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ORIGINAL ARTICLE

Phase I study of the synthetic triterpenoid, 2-cyano-3, 12-dioxoolean-1, 9-dien-28-oic acid (CDDO), in advanced solid tumors

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Abstract
Background The triterpenoid 2-cyano-3,12-dioxoolean-1,9-dien-28-oic Acid (CDDO, previously RTA 401) is a multifunctional molecule that controls cellular growth and differentiation. While CDDO is capable of activating the transcription factor peroxisome proliferator activator receptor-γ (PPARγ), its apoptotic effects in malignant cells have been shown to occur independently of PPARγ. A phase I dose-escalation study was conducted to determine the toxicity, the maximum tolerated dose, and the pharmacokinetics and pharmacodynamics of CDDO, administered as a 5-day continuous infusion every 28 days in patients with advanced cancers.

Methods An accelerated titration design was followed, with one patient per cohort entered, and doses ranging from 0.6 to 38.4 mg/m^2/h. Pharmacokinetics of CDDO was assessed and cleaved poly (ADP-ribose) polymerase (c-PARP), as a marker of apoptosis, was measured in peripheral blood mononuclear cells to assess drug effect.
Results Seven patients, one patient per dose level up to dose level 7 (38.4 mg/m^2/h), were enrolled and received a total of 11 courses of treatment. *Cmax* increased proportionally with dose. Preclinically determined efficacious blood level (1 μM) of drug was attained at the highest dose level. One patient, at dose level 6, experienced grade 2 mucositis, nausea, vomiting, and anorexia. Four patients developed thromboembolic events subsequently considered as dose-limiting toxicity. No antitumor activity was noted.
Conclusion A causal relationship of observed thromboembolic events to CDDO was considered possible but could not be established.

Keywords Triterpenoid · CDDO · Adverse event · Thromboembolism

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Introduction

Synthetic triterpenoids are a class of agents derived from the naturally occurring plant metabolite, triterpene, with the capability of exerting antitumor effects through multiple mechanisms [1, 2]. In preclinical models, they can target tumor cells by inducing apoptosis, inhibiting proliferation, controlling cellular differentiation, and displaying antiangiogenic, anti-inflammatory, and antioxidant effects. Naturally occurring triterpenoids such as oleanolic acid and ursolic acid have been used for medicinal purposes in some Asian countries and have weak

anti-inflammatory, anticarcinogenic, and antiproliferative properties [3].

CDDO (2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid, previously RTA 401) is a synthetic triterpenoid and a potent multifunctional molecule. It induces apoptosis in vitro in malignant cells through both caspase-dependent and caspase-independent pathways [4]. It has been studied as a potential anticancer agent and was demonstrated to induce cell differentiation, growth inhibition, and apoptosis in cell lines from various tumor types including solid tumors such as osteosarcoma [5], breast cancer [6], ovarian, prostate, and colon cancers when combined with TRAIL [7], as well as in human leukemia [4] and lymphoid cells [8]. CDDO also exhibits the ability to inhibit tumor growth in a number of xenograft models, including MDA-MB-435 [6], L1210 [9], and B16 [9]. Although CDDO has been demonstrated to activate PPARγ in vitro, several reports have demonstrated that the growth inhibitory and pro-apoptotic effects of CDDO are PPARγ-independent [10–13].

In preclinical in vitro studies, the IC_{50} for pancreatic and breast cancer cell lines was <1 μM as determined after 48–72 h of treatment with CDDO [6, 14]. In the preparation for defining the clinical dosing schedule, in vivo efficacy was noted to be suboptimal in xenograft models using either q2 or q3 day IV dosing. However, BID dosing in mice xenograft models treated with IV CDDO at 20 and 40 mg/kg q12 h for 10 days resulted in modest, but statistically significant antitumor effects. In vivo pharmacokinetic studies conducted in mice, rats, and dogs showed that the targeted plasma concentration of 1 μM (or higher) was maintained for up to 1–4 h post-administration when given as a bolus. When given as a 5-day continuous intravenous infusion, steady-state plasma concentrations were achieved within 24 h after the start of the infusion in dogs. Steady state plasma concentrations exceeded the target plasma level of 1 μM at doses below the maximum tolerated dose (MTD) in dogs and caused minimal toxicity. Toxicity was reversible and was limited to decreased food consumption, transient decreases in platelet count, and decreased serum albumin (data on file, Toxicology-Pharmacology Branch, DCTD, NCI).

