Cancer Chemother Pharmacol (2006) 57: 789–795
DOI 10.1007/s00280-005-0125-1

ORIGINAL ARTICLE

**Dale Schaar · Lauri Goodell · Joseph Aisner
Xiao Xing Cui · Zheng Tao Han · Richard Chang
John Martin · Stephanie Grospe · Liesel Dudek
Joan Riley · Jacqueline Manago · Yong Lin
Eric H. Rubin · Allan Conney · Roger K. Strair**

# A phase I clinical trial of 12-*O*-tetradecanoylphorbol-13-acetate for patients with relapsed/refractory malignancies

Received: 6 May 2005 / Accepted: 22 August 2005 / Published online: 18 October 2005
© Springer-Verlag 2005

**Abstract** Phorbol esters activate protein kinase C and modulate a variety of downstream cell signaling pathways. 12-*O*-tetradecanoylphorbol-13-acetate (TPA) is a phorbol ester that induces differentiation or apoptosis in a variety of cell lines at low concentrations. A phase I dose escalation trial of TPA was undertaken for patients with relapsed or refractory malignancies. The starting dose was 0.063 mg/m$^2$ and most patients were treated with an intravenous infusion of TPA on days 1–5 and 8–12 followed by a 2-week rest period prior to retreatment. Thirty-five patients were treated. A biological assay was used to monitor levels of TPA-like activity in the blood after treatment. Serious adverse events included individual episodes of gross hematuria, a grand mal seizure, syncope, and hypotension. Many patients had transient fatigue, mild dyspnea, fever, rigors, and muscular aches shortly after the infusion. Dose-limiting toxicities included syncope and hypotension at a dose of 0.188 mg/m$^2$. Only a single patient had evidence of tumor response. These studies establish 0.125 mg/m$^2$ as the maximally tolerated dose when TPA is administered on this schedule.

D. Schaar · J. Aisner · J. Martin · S. Grospe · L. Dudek
J. Riley · J. Manago · Y. Lin · E. H. Rubin · R. K. Strair (✉)
The Cancer Institute of New Jersey,
Robert Wood Johnson Medical School,
University of Medicine and Dentistry of New Jersey,
195 Little Albany Street, New Brunswick, NJ 08901, USA
E-mail: strairrk@umdnj.edu
Tel.: +1-732-2356044
Fax: +1-732-2358098

L. Goodell
Department of Pathology, Robert Wood Johnson Medical School,
University of Medicine and Dentistry of New Jersey,
New Brunswick, NJ, USA

X. X. Cui · R. Chang · A. Conney
Laboratory of Cancer Research, Rutgers University,
Piscataway, NJ, USA

Z. T. Han
Henan Tumor Research Institute, Zheng Zhou,
Henan, People's Republic of China

**Keywords** Phorbol esters · 12-O-tetradecanoylphorbol-13-acetate

## Introduction

12-*O*-tetradecanoylphorbol-13-acetate (TPA) is a phorbol ester with the capacity to induce differentiation and/or apoptosis in multiple cell lines and primary cells [1–8]. Phorbol esters activate protein kinase C (PKC) and modulate the activity of multiple downstream cell signaling pathways, including the mitogen-activated protein kinase (MAPK) pathways [9–12].

The MAPKs are proline directed ser/thr kinases categorized into families based upon sequence homologies [13–21]. Each MAPK phosphorylates downstream substrates, including kinases and transcription factors. Histones, chromatin components, and other transcriptional modulators are phosphorylated secondarily. The MAPK extracellular signal-regulated kinase (ERK) is often associated with cellular proliferation; ERK activation being central to signaling from receptor tyrosine kinases, G-protein coupled receptors, selected integrins, and activated ras and raf. In contrast, activation of c-Jun N terminal-kinases (JNK) and p38 kinases has been associated with stress responses and induction of apoptosis [13–21]. However, there are complex interactions among MAPKs and many factors, including the kinetics of specific MAPK activation and inactivation, can determine phenotypic effects [22–24].

Regulation of MAPK cascades is complex. The subcellular localization of individual kinases, the nature of associated "scaffolding"/interacting proteins, the presence of single and dual specificity MAPK phosphatases which target specific MAPK isoforms, and the duration of kinase activation all modulate MAPK signaling [9–11, 22–26]. Hence, activation of specific MAPKs represents a balance of phosphorylation and dephosphorylation mediated at various subcellular locations in association with various interacting proteins.

