Initial Clinical Trial of the Retinoid Receptor *pan* Agonist 9-*cis* Retinoic Acid[1]

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ABSTRACT

The retinoid response is mediated by families of nuclear receptors, the retinoic acid receptors (RARs), and the retinoid X receptors. All-*trans* retinoic acid (RA) binds only RARs and induces its own metabolism. In contrast, 9-*cis* RA is a newly identified agonist for both RARs and retinoid X receptors. We undertook a dose-ranging study to examine the safety, clinical tolerance, and pharmacokinetics of 9-*cis* RA in patients with advanced cancer. Thirty-four patients received once daily p.o. doses of 9-*cis* RA (administered as LGD1057) ranging from 5 to 230 mg/m^2 for 4 weeks. Pharmacokinetic studies were performed on 28 patients at seven dose levels. 9-*cis* RA was generally well tolerated. Headache was the most common dose-limiting adverse effect. Other prominent reactions included facial flushing, myalgia, dyspnea, hypertriglyceridemia, and hypercalcemia. Relative to other retinoids, mucocutaneous reactions were mild. No major antitumor responses were observed. Pharmacokinetic analysis revealed that the day 1 area under the plasma concentration × time curves (AUCs) were proportional to the dose. Up through doses of 140 mg/m^2, the day 1 AUCs were similar to those on days 15 and 29. At higher doses, however, AUCs tended to decline with repeat dosing. 9-*cis* RA is a novel compound that exploits a newly identified pathway of retinoid receptor biology that may be relevant to tumor cell proliferation and differentiation. We recommend a dose of 140 mg/m^2 for single-agent trials utilizing a once-daily schedule of administration.

INTRODUCTION

The natural retinoids, 9-*cis* RA,[3] 13-*cis* RA, and their geometric isomer, all-*trans* RA, comprise a group of structurally similar compounds with diverse pharmacokinetic, therapeutic, and adverse effect profiles (1). Isotretinoin (13-*cis* RA) has activity in several clinical settings, including the treatment of certain premalignant lesions such as oral leukoplakia (2), as well as some advanced cancers such as squamous cell carcinomas of the skin and cervix (3, 4). Furthermore, all-*trans* RA has been shown to induce remission in a very high proportion of patients with APL (5). The natural retinoids act by binding to intranuclear receptors that interact with specific DNA response elements and ultimately regulate transcriptional activity of retinoid target genes (6).

RARs were originally described as receptors for all-*trans* RA, and *RAR*-α is one of the genes disrupted by the 15;17 translocation in APL (7, 8). In 1990, a new family of receptors, the RXRs was described. These receptors act as cofactors that increase the DNA-binding affinity not only of RARs, but also of the nuclear receptors for VDR, THR, and PPARs (9). In 1992, 9-*cis* RA was identified by two groups as being the natural ligand of the RXRs (10, 11). Unlike all-*trans* RA, which only binds RARs, 9-*cis* RA binds and activates both RARs and RXRs (6).

Preclinical studies have suggested that 9-*cis* RA may have somewhat greater potency than all-*trans* RA in its ability to induce differentiation and inhibit proliferation of acute myelogenous leukemia cells (12, 13). *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.[4] Given this broad spectrum of action, as well as the potentially central role for this newly discovered biological pathway, we conducted a dose-ranging study to determine the toxicity, safety, pharmacokinetics, and metabolic profile of 9-*cis* RA in patients with advanced cancer.

PATIENTS AND METHODS

Patient Selection. Eligible patients had histologically confirmed cancer, were at least 18 years of age, and had an Eastern Cooperative Oncology Group performance status of 0, 1, or 2. Patients who had undergone major surgery within the preceding 3 weeks, who had recently received cytotoxic chemotherapy or other investigational agents, or who had a history of brain metastasis or cardiomyopathy were excluded. Other requirements included: total leukocyte count ≥4,000/mm^3, he-

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[3] The abbreviations used are: RA, retinoic acid; APL, acute promyelocytic leukemia; RAR, retinoic acid receptor; RXR, retinoid X receptor; VDR, vitamin D receptor; THR, thyroid hormone receptor; PPAR, peroxisome proliferation-activating receptor; AUC, area under the plasma concentration × time curve.
[4] R. Heyman, personal communication.

moglobin ≥9 g/dl, platelets ≥100,000/mm^3, and adequate coagulation, hepatic, and renal function. All patients must have failed or not have been candidates for standard therapy when available. Signed informed consent was required, and the protocol was reviewed and approved in advance by this Center's Institutional Review Board.