Therefore, based on its preclinical activity and minimal toxicity observed in dogs, a phase I dose-escalation study to determine MTD, evaluate pharmacokinetics, and assess the toxicity profile of CDDO administered as a continuous infusion for 5 consecutive days in 28-day cycles was conducted. To assess drug effect, cleaved poly (ADP-ribose) polymerase (c-PARP) was measured in peripheral blood mononuclear cells (PBMCs) for the detection of CDDO-induced apoptosis.

Patients and methods

Eligibility criteria

Adult patients (age ≥ 18) with histologically confirmed metastatic or unresectable solid tumor malignancy refractory to standard therapies or for which there were no acceptable standard treatments; Eastern Cooperative Oncology Group performance status ≤2; adequate marrow, hepatic, and renal function defined as leukocytes $\geq 3.0 \times 10^9$/l, absolute neutrophil count $\geq 1.5 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, total bilirubin ≤1.5 × upper limit of normal (ULN), aspartate aminotransferase and alanine aminotransferase <2.5 × ULN and creatinine within normal institutional limits.

There were no limits on prior treatments. Prior anticancer therapy must have been completed at least 4 weeks prior to enrolling on study. Patients were excluded if they had an uncontrolled intercurrent illness, were pregnant or nursing, or had brain metastases within the past 6 months. Other exclusion criteria included patients with a history of symptomatic congestive heart failure, unstable angina pectoris, myocardial infarction within 6 months of study entry, or cardiac arrhythmias. Patients with prior thrombosis on stable doses of an anticoagulant were not excluded from this study.

Written informed consent was obtained from all patients. This trial was conducted under a National Cancer Institute (NCI)-sponsored IND with institutional review board approval. The protocol design and conduct followed all applicable regulations, guidances, and local policies. ClinicalTrials.gov identifier: NCT00352040.

Trial design

This was an open-label, single-arm phase I study of CDDO in patients with advanced malignancies. CDDO (NSC 711193) was supplied by the Division of Cancer Treatment and Diagnosis, NCI under a Collaborative Research and Development Agreement with Reata Pharmaceuticals, Inc.

CDDO was administered as a 5-day continuous intravenous infusion every 28 days. The design was a Simon accelerated titration design, 4B [15] with an initial starting dose of 0.6 mg/m^2/h (1/10th the MTD in the most sensitive species, the rat). Dose levels were increased in 100% increments. One patient per dose level was entered. The accelerated phase was to end when one patient experienced dose-limiting toxicity (DLT) or two different patients experienced grade 2 drug-related toxicity during the first cycle. Higher dose levels were not open to accrual until the patient(s) in the previous cohort had completed at least one cycle. When the accelerated phase ended, the dose level

was to be expanded to three patients, and standard dose-escalation was to proceed using a 3 + 3 modified Fibonacci escalation.

Patients were considered evaluable for toxicity for the purpose of determining cohort dose-escalation if they experienced a DLT or received the total dose of CDDO as planned and remained on study for one full cycle without DLT. Exceptions for infusion pump malfunction for up to 12 h out of the 120-h continuous infusion were permitted, provided that the patient received the total dose of drug by the end of the sixth day of the cycle.

Adverse events were graded according to NCI Common Terminology Criteria for Adverse Events version 3.0. DLT was defined as an adverse event, felt to be related to the study drug, that fulfilled one of the following criteria: (1) grade 3 or greater non-hematologic toxicity (except for nausea/vomiting and diarrhea without maximal symptomatic/prophylactic treatment; grade 3 rise in creatinine or electrolyte toxicities corrected within 24 h; and grade 3 rise in liver function tests that resolved to baseline by the subsequent cycle dosing time) or (2) grade 4 hematologic toxicity.