The effects of TPA on MAPK pathways may be particularly relevant to the differentiating and proapoptotic effects of TPA in certain cells. For example, molecular analysis of AML indicates at least two overlapping classes of interacting genetic alterations. Signaling pathway alterations facilitate proliferation and survival, whereas transcription factor mutations/fusions often alter chromatin remodeling and inhibit differentiation (reviewed in reference [27]). Approximately 30% of AML samples contains a Flt 3 receptor that is constitutively active as a consequence of an internal tandem duplication (ITD) or kinase domain mutation and additional AML samples have mutations in other receptor protein tyrosine kinase genes (e.g., *c-kit*) or *ras* [27–29]. Furthermore, other related myeloid malignancies have translocations (e.g., *BCR-ABL, ABL-TEL, TEL-PDGFR, TEL-JAK2*) which result in fusion kinases that may activate ras and other signaling pathway components [27]. The downstream effects of these alterations often include constitutive ERK activation, present in 51–70% of primary AML, including all samples containing the Flt 3 receptor ITD [30–32]. In addition, pharmacologic inhibition of ERK pathway activation results in growth inhibition of myeloid leukemia cell lines [33]. Recent studies have identified TPA as an agent that modulates ERK activation in primary AML cells. In a subset of primary AML cells studied ex vivo, prolonged exposure to TPA reduced ERK activation at 24 h and induced apoptosis [34].

The capacity of TPA to induce phenotypic changes, characteristic of differentiation and/or apoptosis in hematopoietic cell lines, led investigators in China to undertake a pilot study of TPA in patients with myeloid malignancies [35]. A variety of doses and schedules, in conjunction with cytarabine or vitamin D3, were used. Clinical efficacy, as indicated by reduction in bone marrow myeloblasts and/or improvement of blood counts, was apparent in several treated patients, and the most prominent adverse effects included fevers, chills, dyspnea, hematuria, and phlebitis. These adverse effects were transient and repeated cycles of treatment were administered to most patients. Although the adverse effects were not ascribed to individual components of each patient's therapy, they were not characteristic of cytarabine or vitamin D3, and were felt to be most likely related to TPA.

A subsequent formal phase I study of TPA was initiated in the United States. TPA-induced alterations in AML cell gene expression and immunophenotype in selected patient samples were reported after treatment of the first 14 patients [36]. In addition, a biologic assay for TPA-like differentiating activity in the blood of treated patients was developed and used to determine the kinetics of TPA-like activity in the blood of five treated patients [37]. A biologic assay was required because a physical assay of appropriate sensitivity could not be developed. This report describes the maximally tolerated dose and dose- limiting toxicity profile of TPA after the treatment in 35 patients and completion of the phase I trial.

## Methods

### Clinical trial/study drug

The clinical trial represents an investigator-initiated trial without industry support, other than provision of drug. This was a single institution trial with all patients treated at The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine & Dentistry of New Jersey and Robert Wood Johnson University Hospital. All patients provided informed consent approved by our Institutional Review Board. A New Drug Application was filed with the Food and Drug Administration. Clinical grade TPA was produced by Xichuan Pharmaceutical Co. (Nan Yang, Henan). The purity of TPA was >99% as measured by HPLC, NMR, IR and mass spectrometry.

### Patients

Patients with relapsed/refractory malignancies for which there was no standard therapy anticipated to induce disease response or result in palliation were eligible for the study. Other eligibility criteria included ECOG performance stage 0–2, age >18 years, bilirubin <1.5 times upper limit of normal, serum creatinine <2.0 mg/dl, AST <3.0 times upper limit of normal, left ventricular ejection fraction >40% and pulmonary function FEV1.0 >50% predicted [36].

### Blood levels of TPA

Blood levels of TPA after an IV infusion were measured in 31 patients by a recently developed bioassay that measures organic solvent extractable differentiation activity (toward HL60 myeloid leukemia cells) as previously described [37]. This assay utilizes two extractions of 1 ml of blood with 5 ml of ethyl acetate, redissolving the extraction residue in 50 ul of ethanol and addition of an aliquot to HL60 cells. Adherent cells are measured 48 h later. A blood sample obtained from the patient prior to administration of TPA is used as a negative controls and is mixed with various amounts of TPA to establish a standard curve during side-by-side assay with the patient samples obtained after treatment with TPA.