Treatment Plan. 9-*cis* RA (LGD1057) was administered once daily p.o. for 4 weeks. 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 month. In the absence of progressive disease, patients could be continued on treatment in additional 1-month blocks with reevaluation every 4 weeks. Dose escalation in an individual was not permitted, and treatment proceeded from a starting dose level of 5 mg/m^2. Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria. During the study, if one of three patients experienced any prespecified grade of toxicity, that level was to be expanded to total six patients. The maximum tolerated dose was defined as the highest dose level that resulted in not more than one patient experiencing a dose-limiting toxicity among at least six patients who completed 4 weeks of treatment at that dose level. Various laboratory parameters were followed during the study, including serial blood counts, coagulation tests, lipid profiles, thyroid function tests, and urinalyses. Imaging studies were obtained as clinically indicated to evaluate possible antitumor effects.

Pharmacokinetic Studies. Selected patients underwent pharmacokinetic studies on days 1, 15, and 29. In these patients, following an overnight fast, 9-*cis* RA was taken immediately after ingestion of a liquid formula of defined lipid content (Ensure, 250 ml of an 8.8 g fat/8 fluid ounces; Abbott Laboratories, Columbus, OH). Heparinized whole-blood samples were collected prior to dosing and at 0.5, 1, 2, 4, and 6 h after the dose was taken. Plasma samples were obtained after centrifugation and were stored at −20°C. Samples were protected from direct light and transported in amber-colored bags.

Analytical Methods. Prior to assay, an equal volume of isopropanol was added to the plasma, and the protein precipitate was removed by centrifugation. The supernatant was then analyzed with high-performance liquid chromatography without further modification using a Spherisorb ODS-1 column and the methods previously described by Creech-Kraft *et al.* (14). A gradient elution was set up with 50% methanol/40 mM ammonium acetate (pH = 7.0) as solvent A and 100% methanol as solvent B. The gradient ranged from 30% B:70% A to 100% B in 50 min, with a flow rate of 1 ml/min, and was monitored at 354 nm. The retention times for 13-*cis* RA, 9-*cis* RA, and all-*trans* RA were approximately 29, 30, and 31 min, respectively. With a 200-ml injection volume, the detection limit is about 10 ng/ml. The AUC was then calculated according to the trapezoidal method from $t = 0$–∞. The proportion of the AUC that was extrapolated from $t = 6$–∞ varied from patient to patient based on that individual's measured concentrations at the different sampling points (15).

RESULTS

Patient Characteristics. Thirty-four patients were treated with varying doses of 9-*cis* RA. Relevant patient characteristics are listed in Table 1. Twelve patients had not received previous chemotherapy, and the median number of previous chemotherapy regimens was one.

Table 1 Pretreatment characteristics of patients treated with 9-*cis* RA

	No.	%
Patients treated	34	
Age (yr)		
Median	62	
Range	35–71	
Eastern Cooperative Oncology Group performance status		
0	8	24
1	20	59
2	6	17
Sex		
Female	11	32
Male	23	68
Malignancy		
Non-small cell lung cancer	16	47
Sarcoma	5	15
Other	13	38
Previous chemotherapy regimens		
None	12	35
One	11	32
Two	5	15
Three or more	6	18

Response and Response Duration. No major antitumor responses were observed. Cutaneous disease in one patient with cutaneous T-cell lymphoma had improved somewhat at day 29; however, this patient was removed from the study due to unrelated worsening of preexisting congestive heart failure. Six patients had disease stabilization for 3, 3, 4, 4+, 5, and 5 months, respectively.

