 mM in patients who experienced CNS toxicity compared with 0.21 mM in those who did not (P = 0.019, Wilcoxon rank sum test). PB and PAG concentrations did not correlate with CNS complications. No clear relationships between PB, PA, or PAG exposure and changes in biological or clinical end points were found.

Clinical Outcomes. No patients achieved a complete or partial remission. Three patients achieved HI on the basis of increased neutrophil count. One MDS patient who was treated for 10 cycles at 125 mg/kg/day sustained neutrophil improvement (pre- and posttreatment absolute neutrophil count (ANC) 324 to 2414/μl). This patient subsequently declined additional therapy because of inconvenience; his neutrophils continued in the improved range until his death 7 months later from complications of obstructive and interstitial lung disease. Two MDS patients treated at 375 mg/kg/day demonstrated HI based on increased neutrophils (pre- and posttreatment ANC 703 to 1920/μL and 646 to 4705/μL). One received four cycles of the drug, discontinuing because of inconvenience. The other patient received two cycles and discontinued because of interstitial lung disease. Nonsustained improvement in neutrophil counts were frequent and seen at all doses: 6 of 15 granulocytopenic patients with AML and 4 of 8 granulocytopenic MDS patients developed neutrophil counts >1000/μl *(*data not shown).

One patient with relapsed AML (500 mg/kg/day) demonstrated improvement based on the achievement of platelet transfusion-independence (pretreatment value, <20,000/μl; posttreatment, 50,000–80,000/μl). This patient received five cycles of PB before developing infections that led to her demise. Infections were not related to her indwelling catheter and could not be attributed to PB administration. Unsustained improvements in platelets were seen in three additional patients: two

Table 3 Pharmacokinetic parameters derived from the model

Dosage (mg/kg/day)	n	V_{PB}[a] (liters)	V_{PA} (liters)	V_{PAG} (liters)	CL_{int} (liters/h)	CLt1 (liters/h)	CLt2 (liters/h)	CL_m (liters/h)	K_m (μmol)	V_{max} (μmol/h)	R^2-PB	R^2-PA
125	5	20.49	21.00	10.86	22.26	42.13	10.38	11.55	159.52	3,623.51	0.86	0.83
		(9.62)	0.00	(2.83)	(4.65)	(17.09)	(3.01)	(3.31)	(33.15)	(1,341.67)	(0.06)	(0.06)
250	6	11.38	21.00	11.30	28.26	15.68	8.52	10.10	183.33	5,273.21	0.88	0.79
		(2.32)	0.00	(3.21)	(6.42)	(6.48)	2.16	5.20	27.16	1,774.46	0.06	0.10
375	6	11.03	21.00	11.45	43.71	12.21	9.08	6.97	203.93	9,192.15	0.91	0.81
		(3.05)	0.00	(2.46)	(15.35)	(9.32)	(2.01)	(3.97)	(33.79)	(4,346.72)	(0.08)	(0.06)
440	3	11.54	21.00	4.93	49.47	1.68	9.49	3.87	248.87	12,677.73	0.87	0.77
		(7.51)	0.00	(0.70)	(11.29)	(0.20)	(4.14)	(1.01)	(66.26)	(5,320.07)	(0.08)	(0.09)
500	3	16.83	21.00	4.86	28.64	2.79	6.04	4.09	245.57	7,142.36	0.70	0.78
		(14.35)	0.00	(1.51)	(11.18)	(1.73)	(1.82)	(1.72)	(116.02)	(4,351.33)	(0.20)	(0.14)
Mean		14.00	21.00	9.57	33.80		8.88	8.00	200.20	7,146.52	0.86	0.80
SD		7.73	Fixed	3.68	13.87		2.68	4.56	57.54	4,326.94	0.11	0.08

[a] V_{PB}, volume of distribution for PB; V_{PA}, volume of distribution for PA; V_{PAG}, volume distribution for PAG; CL_{int}, intrinsic clearance; CLt1, formation clearance of PA to PAG; CLt2, clearance of PAG into the urine; CL_m, clearance of PB out of central compartment. Data are expressed as mean and SD. The volume of distribution of PA was fixed at 21 liter based on previous phase 1 study (15) and model fitting (15).