### Treatment

Initially patients were treated with a 1-h infusion of TPA in 200 ml 0.9% sodium chloride on days 1 and 8, followed by re-treatment on day 29. Based upon ex vivo

studies that demonstrated TPA-induced ERK pathway modulation and induction of apoptosis after prolonged exposure to TPA [34], the clinical trial was amended after the eleventh patient to a modified treatment schedule incorporating a 1-h infusion each day for 5 days on two consecutive weeks. The starting TPA dose on this amended schedule was 0.063 mg/m$^2$ and repeat cycles were allowed after a 2-week rest if the patient was clinically stable and all toxicities had reversed. The starting dose of 0.063 mg/m$^2$ was empirically chosen based upon results of studies in China indicating biological effects in humans at total daily doses of 0.125–1.0 mg, with no information about lower doses [35, 38]. The patients were hospitalized for their first dose of TPA. All subsequent doses could be administered as an outpatient. Dose escalation in increments of 0.063 mg/m$^2$ was undertaken when three consecutive patients did not experience a TPA-related dose-limiting toxicity (irreversible grade 2 or any treatment-related grade 3–4 nonhematologic toxicity). An additional three patients were treated at the same dose if a single patient had dose-limiting toxicity. The maximally tolerated dose was the highest administered dose that did not cause a TPA-related dose-limiting toxicity in more than one patient. Patients were monitored on days 1, 2, 8, 12, and 29 with history, physical examination, toxicity assessment, routine laboratory studies (hemogram, standard chemistry analysis, BUN, creatinine, LDH, liver transaminases, ECG, and urinalysis). Disease assessment with physical examination, hemogram, bone marrow aspiration, and biopsy and/or radiographic studies was done on day 29. Clinical efficacy for patients with solid tumors, including non-Hodgkin's lymphoma and Hodgkin's lymphoma, after patient 15 was determined using RECIST criteria [39]. Efficacy for patients with hematologic malignancies was assessed using the following criteria: (i) complete response: normalization of the marker and/or abnormal cell counts and differential counts in blood and bone marrow for at least 4 weeks; (ii) partial response: a decline in the marker by 80% from the baseline value for at least 4 consecutive weeks or an improvement in cell counts and differential counts in blood and/or bone marrow by at least 80% for at least 6 weeks; (iii) stable disease: less than a 25% change in the marker or cell counts from the baseline value over a period of 8 weeks; (iv) progression: $>50\%$ increase in the marker from the baseline value over a period of 8 weeks or a worsening of cell counts in blood or bone marrow.

**Table 1** Patient characteristics. Thirty-five patients with relapsed/refractory malignancies were treated in a phase study of TPA

| Patient | Age | Sex | TPA dose (mg/m$^2$) | Diagnosis |
|---|---|---|---|---|
| 001 | 73 | F | 0.063 | AML |
| 002 | 73 | M | 0.063 | 2° AML |
| 003 | 60 | M | 0.063 | 2° AML |
| 004 | 72 | F | 0.125 | 2° AML |
| 005 | 63 | F | 0.125 | 2° AML |
| 006 | 74 | M | 0.125 | 2° AML |
| 007 | 44 | F | 0.125 | 2° AML |
| 008 | 68 | F | 0.125 | 2° AML |
| 009 | 64 | F | 0.125 | 2° AML |
| 010 | 76 | M | 0.125 | 2° AML |
| 011 | 69 | M | 0.125 | AML |
| 012 | 61 | M | 0.063 | 2° AML |
| 013 | 41 | M | 0.063 | CML–myeloid blast crisis |
| 014 | 29 | F | 0.063 | Hodgkin's lymphoma |
| 015 | 21 | F | 0.063 | Ovarian teratocarcinoma |
| 016 | 52 | F | 0.063 | Subcutaneous adenocarcinoma |
| 017 | 42 | M | 0.063 | Non-Hodgkin's Lymphoma |
| 018 | 34 | F | 0.063 | Hodgkin's lymphoma |
| 019 | 38 | M | 0.063 | AML |
| 020 | 54 | M | 0.063 | AML |
| 021 | 78 | M | 0.063 | Squamous cell carcinoma–skin |
| 022 | 53 | F | 0.125 | AML |
| 023 | 75 | F | 0.125 | AML |
| 024 | 56 | F | 0.125 | Renal cell carcinoma |
| 025 | 71 | M | 0.125 | Acute Lymphocytic Leukemia |
| 026 | 68 | F | 0.125 | AML |
| 027 | 78 | M | 0.125 | AML |
| 028 | 70 | M | 0.125 | AML |
| 029 | 29 | F | 0.188 | Hodgkin's lymphoma |
| 030 | 36 | M | 0.188 | Non-Hodgkin's lymphoma |
| 031 | 45 | M | 0.188 | Non-Hodgkin's lymphoma |
| 032 | 81 | M | 0.188 | Prostate cancer |
| 033 | 59 | M | 0.188 | Prostate cancer |
| 034 | 59 | M | 0.188 | AML |
| 035 | 64 | F | 0.188 | Non-Hodgkin's lymphoma |