Safety and efficacy evaluations

A complete patient history, physical exam, CBC with differential, PT, PTT, complete chemistry panel, urinalysis, EKG, CXR, HIV, hepatitis testing, and pregnancy test for women of childbearing potential were performed at baseline. CBC and chemistry panels were obtained daily during the infusion on cycle 1, then weekly, and before each subsequent cycle. Radiologic evaluation (CT scan of chest, abdomen, and pelvis) was performed at baseline and every 2 cycles to assess for tumor response. Assessments were made based per the Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 guidelines [16].

Pharmacokinetics

Blood samples for pharmacokinetic analysis were collected during the first cycle. Blood (7 ml) was collected in heparin-containing tubes prior to the start of infusion, and at 2, 4, 8, and 12 h after start of infusion on day 1, and once within 60 min prior to an infusion bag change on days 2, 3, 4, and 5. Blood samples were also collected within 60 min prior to the end of infusion on day 6, and 1, 2, 4, 8, and 12 h after discontinuation of the CDDO infusion. Blood was drawn from a site distant from the site of CDDO infusion to avoid contamination with study drug. Plasma was separated by centrifugation at 3,000 rpm for 15 min in a refrigerated centrifuge and was transferred to polypropylene storage tubes and frozen at −80°C until time of analysis. Urine was collected in each subject over a 24-h period of treatment starting on day 5. Aliquots were frozen until time of analysis. The concentration of CDDO in plasma and urine was determined by high performance liquid chromatography (HPLC) with tandem mass spectrometric detection (further described in the online supplement).

Pharmacodynamics

Peripheral blood mononuclear cells were collected before CDDO administration and 48 h after the initiation of CDDO infusion for the assessment of evidence of apoptosis through the detection and quantitation of PARP cleavage products by western and electrochemiluminescence (ECL) immunoassay. Patients' blood samples (7 ml) were collected in Vacutainer Cell Preparation Tubes (CPT, BD, Franklin Lakes, NJ, USA). The CPT tubes were processed at the collection site within 2 h, according to the manufacturer's instructions. Cell lysates were prepared with MDS lysis buffer (Meso-Scale, Gaithersburg, MD, USA), and protein concentrations were determined by using Pierce BCA kit (Thermo Scientific, Rockford, IL, USA). Immunoblot was performed with an antibody against PARP (Cell Signal, Danvers, MA, USA). Quantitative ECL assay for c-PARP was performed using 25 μg cell lysates with the c-PARP assay kit from Meso-Scale. The test has a sensitivity to detect approximately 1% of the c-PARP seen in the control CDDO-treated Jurkat cells.

Jurkat cells maintained in RPMI with 10% fetal calf serum were used to generate controls for the assays. Cell viability assay was performed using ATPlite assay as previously described [17] for the determination of the activity of CDDO. The LD_{50} for Jurkat cells was determined to be 0.36 μM and less than 25% of cells were viable after 3 days of treatment with CDDO at 1 μM (data not shown). For generating the positive control, Jurkat cells were treated with 3 μM CDDO for 24 h. Cell lysates were prepared and used for immunoblots and ECL assays.

Results

Patients

Patient characteristics are detailed in Table 1. Seven patients were enrolled between May 2006 and January 2007. All patients had at least three prior chemotherapy regimens (range, 3–11 regimens).

