A Phase I Study of Pivaloyloxymethyl Butyrate, a Prodrug of the Differentiating Agent Butyric Acid, in Patients with Advanced Solid Malignancies[1]

Amita Patnaik,[2] Eric K. Rowinsky, Miguel A. Villalona, Lisa A. Hammond, Carolyn D. Britten, Lillian L. Siu, Andrew Goetz, Sally A. Felton, Susan Burton, Frank H. Valone, and S. Gail Eckhardt

Institute for Drug Development, Cancer Therapy and Research Center and The University of Texas Health Science Center at San Antonio, Texas 78229 [A. P., E. K. R., M. A. V., L. A. H., C. D. B., L. L. S., A. G., S. A. F., S. G. E.], and Titan Pharmaceuticals, Inc., South San Francisco, California 94080 [S. B., F. H. V.]

ABSTRACT

Pivaloyloxymethyl butyrate (AN-9), an acyloxyalkyl ester prodrug of butyric acid (BA), has demonstrated greater potency than BA at inducing malignant cell differentiation and tumor growth inhibition and has demonstrated more favorable toxicological, pharmacological, and pharmaceutical properties than BA in preclinical studies. The principal objective of this study was to determine the feasibility of administering AN-9 as a 6-h i.v. infusion daily for 5 days every 3 weeks in patients with advanced solid malignancies. The study also sought to determine the principal toxicities and maximum tolerated dose of AN-9 on this intermittent schedule, as well as the effects of AN-9 on fetal hemoglobin production, a parameter indicative of RBC differentiation. None of the 28 patients treated with 85 total courses of AN-9 at dosages ranging from 0.047 to 3.3 g/m^2/day every 3 weeks experienced dose limiting toxicity. Mild to moderate nausea, vomiting, hepatic transaminase elevation, hyperglycemia, fever, fatigue, anorexia, injection site reaction, diarrhea, and visual complaints were observed. Dose escalation of AN-9 was limited by the maximum feasible volume of its intralipid formulation vehicle that could be administered safely on this schedule, resulting in a maximum deliverable dose of 3.3 g/m^2/day. There was no consistent increase in fetal hemoglobin with AN-9 treatment. A partial response was observed in a previously untreated patient with metastatic non-small cell lung cancer. Additional disease-directed clinical evaluations of AN-9 are necessary to establish the breadth of its antitumor activity and to assess its role as an effective differentiating agent.

INTRODUCTION

BA,[3] a potent differentiation agent, inhibits proliferation of a wide variety of cancer cells *in vitro* and *in vivo* (1). In addition to its differentiating and antiproliferative activities, BA affects gene expression and cell cycle protein regulation, and induces apoptosis, all of which appear to be mediated through several mechanisms (2–4). One potentially important mechanism is the inhibitory effects of BA on nuclear deacetylases that result in the hyperacetylation of histones and, ultimately, gene transcription and differentiation (5–7). In addition, BA can induce DNA hypermethylation, affecting the expression of proliferation-related genes, such as *p53*, *bcl-2*, and *c-Myc* (8–10). By activating cysteine proteases, specifically caspase-3, BA also promotes apoptosis (11).

The wide range of pharmacological actions of BA and its prominent anticancer effects in preclinical studies have suggested that the agent might play a role in treating human malignancies, and, therefore, clinical development along many avenues was begun (2–4). Several clinical evaluations of sodium butyrate, particularly in hematological malignancies and premalignant disorders have been performed; however, evidence of differentiating activity and anticancer activity in the clinic have been transient and largely unimpressive (12–14). Factors that have contributed to the lack of significant antitumor activity for butyrate and its derivatives include their relatively low (millimolar) potency, inability to sustain biologically relevant *in vivo* drug concentrations, a rapid clearance rate ($t_{1/2}$, 6 min), and a noxious odor (15). For these reasons, many BA derivatives and precursors have been synthesized and screened, and the acyloxyalkyl ester derivatives, particularly the BA prodrug AN-9 (Pivanex; Fig. 1), have demonstrated impressive anticancer activity and pharmacological attributes in preclinical studies (1, 16).

In contrast to BA, AN-9 is rapidly and extensively transported intracellularly because of its high lipophilicity, where it undergoes esterase-mediated hydrolysis to form pivalic acid, formaldehyde, and BA (1). Although the differentiating and

Received 1/10/02; revised 4/19/02; accepted 4/22/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Some patients were treated at the Frederic C. Barter Clinical Research Unit of the Audie Murphy Veterans Administration Hospital (San Antonio, TX), the clinical unit and patient treatment were supported in part by NIH Grant MO1 RR01346. Presented, in part, at the 89th Annual Meeting of the American Association for Cancer Research. New Orleans, LA, March 1998.
[2] To whom requests for reprints should be addressed, at Institute for Drug Development, Cancer Therapy and Research Center, 8122 Datapoint Drive, Suite 700, San Antonio, TX 78229. Phone: (210) 949-5069; Fax: (210) 692-7502; E-mail: apatnaik@saci.org.

[3] The abbreviations used are: BA, butyric acid; HbF, fetal hemoglobin; F-cells, whole blood HbF-bearing red cells; F-reticulocyte, fetal reticulocyte; AN-9, pivaloyloxymethyl butyrate.