## Results

### TPA-associated adverse effects

Patient characteristics are presented in Table 1. Adverse events and serious adverse events are summarized in Table. 2 and 3. Transient fevers, chills, dyspnea, mild hypoxia (decline in oxygen saturation by pulse oximetry not requiring supplemental oxygen at rest), muscle aches, and pain, and fatigue were common. These were grade 1 or 2 toxicities by NCI Common Toxicity Criteria v 2.0. The symptoms generally lasted $<4$ h. The dyspnea and hypoxia did not have any radiographic correlate on chest X-ray. Phlebitis, previously described in association with TPA infusion [35, 36], was not seen in this study after central venous access was mandated. No clinical abnormalities suggestive of renal or hepatic dysfunction were detected. Gross hematuria requiring cystoscopy for evacuation of clots occurred in one patient. Cystoscopy demonstrated multiple mucosal hemorrhagic lesions. A grand mal seizure without associated metabolic, electroencephalographic, or radiographic findings occurred in another patient treated with TPA. Hypotension or syncope that was not readily reversible occurred in 2 patients treated at 0.188 mg/m2 on days 1–5, 8–12. These grade 3 nonhematologic toxicities were dose-limiting toxicities and were used to define the maximally tolerated dose as 0.125 mg/m$^2$ intravenously on days 1–5, 8–12. The adverse events and serious adverse events are summarized in Table. 2 and 3

**Table 2** Adverse events for all grades and cycles are listed by frequency

| Adverse events | *n* | Percentage |
|---|---|---|
| Anemia | 23 | 65.7 |
| Thrombocytopenia | 20 | 57.1 |
| Fever | 16 | 45.7 |
| Fatigue | 15 | 42.9 |
| Neutropenia | 14 | 40.0 |
| Pain | 13 | 37.1 |
| Hyperglycemia | 12 | 34.3 |
| Rigors | 10 | 28.6 |
| Dyspnea | 9 | 25.7 |
| Nausea | 9 | 25.7 |
| Chills | 8 | 22.9 |
| Anorexia | 6 | 17.4 |
| Infection | 4 | 11.4 |
| Increased alkaline phosphatase | 4 | 11.4 |
| Cough | 4 | 1.4 |
| Hematuria | 4 | 1.4 |
| Hyponatremia | 4 | 1.4 |
| Diarrhea | 3 | 8.6 |
| Hypoxia | 3 | 8.6 |
| Petechiae | 3 | 8.6 |
| Vomiting | 3 | 8.6 |
| Weakness | 3 | 8.6 |
| Chest pain | 3 | 8.6 |
| Edema | 2 | 5.7 |
| Epistaxis | 2 | 5.7 |
| Extravasation | 2 | 5.7 |
| Headache | 2 | 5.7 |
| Hives | 2 | 5.7 |
| Hypoalbuminemia | 2 | 5.7 |
| Rash | 2 | 5.7 |
| Rhinitis | 2 | 5.7 |
| Sore throat | 2 | 5.7 |
| Syncope | 2 | 5.7 |
| Hypotension | 2 | 5.7 |
| Myalgia | 2 | 5.7 |
| Night sweats | 2 | 5.7 |
| Death | 2 | 5.7 |
| Dizziness | 2 | 5.7 |

### Blood level studies

Blood levels of TPA biological activity were measured before infusion and immediately after a 1-h infusion in 31 patients and at several times after the infusion in 28 of these patients (Table 4). The average blood level of TPA-associated biological activity after a 1-h infusion of 0.063, 0.125 or 0.188 mg/m$^2$ on days 1 and 8 was $1.20 \pm 0.26$ ng/ml ($n=18$), $1.68 \pm 0.22$ ng/ml ($n=27$), and $4.08 \pm 0.77$ ng/ml ($n=11$) of TPA equivalents in blood, respectively. Patients receiving 0.188 mg/m$^2$ had blood levels also measured at 5 and 11 h after the start of infusion. In those patients the blood half-life for TPA equivalents was ~3–4 h between 5 and 11 h after infusion (Table 4).