Toxicity

A total of 11 courses of treatment were administered at 7 different dose levels (Table 2) ranging from 0.6 to

Table 1 Baseline characteristics of the seven study patients

Sex	
Male	2
Female	5
Age (years)	
Mean	52
Median	49
Range	47–62
Tumor types	
Colorectal	4
Bladder	1
Uterine sarcoma	1
Ovarian	1
ECOG performance status	
1	7
No. of previous chemotherapy regimens (range 3–11)	
3	3
>3	4

ECOG Eastern cooperative oncology group

38.4 mg/m^2/h delivered via continuous infusion on days 1–5 of a 28-day cycle. All patients completed at least the first course of treatment; four patients completed two courses. Aside from venous thromboembolism (VTE), discussed below, no other drug-related toxicities were noted in patients treated at the first 5 dose levels. Drug-related grade 2 mucositis, nausea, vomiting, and anorexia were experienced by the patient treated at dose level 6 (19.2 mg/m^2/h). The MTD was not determined.

A patient with colon cancer treated at dose level 4 (4.8 mg/m^2/h) presented shortly prior to cycle 3 with neurological symptoms of the lower extremities. A CT scan revealed progressive pelvic disease with sacral involvement, as well as a new finding of a pelvic and thigh deep venous thrombosis (DVT) not present on baseline scans. The patient was positive for lupus anticoagulant. This DVT was felt to be secondary to progression of the underlying malignancy and presence of lupus anticoagulant.

A patient at dose level 5 (9.6 mg/m^2/h) with leiomyosarcoma experienced tachycardia on day 6 of cycle 1. A CT angiogram indicated the presence of multiple bilateral pulmonary emboli (PE). The patient was positive for lupus anticoagulant and this adverse event was attributed to the underlying malignancy and presence of lupus anticoagulant. The patient developed disease progression with brain metastases prior to the next cycle, and therefore received no further treatment with CDDO.

As the previous VTEs were thought to be disease related, the following patient, who had ovarian cancer, was treated at dose level 6 (19.2 mg/m^2/h). This patient also experienced tachycardia on day 3/4 of cycle 1; the test for lupus anticoagulant was positive, however, an angiogram performed at that time was negative for PE. The patient received a second cycle of treatment at the same dose level. Tachycardia in the context of fever developed on days 3/4 of cycle 2, and the patient later developed right upper quadrant abdominal pain. A CT scan performed to assess disease progression on day 12 of cycle 2 revealed bilateral PEs. This patient also developed disease progression prior to the next cycle and received no further treatment with CDDO.

As the above patient had cleared cycle 1 without DLT, the next patient with rectal cancer was started on dose level 7 (38.4 mg/m^2/h) before the PE was diagnosed in the sixth patient on cycle 2. On days 4/5, this patient experienced tachycardia. A CT angiogram revealed a left pulmonary artery PE; lower extremity DVT was also noted. This patient had a history of DVT and was on prophylactic anticoagulation (warfarin 1 mg/day). After the diagnosis of DVT and PE, he tested positive for lupus anticoagulant though baseline status was unknown. It is unknown whether DVT was present at baseline prior to initiation of treatment with CDDO. Patient received no further treatment.

Antitumor activity

No antitumor activity was seen in any of the patients treated in this study. All developed progressive disease,

Table 2 Dose levels and observed adverse events at least grade 2 and possibly related to study drug

Dose level (No. of patients)	Dose (mg/m^2/h)	Non-thrombotic adverse events	Thrombotic adverse events
1 (1)	0.6		
2 (1)	1.2		
3 (1)	2.4		
4 (1)	4.8		DVT (grade 3)[a]
5 (1)	9.6		Pulmonary embolism (grade 4)[a]
6 (1)	19.2	Anorexia, nausea, vomiting, mucositis (grade 2)	Pulmonary embolism (grade 4)[b]
7 (1)	38.4		Pulmonary embolism (grade 4)[b], DVT (grade 3)[b]