Table 4 AUC and half-life of phenylbutyrate and phenylacetate

Dosage (mg/day)	*n*	PB		PA	
		AUC[a]	$t_{1/2}$	AUC	$t_{1/2}$
125	5	13,605 (3,002)	0.9 (0.84)	6,053 (1,682)	0.80 (0.35)[b]
		14,723 (8,368–15,609)	0.49 (0.44–2.16)	6,003 (3,916–8,292)	0.64 (0.53–1.39)[c]
250	6	38,838 (21,081)	0.81 (0.75)	52,505 (63,211)	1.25 (0.79)
		37,639 (17,322–72,812)	0.55 (0.35–2.31)	28,704 (17,453–180,891)	0.95 (0.83–2.86)
375	6	40,817 (18,848)	0.52 (0.25)	104,366 (77,290)	1.38 (0.44)
		34,082 (20,163–68,000)	0.49 (0.17–0.81)	104,933 (17,694–216,620)	1.28 (0.84–2.05)
440	3	51,263 (8,922)	0.32 (0.07)	609,790 (312,248)	4.11 (3.25)
		55,070 (41,069–57,652)	0.32 (0.27–0.38)[d]	485,181 (379,087–965,102)	3.23 (1.39–7.7)
500	3	96,006 (28,170)	2.19 (2.08)	460,092 (320,809)	4.40 (3.77)
		102,417 (65,183–120,419)	2.06 (0.18–4.33)	560,338 (101,130–718,810)	3.01 (1.53–8.67)

[a] AUC values are represented as μmol h/liter.
[b] Mean and SD.
[c] Median and range.
[d] $n = 2$.

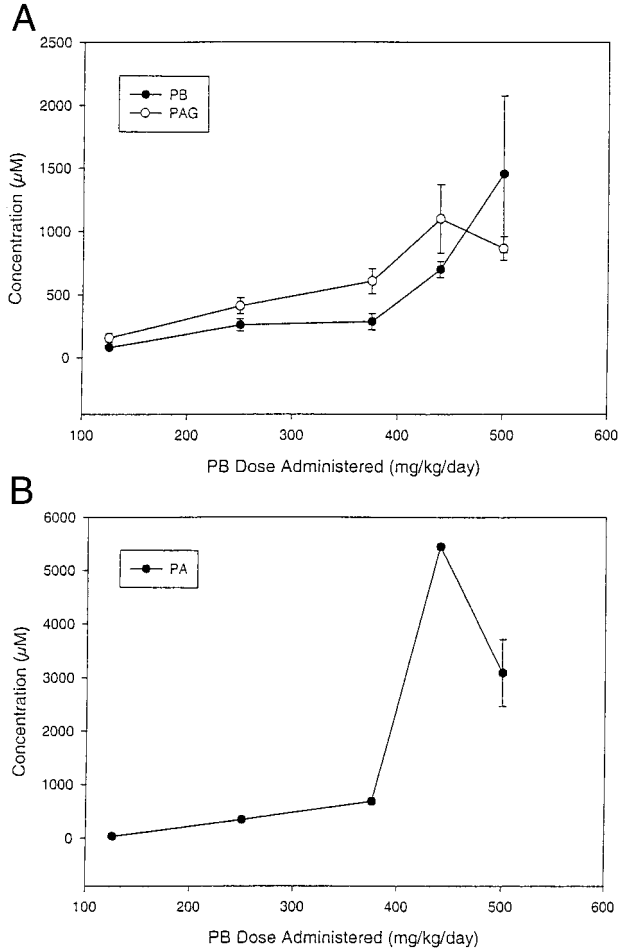

Fig. 2 Steady-state plasma concentrations of PB, PAG (*A*), and PA (*B*). Values represent mean ± SE.

MDS patients treated at 375 and 500 mg/kg/day (change in platelet counts 40,000–58,000 and 110,000–150,000, respectively) and one patient with untreated AML (500 mg/kg/day; 100,000–270,000). This latter patient had a simultaneous increase in WBC, including neutrophils but primarily blasts, which required discontinuation of PB.

