Phase I Study of 9-cis-Retinoic Acid (ALRT1057 Capsules) in Adults with Advanced Cancer

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ABSTRACT

9-cis-Retinoic acid (9-cis-RA) and all-trans-RA (ATRA) are naturally occurring hormones. The nuclear receptors that mediate the effects of retinoids are the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). ATRA binds RAR with high affinity but does not bind to RXR, whereas 9-cis-RA, an isomer of ATRA, is a ligand that binds and transactivates both RARs and RXRs. The goals of this study were to determine the safety, tolerability, pharmacokinetics, and metabolic profile of 9-cis-RA in advanced cancer patients. Forty-one patients received oral 9-cis-RA (ALRT1057; Panretin capsules) at doses ranging from 5–140 mg/m²/day. Twenty-six patients were treated once daily with up to 140 mg/m²; a subsequent cohort of 15 patients were treated twice daily (b.i.d.) at 100–140 mg/m²/day (50, 60, and 70 mg/m² b.i.d.) to evaluate a b.i.d. dosing regimen. Headache was the most frequent adverse event and was dose limiting in 3 of 41 patients. Skin toxicity was the next most common toxicity and was seen in 11 of 41 patients; it was typically mild and limited to skin dryness and erythema. Other toxicities included conjunctivitis, flushing, diarrhea, transaminitis, hypercalcemia, and asymptomatic hypertriglyceridemia. Toxicities were typically dose related, occurred primarily above 83 mg/m²/day, and were not ameliorated by b.i.d. dosing. No tumor responses were observed. The mean day 1 area under the plasma concentration-time curve and peak plasma concentration values were dose-proportional over all dose levels, whereas day 15 area under the plasma concentration-time curve and peak plasma concentration values were nonlinear above 83 mg/m²/day, suggesting that 9-cis-RA induced its own metabolism at doses equal to and above 140 mg/m²/day. 9-cis-RA is a retinoid receptor pan agonist with a more favorable pharmacokinetic and toxicity profile than that observed with previously studied retinoids and merits further investigation.

INTRODUCTION

Retinoids, including vitamin A and its analogues, regulate the morphogenesis, development, growth, and differentiation of cells (1). Retinoids and their precursors suppress carcinogenesis in experimental animals (2) and have shown promise as chemopreventive agents in epithelial tumors (3, 4) and as therapeutic agents in APML2 (5).

Retinoids modulate normal, premalignant, and malignant cell phenotypes by changes in gene expression that are mediated through binding to two classes of nuclear hormone receptors, the RARs and the RXRs. There are six known retinoid IR subtypes, RAR α, β, and γ and RXR α, β, and γ, which are members of the steroid receptor superfamily (6). These retinoid IRs form heterodimers that bind to specific DNA sequences and act as ligand-dependent transcriptional regulators for RA-responsive genes. The RARs and RXRs coexist in most cells, and the effects of RA on cellular differentiation and death may reflect selective activation of RARs and/or RXRs (1, 7, 8). RAR, vitamin D receptor, thyroid hormone receptor, peroxisome proliferator-activated receptor, and LXR-α, an orphan member of the nuclear receptor superfamily, preferentially bind to their hormone response elements in vitro as heterodimers complexed with RXR (9–11). Thus, RXRs seem to be essential pleiotropic regulators of several signaling pathways.

Naturally occurring and synthetic ligands have been described that have distinctive binding properties and transactivation effects on the various RAR and RXR subtypes, thereby allowing differential modulation of retinoid receptor gene expression. ATRA binds RAR with high affinity but does not bind to RXR, whereas 9-cis-RA, an isomer of ATRA, is a bifunctional ligand that binds and transactivates both RARs and RXRs (1, 12). Because 9-cis-RA is known to interact with all known retinoid IR subtypes (unlike ATRA), it may lead to a broader or a different spectrum of activity than previously described retinoids; 9-cis-RA also seems to have substantially less affinity for the CRABP, a RA carrier protein that does not transduce retinoid signals, which may also lead to a unique activity profile (13).

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9-cis-RA inhibited the growth of tumor cell lines of both hematological and squamous epithelial origin.\(^3\) In vitro pharmacological testing has shown that 9-cis-RA induces differentiation of HL-60, a human leukemic cell line, and inhibits proliferation in a number of murine and human tumor cell lines with responses equal to or better than those seen with ATRA. Furthermore, 9-cis-RA has also been shown to induce apoptosis in HL-60 and squamous epithelial carcinoma cell lines. In vivo, 9-cis-RA blocks the formation of papillomas in a two-stage model of carcinogenesis, and it can retard the growth of primary human head and neck tumors in a nude mouse xenograft model. These data indicate that 9-cis-RA may differentially regulate cellular differentiation, proliferation, and cell death with a retinoid IR activation profile, unlike previously described retinoids. This Phase I clinical trial of 9-cis-RA was conducted as a dose-finding study to determine the safety, tolerability, PKs, and metabolic profile of this agent in patients with advanced cancer.