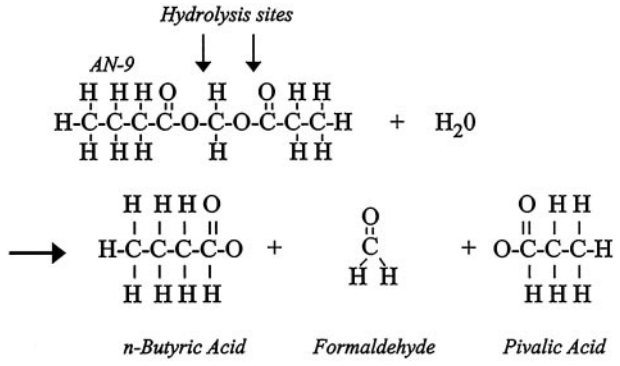

Fig. 1 Chemical reaction demonstrating the hydrolysis of AN-9 to BA, pivalic acid, and formaldehyde

antiproliferative activities of AN-9 are likely caused by BA, 10-fold lower AN-9 concentrations and exposure periods are required for equivalent *in vitro* activity compared with BA (1–4). In the human tumor cloning assay, in which fresh human tumor cells were treated with AN-9 and BA for 14 days, substantial inhibition of tumor growth occurred in 37 (77%) and 10 (21%) of 48 human tumor specimens, respectively ($P < 0.001$). Prominent inhibition of growth of fresh human breast, ovarian, colorectal, and non-small cell lung cancers occurred in 63, 67, 62, and 60% of tumor specimens treated with AN-9, respectively, whereas 0, 40, 20, and 10% responded to BA (16). AN-9 also demonstrated impressive activity against both murine tumors *in vivo* and human tumor xenografts. Treatment of mice bearing Lewis lung carcinoma with AN-9 resulted in superior survival over that achieved with cyclophosphamide, with more protracted treatment schedules generally resulting in greater activity (1).

The toxicological effects of AN-9 have been evaluated in mice, rats, and dogs. The incidence and severity of the principal toxicities, including lethargy, vomiting, weight loss, and decreases in blood glucose, were generally associated with AN-9 treatment schedules that resulted in higher peak plasma concentration and drug exposure. For example, the maximum sublethal plasma concentration was 1.25 ng/ml when AN-9 was administered to rats over a period of 24 h, whereas lethality occurred with AN-9 on continuous 7- and 14-day infusion schedules when plasma concentrations exceeded 0.55 and 0.275 ng/ml, respectively. Toxicity was also noted to be species dependent, with decrements in platelet counts, albeit not clinically significant, observed predominantly in rats and dogs; decrements in blood glucose concentrations principally in rats; and decrements in both serum sodium and potassium concentrations and increments in both serum cholesterol and triglyceride concentrations, mainly in dogs (17).

The rationale to pursue clinical evaluations of AN-9 was largely based on its potent tumor differentiating and antiproliferative activities; broad spectrum of anticancer activity; and favorable toxicity, and pharmacological and pharmaceutical profiles in preclinical studies. It was elected to evaluate AN-9 as a 6-h i.v. infusion daily for 5 days every 3 weeks. This schedule was projected to permit treatment with high total doses of AN-9 without inordinately high plasma concentrations. The main objectives of this Phase I study were to: (*a*) characterize the toxicities of AN-9 administered as a 6-h i.v. infusion daily for 5 days every 3 weeks in patients with advanced solid malignancies; (*b*) determine the maximum-tolerated dose and recommend a dose for subsequent disease-directed evaluations of AN-9; (c) evaluate potential drug-induced biological effects indicative of differentiation in hematopoietic cells of erythrocyte lineage such as HbF synthesis; and (*d*) seek preliminary evidence of anticancer activity in patients with advanced solid tumors.

PATIENTS AND METHODS

Patient Eligibility. Patients with histologically confirmed advanced or metastatic cancer refractory to conventional therapy or for whom there was no known effective therapy were candidates for this study. Eligibility criteria included (*a*) age, ≥18 years; (*b*) Zubrod performance status, ≤2 (ambulatory and capable of self-care); (*c*) no chemotherapy, radiotherapy, or investigational therapy within the previous 4 weeks (6 weeks for nitrosoureas or mitomycin C); (*d*) adequate hematopoietic (WBC count, >2500/μl; absolute neutrophil count, >1500/μl; platelets, ≥100,000/μl; and hemoglobin, ≥9 g/day), hepatic (total bilirubin level, ≤1.5 times the institutional upper normal limit; aspartate aminotransferase, alanine aminotransferase, ≤2.0 times the upper limit of normal), renal (serum creatinine within upper limit of normal), and cardiac (New York Heart Association Class I or II, moderate to no symptoms) functions; (*e*) serum amylase, <1.5 times the upper normal limits; (*f*) serum triglyceride <400 mg/day; and (*g*) no coexisting medical conditions likely to interfere with study procedures or treatment. Written informed consent was obtained according to federal and institutional guidelines.