Peripheral blasts declined in four patients with AML (three relapsed, one untreated). Two of these patients received 250 mg/kg/day, one 440 mg/kg/day, and one 500 mg/kg/day. Decrement in peripheral blood blast percentage were as follows: 49% to 3%, 49% to 12%, 30% to 3%, and 30% to 3%. Bone marrow blasts declined in the patient treated at 500 mg/kg/day (49% to 21%). Another patient treated at 500 mg/kg/day had a decrease in marrow blast percentage from 48% to 22%. Two other AML patients had minor decrements in bone marrow blast percentage (73% to 63% and 92% to 85%, 375 mg/kg/day). The MDS patient treated at 125 mg/kg/day with neutrophil improvement showed decline in marrow blasts from 23% to 14%. Blasts did not significantly decline in other MDS patients. Four MDS patients progressed to AML (one at 125 mg/kg/day; one at 250 mg/kg/day; and two at 375 mg/kg/day).

One patient with refractory anemia had an extensive nodular erythematous skin eruption with onset around the time of diagnosis of MDS. Biopsy of the rash revealed superficial perivascular T cell infiltration. This rash showed marked improvement during the first week of PB therapy (500 mg/kg/day) and completely resolved before the second cycle (administered at 375 mg/kg/day). The patient received only two cycles of the drug. The rash showed evidence of early recurrence 1 month after cessation of PB therapy.

Bone Marrow Correlative Studies

Differentiation. No consistent changes were seen in the CD34+ compartment. Among the four patients who developed HI, one patient showed a progressive rise in CD34+ cells over 29 days (125 mg/kg/day; 9% to 26%), two patients showed transient increases in CD34+ cells on day 8 (500 mg/kg/day; 5.4% to 19.2% and 12.4% to 32.5%, both returning toward baseline by day 29), and one patient had no significant changes. In five patients, CD14+ cells increased by at least 1.5-fold on day 8 compared with day 0 (two patients treated with 500 mg/kg/day, one each treated with 250, 375, and 440 mg/kg/day). Changes in CD15 expression of a similar magnitude were ob-

Table 5 Impact of PB administration on hemoglobin F

Dose (mg/kg/day)	A. MDS F-cells Mo 0	F-cells Mo 1	F-cells Mo 2	F-reticulocytes Mo 0	F-reticulocytes Mo 1	F-reticulocytes Mo 2
125	8	14.5	2.5	10	24	0
125	4.4	1.2	2.1	0.7	0	0
125	5.9	5.3	3.9	0.7	0	0
250	26.9	29.7	nd[a]	36.8	26	nd[a]
250	29.9	36.1	38.8	36	37	nd[a]
375	3.1	nd[a]	70.1	3.3	nd[a]	34
375	11.8	8.5	7.7	13.3	10	2
375	3.3	2.9	nd[a]	0.7	0	nd[a]
375	2.2	2.5	2.7	0.7	0.7	2
500	1.1	1.2	1.2	0	0	0
500	14.1	16.8	21	10.7	12	14
	B. AML					
125	20.2	27	Off study	0	5.3	Off study
125	62.4	60.2	Off study	36	40	Off study
125	16.2	33.6	16	9.3	16.7	2
250	5.3	3.1	3.6	nd[a]	0.7	0
250	3.8	2.4	3.2	0	0	0
250	3.4	2.8	3.3	0.7	0	0
375	22.3	21.7	22.8	17.3	20.7	24.7
375	65.5	70	72.4	10	14.3	22.7
375	3	3		0	9.1	
375	1.5	1.6	2.6	0	0	0
400	6	nd[a]	7.2	0	nd[a]	0
400	3.8	Off study		0	Off study	
400	nd[a]			nd[a]		
400	nd[a]			nd[a]		
500	nd[a]					
500	18.3	15.6	19.6	2	16.7	2

[a] nd, not done.

served in seven patients (two each at 375 and 500 mg/kg/day; one each at 125, 250, and 400 mg/kg/day). No consistent pattern of changes of these latter two antigens was seen among patients who demonstrated HI.

Expression of a variety of antigens associated with differentiation stage of progenitor cells was studied in isolated CD34+ cells. No consistent pattern of change in expression of c-kit, CD13, CD33, HLA-DR, or CD38 or glycophorin was seen between protocols day 1, 8, and 29 (data not shown).