Adverse Effects. Adverse effects that were observed with 9-*cis* RA are displayed in Table 2. Preexistent baseline laboratory abnormalities included ten cases of grade 1 and two cases of grade 2 anemia, seven cases of grade 1, and one case each of grade 2 and 3 elevations in alkaline phosphatase (both in patients with bony metastasis from prostate cancer), three cases of grade 1 elevations of serum creatinine, and two cases of grade 1 hypercalcemia. All patients at each of the first six dose levels (5 through 140 mg/m^2) completed the 4-week study period, and none required dose attenuation because of toxicity. The first patient treated at a dose of 230 mg/m^2 developed hypercalcemia (serum calcium, 12.6 mg/dl), and treatment was discontinued until this abnormality was corrected. A second patient at this level developed a severe headache (grade 3), fever to 101°F, and generalized arthralgia. The drug was discontinued for 24 h with resolution of symptoms, after which the drug was restarted at two thirds of the previous dose. Only a mild headache recurred, and the dose was escalated back to 230 mg/m^2 without further complications. A third patient with lung cancer developed increasing dyspnea that improved after interruption of therapy, but this problem recurred after rechallenge. Three more patients were enrolled at this level, two of whom developed moderately severe headaches (grade 2). Symptoms resolved upon drug withdrawal; however, this dose was believed to be in excess of the maximum tolerated dose, and no further dose escalation was attempted.

Table 2 Adverse reactions observed with 9-*cis* RA

	Dose level (mg/m²/day)																										
	5 (*n* = 3)			15 (*n* = 3)			30 (*n* = 3)			50 (*n* = 3)			83 (*n* = 3)			140 (*n* = 6)			180 (*n* = 7)			230 (*n* = 6)			Cumulative (*n* = 34)		
Toxicity grade	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Headache	0	0	0	2	0	0	1	0	0	2	0	0	1	0	0	4	0	0	3	2	0	2	3	1	15	5	1
Skin (mucocutaneous)	0	0	0	1	0	0	0	0	0	2	0	0	3	0	0	3	1	0	2	1	0	1	3	0	12	5	0
Pulmonary	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	2	0	0	0	0	0	1	0	0	6	0
Hematological																											
Hemoglobin	0	0	0	1	0	0	1	1	0	3	0	0	3	0	0	3	1	0	3	2	2	3	1	0	17	5	2
Leukocytes	0	0	0	0	0	0	1	0	0	1	0	0	2	0	0	2	0	0	0	1	1	3	1	0	9	2	1
Platelets	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0
Biochemical																											
Alkaline phosphatase	1	0	0	3	0	0	3	0	0	2	0	0	1	0	0	4	0	0	3	1	1	2	0	0	19	1	1
Calcium	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	3	1	0	4	0	1	2	0	1	12	1	2
Creatinine	0	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	2	0	0	0	0	0	5	1	0
Serum glutamate oxaloacetate transferase	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	3	0	0	2	1	0	2	0	0	10	1	0
Hematuria	3	0	0	1	0	0	2	0	0	2	0	0	3	0	0	1	0	0	3	0	0	2	0	0	17	0	0
Proteinuria	0	0	0	1	0	0	1	0	1	0	1	0	1	0	0	0	0	0	2	0	1	1	0	0	6	1	2

Table 3 Mean maximum percentage of change from initial values in serum concentrations of total cholesterol and triglycerides

Dose (mg/m²)	No. of patients	Cholesterol	Triglyceride
5	3	+10.4	+49.8
15	3	+11.7	−5.0
30	3	+19.5	+65.9
50	3	+45	+144
83	3	+43	+301
140	5	+47	+621
180	6	+43	+353
230	5	+106	+902

Table 4 Mean day 1 pharmacokinetic parameters of 9-*cis* RA

Dose (mg/m²)	No. of patients	$t_{1/2}$ (h)	C_{pmax} (ng/ml)	t_{max} (h)
5	3	1.8	26	1.3
15	3	1.7	64	2
30	3	1.1	145	1.7
83	3	1.1	267	2.3
140	4	1.3	183	2.5
180	6	1.5	728	1.8
230	6	1.5	1003	2.0

Seven patients were then studied at 180 mg/m². Two patients developed increased pain in known areas of disease shortly after starting and refused to continue. One patient developed a serum calcium of 12.5 mg/dl, which normalized after drug discontinuation. Three other patients completed the 4-week study period with no toxicity greater than grade 1. Since only four of the seven patients treated at 180 mg/m² were able to complete the planned 4 weeks of treatment, 140 mg/m² was recommended as the Phase II dose.