Drug Administration. AN-9 was supplied by Titan Pharmaceuticals, Inc. (South San Francisco, CA) in 5-ml ampuls that contained 1.5 g of AN-9 (0.97 g/ml) in liquid form. Three ml of dehydrated ethanol was aseptically added to the ampul containing AN-9, and the AN-9/ethanol mixture was aseptically added to 95 ml of 20% Intralipid. The emulsion was infused at a continuous rate over 6 h through a central vein using an infusion pump. The maximum concentration of the AN-9/Intralipid mixture was not to exceed 20 mg/ml, and the maximum infusion volume of formulated Intralipid administered was 250 ml over 6 h.

Dosage and Dose Escalation Scheme. The starting dose of AN-9 was 0.047 g/m^2/day administered as a 6-h continuous i.v. infusion daily for 5 days every 3 weeks. This dosing regimen was selected as a compromise between predicted safety for AN-9 infusions based on animal toxicology studies and clinical convenience for AN-9 infusions administered on an outpatient basis. The starting clinical dose of AN-9 was equivalent to one-tenth of the rat daily dose that was judged as the maximum tolerated dose on a 7-day continuous infusion schedule and adjusted for human infusions at only 6 h daily. Three patients were treated at the first dose level, and a single patient was to be treated at each subsequent dose level in which moderate or more severe drug-related toxicity was not noted. The occurrence of moderate or more severe toxicity required that at least three new patients be treated at the specific dose level. If there were no additional toxicities of at least moderate severity, dose escala-

tion, using one patient per dose level, was to resume. Initially, dose escalation was to proceed in 100% increments until 0.375 g/m^2/day, which approached the rat maximum tolerated dose adjusted for the length of infusion. The subsequent increments of dose escalation were guided by a modified version of the Continual Reassessment Method (mCRM; Ref. 18). The mCRM incorporated updated Bayesian estimates of the maximum tolerated dose, which was defined *a priori* as the highest dose at which a maximum of 30% of patients experienced dose-limiting toxicity during the first treatment course. The maximum dose was set at 3.3 g/m^2/day because of drug formulation and administration volume considerations. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (Version 1.0). Dose limiting toxicity was defined as the development during course 1, of a new-grade 3 or 4 nonhematological toxicity, grade 4 hematological toxicity, or grade $\geq$2 loss of cardiac function. Intrapatient dose-escalation was performed only if another patient had already been enrolled at the next higher dose level and had completed at least two courses of therapy without any serious adverse events.

Pretreatment Assessment and Follow-Up Studies. Interval histories, and physical examinations that included fundoscopic and Snellen visual acuity assessments, and routine laboratory studies were performed pretreatment, daily on days 2–6, and weekly. Change in vision was assessed as a clinical indicator of potential formaldehyde or formic acid toxicity. Direct measure of these toxic compounds was considered unlikely to succeed because of the rapid turnover of AN-9 and its metabolites, yielding CO_2 and water. Routine laboratory evaluations included complete blood counts, differential WBC counts, serum electrolytes, renal and hepatic function tests, prothrombin time, and urinalysis. HbF, measured as F-cells, and F-reticulocytes were assessed during the first course of treatment on day 1 before commencing AN-9, on day 5 at the end of AN-9 infusion, and also on day 8. Pretreatment studies also included an electrocardiogram, and relevant radiological studies for evaluation of all measurable or evaluable sites of malignancy, as well as an assessment of relevant tumor markers. Radiological studies for disease status assessments were repeated after every other course or as needed to confirm response. Patients were able to continue treatment if they did not develop progressive disease, which was defined as an increase in the sum of the bidimensional product of measurable disease by at least 25% or the appearance of new lesions. A complete response was scored if there was disappearance of all active disease on two measurements separated by a minimum period of 4 weeks, and a partial response required at least a 50% reduction in the sum of the product of the bidimensional measurements of all of the lesions documented, separated by at least 4 weeks.

HbF Studies. F-cells and F-reticulocytes were identified using a monoclonal anti-human HbF antibody and immunofluorescence, respectively, at the Johns Hopkins School of Medicine (Baltimore, MD) and reported as a percentage according to published methods (19). The percentage of HbF cells was determined with washed RBCs fixed in dimethyl 3,3′-dithiobispropionimidate at 37°C for 30 min. Fixed RBCs were washed in 0.6% diethanolamine in borate saline (pH 8.4), resuspended in 3% BSA in PBS (pH 7.4), and then successively rinsed in 2.5% Triton X-100 (Sigma/Aldrich, St. Louis, MO) in PBS (pH 7.4), 2.5% Triton X-100 in PBS (pH 7.4) with an equal volume of 100% isopropanol, and then in 2.5% Triton X-100 in PBS (pH 7.4). The rinsed RBCs were then resuspended in 2.5% Triton X-100, 3% BSA in PBS (pH 7.4) and sonicated before reaction with a primary mouse anti-human HbF monoclonal antibody (clone 15.3.1). Secondary staining was accomplished with an FITC-labeled rabbit antimouse IgG F(ab′)$_2$ (Cappel Laboratories, Westchester, NY), and stained cells were enumerated under a microscope with appropriate filtration (FITC λ_{ex} = 494 nm, λ_{em} = 519 nm).