Clonogenic Assay. The results of clonogenic assays were variable. Among MDS patients, changes in CFU-L and CFU-GM were variable, with some patients showing increased CFU and some declining CFU. Of the patients with improved neutrophil counts, one developed significant increases in CFU-GM and CFU-L (day 1/day 8 CFU-L, 272 ± 41.5/725 ± 48.5; day 1/day 8, CFU-GM, 928 ± 37/1438 ± 155), one trended to increased CFU-GM and CFU-L, and a third grew no colonies at any time.

Changes in CFU were also variable among AML patients. Of interest, the three patients with reduced blasts all had increased CFU-L on day 29; 2 of 3 had increased CFU-GM as well (data not shown).

Cell Cycle Analysis and Apoptosis. PB did not impact on the percentage of isolated CD34+ cells in S-phase on either day 8 or 29 (data not shown). The median percentage of S-phase in isolated CD34+ cells was 4.8% for MDS (range 0% to 13%) and 1.2% for AML (range 0% to 8.5%). Apoptosis among isolated CD34+ cells, assessed using a TUNEL technique, was also not significantly impacted by PB therapy on day 8 or 29 (median percent of CD34+ cells positive in TUNEL assay was 2% in MDS and 0.9% in AML, data not shown).

Fetal Hemoglobin. The impact of PB infusion on fetal hemoglobin is displayed in Table 5. The number of reticulocytes and/or erythrocytes expressing fetal hemoglobin increased over time in 4 of 11 patients with MDS and 8 of 12 evaluable patients with AML.

Clonality. Twenty of 27 patients studied had clonal cytogenetic abnormalities. Fourteen of these were appropriate for monitoring by FISH and had monthly samples of blood and/or bone marrow available for monitoring of change in clonality while receiving PB. The results are displayed in Table 6. Of the four patients followed longitudinally who developed HI, the percentage of clonal cells increased over time in two, remained stable in one, and transiently decreased (over 2 months) in the fourth. Of the remaining patients, the percentage of clonal cells remained stable or increased over time in eight patients. Two patients with AML were exceptions. One patient treated at 250 mg/kg/day was monitored using bone marrow metaphase cytogenetics. Although he did not have a hematological response, clonal cells decreased from 63% to zero detectable at 1 month, and to 11% at the end of 2 months. Another patient with AML

Table 6 Clonality studies

Percentage of clonal cells studied by FISH or classical cytogenetics documented by study month. Bold numbers indicate study performed on bone marrow rather than peripheral blood. Italicized row indicates patient scored as "hematologic improvement."

Dose	Abnormality studied	FISH[a]/ Cytogenetics (F/C)	Percentage clonal cells (Mo) 0	1	2	3
MDS						
125	+21	F	5	11	14	
125	*+8*	*F*	*56*	*66*	*73*	
125	+8	F	**4**	6	8	
375	*+8*	*F*	*29*	*27*	*52*	
375	+8	F	57	64	51	
375	*−y*	*F*	***74***	*65*	*59*	*89*
AML						
250	+8	F	22	10	15	
250	+6	C	**63**	**0**	**11**	
250	+11	F	61	52	61	
375	+9	F	57	69	79	
375	−y	F	37	17	18	
400	t(x;12)	C	**100**	**100**	nd	
500	*+8*	*F*	*6*	*7*	*6*	*9*
500	−7,−16	F	16	23	27	

[a] FISH, fluorescein *in situ* hybridization.

treated at 375 mg/kg/day demonstrated a decrement in peripheral blood clonal cells over time (37% to 18%).

DISCUSSION

The current Phase I trial confirms the safety and tolerability of 7-day continuous i.v. infusions of sodium PB and provides preliminary evidence of hematological activity in myeloid disorders. At the MTD, plasma concentrations (median 0.3 mM) were significantly less than those targeted based on *in vitro* studies. The clinical activity of PB at these lower concentrations may be explained by its effect on histone acetylation at these doses (see below). Administration of higher doses was not feasible because of the development of dose-limiting CNS toxicity, apparently attributable to accumulation of the metabolite PA. Phase I studies of PA documented CNS toxicity as dose limiting (24, 25). CNS toxicity was associated with hyperammonemia. This has not been studied in previous trials of PA and may play a role in the pathophysiology of PA toxicity. Whereas the conversion of PA to PAG appeared nonlinear as PB dose increased from 375 to 440 mg/kg/day, this should not influence further studies at the MTD.