Among other prominent effects, facial flushing was observed in 13 patients. This was seen more commonly, but not exclusively, at the higher dose levels and characteristically occurred 2–4 h after drug ingestion, frequently in association with headache. These effects tended to become less severe with extended treatment. Myalgias and arthralgias, usually transient and beginning shortly after initiation of treatment, were seen in 12 patients, 2 of whom briefly required narcotic analgesics. Of the 16 patients treated who had non-small cell lung cancer, six developed worsening dyspnea during treatment. This effect was not dose related nor associated with disease progression at the time, and these symptoms usually improved or resolved after discontinuation of the drug. Notably, this effect was not seen in patients with other diseases. Hyperlipidemia was characterized by mild hypercholesterolemia and moderate to marked hypertriglyceridemia (Table 3). This effect was noted in almost all patients, and it appeared to be both dose and time related, increasing with protracted use.

Pharmacokinetics. Pharmacokinetic data were obtained from 28 patients. Of these, 16 patients were studied on days 1, 15, and 29. Seven patients failed to complete all three phases because of drug toxicity or progression of disease. Pharmacokinetic results are presented in Tables 4 and 5. Notably, AUCs on day 1 were proportional to the dose except at the highest dose level. At this dose (230 mg/m²) the day 1 AUC is significantly greater than expected. Up through 140 mg/m², the AUCs changed little with repeat dose administration. However, at doses of 180 and 230 mg/m², AUCs on days 15 and 29 were generally lower than on day 1, a result that suggested that the pharmacokinetics may be time and dose dependent. Metabolites of 9-*cis* RA that were detectable in plasma only at the two highest dose levels included all-*trans* RA and 13-*cis* RA.

DISCUSSION

This study sought to determine the dose-limiting toxicity, safety, pharmacokinetic parameters, metabolic profile, and potential antitumor activity of 9-*cis* RA. We observed a pattern of adverse effects somewhat different from our recent experience

Table 5 Mean and range of AUC of 9-*cis* RA as a function of time and dose

Dose (mg/m^2)	Day	No. of patients	Mean 9-*cis* RA AUC (ng · h/ml)	Range of 9-*cis* RA AUC (ng · h/ml)
5	1	3	74	36–103
	15	3	48	12–102
15	1	3	212	98–423
	15	3	155	41–265
	29	2	163	155–170
30	1	3	363	248–439
	15	3	308	193–474
	29	3	378	185–518
	≥43	1	219	
83	1	3	775	604–1005
	15	3	556	300–718
	29	3	546	394–749
	≥43	1	1027	
140	1	4	573	290–857
	15	4	534	162–991
	29	4	550	205–745
180	1	6	2111	834–3517
	15	3	1036	324–2049
	29	3	828	640–1115
230	1	6	3841	1171–8510
	15	3	1558	423–2968
	29	2	982	433–1531

with other retinoids, primarily all-*trans* RA (16, 17). Skin reactions, especially, cheilitis, xeroderma, or skin peeling, have occurred in up to 90% of patients treated in a Phase I trial with all-*trans* RA (18). However, skin and mucous membrane toxicity was generally mild during treatment with 9-*cis* RA and was observed in less than half of our patients.

Hyperlipidemia, particularly hypertriglyceridemia, has been reported in approximately 25% of patients treated with all-*trans* RA or 13-*cis* RA (18–20). When one applies the same grading system for triglycerides as suggested by Lee *et al.* (18), hypertriglyceridemia was seen in 42% of our patients. Recent work has shown that PPARs, like RARs, are members of the steroid/thyroid nuclear receptor superfamily (21) which heterodimerize with RXRs. Addition of either all-*trans* or 13-*cis* RA further enhances PPAR-driven transcription of the *acyl-CoA* oxidase gene, the rate-limiting step in fatty acid oxidation. Therefore, use of 9-*cis* RA might be expected to result in a more favorable lipid profile than that of a pure RAR agonist such as all-*trans* RA. Thus, the paradoxical adverse effect observed on lipid profiles associated with 9-*cis* RA use is likely related to other, poorly understood, effects of this retinoid on lipoprotein synthesis or clearance (22).