The percentage F-reticulocyte assay for patient samples used the Katsura reaction (20). One volume of an RBC suspension in borate saline (pH 8.4) was added to 7 volumes molten agarose (40°C), 4 volumes mouse antihuman HbF antibody and one volume methylene blue. The mixture was immediately flowed onto a microscope slide and allowed to gel at 4°C for 3 min. The gel was then covered with 2% Triton X-100 in water for 5 min, and the percentage of reticulocytes with a white cloud of precipitate around them was quantified. Percentage (absolute) change in HbF parameters was calculated by subtracting baseline values from day 5 and day 8 values, respectively. Relative percent changes could not be calculated, as some patients had no measurable F-cells or F-reticulocytes at baseline.

Table 1 Patient characteristics

Characteristic		No. of patients
Gender		
Male		20
Female		8
Age, yr, median (range)	61 (33–76)	
Zubrod performance status		
0		14
1		10
2		4
Previous treatment		
Extensive[a]		14
Minimal		14
Cancer type		
Colorectal		11
Non-small cell lung		7
Breast		2
Soft tissue sarcoma		2
Osteosarcoma, pancreas, ovary, cervix, esophagus, thymus		1 each

[a] Prior treatment with more than six courses of an alkylating agent, more than two courses of carboplatin, irradiation to >25% of bone marrow, mitomycin C, nitrosoureas, or high-dose therapy requiring stem cells.

RESULTS

General. Twenty-eight patients, whose pertinent characteristics are listed in Table 1, were treated with 85 total courses (425 infusions) of AN-9 at doses ranging from 0.047 to 3.3 g/m^2/day for 5 days every 3 weeks. All of the patients were fully evaluable for toxicity. Twenty-six of the 28 patients had previously received chemotherapy, radiotherapy, or both. The dose escalation scheme, as well as the number of patients and courses administered as a function of dose level, is listed in Table 2. No

Table 2 Dose escalation scheme

AN-9 dose (g/m^2/day)	No. of patients			Total Courses
	New	Escalated to this dose	Total	
0.047	3	0	3	7
0.094	1	0	1	2
0.1888	1	0	1	2
0.376	1	0	1	4
0.564	1	0	1	2
0.75	3	0	3	6
1.0	1	1	2	4
1.2	1	1	2	8
1.5	3	1	4	8
1.875	1	0	1	6
2.34	1	0	1	2
2.9	2	0	2	6
3.3	9	0	9	28

patient required dose reduction, and one patient who received 13 courses underwent dose escalation three times from 0.376 g/m^2/day (four courses) to 1.0 g/m^2/day (two courses), then to 1.2 g/m^2/day (six courses), and finally to 1.5 g/m^2/day (one course). The median number of courses administered per patient was two (range, 1–13).

No patient experienced dose-limiting toxicity. Because of solubility limitations of AN-9 in the formulation vehicle that resulted in volume administration constraints, the maximum feasible dose was determined to be 3.3 g/m^2/day.

Nonhematological Toxicity. The principal nonhematological toxicities of AN-9 included nausea, vomiting, fever, and fatigue, which were predominantly mild to moderate (grade 1–2) in severity. Grade 1 or 2 nausea and/or vomiting occurred in 15 and 11% of courses, respectively. These adverse effects were typically noted in the peritreatment period and did not appear to be related to dosage. Nausea and vomiting were also prevented and/or managed successfully with prochlorperazine or serotonin $5HT_3$ receptor antagonists, but routine premedication was not necessary because most events were mild and/or sporadic. In addition, patients did not complain of delayed emesis. Mild or moderate fever, which usually occurred in the peritreatment period, was experienced in 12% of courses. In 7% of courses, antipyretics were administered for symptom management. Grade 1–2 fatigue, possibly attributable to AN-9, occurred in 14% of the courses. In two patients, grade 3 fatigue was observed in two courses, but both events occurred concomitant with disease progression and were thought to be disease related.

Other mild to moderate toxicities included diarrhea, elevations in hepatic transaminases, hyperglycemia, injection site reactions, and anorexia. Of these toxicities, only hyperglycemia seemed to be more common at higher dosages. Diarrhea of grade 1 or 2 severity was experienced by seven patients in seven (8%) courses, typically occurring between days 3 and 7 and ameliorated by loperamide. Grade 1–2 hepatic transaminase elevations were typically noted between days 1 and 10 and resolved spontaneously within 4–6 days in four patients during six (7%) courses. Grade 1–2 hyperglycemia was experienced by three patients in five (6%) courses. Grade 3 hyperglycemia occurred in a patient with preexisting diet-controlled non-insulin-dependent diabetes mellitus whose serum glucose was minimally elevated prior to treatment. However, this event was not considered dose limiting, as it was first noted on day 1 of course 2 and then intermittently throughout this course. Inflammation and/or burning along the course of the injected vein occurred in five (6%) courses, all of which resolved after application of heat or other simple supportive measures. Anorexia of grade 1 or 2 severity was also experienced by seven patients in seven (8%) courses, but the relative contributions of AN-9 and malignant disease in the etiology of this toxicity is not known.