### Tumor response

One of the patients (patient 014—relapsed/refractory Hodgkin's lymphoma) had a reduction in a palpable chest wall mass (7–2.5-cm-long dimension) during treatment. This patient had relapsed refractory disease with a biopsy-proven chest wall recurrence within a prior radiation port. Previous treatment for relapsed Hodgkin's lymphoma included high-dose chemotherapy followed by autologous hematopoietic stem cell rescue. A reduction in palpable tumor mass was noted 3 weeks after initiation of TPA, but after two cycles of therapy (2 months), there was an increase in tumor size and the patient was removed from the study to undergo allogeneic hematopoietic stem cell transplantation. None of the other patients had evidence of disease response during the treatment.

## Discussion

TPA is a potent modulator of signal transduction and cell differentiation in primary AML cells and multiple cell lines [1–8, 34]. The best-characterized cellular receptor for TPA is protein kinase C (PKC), which once activated, induces substrate phosphorylation that propagates signals to the MAPK cascades [9–26]. TPA modulation of these pathways is cell type-specific and dependent upon the activity of interacting signaling pathways. MAPK modulation of immunophenotype, proliferation, adhesion, phagocytosis, differentiation,

**Table 3** Serious adverse events are listed by grade, likelihood of relationship to TPA and patient number

| Patient number | Serious adverse events | Relationship to study therapy |
|---|---|---|
| 01 | Increasing WBC count | Not related |
| 02 | Hospitalization for hydration | Not related |
| | Poor appetite | Not related |
| 04 | Neutropenic fever | Probably |
| 08 | Fever | Possibly |
| | Pneumonia | Not related |
| 09 | Syncope | Possibly |
| | Seizure | Possibly |
| 10 | Cellulitis | Possibly |
| | Neutropenic fever | Possibly |
| | Hematuria (hemorrhagic cystitis) | Possibly |
| 11 | Cellulitis | Definitely |
| | Vomiting (aspiration) | Not related |
| 12 | Chest pain | Not related |
| | Neutropenic fever | Not related |
| | Erythematous, multiform macules | Unlikely |
| | Fever | Unlikely |
| | Abdominal pain | Unlikely |
| | Diarrhea | Unlikely |
| | Chills | Unlikely |
| 13 | Fever | Possibly |
| 15 | Pain (lower back) | Not related |
| | Pain (l-thorax) | Not related |
| | Abdominal pressure/distention | Not related |
| 16 | Right hip pain (hospitalized for right orbital fracture) | Unlikely |
| 17 | Hospitalization for observation after liver biopsy | Not related |
| | Febrile neutropenia | Not related |
| 19 | Respiratory distress | Possibly |
| 20 | Febrile neutropenia | Possibly |
| | Shortness of breath | Possibly |
| | Neutropenic fever | Unlikely |
| 21 | Hyperkalemia | Unlikely |
| 22 | Neutropnic fever | Unlikely |
| | Cellulitis | Unlikely |
| 25 | Herpes zoster infection (without neutropenia) | Not related |
| 26 | Fever | Possibly |
| 27 | Fever | Possibly |
| | Death | Not related |
| 32 | Hypotension | Definitely |
| | Hypoxia | Definitely |
| 33 | Dyspnea | Possibly |
| | Deep vein thrombosis | Possibly |
| 35 | Dyspnea | Probably |
| | Syncope | Probably |
| | Abdominal pain | Unlikely |
| | Death (disease progression) | Not related |

apoptosis, and gene expression have been studied in primary hematopoietic cells and hematopoietic cell lines [1–9, 34].