Hypercalcemia was seen in 44% of the patients. Although the reaction was mild in all but two cases, this reaction is uncommon during treatment with all-*trans* RA. Since 9-*cis* RA is a high-affinity ligand for RXRs, and since VDR-RXR heterodimers can activate vitamin D response elements, increased intracellular concentrations of 9-*cis* RA could plausibly amplify transcription of 1,25-dihydroxyvitamin D_3 target genes that might lead to accelerated bone resorption or reduced urinary calcium excretion. However, VDR elements may also be activated by monomeric VDR or VDR-VDR homodimers; thus, the relative amounts of both ligands present, as well as the receptor levels expressed in a given cell type, may contribute to the presence or absence of hypercalcemia in a given individual (23, 24).

RXRs also form heterodimers with THR, and this coupling has been shown to increase the affinity of THR for their response elements (25–27). However, we observed no significant change in thyrotropin levels even at the highest doses. There are several plausible explanations for this finding. *In vitro*, the addition of T3 enhances formation of the thyroid receptor/RXR preinitiation complex; however, the effect of the addition of 9-*cis* RA on the T3 response is variable (27). Moreover, the results of clinical thyroid function tests can be significantly affected by chronic illnesses such as malignancy (28). Finally, other gene products regulated by T3 are not readily measured clinically (28).

All-*trans* RA has engendered significant interest for its ability to induce remission in patients with APL (5). However, continuous p.o. dosing with this agent is associated with a progressive decline in plasma concentrations (29) that has been linked to relapse and resistance in APL. In preclinical studies, plasma levels of 9-*cis* RA appeared (30, 31) to be sustained with chronic dosing and this feature, plus its potentially unique biological characteristics, made it appealing for clinical exploration. In our study this pharmacokinetic pattern appeared to be preserved at doses up through 140 mg/m^2. However, at higher doses substantial declines in the AUCs of 9-*cis* RA were observed after 15 and 29 days of treatment compared to day 1. We also detected increased plasma levels of the isomers, all-*trans* RA and 13-*cis* RA, in plasma at these doses. Simple conversion of 9-*cis* RA to these isomers does not fully explain these pharmacokinetics since measured concentrations of all-*trans* and 13-*cis* RA were approximately 10 ng/ml even at the highest dose level studied. Conceivably, enzyme(s) responsible for 9-*cis* RA metabolism may be saturated at these higher doses, and the parent compound may be directed to other, normally secondary, metabolic pathways of isomerization or oxidation that are potentially inducible by 9-*cis* RA. This hypothesis is supported by our observation that the day 1 AUC at the 230-mg/m^2 dose level was significantly greater than that predicted by simple dose proportionality. Recently, glucuronidation has been suggested to be a major pathway in the metabolism of all-*trans* and 13-*cis* RA, and future studies will attempt to measure the formation of the glucuronide metabolites of 9-*cis* RA (32).

No major antitumor responses were seen in this trial. However, we have recently noted in a parallel study that 9-*cis* RA induced remission in a relapsed patient with APL (no patients with leukemia were treated in this study) who had been previously treated with all-*trans* RA (33). Preclinical data and results from clinical trials with other retinoids suggest this compound should be tested further both as a single agent and in combination with other agents (3, 4, 34). For single-agent Phase II trials using once-daily administration, we recommend a dose of 140 mg/m^2 given p.o.

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REFERENCES

1. Chytil, F. Retinoic acid: Biochemistry, pharmacology, toxicology, and therapeutic use. Pharmacol. Rev., *36:* 93S–100S, 1984.

2. Lippman, S., Batsakis, J., Toth, B. B., Weber, R. S., Lee, J. J., Martin, J. W., Hays, G. L., Goepfert, H., and Hong, W. K. Comparison of low-dose isotretinoin with beta carotene to prevent oral carcinogenesis. N. Engl. J. Med., *328:* 15–20, 1993.

3. Lippman, S. M., Parkinson, D. R., Itri, L. M., Weber, R. S., Schantz, S. P., Ota, D. M., Schusterman, M. A., Krakoff, I. H., Gutterman, J. U., and Hong, W. K. 13-*cis*-retinoic acid and interferon α-2a: effective combination therapy for advanced squamous cell carcinoma of the skin. J. Natl. Cancer Inst., *84:* 235–241, 1992.