DVT deep vein thrombosis

[a] Thrombotic events initially attributed to underlying malignancy

[b] Dose-limiting toxicities attributed to study drug

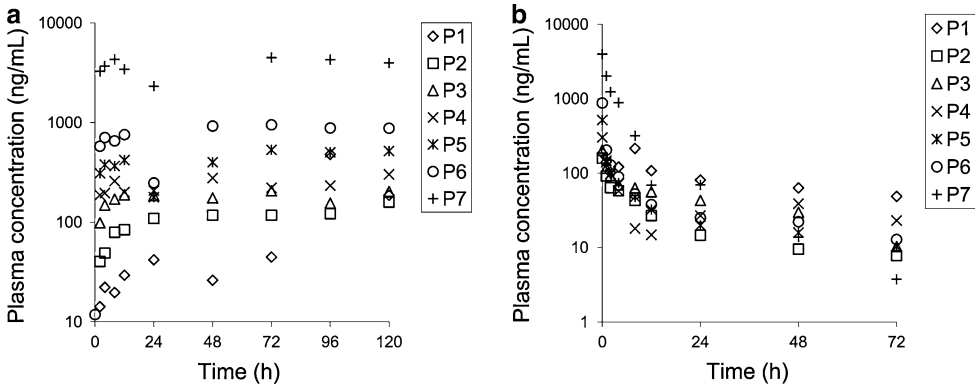

Fig. 1 Individual plasma concentration–time profile for all seven patients treated during infusion (**a**) and after infusion (**b**). Each *symbol* represents a single sample from a single patient at a given time point

except for one patient, at dose level 7 (38.4 mg/m^2/h) who had radiographically stable disease after one cycle of treatment. However, this patient did not receive further treatment with CDDO as stated previously.

Pharmacokinetics

The plasma concentration versus time profiles showed a dose-dependent increase in plasma concentration over the 64-fold range of doses investigated (Fig. 1a). There was a relatively rapid increase in concentration initially, followed by an approach to steady-state, which was reached by 48 h at all dose levels. Total body clearance calculated as the ratio of infusion rate/Css was 12–36 l/h/m^2. The qualitative and quantitative patterns were similar across the dosage range, with only isolated outlying time points. Following the end of the 120-h infusion, CDDO plasma concentrations decreased relatively rapidly for the first 12 h and then exhibited a second elimination phase consistent with a terminal half-life of 2 days (Fig. 1b). Less than 1% of CDDO was excreted in the urine unchanged. The patient at the 7th dose level (38 mg/m^2/h) achieved a plasma concentration >1 μM, the target blood level determined from preclinical studies.

Pharmacodynamics

Peripheral blood mononuclear cells were obtained both prior to and at 48 h post-CDDO therapy. Lysates were normalized for protein concentration and analyzed with both immunoblot and a quantitative ECL assay for c-PARP. The results showed that c-PARP fragment was absent in all seven patients tested on immunoblot (Fig. 2a). The quantitative ECL assay for c-PARP also confirmed that no significant c-PARP was seen. Lack of detectable c-PARP in all samples suggests the absence of induction of apoptosis in PBMCs from patients treated with CDDO for 48 h (Fig. 2b).