Exposure of primary AML cells to TPA ex vivo may result in differentiation or apoptosis, cellular outcomes anticipated to be associated with clinical response [34]. In fact, reduction in leukemic blasts and improvement in blood counts were seen in a pilot trial of TPA for patients with myeloid malignancies performed in China [35]. In that study, patients received higher doses of TPA

**Table 4** Blood levels. Patients were given a 1-h infusion of TPA and blood levels determined as described [37]. Each value represents the mean ± SD from patients who had three blood level measurements (0.063 mg/m$^2$, and 0.125 mg/m$^2$) or five blood level measurements (0.188 mg/m$^2$) on day 1

| Dose (mg/m$^2$) | Number | Time after the end of infusion | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Preinfusion | 0 h | 1 h | 2 h | 3 h | 5 h | 11 h |
| 0.063 | 8 | 0 | 1.09 ± 0.24 | 0.22 ± 0.07 | – | 0.12 ± 0.05 | – | – |
| 0.125 | 13 | 0 | 1.66 ± 0.20 | 0.62 ± 0.16 | – | 0.28 ± 0.05 | – | – |
| 0.188 | 7 | 0 | 4.93 ± 1.06 | 2.56 ± 1.15 | 1.94 ± 0.93 | – | 1.40 ± 0.90 | 0.40 ± 0.15 |

administered on varying schedules and as a component of combination therapy with cytarabine or vitamin D3. In contrast to that study, TPA was used as a single agent in this study and the dosing regimen was based upon anticipated benefit with prolonged exposure to TPA [34]. However, tumor reduction was seen only in one patient (patient 014, Hodgkin's lymphoma).

The toxicity profile seen in this study was predominated by reversible pulmonary toxicity, fever rigors, and muscle aches and pains, generally occurring within 4 h after the completion of the infusion. Prior to mandating the use of central venous access, phlebitis and pain with the infusion were common. The dose-limiting toxicities (treatment-related grade 3 or greater toxicities ascribed to TPA) were variable and included hypotension and syncope at the highest dose tested (0.188 mg/m$^2$). Other toxicities included seizure in a single patient (in association with a platelet transfusion and without metabolic, radiographic or electroencephalographic correlate) and gross hematuria in a single patient (with cystoscopy required for clot evacuation).

The maximally tolerated TPA dose in our study was 0.125 mg/m$^2$ when administered daily for five consecutive days on two consecutive weeks. This is in contrast to doses of 0.125 mg, 0.25 mg, 0.5 mg, and 1.0 mg administered in various schedules and combinations in China [35, 38]. The ability of patients in China to tolerate daily doses as high as 1.0 mg may be related to the schedule (once weekly for most patients in China as opposed to daily for five consecutive days on consecutive weeks in this study); the associated components of therapy (many patients in China received combination therapy with cytarabine and/or vitamin D3), or other uncharacterized parameters impacting on metabolism or toxicity.

A physical assay of TPA and metabolites with appropriate sensitivity could not be developed. Hence, pharmacokinetic studies were undertaken using a biological assay that measured the capacity of an organic extract of blood to induce differentiation of HL60 cells [37]. Advantages of a biological assay include the potential for a single measurement of the activity of several biologically "active" metabolites and the ability to assess activity in the context of patient-specific physiologic parameters. Disadvantages to a bioassay include the need to select a specific restricted biological parameter to study, the presence of physiologic variables that might affect drug activity in the assay, and uncertainty about what is actually being measured. In this study, there was a nonlinear response in blood levels of TPA biological activity immediately after the infusion as the dose of TPA was increased. In addition, significant variability in blood levels was determined between different patients receiving the same dose. Some of this variability may be the consequence of the biological nature of the assay. Despite the appearance of TPA-like activity in the blood of most treated patients, there was no correlation of blood levels with toxicity or clinical features.

The majority of treated patients had relapsed/refractory AML or other advanced malignancies. Eligibility criteria included an ECOG performance status of two or lower and an estimated life expectancy of $>1$ month, but many patients did not complete a course of therapy because of disease progression or disease-related adverse effects that were not ascribed to the drug. TPA administered as a single agent in dose and schedule described in this report resulted in a reduction in palpable disease in a single patient with Hodgkin's lymphoma and no durable clinical benefit in any patient, and resulted in adverse effects at a dose of 0.188 mg/m$^2$. Given the reduction in leukemic blasts and improvement in blood counts seen in some patients with myeloid leukemias in China [35] and synergistic differentiation of HL60 cells in combination with all-trans retinoic acid [40], combination therapies, or alternate dosing regimens may be developed clinically for patients with AML. In addition, TPA, alone or in combination, may be studied in patients with other malignancies. For example, TPA in combination with all-trans retinoic acid results in enhanced apoptosis of LNCap prostate cancer cells in immunodeficient mice [41].

**Acknowledgements** These studies were supported by the National Institutes of Health (NCI R3CA85201) and the Leukemia and Lymphoma Society.