Other toxicities of PB were minimal. The occurrence of usual interstitial pneumonitis, a relatively rare lung disease, in two patients (both with underlying lung disease) makes ongoing observation for pulmonary toxicity of chronic PB administration mandatory. Both patients who developed this complication had sustained improvement in neutrophil counts. It is possible that the exacerbation of lung disease may have been related to changes in neutrophil physiology induced by PB, analogous to retinoid-induced changes in promyelocytic leukemia cells, which have been etiologically implicated in the so-called "all-*trans* retinoic acid syndrome" (26, 27).

Indications of hematological activity were seen at all doses studied, including improvement in neutrophil and platelet counts, and decrements in circulating blast cells. Prolonged *in vitro* exposures to lower concentrations of PB, such as those achieved in the present study, have led to changes in proliferation and differentiation of myeloid leukemia cells, which approximate those of higher concentrations (7). Thus, it is possible that prolonged exposure to achievable concentrations of PB may have more significant hematological benefit than the 7-day infusion repeated every 28 days that is currently reported. Improved hematopoiesis remained clonal, although suppression of cytogenetically abnormal cells was found in two patients. The remarkable improvement of the T cell infiltrates in one MDS patient is unexplained; however, it is possible that this skin abnormality was a paraneoplastic phenomenon, based on its temporal concurrence with the development of symptomatic MDS.

Correlative studies were unable to clarify the mechanism of improved hematopoiesis in responding patients. Hematological responses were usually associated with increased numbers of CFU-GM as well as CFU-L. This suggests the possibility that PB may nonspecifically support proliferation of myeloid progenitor cells. Recent data has shown that low concentrations of PB in the range of those achieved at the MTD increase proliferation of myeloid cells (7). Although no change in percentage of apoptosis in the CD34+ cell compartment was found, it is possible that such apoptotic cells may have higher density and thus may have been underestimated by examination of CD34+ cells isolated from the mononuclear cell fraction (28). Nonetheless, the plasma concentrations achieved were substantially less than those that induce apoptosis *in vitro*.

PB, like butyrate, is known to promote expression of the hemoglobin γ gene and has been used effectively as treatment for patients with sickle cell anemia and thalassemia (9–11). The current study demonstrates the induction of fetal hemoglobin in patients with MDS and AML receiving PB at all doses administered. Thus, at the plasma concentrations achieved, PB impacts on expression of at least one surrogate gene involved in hematopoiesis.

Recent demonstrations of the important role that histone acetylation plays in the regulation of gene expression (29) and the frequent recruitment of histone deacetylase enzymes by fusion genes involved in acute leukemias have led to the speculation that agents that inhibit histone deacetylase may be useful in the treatment of neoplasms. Like butyric acid, PB has substantial activity in the inhibition of this enzyme. Significantly, concentrations of 0.25–0.5 mM, which were sustained in the present study, effectively induce acetylation of histones H3 and H4 (30). Like other histone deacetylase inhibitors (31, 32), PB synergizes with retinoids in the induction of differentiation and cell cycle arrest of myeloid leukemia cells (30). Inhibition of histone deacetylase, not measured in the present study, may explain the changes in bone marrow CFU, hemograms, and F-cells seen in patients in this study despite submillimolar plasma concentrations. The outstanding toxicity profile of PB makes it an excellent candidate for early studies of clinical inhibition of histone deacetylase in both hematological and nonhematological malignancies.

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REFERENCES

1. Estey, E., Thall, P., Beran, M., Kantarjian, H., Pierce, S., and Keating, M. Effect of diagnosis (refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, or acute myeloid leukemia [AML]) on outcome of AML-type chemotherapy. Blood, *90:* 2969–2977, 1997.

2. Heinemann, V., and Jehn, U. Acute myeloid leukemia in the elderly: biological features and search for adequate treatment. Ann. Hematol., *63:* 179–188, 1991.