Nine patients experienced visual complaints, characterized principally by blurred vision or photophobia, at some time during treatment. The events occurred more frequently at the higher AN-9 dose levels. Visual toxicity was mild (grade 1) or moderate (grade 2) in severity in four patients (four courses) and five patients (six courses), respectively. The complaints were brief, noncumulative, experienced in the peritreatment period, and were not usually associated with objective findings on ophthalmological and visual acuity examinations. However, brief and reversible decrements in visual acuity were also documented in two patients. The visual acuity of one patient, who complained of blurred vision, decreased from pretreatment values on Snellen examination from 20/30 and 20/40 in the left and right eyes, respectively, to 20/70 in both eyes on day 2 of course 1 of AN-9 administered at the 0.047 g/m^2/day dose-level. These changes were noted at the end of AN-9 infusion on day 2. A fundoscopic examination was unremarkable, and the patient's visual acuity normalized on day 3. No interruption occurred in drug administration. No visual effects were noted on retreatment of this patient with a second course of AN-9 at the same dose level. The visual acuity of a second patient, treated with AN-9 at a dose of 3.3 g/m^2/day decreased from a pretreatment value of 20/25 bilaterally to 20/70 bilaterally on day 22 of course 1. An ophthalmological evaluation, which included a slit-lamp examination, was unremarkable and visual acuity returned to baseline within 1 week. No further treatment with AN-9 was administered.

Hematological Toxicity. Hematological toxicity was minimal. Grade 1 or 2 anemia was observed in five (6%) courses, and grade 1 leukopenia occurred in two (2%) courses. Grade 3 leukopenia and grade 2 neutropenia were experienced by a heavily pretreated subject with an advanced leiomyosarcoma on day 6 of course 1 at the 0.75 g/m^2/day-dose level of AN-9. Complete recovery occurred by day 11. Two patients experienced transient, uncomplicated grade 4 lymphocytopenia during their first course of AN-9 at the 0.75 and 1.875 g/m^2/day-dose levels.

Anticancer Activity. A partial response occurred in a 41-year-old female with metastatic squamous cell carcinoma of the lung and a history of laryngeal papillomatosis, who had not received any prior systemic therapy. The patient experienced a 54% reduction in the size of her pulmonary metastases after treatment with two courses of AN-9 at the 0.376 g/m^2/day-dose level. Thereafter, the patient received escalating doses of AN-9 to a maximum of 1.5 g/m^2/day and experienced a 90% reduction in the size of her measurable disease after eight courses. The patient developed progressive disease after receiving 13 total courses over 14 months. Treatment of this patient was delayed

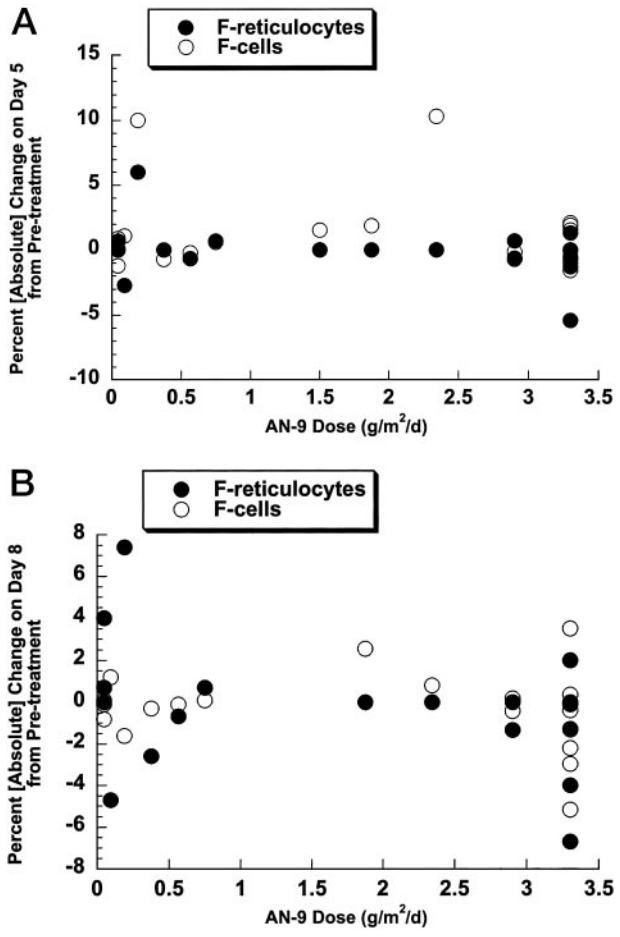

Fig. 2 *A,* scatter plot illustrating absolute percentage of change in F-cells and F-reticulocytes on day 5 from pretreatment. *B,* scatter plot illustrating absolute percentage of change in F-cells and F-reticulocytes on day 8 from pretreatment.

in part because of disease-related clinical complications. Six other patients with various malignancies experienced stable disease lasting 4–10 months as their best response.

HbF and F-Reticulocyte Studies. The percentages of F-reticulocytes and F-cells were determined in serial blood samples collected pretreatment, and on days 5 and 8 of course 1 in 22 patients. Scatter plots of the percentage (absolute) changes in these parameters on days 5 and 8 compared with pretreatment values as a function of AN-9 dose are displayed in Fig. 2. It was noted that the one patient with an objective tumor response in this clinical trial had an incremental increase in both F-reticulocytes (day 1, 0.7%; day 8, 3.3%) and F-cells (day 1, 1.3%; day 5, 2.0%). Of the six patients with stable disease, two were inevaluable for HbF studies, two had slight increases in either F-reticulocytes or F-cells, and two showed no significant change. Separately, two patients with transient visual loss (one of whom was the patient with an objective tumor response) experienced increases in F-cells or F-reticulocytes. Overall, there was no consistent pattern of change in these parameters.