## References

1. Huberman E, Callaham MF (1979) Induction of terminal differentiation in human promyelocytic leukemia cells by tumor promoting agents. Proc Natl Acad Sci USA 76:1293–1297
2. Lotem J, Sachs L (1979) Regulation of differentiation in mouse and human myeloid leukemic cells by phorbol esters and the mechanism of tumor promotion. Proc Natl Acad Sci USA 76:5158–5162
3. Rovera G, Santoli D, Damsky C (1979) Human promyelocytic cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. Proc Natl Acad Sci USA 76:2779–2783
4. Koeffler HP (1980) Phorbol diester-induced macrophage differentiation of leukemic blasts from patients with human myelogenous leukemia. J Clin Invest 66:1101–1108
5. Koeffler HP, Bar-Eli M, Territo MC (1981) Phorbol ester effect on differentiation of human myeloid leukemia cell lines blocked at different stages of maturation. Cancer Res 41:919–926
6. Polliack A, Leizerowitz R, Korkesh A, Gurfel D, Gamliel H, Galili U (1982) Exposure to TPA in vitro as an aid in the classification of blasts in human myelogenous and lymphoid leukemias. Am J Hematol 13:199–211
7. Shkolnick T, Schlossman SF, Griffin JD (1985) Acute undifferentiated leukemia: induction of partial differentiation by phorbol ester. Leuk Res 9:11–17
8. Abrahm JL, Gerson SL, Hoxie JA, Tannenbaum SH, Cassileth PA, Cooper RA (1986) Differential effects of phorbol esters on normal myeloid precursors and leukemic cells. Cancer Res 46:3711–3716
9. Hofmann J (1997) The potential for isoenzyme-selective modulation of protein kinase C. FASEB J 11:649
10. Mochly-Rosen D, Kauvar LM (1998) Modulating protein kinase C signal transduction. Adv Pharmacol 44:91
11. Newton AC (1995) Protein kinase C structure, function and regulation. J Biol Chem 270:28495