3. Geller, R. B., Burke, P. J., Karp, J. E., Humphrey, R. L., Braine, H. G., Tucker, R. W., Fox, M. G., Zahurak, M., Morrell, L., Hall, K. L., *et al.* A two-step timed sequential treatment for acute myelocytic leukemia. Blood, *74:* 1499–1506, 1989.

4. Geller, R. B., Zahurak, M., Hurwitz, C. A., Burke, P. J., Karp, J. E., Piantadosi, S., and Civin, C. I. Prognostic importance of immunophenotyping in adults with acute myelocytic leukaemia: the significance of the stem-cell glycoprotein CD34 (My10). Br. J. Haematol., *76:* 340–347, 1990.

5. Edenfield, W. J., and Gore, S. D. Stage-specific application of allogeneic and autologous marrow transplantation in the management of acute myeloid leukemia. Semin. Oncol., *26:* 21–34, 1999.

6. Warrell, R. P., Frankel, S. R., Miller, W. H., Jr., Scheinberg, D. A., Itri, L. M., Hittelman, W. N., Vyas, R., Andreeff, M., Tafuri, A., Jakubowski, A., Gabrilove, J., Gordon, M. S., and Dmitrovsky, E. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans*-retinoic acid). N. Engl. J. Med., *324:* 1385–1393, 1991.

7. DiGiuseppe, J. A., Weng, L-J., Yu, K. H., Fu, S., Kastan, M. B., Samid, D., and Gore, S. D. Phenylbutyrate-induced G_1 arrest and apoptosis in myeloid leukemia cells: structure-function analysis. Leukemia, *13:* 1243–1253, 1999.

8. Gore, S. D., Samid, D., and Weng, L-J. Impact of the putative differentiating agents sodium phenylbutyrate and sodium phenylacetate on proliferation, differentiation, and apoptosis of primary neoplastic myeloid cells. Clin. Cancer Res., *3:* 1755–1762, 1997.

9. Dover, G., Brusilow, S., and Samid, D. Increased fetal hemoglobin in patients receiving sodium 4-phenylbutyrate. N. Engl. J. Med., *327:* 569–570, 1992.

10. Dover, G. J., Brusilow, S., and Charache, S. Induction of fetal hemoglobin production in subjects with sickle cell anemia by oral sodium phenylbutyrate. Blood, *84:* 339–343, 1994.

11. Collins, A. F., Pearson, H. A., Giardina, P., McDonagh, K. T., Brusilow, S. W., and Dover, G. J. Oral sodium phenylbutyrate therapy in homozygous β thalassemia: a clinical trial. Blood, *85:* 43–49, 1995.

12. Silverman, L. R., Holland, J. F., Weinberg, R. S., Alter, B. P., Davis, R. B., Ellison, R. R., Demakos, E. P., Cornell, C. J., Jr., Carey, R. W., Schiffer, C., *et al.* Effects of treatment with 5-azacytidine on the *in vivo* and *in vitro* hematopoiesis in patients with myelodysplastic syndromes. Leukemia, *7* (Suppl. 1)*:* 21–29, 1993.

13. Maestri, N. E., Hauser, E. R., Bartholomew, D., and Brusilow, S. W. Prospective treatment of urea cycle disorders. J. Pediat., *119:* 923–928, 1991.

14. Brusilow, S. W. Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion. Pediat. Res., *29:* 147–150, 1991.

15. Piscitelli, S. C., Thibault, A., Figg, W. D., Tompkins, A., Headlee, D., Lieberman, R., Samid, D., and Myers, C. E. Disposition of phenylbutyrate and its metabolites, phenylacetate and phenylacetylglutamine. J. Clin. Pharmacol., *35:* 368–373, 1995.

16. Gore, S. D., Weng, L. J., and Burke, P. J. Identification of growth factor-responsive acute myelogenous leukemia based on factor-dependence for survival and proliferation. Leukemia, *8:* 1854–1863, 1994.

17. Loken, M. R., Shah, V. O., Dattilio, K. L., and Civin, C. I. Flow cytometric analysis of human bone marrow: II. Normal B lymphocyte development. Blood, *70:* 1316–1324, 1987.