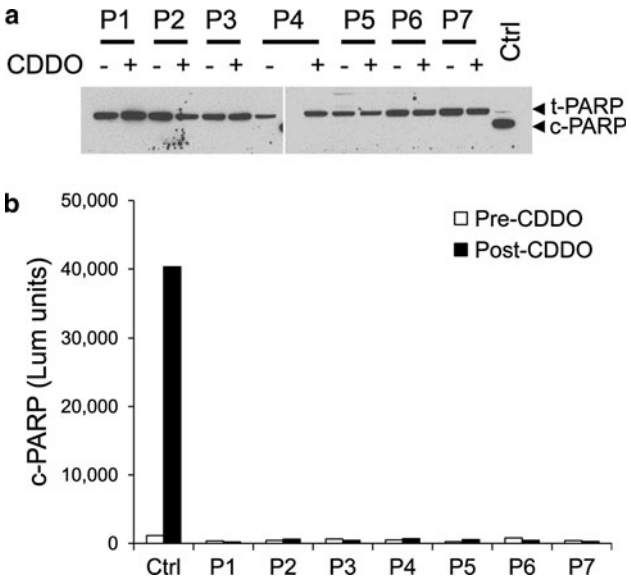

Fig. 2 Lack of detectable cleaved PARP (c-PARP) in PBMCs of CDDO-treated patients at 48 h. **a** Western blot analysis of the effect of CDDO on PARP cleavage from patients' PBMCs collected at time 0 and 48 h. Control (Ctrl): Jurkat cells treated with 3 μM CDDO for 24 h. **b** Quantitative immunoassay analysis of the effect of CDDO on c-PARP from patients' PBMCs pre- and 48 h post-CDDO; Ctrl: Jurkat cells pre- and post-treatment with 3 μM CDDO for 24 h

Discussion

CDDO, a potent multifunctional triterpenoid, held considerable promise as a novel antitumor agent against a wide range of malignancies by concurrently targeting multiple pathways leading to cancer. This study was a clinical phase I trial of increasing doses of CDDO escalated from 0.6 to 38.2 mg/m^2/h, in patients with advanced solid tumors, in which we investigated the proapoptotic effects of CDDO. No evidence of nonlinearity in the pharmacokinetics of CDDO was found. No antitumor activity was seen by imaging in any of the seven patients treated in this study. CDDO failed to induce c-PARP in PBMCs of all the patients indicating lack of apoptosis in this potential

surrogate tissue within the dose ranges tested. Without tumor tissue, however, this finding cannot be extrapolated to indicate lack of effect on tumor cells by CDDO. In addition, the targeted dose level of 1 μM was not reached except in the highest dose level tested (38.4 mg/m^2/h).

The study was closed based on the sequential occurrences of four thromboembolic events. The sixth patient on study developed a PE during the second cycle of therapy, at approximately the same time the seventh patient developed a PE on the first cycle of treatment. Both of these were considered to be related to CDDO due to the noted frequency of thromboembolism on study. A PE documented earlier in patient 5, and DVT in patient 4, believed to be related to the underlying malignancy at the time of occurrence, were retrospectively reassessed as possibly related to the study drug. The relationship of VTE to CDDO, though, is uncertain due to the high rate of thromboembolism in patients with metastatic solid tumors.

There is a strong association between the presence of cancer and the development of acute venous thromboembolic disease. There is considerable variability, but reported incidence rates of VTE range from approximately 8% [18] to 20% of patients with cancer before death, and up to 50% at the time of post-mortem examinations [19]. The four patients on study in whom DVT or PE developed had metastatic leiomyosarcoma (1), ovarian cancer (1), and colorectal cancer (2). The incidence of VTE in patients with ovarian malignancy has been reported as 16.6% across all phases of the disease [20], whereas in non-metastatic colorectal cancer patients, the prevalence of DVT has been noted as 7.8% [21]. While the incidence rate is highest with more aggressive tumors (in particular pancreatic, brain, stomach, and ovary); it increases with increased rate of metastatic spread in all cancers [19, 22]. Systemic chemotherapy and newer targeted agents like bevacizumab have also been associated with increased incidence of VTE in cancer patients [19].

In preclinical toxicology studies of continuous CDDO infusion over 5 days, rats dosed via a femoral vein catheter developed femoral vein thromboses, including those which received vehicle control, thus believed to be catheter-related. Rare lung thromboses were noted in few rats. In dog studies, CDDO was delivered via jugular vein catheters; no thromboses or drug-related changes in coagulation parameters were observed. Triterpenoids comprise a large number of compounds. The naturally occurring triterpenoid, oleanolic acid, has been shown to induce concentration-dependent platelet aggregation, possibly through a phospholipase C-mediated calcium mobilization mechanism [23]. On the other hand, several other naturally occurring triterpenoids have demonstrated antiplatelet aggregation activity [24–27] possibly through down-regulation of phosphorylated ERK [26]. In hepatocellular stellate cells, triterpenoid appears to decrease phosphorylation of PDGF-R and AKT [28]. To the best of our knowledge, there are no reports of other synthetic triterpenoids or CDDO derivatives describing an association with increased clotting susceptibility. Thus, toxicology studies did not suggest VTE as a possible expected adverse event in the clinical trial. In an oncology phase I study population, the incidence of VTE is high, leading to the first two occurrences (on dose level 4 and 5) being attributed to underlying disease.