4. Lippman, S. M., Kavanagh, J. J., Paredes-Espinoza, M., Delgadillo-Madrueno, F., Paredes-Casillas, P., Hong, W. K., Holdener, E., and Krakoff, I. H. 13-*cis*-retinoic acid plus interferon α-2a: highly active systemic therapy for squamous cell carcinoma of the cervix. J. Natl. Cancer Inst., *84:* 241-245, 1992.

5. Warrell, R. P., Jr., de The, H., Wang, Z. Y., and Degos, L. Acute promyelocytic leukemia. N. Engl. J. Med., *329:* 177–189, 1993.

6. Mangelsdorf, D. J. Vitamin A receptors. Nutr. Rev., *52:* S32–S44, 1994.

7. Borrow, J., Goddard, A. D., Sheer, D., and Solomon, E. Molecular analysis of acute promyelocytic leukemia breakpoint cluster region on chromosome 17. Science (Washington DC), *249:* 1577–1580, 1990.

8. de The, J., Chomienne, C., Lannote, M., Degos, L., and Dejean, A. The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor a gene to a novel transcribed locus. Nature (Lond.), *347:* 558–561, 1990.

9. Mangelsdorf, D. J., Ong, E. S., Dyck, J. A., and Evans, R. M. Nuclear receptor that identifies a novel retinoic acid response pathway. Nature (Lond.), *345:* 224–229, 1990.

10. Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M., and Thaller C. 9-*cis* retinoic acid is a high affinity ligand for the retinoid X receptor. Cell, *68:* 397–406, 1992.

11. Levin, A. A., Sturzenbecker, L. J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C., Rosenberger, M., Lovey, A., and Grippo, J. F. 9-*cis* retinoic acid stereoisomer binds and activates the nuclear receptor RXRα. Nature (Lond.), *355:* 359–361, 1992.

12. Sakashita, A., Kizaki, M., Pakkala, S., Schiller, G., Tsuruoka, N., Tomosaki, R., Cameron, J. F., Dawson, M., and Koeffler, H. P. 9-*cis*-retinoic acid: effects on normal and leukemic hematopoiesis *in vitro*. Blood, *81:* 1009–1016, 1993.

13. Bollag, W., Majewski, S., and Jablonska, S. Cancer combination chemotherapy with retinoids: experimental rationale. Leukemia (Baltimore), *8:* 1453, 1994.

14. Creech-Kraft, J., Echoff, C., Kuhnz, W., Lofberg, B., and Nau, H. Automated determination of 13-*cis*- and all-*trans*-retinoic acid, their 4-oxo metabolites and retinol in plasma, amniotic fluid and embryo by reversed-phase high-performance liquid chromatography with a precolumn switching technique. J. Liq. Chromat., *11:* 2051–2069, 1988.

15. Gibaldi, M., and Perrier, D. Pharmacokinetics, 2nd ed., pp. 480–496. New York: Marcel Dekker, 1982.

16. Miller, V. A., Rigas, J. R., Muindi, J. R. F., Tong, W. P., Venkatraman, E., Kris, M. G., and Warrell, R. P., Jr. Modulation of all-*trans* retinoic acid pharmacokinetics by liarozole. Cancer Chemother. Pharmacol., *34:* 522–526, 1994.

17. Rigas, J. R., Francis, P. A, Muindi, J. R. F., Huselton, C., DeGrazia, F., Kris, M. G., Young, C. W., and Warrell, R. P., Jr. Constitutive variability in the pharmacokinetics of the natural retinoid, all-*trans* retinoic acid, and its modulation by ketoconazole. J. Natl. Cancer Inst., *85:* 1921–1926, 1993.

18. Lee, J. S., Newman, R. A., Lippman, S. M., Huber, M. H., Minor, T., Raber, M. N., Krakoff, I. H., and Hong, W. K. Phase I evaluation of all-*trans*-retinoic acid in adults with solid tumors. J. Clin. Oncol., *11:* 959–966, 1993.

19. Bershad, S., Rubinstein, A., Paterniti, J. R., Jr., Le, N. A., Poliak, S., Heller, B., Ginsberg, H. N., Fleischmajer, R., and Brown, W. V. Changes in plasma lipids and lipoproteins during isotretinoin therapy for acne. N. Engl. J. Med., *313:* 981–985, 1985.