**PATIENTS AND METHODS**

**Patient Selection.** Key eligibility criteria included: (a) histologically confirmed advanced cancer having failed standard therapy; (b) 18 years of age or older; (c) Eastern Cooperative Oncology Group performance status of 0–2; (d) adequate hematological, hepatic, and renal function; and (e) negative pregnancy test and effective means of contraception. Exclusion criteria included: (a) surgery, chemotherapy, radiotherapy, or investigational therapy within 21 days of the study; (b) brain metastases; and (c) concurrent vitamin A (or other retinoid) use.

**Treatment Plan.** This study was an open-label, uncontrolled, multiple-dose, dose escalation, safety evaluation study of oral 9-cis-RA (ALR1057; Panretin capsules) in patients with advanced cancer. The drug was supplied by Ligand Pharmaceuticals, Inc. as 10- and 25-mg soft-gelatin capsules, and doses were rounded to the nearest 5 mg. Patients were seen weekly for the first 4 weeks. In the absence of progressive disease, patients were allowed to continue on treatment in 4-week intervals. Dose escalation was not permitted within individual patients, and the starting dose was 5 mg/day. Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria. If DLT was not observed in three patients, the dose was escalated to the next dose level using a modified Fibonacci schedule. If DLT was observed in one of three patients, three additional patients were accrued at that dose level. The MTD was defined as the highest dose level that resulted in no more than one of six patients experiencing DLT. Standard laboratory tests including complete blood counts, chemistry survey, coagulation tests, lipid profiles, thyroid tests, and urinalysis were followed during the study. Imaging studies to evaluate possible tumor response were performed as clinically indicated during the study.

**PK Studies.** Patients underwent PK studies on days 1 and 15. After an overnight fast, 9-cis-RA was taken after ingestion of a liquid formula of defined lipid content (Ensure; 250 ml; Abbott Laboratories, Columbus, OH). Heparinized whole-blood samples were collected before dosing and at the 0.5, 1, 2, 4, and 6 hour time points. Plasma samples were obtained after centrifugation and stored at −20°C.

**Analytical Methods.** Plasma 9-cis-RA and ATRA concentrations were determined by a validated high-performance liquid chromatography method as described previously (14). 9-cis-RA PK parameter values were determined by noncompartmental methods using the WinNonlin Version 1.1 PK analysis program (Scientific Consulting, Inc.). \(C_{\text{max}}\) and \(t_{\text{max}}\) values were recorded as observed. Apparent terminal elimination rate constants (\(\lambda_z\)) were determined as the slope of the terminal log-linear portion of the plasma concentration-time profile, and terminal elimination \(t_{1/2}\) values were determined as 0.693/\(\lambda_z\). The AUC value from time 0 to 6 hours [\(\text{AUC}_{0-6}\)] was determined by linear trapezoidal approximation. \(\text{AUC}_{0-\infty}\) was determined by summing \(\text{AUC}_{0-6}\) and (C(last)/\(\lambda_z\)) parameter values. Ratios of repeat dose (day 15–34) \(\text{AUC}_{0-\infty}\), divided by day 1 \(\text{AUC}_{0-\infty}\) were determined.

**RESULTS**

**Patient Characteristics and Responses.** A total of 41 patients were treated with 9-cis-RA over 6 q.d. dose levels and subsequently three b.i.d. dose levels. Patient characteristics are shown in Table 1. No major antitumor responses were observed.

**Adverse Events.** Headache was the most frequent adverse event and was observed in 26 of 41 patients (Table 2). Headaches typically began on the first day of treatment with 9-cis-RA, and in the majority of cases, they were controlled with medications (including nonsteroidal anti-inflammatory drugs, acetaminophen, and/or narcotics). Headaches were moderate or severe but controllable (grade 2) in 8 of 41 patients and were unrelenting and severe (grade 3) in 3 of 41 patients. Skin toxicity was observed in 11 of 41 patients and was the next most common toxicity. Grade 1 skin toxicity was observed in 10 of 41 patients. Headache was the most frequent adverse event and was observed in 26 of 41 patients.