DISCUSSION

The rationale for selecting AN-9 for clinical development was because of its unique capabilities as an inducer of both differentiation and apoptosis in preclinical studies (2–4). In the present study, administration of AN-9 as a 6-h i.v. infusion daily for 5 days every 3 weeks was associated with several types of hematological and nonhematological effects; however, neither dose reduction nor treatment delay was required. Transient visual disturbances, in the absence of pathological changes on fundoscopic examination or metabolic disturbances, occurred in 12% of courses. Although two patients experienced transient visual blurring accompanied by worsening visual acuity, these events resolved completely and did not recur after additional treatment. Instead, the volume of the Intralipid that was required to formulate AN-9 precluded dose escalation above 3.3 g/m^2/day. At this dose level, at least 250 ml of Intralipid is required to treat patients who have body surface areas exceeding 2.0 m^2. However, the administration of 20% Intralipid emulsion at volumes exceeding 250 ml over 6 h is associated with a high risk of acute complications related to fat overload (21, 22). On the basis of these observations, the maximum tolerated dose and recommended dose for subsequent disease-directed studies for AN-9 is 3.3 g/m^2/day. This recommendation stems from both volume limitations on the Intralipid vehicle and the observed AN-9 dose intensity for patients who either had an objective tumor response or durable stable disease. The one patient with a partial tumor response received up to 1.5 g/m^2/day of AN-9, whereas the six patients with stable disease received daily AN-9 doses that ranged from 1.5 to 3.3 g/m^2/day. In the absence of instructive pharmacodynamic data for AN-9 linked to AN-9 pharmacokinetics, a traditional Phase I trial paradigm that uses the highest tolerable dose of AN-9 was chosen.

From a drug development standpoint, the production and accumulation of formaldehyde as a potential toxicant after degradation of AN-9 by plasma esterases may be problematic. The principal toxicities of formaldehyde ingestion are related to the irritating properties of the agent on gastrointestinal and respiratory tract mucosa (23). By contrast, the toxic effects of formaldehyde produced by AN-9 degradation may be similar to those caused by methanol ingestion, which leads to the formation and systemic accumulation of both formaldehyde and formic acid (24). Accumulation of these catabolites can produce central nervous system depression, toxic encephalopathy, abdominal pain, nausea, vomiting, metabolic acidosis, and hypokalemia. Visual manifestations, characterized by blurred vision, loss of peripheral vision, visual hallucinations, and blindness, along with hyperemia or edema of the optic discs, may also occur. Concentrations of formaldehyde or formic acid produced by the metabolism of AN-9 during prolonged infusions were expected to be at or below limits of detection, and the measurement of formic acid is further complicated because formate is an intermediate in the folate pathway. Because of these difficulties, visual acuity examinations were undertaken to detect the toxic effects of formaldehyde or formic acid. Although it is possible that formaldehyde released during metabolism of AN-9 may account for visual changes documented in this study, there were no other associated findings of formaldehyde toxicity, such as metabolic acidosis, thus making this phenomenon less likely. It

Table 3 Studies of butyrate derivatives in clinical development

Drug	Schedule	Dose-limiting toxicity	Recommended dose	Side effects at recommended dose	Ref.
TB[a]	p.o./o.d. days 1–21 every 4 weeks	N/V, myalgia		??	28
PA	i.v. bolus, followed by CIV days 1–14 every 6 weeks	N/V, neurocortical toxicity		??	29
PA	i.v./bid, days 1–14 every 4 weeks	Neurocortical toxicity	125 mg/kg	N/V, neurocortical toxicity, edema, rash	30
SB	p.o./o.d. continuous	N/V, hypocalcemia, fatigue, neurocortical toxicity, edema	27 g/day	N/V, dyspepsia, fatigue, neurocortical toxicity, odor	31
PB	CIV, days 1–7 every 4 weeks	Neurocortical toxicity, hypocalcemia, fever	375 mg/kg/day	N/V, neurocortical toxicity, skin, hypocalcemia, fever	32

[a] TB, tributyrin; PA, phenylacetate; PB, phenylbutyrate; SB, sodium butyrate; o.d., once daily; b.i.d., twice daily; CIV, continuous i.v. infusion; N/V, nausea and vomiting.

is realized that the results of this study are insufficient to either substantiate or refute the presence of formaldehyde-related ocular toxicity. A subsequent Phase II study of AN-9 in patients with non-small cell lung cancer was not associated with ocular symptoms or other manifestations of formaldehyde toxicity (25).