20. Rigas, J. R., Miller, V. A., Levine, D. M., Venkatraman, E. S., Gill, G. M., Truglia, J. A., and Warrell, R. P., Jr. Lipoprotein alterations in patients treated with novel retinoids. Proc. Am. Assoc. Cancer Res., *36:* 506, 1995.

21. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K., and Wahli, W. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. Proc. Natl. Acad. Sci. USA, *90:* 2160–2164, 1993.

22. Marsden, J. R. Lipid metabolism and retinoid therapy. Pharmacol. Ther., *40:* 55–65, 1989.

23. Yu, V. C., Delsert, C., Andersen, B., Holloway, J. M., Devary, O. V., Naar, A. M., Kim, S. Y., Boutin, J. M., Glass, C. K., and Rosenfeld, M. G. RXRβ: A coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. Cell, *67:* 1251–1266, 1991.

24. Carlberg, C., Bendik, I., Wyss, A., Meier, E., Sturzenbecker, L. J., Grippo, J. F., and Hunziker, W. Two nuclear signaling pathways for vitamin D. Nature (Lond.), *361:* 657–660, 1993.

25. Hallenbeck, P. L., Marks, M. S., Lippoldt, R. E., Ozato, K., and Nikodem, V. M. Heterodimerization of thyroid hormone (TH) receptor with H-2RIIBP (RXRβ) enhances DNA binding and TH-dependent transcriptional activation. Proc. Natl. Acad. Sci. USA, *89:* 5572–5576, 1992.

26. Zhang, X. K., Hoffman, B., Tran, P. B. V., Graupner, G., and Pfahl, M. Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. Nature (Lond.), *355:* 441–446, 1992.

27. Lee, I. J., Driggers, P. H., Medin, J. A., Nikodem, V. M., and Ozato, K. Recombinant thyroid hormone receptor and retinoid X receptor stimulate ligand-dependent transcription *in vitro*. Proc. Natl. Acad. Sci. USA, *91:* 1647–1651, 1994.

28. Brent, G. A. The molecular basis of thyroid hormone action. N. Engl. J. Med., *331:* 847–853, 1994.

29. Muindi, J., Frankel, S., Miller, W., Jakubowski, A., Scheinberg, D. A., Young, C. W., Dmitrovsky, E., and Warrell, R. P., Jr. Continuous treatment with all-*trans* retinoic acid causes a progressive reduction in plasma drug concentrations: implications for relapse and retinoid "resistance" in patients with acute promyelocytic leukemia. Blood, *79:* 299–303, 1992.

30. Adamson, P. C., Murphy, R. F., Godwin, K. A., Ulm, E. H., and Balis, F. M. Pharmacokinetics of 9-*cis*-retinoic acid in the rhesus monkey. Cancer Res., *55:* 482–485, 1995.

31. Achkar, C. C., Bentel, J. M., Boylan, J. F., Scher, H. I., Gudas, L. J., and Miller, W. J. Differences in the pharmacokinetic properties of orally administered all-*trans*-retinoic acid and 9-*cis*-retinoic acid in the plasma of nude mice. Drug Metab. Dispos., *22:* 451–458, 1994.

32. Sass, J. O., Forster, A., Bock, K. W., and Nau, H. Glucuronidation and isomerization of all-*trans*- and 13- *cis*-retinoic acid by liver microsomes of phenobarbital or 3-methylcholanthrene-treated rats. Biochem. Pharmacol., *47:* 485–492, 1994.

33. Miller, W. H., Jr., Jakubowski, A., Tong, W. P., Miller, V. A., Rigas, J. R., Benedetti, F., Gill, G. M., Truglia, J. A., Ulm, E., Shirley, M., and Warrell, R. P., Jr. 9-*cis* retinoic acid induces complete remission but does not reverse clinically required retinoid resistance in acute promyelocytic leukemia. Blood, *85:* 3021–3027, 1995.

34. Lotan, R., Dawson, M. I., Zou, C. C., Jong, L., Lotan, D., and Zou, C. P. Enhanced efficacy of combinations of retinoic acid- and retinoid X receptor-selective retinoids and α-interferon in inhibition of cervical carcinoma cell proliferation. Cancer Res., *55:* 232–236, 1995.

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