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\(^3\) R. Heyman, unpublished observations.
patients and was characterized by skin dryness and erythema. Grade 2 skin toxicity was observed in only one patient; it was seen at the 140 mg/m²/day dose level and was characterized by mild peeling at the fingers. Facial flushing was experienced by 10 of 41 patients. The flushing was typically mild (grade 1/2) and not dose limiting. Triglyceride elevation occurred in 6 of 41 patients: 4 patients with WHO grade 1 (2.5–5 × upper limit of normal); 1 patient with grade 2 (5–10 × upper limit of normal); and 1 patient with grade 3 (10–20 × upper limit of normal) triglyceride elevation. There were no cases of symptomatic hypertriglyceridemia.

At doses of 5–50 mg/m²/day, most patients had either no clinical toxicity or toxicity limited to mild (grade 1) facial flushing and skin dryness. The 83 mg/m²/day dose level was expanded to six patients, because one patient experienced a grade 3 transaminase elevation that was dose limiting. Two patients were added to the 83 mg/m²/day dose level to replace patients that did not complete the full 29 days due to progressive disease. Of these eight patients, the majority of the toxicities were grade 1. At the 140 mg/m²/day dose level, headaches became more problematic; all of the patients experienced grade 1 or 2 headaches. At this dose level, one patient experienced an ocular DLT characterized by retinal pigment epithelial detachments in the left eye and retinal hemorrhages in the right eye, and another patient had dose-limiting transaminase elevation (grade 3). The study was extended to determine the PKs and tolerability profile of b.i.d. dosing. All of the patients treated at 70 mg/m² b.i.d. experienced headache, and in two patients, it was dose limiting (grade 3); also, one patient at this dose level had dose-limiting hyperbilirubinemia. Dose levels of 60 b.i.d. and 50 mg/m² b.i.d. were subsequently studied. At 50 mg/m² b.i.d., two patients experienced grade 4 hypercalcemia that was probably related to 9-cis-RA treatment; one patient with hypercalcemia developed associated renal failure, seizures, sepsis, and respiratory failure and ultimately died.

**PKs.** PKs of 9-cis-RA were examined on days 1 and 15 for all dose levels. After daily administration on day 1, the mean AUCₜₒ₋₆ₜ and Cₘₐₓ increased dose proportionally (Fig. 1), and tₘₐₓ values ranged between 1 and 6 h after dose administration. With b.i.d. administration, the mean AUCₜₒ₋₆ₜ and Cₘₐₓ also increased dose proportionally; however, 140 mg/m² q.d. resulted in higher AUCₜₒ₋₆ₜ and Cₘₐₓ values as compared to 70 mg/m² b.i.d.

After repeat daily doses of 5–83 mg/m² q.d., 9-cis-RA AUCₜₒ₋₆ₜ and Cₘₐₓ values were comparable to day 1 values. After repeat daily dosing of 140 mg/m² q.d., 9-cis-RA AUCₜₒ₋₆ₜ...
and \( C_{\text{max}} \) values were consistently lower (70% reduction in mean \( \text{AUC}_{(0-6)} \)) than day 1 values. After repeat b.i.d. dosing of 50–70 mg/m², mean repeat AUC values were 48–77% lower than day 1 values (Fig. 2). The magnitude of the reduction in AUC values after repeat b.i.d. dosing tended to be greater than that observed after repeat q.d. dosing.

When determinable, apparent 9-cis-RA terminal elimination \( t_{1/2} \) values were generally between 1 and 2 h. Detectable day 15 predose 9-cis-RA concentrations (≥2.5 ng/ml) were only sporadically observed after doses up to 83 mg/m²/day but were frequently observed at doses of 140 mg/m²/day. 4-oxo-9-cis-RA was identified as a major metabolite of 9-cis-RA (14). No isomerization or minimal isomerization of 9-cis-RA to ATRA, 13-cis-RA, or 9,13-di-cis-RA was observed. In patients for whom a reduction in 9-cis-RA concentrations was observed, a similar magnitude reduction in 4-oxo-9-cis-RA concentrations was also observed during repeat administration.

**DISCUSSION**

The goals of this study were to determine the safety, tolerability, PKs, and metabolic profile of 9-cis-RA in advanced cancer patients. In our study, the MTD was exceeded at the 140 mg/m² dose level, with two of three patients experiencing DLT (ocular toxicity and transaminitis). As a result, the MTD for 9-cis-RA AUC\(_{(0-6)}\) values were comprised to day 1 values. After repeat daily doses of 50–140 mg/m² q.d. (○), 9-cis-RA AUC\(_{(0-6)}\) values were consistently lower (70% reduction in mean AUC\(_{(0-6)}\)) than day 1 values. After repeat daily doses of 140 mg/m² q.d. (○), 9-cis-RA AUC\(_{(0-6)}\) values were consistently lower (70% reduction in mean AUC\(_{(0-6)}\)) than day 1 values. After repeat b.i.d. dosing of 50–70 mg/m² (□), mean repeat AUC values were 48–77% lower than day 1 values.