This study evaluated HbF production as an alternative surrogate biological end point; pharmacokinetic studies could not be performed because of the short half-life of AN-9 (<2 min). The percentage of F-reticulocytes and F-cells on days 1, 5, and 8 of the first course of treatment was assayed. The BA analogues have been associated with the induction of HbF in cultures of adult blood and with stimulation of HbF production in primates (25). A pilot study showed that short-term infusions of arginine butyrate increased the number of F-reticulocytes as well as the synthesis of γ-globin mRNA in a small number of patients with β-hemoglobinopathies (26). In the present study, the percentage of F-cells increased in eight patients at dose levels ranging from 0.047 g/m^2 to 3.3 g/m^2, suggesting the possibility of butyrate-induced effects at a cellular level. However, there was no consistent increase in parameters of HbF synthesis when reviewing all of the patients. Serial assessments of F-cells and F-reticulocytes with progressive courses of treatment were not performed, but such additional studies may improve the determination of biological activity, particularly with repeated or prolonged drug administration. Future studies of AN-9 as an anticancer agent will further evaluate the use of HbF as a pharmacodynamic end point in larger patient cohorts. Such studies should also more carefully evaluate any possible link between this surrogate end point and other clinical parameters, such as ocular toxicity.

Although the clinical development of BA derivatives has focused on indications such as hemoglobinopathies and gastrointestinal diseases, there has been significant interest in their antineoplastic properties. The first-generation BA derivatives, including sodium butyrate, phenylbutyrate, and phenylacetate were associated with *in vitro* antitumor activity, albeit at millimolar concentrations (27). Summarized in Table 3 are the clinical studies of butyrate derivatives in clinical development (28–32). Neurocortical and gastrointestinal toxicities have typically been dose limiting, along with the demonstration of nonlinear kinetics and rapid clearance. Overall, the development of butyrate derivates has been limited by the requirement for large amounts of drug infused over extended periods of time, paralleled by millimolar concentrations *in vitro* which are ostensibly a reflection of poor transmembrane transport and rapid cellular metabolism. It is anticipated that AN-9, which has better cell membrane permeability will result in improved intracellular delivery of BA, given its activity *in vitro* at micromolar concentrations and more rapid action when compared with its metabolite BA (1, 3).

The present study lends support to the feasibility of administering AN-9 as a single agent. Given the unique mechanism of action of this cytostatic agent, there is a distinct possibility that greater antitumor activity may be observed if combined with cytotoxic chemotherapy. *In vitro* studies demonstrate that combinations of AN-9 and docetaxel, gemcitabine, or cisplatin have more than additive cytotoxic effects against a variety of cell lines (33). AN-9 also overcomes *in vitro* resistance to paclitaxel and decreases expression of the c-*Myc* oncogene. (33). The observation of a durable response as well as disease stabilization suggests that it may be worthwhile to pursue additional disease-directed evaluations of AN-9 alone or in combination with cytotoxic agents. Future studies of AN-9 should also investigate possible synergistic interactions with novel, selective, and targeted therapies.

ACKNOWLEDGMENTS

We thank Dr. Jon Williams for his editorial assistance.

REFERENCES

1. Rephaeli, A., Rabizadeh, E., Aviram, A., Shaklai, M., Ruse, M., and Nudelman, A. Derivatives of butyric acid as potential anti-neoplastic agents. Int. J. Cancer, *49:* 66–72, 1991.

2. Aviram, A., Zimrah, Y., Shaklai, M., Nudelman, A., and Rephaeli, A. Comparison between the effect of butyric acid and its prodrug pivaloyloxymethylbutyrate on histones hyperacetylation in an LH-60 leukemic cell line. Int. J. Cancer, *56:* 906–909, 1994.

3. Rabizadeh, E., Shaklai, M., Nudelman, A., Eisenback, L., and Rephaeli, A. Rapid alteration of c-myc and c-jun expression in leukemic

cells induced to differentiate by a butyric acid prodrug. FEBS Lett., *328:* 225–229, 1993.

4. Zimra, Y., Wasserman, L., Maron, L., Shaklai, M., Nudelman, A., and Rephaeli, A. Butyric acid and pivaloyloxymethyl butyrate. AN-9, a novel butyric acid derivative, induce apoptosis in HL-60 cells. J. Cancer Res. Clin. Oncol., *123:* 152–160, 1997.

5. Saunders, N., Dicker, A., Popa, C., Jones, S., and Dahler, A. Histone deacetylase inhibitors as potential anti-skin cancer agents. Cancer Res., *59:* 399–404, 1999.

6. Archer, S. Y., and Hodin, R. A. Histone acetylation and cancer. Curr. Opin. Genet. Dev., *9:* 171–174, 1999.

7. Bajenova, O. V., Childs, B. H., Pearse, R. N., and Michaeli, J. Histone deacetylase inhibitors induce programmed cell death in human myeloma cells. Proc. Amer. Assoc. Cancer Res., *40:* 3218, 1999.

8. Liu, W. H., and Yung BYM. Mortalization of human promyelocytic leukemia HL-60 cells to be more susceptible to sodium butyrate-induced apoptosis and inhibition of telomerase activity by down-regulation of nucleophosmin/B23. Oncogene, *17:* 3055–3064, 1998.

9. Thompson, M. A., Rosenthal, M. A., Ellis, S. L., Friend, A. J., Zorbas, M. I., Whitehead, R. H., and Ramsay, R. G. c-Myc down-regulation is associated with human colon cell differentiation, apoptosis, and decreased Bcl-2 expression. Cancer Res., *58:* 5168–5175, 1998.

10. Schwartz, B., Avivi-Green, C., and Polak-Charcon, S. Sodium butyrate induces retinoblastoma protein dephosphorylation, p16 expression and growth arrest of colon cancer cells. Mol. Cell Biochem., *188:* 21–30, 1998.