12. Kazanietz MG (2000) Eyes wide shut: protein kinase C isoenzymes are not the only receptors for the phorbol ester tumor promoters. Mol Carcinog 28:5
13. Hunter T (2000) Signaling 2000 and beyond. Cell 100:113
14. Meinhardt G, Roth J, Hass R (2000) Activation of protein kinase C relays distinct signaling pathways in the same cell type: differentiation and caspase-mediated apoptosis. Cell Death Differ 7:795–803
15. Garrington TP, Johnson GL (1999) Organization and regulation of MAPK signaling pathways. Curr Opin Cell Biol 11:211
16. Vojtek AB, Der CJ (1998) Increasing complexity of the ras signaling pathway. J Biol Chem 273:19925
17. Hill CS, Treisman R (1995) Transcriptional regulation by extracellular signals: mechanisms and specificity. Cell 80:199
18. Treisman R (1996) Regulation of transcription by MAP kinase casacades. Curr Opin Cell Biol 8:205
19. Schwartz MA, Baron V (2000) Interactions between mitogenic stimuli, or, a thousand and one connections. Curr Opin Cell Biol 11:197
20. Chang F, Steelman LS, Lee JT, Shelton JG, Navolanic PM, Blalock WL, Franklin RA, McCunbrey JA (2003) Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention. Leukemia 17:1263–1293
21. Platanias LC (2003) Map kinase signaling pathways and hematologic malignancies. Blood 101:4667–4679
22. Marshall CJ (1995) Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal regulated kinase activation. Cell 80:179
23. Keyse SM (2000) Protein phosphatases and the regulation of MAPK signaling. Curr Opin Cell Biol 12:186
24. Bhalla US (2004) Models of cell signaling pathways. Curr Opin Genet Dev 14:375–381
25. Mochly-Rosen D, Kauvar LM (2000) Pharmacologic regulation of network kinetics by protein kinase C localization. Semin Immunol 12:55
26. Mochly-Rosen D, Gordon AS (1998) Anchoring proteins for protein kinase C: a means for isozyme specificity. FASEB J 12:35
27. Kelly L, Clark J, Gilliland DG (2002) Comprehensive genotypic analysis of leukemia:clinical and therapeutic implications. Curr Opin Oncol 14:10
28. Kiyoi H, Naoe T, Nakano Y, Yokota S, Minami S, Miyawaki S, Asou N, Kuriyama K, Jinnai I, Shimazaki C, Akiyama H, Saito K, Oh H, Motoji T, Omoto E, Saito H, Ohno R, Ueda R (1999) Prognostic implications of Flt-3 and N-ras gene mutations in acute myeloid leukemia. Blood 93:3074–3080
29. Beaupre DM, Kuzrock R (1999) RAS and leukemia. J Clin Oncol 17:1071–1079
30. Towatari M, Iida H, Tanimoto M, Iwata H, Hamaguchi M, Saito H (1997) Constitutive activation of the mitogen-activated protein kinase pathway in acute leukemia cells. Leukemia 11:479–484
31. Kim S-C, Hahn J-S, Min Y-H, Yoo N-C, Ko Y-W, Lee W-J (1999) Constitutive activation of extracellular signal-regulated kinase in human acute leukemias: combined role of activation of MEK, hyperexpression of extracellular signal regulated kinase, and down-regulation of a phosphatase, PAC1. Blood 93:3893
32. Hayakawa F, Towatari M, Kiyoi H, Tanimoto M, Kitamura T, Saito H, Naoe T (2000) Tandem duplicated Flt-3 constitutively activates STA5 and MAP kinase and introduces autonomous cell growth in IL-3-dependent cell lines. Oncogene 19:624–631
33. Morgan MA, Dolp O, Reuter WM (2001) Cell cyle dependent activation of mitogen-activated protein kinase kinase (MEK-1/2) in myeloid leukemia cell lines and induction of growth inhibition and apoptosis by inhibitors of RAS signaling. Blood 97:1823
34. Schaar DG, Liu H, Sharma S, Ting Y, Martin J, Krier C, Ciardella M, Osman M, Goodell L, Notterman DA, Strair RK (2005) 12-O-Tetradecanoylphorbol-13-acetate (TPA)-induced dual-specificity phosphatase expression and AML cell survival. Leuk Res 29:1171–1179
35. Han ZT, Zhu XX, Yang RY, Sun JZ, Tian GF, Liu XJ, Cao GS, Newmark HL, Conney AH, Chang RL (1998) Effect of intravenous 12-O-tetradecanoylphorbol-13-acetate (TPA) in patients with myelocytic leukemia:preliminary studies on therapeutic efficacy and toxicity. Proc Natl Acad Sci USA 95:5357–5361
36. Strair RK, Schaar D, Goodell L, Aisner J, Chin K-V, Eid J, Senzon R, Knox B, Cui XX, Han ZT, Newmark HL, Rabson AB, Chang RL, Conney A (2002) Administration of a phorbol ester to patients with hematologic malignancies: Preliminary results from a phase I clinical trial of 12-*O*-tetradecanoylphorbol-13-acetate. Clin Cancer Res 8:2512–2518
37. Cui XX, Chang RL, Zheng X, Woodward D, Strair R, Conney A (2002) A sensitive bioassay for measuring blood levels of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in patients: preliminary pharmacokinetic studies. Oncol Res 13:169–174
38. Han ZT, Tong YK, He LM, Zhang Y, Sun JZ, Wang TY, Zhang H, Cui YL, Newmark H, Conney AH, Chang R (1998) 12-O-Tetradecanoylphorbol-13-acetate (TPA)-induced increase in depressed white blood cell counts in patients treated with cytotoxic cancer chemotherapeutic drugs. Proc Natl Acad Sci USA 95:5362–5365
39. Therasse P, Arbuck SG, Eisenhauer EA (2000) New Guidelines to Evaluate the Response to Treatment in Solid Tumors. J Natl Cancer Inst 92:205–216
40. Zheng X, Chang RL, Cui XX, Kelly KA, Shih WJ, Lin Y, Strair R, Suh J, Han ZT, Rabson A, Conney AH (2001) Synergistic effects of clinically achievable concentrations of 12-O-tetradecanoylphorbol-13-acetate in combination with all-trans retinoic acid, 1alpha,25-dihydroxyvitamin D3, and sodium butyrate on differentiation in HL-60 cells. Oncol Res 12:419–427
41. Zheng X, Chang RL, Cui XX, Avila GE, Lee S, Lu YP, Lou YR, Shih WJ, Lin Y, Reuhl K, Newmark H, Rabson A, Conney AH (2004) Inhibitory effect of 12-O-tetradecanoylphorbol-13-acetate alone or in combination with all-trans-retinoic acid on the growth of LNCaP prostate tumors in immunodeficient mice. Cancer Res 64:1811–1820