![Graph showing the ratio of AUC\(_{(0-6)}\) values to day 1 AUC values.](https://cancerres.aacrjournals.org/content/72/4/1440/F2.large.jpg)

**Fig 2**: Ratio of day 15/day 1 AUC\(_{(0-6)}\). After repeat daily doses of 5–83 mg/m² q.d. (○), 9-cis-RA AUC\(_{(0-6)}\) values were comparable to day 1 values. After repeat daily doses of 140 mg/m² q.d. (○), 9-cis-RA AUC\(_{(0-6)}\) values were consistently lower (70% reduction in mean AUC\(_{(0-6)}\)) than day 1 values. After repeat b.i.d. dosing of 50–70 mg/m² (□), mean repeat AUC values were 48–77% lower than day 1 values.

Another major issue in retinoid therapy is maintenance of drug levels. ATRA has been shown to induce remission in patients with APML (5). Unfortunately, continuous dosing with ATRA is associated with a gradual decline in plasma levels and relapse of disease (19). In preclinical studies, 9-cis-RA plasma levels were maintained with continuous dosing (20, 21), and in this study, day 15 levels were maintained (as compared to day 1) up to the 83 mg/m² dose level. In Miller’s study, 9-cis-RA levels were maintained on days 15 and 29 up to the 140 mg/m² dose level. The reasons for this difference in drug levels are not entirely known but may be due to the increased binding of RA to cytoplasmic proteins that may be up-regulated or the induction of enzymes that catabolize RA. ATRA is known to have a high affinity for CRABPs, and binding to CRABPs can result in the lowering of plasma and intracellular levels of active retinoids. It has been reported that CRABP expression is increased at the time of APML relapse as compared to the levels before...
the initiation of ATRA treatment (22, 23). 9-cis-RA is thought to have substantially less affinity for CRABP, therefore it may not be subject to increased carrier protein binding (13, 24). Also, because CRABP may facilitate the delivery of RA to the microsomal oxidases that catalyze its degradation, and oxidation of RA by cytochrome P-450 monooxygenase is the major pathway of RA inactivation, 9-cis-RA may be less vulnerable to oxidative degradation (25, 26). Other potential mechanisms that have been suggested to play a role in plasma and cellular levels of RA include altered glucuronidation (a major pathway of metabolism of RA; Ref. 27) and overexpression of multidrug resistance (28). It is possible that 9-cis-RA is bound to CRABP and/or metabolized differently from ATRA, with 9-cis-RA levels being more effectively maintained. Because 9-cis-RA has shown activity in APLM and may not be as susceptible to perturbations in retinoid metabolism, it merits further study in APLM.

Retinoids have shown more promise as cancer-preventive agents than any other class of drug (3, 4). For a chemopreventive or differentiating agent, the therapeutic (efficacy:toxicity) ratio is an important consideration, because prolonged administration is likely to be required. 9-cis-RA has much less cutaneous and mucous membrane toxicity than the previously described retinoids and was very well tolerated at 83 mg/m²/day, so that compliance may not be as limiting a factor with its chronic administration.

As our experience with retinoids grows, so does the ability to better manage the toxicities associated with retinoid use. Stepwise escalation of retinoid therapy may avoid the development of headaches and other neurological symptoms (29). Another potential way suggested to ameliorate toxicity is vitamin E (α-tocopherol) administration. Dietary vitamin A reduces the absorption of α-tocopherol in the gastrointestinal tract and increases α-tocopherol clearance from the plasma in a chick model (30). Chronic oral β-carotene administration resulted in a 40% reduction in α-tocopherol (31), and patients receiving both 13-cis-RA and α-tocopherol (rather than 13-cis-RA alone) had a reduction in the major toxic effects of 13-cis-RA without affecting 13-cis-RA plasma concentrations (32, 33). It has been postulated that reduction of the α-tocopherol antioxidant effect leaves the membranolytic activity of retinoids unopposed, and supplementation of α-tocopherol may restore this balance (29).

9-cis-RA is a natural retinoid that has been recently characterized (12), and this study provides data demonstrating our progress in the development of safer and more effective retinoids. Currently, 9-cis-RA is being studied in a number of Phase II clinical trials, including AIDS-related Kaposis’s sarcoma, hormone-refractory prostate cancer, and APLM. A Phase III study is also underway to study the antitumor effects of 9-cis-RA topical gel in AIDS-related Kaposis’s sarcoma.

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