11. Mandal, M., Adam, L., and Kumar, R. Redistribution of activated caspase-3 to the nucleus during butyric acid-induced apoptosis. Biochem. Biophys. Res. Commun., *260:* 775–780, 1999.

12. Kruh, J. Effects of sodium butyrate, a new pharmacological agent, on cells in culture. Mol. Cell. Biochem., *42:* 65–82, 1982.

13. Novogrodsky, A., Dvir, A., Ravid, A., Shkolnik, T., Stenzel, K. H., Rubin, A. L., and Zairov, R., Effect of polar organic compounds on leukemic cells: butyrate-induced partial remission of acute myelogenous leukemia in a child. Cancer (Phila.), *51:* 9–14, 1983.

14. Rephaeli, A., Nordenberg, J., and Aviram, A. Butyrate-induced differentiation in leukemic myeloid cells: *in vitro* and *in vivo* studies. Int. J. Oncol., *4:* 1387–1391, 1994.

15. Rephaeli, A., Zhuk, R., and Nudelman, A. Prodrugs of butyric acid from bench to bedside: synthetic design. Mechanisms of action, and clinical applications. Drug Dev. Res., *50:* 370–391, 2000.

16. Siu, L. L., Von Hoff, D. D., Rephaeli, A., Izbicka, E., Cerna, C., Gomez, L., Rowinsky, E. K., and Eckhardt, S. G. Activity of Pivaloyloxymethyl butyrate, a novel anticancer agent, on primary human tumor colony-forming units. Investig. New Drugs, *16:* 113–119, 1998.

17. Titan Pharmaceuticals, Inc. Clinical Investigator's brochure. South San Francisco, CA: Titan Pharmaceuticals Inc, 1997.

18. O'Quigley, J., and Shen, L. Z. Continual reassessment method: a likelihood approach. Biometrics, *52:* 673–684, 1996.

19. Dover, G. J., Boyer, S. H., and Bell, W. R. Microscopic method for assaying F cell production: ultrastructure changes during infancy and in aplastic anemia. Blood, *52:* 664–672, 1978.

20. Kurokawa, H., Katsura, S., Kamada, M., Matsuo, W., and Tokiwa, K. Increase of fetal hemoglobin and loss of blood group antigen B in a patient with leukemia. Tohoku J. Exp. Med., *99:* 35–44, 1969.

21. Product Information, Liposyn II 10% and 20%. North Chicago, IL: Abbott Laboratories, August 1991.

22. Anon. Intravenous fat. Lancet, *1:* 1059–1060, 1976.

23. Kaye, S. Handbook of Emergency of Toxicology. Charles C Thomas Publishing, 1986.

24. Jefferys, D. B., and Wiseman, H. M. Formic acid poisoning. Postgrad. Med. J., *56:* 761–762, 1980.

25. Keer, H. N., Reid, T., Sreedharan, S. Pivanex activity in refractory non-small cell lung cancer, a Phase II study. Proc. Am. Soc. Clin. Oncol., *21:* 3149, 2002.

26. Blau, C. A., Constantoulakis, P., Shaw, C. M., and Stamatoyannopoulos, G. Fetal hemoglobin induction with butyric acid: efficacy and toxicity. Blood, *81:* 529–537, 1993.

27. Sher, G. D., Ginder, G. D., Little, J., Yang, S., Dover, G. J., and Olivieri, N. F. Extended therapy with intravenous arginine butyrate in patients with β-hemoglobinopathies. N. Engl. J. Med., *332:* 1606–1610, 1995.

28. Gore, S., D., and Carducci, M. A. Modifying histones to tame cancer: clinical development of sodium phenylbutyrate and other histone deacetylase inhibitors. Exp. Opin. Investig. Drugs, *9:* 2923–2934, 2000.

29. Conley, B. A., Egorin, M. J., Tait, N., Rosen, D. M., Sausville, E. A., Dover, G., Fram, R. J., and Van Echo, D. A. A Phase I study of the orally administered butyrate prodrug, tributyrin, in patients with solid tumors. Clin. Cancer Res., *4:* 629–634, 1998.

30. 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., and Myers. C. E. Phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. Cancer Res., *54:* 1690–1694, 1994.

31. 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.

32. Gilbert, J., Baker, S. D., Bowling, M. K., Growchow, L., Figg, W. D., Zabelina, Y., Donehower, R. C., and Carducci, M. A. A Phase I dose escalation and bioavailability study of oral sodium phenylbutyrate in patients with refractory solid tumor malignancies. Clin. Cancer Res., *7:* 2292–2300, 2001.

33. Gore, S. D., Weng, L-J. Zhai, S., Figg, W. D., Donehower, R. C., Dover, G. J., Grever, M., Griffin, C. A., Grochow, L. B., Rowinsky, E. K., Zabalena, Y., Hawkins, A. L., Burks, K., and Miller, C. B. Impact of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia. Clin. Cancer Res., *7:* 2330–2339, 2001.

34. Reid, T., Lee, Y-L., Eng., L., Gadol, N., Sreedharan, S., Keer, H., and Williams, J. Pivanex represses oncogene expression and sensitizes tumor cells to chemotherapeutic agents. Proc. Amer. Assoc. Cancer Res., *43:* 944, 2002.