18. Dolbeare, F., Gratzner, H., Pallavicini, M. G., and Gray, J. W. Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. Proc. Natl. Acad. Sci. USA, *80:* 5573–5577, 1983.

19. Gore, S. D., Weng, L-J., and Burke, P. J. Validation of flow-cytometric determination of Ki67 expression as a measure of growth factor response in acute myelogenous leukemia. Exp. Hematol., *21:* 1702–1708, 1993.

20. Mundle, S., Venugopal, P., Shetty, V., Ali, A., Chopra, H., Handa, H., Rose, S., Mativi, B. Y., Gregory, S. A., Preisler, H. D., and Raza, A. The relative extent and propensity of CD34+ *vs.* CD34-cells to undergo apoptosis in myelodysplastic marrows. Int. J. Hematol., *69:* 152–159, 1999.

21. Parker, J. E., Fishlock, K. L., Mijovic, A., Czepulkowski, B., Pagliuca, A., and Mufti, G. J. "Low-risk" myelodysplastic syndrome is associated with excessive apoptosis and an increased ratio of pro- *versus* antiapoptotic bcl-2-related proteins. Br. J. Haematol., *103:* 1075–1082, 1998.

22. Tsoplou, P., Kouraklis-Symeonidis, A., Thanopoulou, E., Zikos, P., Orphanos, V., and Zoumbos, N. C. Apoptosis in patients with myelodysplastic syndromes: differential involvement of marrow cells in "good" *versus* "poor" prognosis patients and correlation with apoptosis-related genes. Leukemia, *13:* 1554–1563, 1999.

23. Raza, A., Maheshwari, Y., and Preisler, H. D. Differences in cell cycle characteristics among patients with acute nonlymphocytic leukemia. Blood, *69:* 1647–1653, 1987.

24. Thibault, A., Cooper, M. R., Figg, W. D., Venzon, D. J., Sartor, A. O., Tompkins, A. C., Weinberger, M. S., Headlee, D. J., McCall, N. A., Samid, D., Myers, C. E., *et al.* A Phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. Cancer Res., *54:* 1690–1694, 1994.

25. Thibault, A., Samid, D., Cooper, M. R., Figg, W. D., Tompkins, A. C., Patronas, N., Headlee, D. J., Kohler, D. R., Venzon, D. J., and Myers, C. E. Phase I study of phenylacetate administered twice daily to patients with cancer. Cancer (Phila.), *75:* 2932–2938, 1995.

26. Di Noto, R., Lo, Pardo, C., Schiavone, E. M., Ferrara, F., Manzo, C., Vacca, C., and Del Vecchio, L. All-*trans* retinoic acid (ATRA) and the regulation of adhesion molecules in acute myeloid leukemia. Leuk. Lymphoma, *21:* 201–209, 1996.

27. Fenaux, P., and De Botton, S. Retinoic acid syndrome: recognition, prevention and management. Drug Saf., *18:* 273–279, 1998.

28. Preisler, H. D., and Raza, A. Assessment of cell-cycle effects of cytokines *in vivo*. J. Clin. Oncol., *10:* 673–674, 1992.

29. Redner, R. L., Wang, J., and Liu, J. M. Chromatin remodeling and leukemia: new therapeutic paradigms. Blood, *94:* 417–428, 1999.

30. Yu, K. H., Weng, L. J., Fu, S., Piantadosi, S., and Gore, S. D. Augmentation of phenylbutyrate-induced differentiation of myeloid leukemia cells using all-*trans* retinoic acid. Leukemia, *13:* 1258–1265, 1999.

31. Guidez, F., Ivins, S., Zhu, J., Soderstrom, M., Waxman, S., and Zelent, A. Reduced retinoic acid-sensitivities of nuclear receptor corepressor binding to PML- and PLZF-RAR α underlie molecular pathogenesis and treatment of acute promyelocytic leukemia. Blood, *91:* 2634–2642, 1998.

32. Breitman, T. R., and He, R. Combinations of retinoic acid with either sodium butyrate, dimethyl sulfoxide, or hexamethylene bisacetamide synergistically induce differentiation of the human myeloid leukemia cell line HL60. Cancer Res., *60:* 6268–6273, 1990.