Attribution of the study drug to the adverse event is a process that can be altered if more information becomes available after the initial decision is made. This requires vigilance on the part of the investigator and IND sponsor. When a patient on a clinical trial experiences a DLT or a serious adverse event, the investigator must determine whether the event is attributable to the investigational drug. A recent qualitative study evaluating causality assessment during early-phase oncology trials found that it is a complex process, often without complete clinical and investigational data available to the investigator [29]. In early-phase oncology clinical trials (phase I), the toxicities of the study medication are have not been well characterized and often occur in the context of advanced cancer and multiple co-morbidities. The drug may mimic the disease process (e.g., rising LFTs may be as a result of investigational agent toxicity or secondary to liver failure from advanced liver metastases) or the toxicity (e.g., anorexia or VTE) may be an expected event of the cancer itself. It is often difficult to assess the contributions of a new agent to the toxicity. Investigators may follow a "precautionary principle" and err on the side of caution by attributing the toxicity to the study drug [29]. To ensure patient safety, a potentially active new agent may be labeled toxic and not evaluated further.

Although this report is the only clinical experience with this compound in solid tumors, CDDO was also studied in a clinical trial of nine patients with refractory or relapsed acute myeloid leukemia [30]. MTD was not reached and patients did not meet the trial response criteria; systemic exposure and adverse events were not reported. Four out of nine patients showed increased expression of differentiation markers, and three patients showed induction of apoptosis in leukemic blasts, documented as loss of mitochondrial membrane potential. Lack of observed apoptosis in our study could be due to insensitivity of PBMCs to low plasma concentrations of CDDO in most of the patients, or unsuitable surrogate tissue selection. In preclinical work, efficacy in ex vivo patient samples indicated that the effects of CDDO may be selective for malignant cells over normal cells; CDDO-induced apoptosis in samples from patients with acute myeloid leukemia [31], chronic lymphocytic leukemia [32], or cutaneous T-cell lymphoma

[12], but was not toxic to normal peripheral blood lymphocytes [31, 32]. Similarly, a differential effect on malignant solid tumor cell lines versus normal monkey hepatocytes or human umbilical vein endothelial cells has been demonstrated [7].

The accelerated titration phase I design was efficient in this study with respect to minimizing the number of patients treated at very low and potentially ineffective doses. With one patient per cohort and doubling the dose at each level, only seven patients were required to span a range of 64-fold in dose. The more traditional design of 3 patients per cohort and a more conservative escalation strategy (40% escalation steps) would have required up to 42 patients to cover the same dosage range. The more traditional design may have allowed for better assessment of VTE causality; however, more patients would have been treated at subtherapeutic levels since efficacy was expected at doses achieving >1 μM as determined in preclinical models. This was only achieved in the highest dose level tested.

The above issues highlight some of the difficulties and unpredictable nature of early-phase oncology trials. As noted, it can be exceedingly difficult to discriminate between disease and study drug when considering the cause of a common adverse event in cancer patients. The rate of development of venous thromboembolic complications in patients with advanced refractory cancer in a phase I clinic at the M. D. Anderson Cancer Center was reported as 11.8% of patients, with a median time to occurrence of 5.1 months [33]. The elevated frequency, i.e., four patients who developed VTE in our study (57%), raised suspicion that the event was drug-related. However, whether the advanced cancer patients, who all tested positive for lupus anticoagulant, were inherently predisposed to the event, or whether CDDO truly contributed, could not be clearly established. The manufacturer decided to discontinue development of CDDO for reasons unrelated to this study. The study was terminated after seven patients; therefore, further evaluation of the relationship between thrombosis and CDDO was not possible.

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