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NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN: 9-*cis*-RETINOIC ACID

DRUG IDENTIFICATION

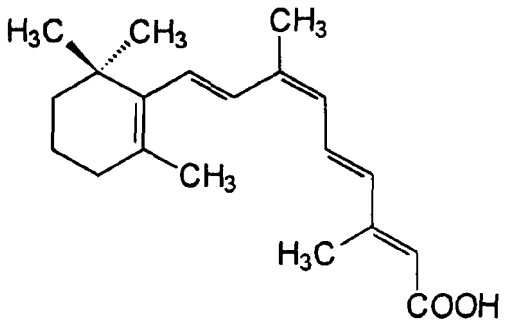
CAS Registry No.: 5300-03-8

CAS Name (9CI): 9-*cis*-Retinoic Acid
Synonyms: 9-*cis*,13-*trans*-Retinoic Acid
9-*cis*-Tretinoin
LGD1057
ALRT1057

Related Compounds:
all-*trans*-Retinoic Acid (CAS No. 302-79-4)
13-*cis*-Retinoic Acid (CAS No. 4759-42-2)

Molecular Wt.: 300.4

Structure:



EXECUTIVE SUMMARY

9-*cis*-Retinoic acid (9-*cis*-RA) is a stereo- and photoisomer of all-*trans*-retinoic acid (all-*trans*-RA) [1]. The cancer chemopreventive activities of the all-*trans* and 13-*cis* (13-*cis*-RA) isomers of retinoic acid [2] are well recognized. The biological effects of retinoids are mediated by intracellular receptors, which function as ligand-dependent transcription factors. Retinoid receptors are classified into two subfamilies, RARs and RXRs, based on differences in primary structure, sensitivity to synthetic retinoid ligands, and ability to regulate expression of different target genes [3]. all-*trans*-RA binds directly to, and transcriptionally activates, RARs; however, although it can activate RXRs to regulate expression of target genes, it is not a ligand for the RXR subfamily of receptors [1].

Recently, 9-*cis*-RA was identified as a high affinity ligand for the RXRs [3,4]; it is up to 40 times more potent than all-*trans*-RA in transactivating RXRs. 9-*cis*-RA also binds to and transactivates RARs, serving as a "bifunctional" ligand [1].

RXRs can form homodimers and are capable of acting independently; however, they also form stable

solution heterodimers with vitamin D, thyroid hormone and peroxisome proliferation-activating receptors (PPAR) [5], as well as RARs. These interactions result in positive or negative regulation of transcription. The ability of RXRs to heterodimerize with receptors responsive to several ligands suggests a central role for RXRs in hormonal signaling. Thus, RXRs define a retinoid signaling pathway distinct from that mediated by the RARs [1]. Together with the observation that the tissue distribution of RXRs and RARs differs [1], this suggests that 9-*cis*-RA, as the high-affinity ligand for the RXRs, might have distinct chemopreventive properties.

9-*cis*-RA can be delivered exogenously. It is also an *in vitro* and *in vivo* metabolite of all-*trans*-RA and has been identified as an endogenous component of mouse liver and kidney [3]. Interconversion between 9-*cis*-RA and all-*trans*-RA could afford a novel method for differential, cell-specific regulation of the two retinoid signaling pathways [1].

The first report of the chemopreventive activity of 9-*cis*-RA was published by NCI in 1994 [4]. The retinoid was a highly effective inhibitor of rat mammary tumorigenesis in the standard MNU model; furthermore, it significantly enhanced the efficacy of suboptimal doses of the antiestrogen tamoxifen. Activities that could contribute to its chemopreventive efficacy include inhibition of proliferation [4,6] and angiogenesis [7], and induction of differentiation [4,8,9] and apoptosis [10,11]. In mammary tissue specifically, the mechanism may be down-regulation of the estrogen receptor itself [12] or inhibition of estrogen-responsive gene transcription [13], suggesting a reason for enhanced efficacy when administered with an antiestrogen. This is mechanistically explained by the fact that retinoid and estrogen receptors may share the same co-activators. The Chemoprevention Branch is currently evaluating the preclinical efficacy of 9-*cis*-RA in rat colon (AOM), mammary (MNU, 50- and 100-day old rats) and prostate (CA/TP/MNU/TP) and mouse bladder (OH-BBN), lung (B(*a*)P) and lymphatic (ENU in transgenic *pim*) tumor models. Other agent combinations with 9-*cis*-RA are being investigated as chemopreventive strategies for the mammary gland in 100-day old rats, such as DFMO, DHEA, 4-HPR, vorozole, and tamoxifen. The 100-day old rats perhaps provide a better model of human breast cancer.

Identification and characterization of intermediate biomarkers as potential surrogates for cancer is a high priority of the Chemoprevention Branch. 9-*cis*-RA has modulated two histological intermediate biomarkers—aberrant crypt foci in rat colon and skin papillomas in mice.

Results of preclinical and clinical toxicity and ADME studies may be available from manufacturers of 9-*cis*-RA, including Allergan Ligand Retinoid Therapeutics, Inc. (ALRT) and Hoffman-LaRoche. Published data indicate that the drug decreased serum retinol levels in mice, a finding that was clinically significant with 4-HPR. Also in common with other retinoids, 9-*cis*-RA was teratogenic in two species. In the NCI-funded efficacy study in female rats, no signs of gross toxicity were observed at doses up to 120 mg/kg diet (*ca.* 20 μmol/kg-bw/day) for 4.5 months. Limited published pharmacokinetic data in mice and monkeys suggest that 9-*cis*-RA is absorbed ($t_{max} \leq 40$ min) and cleared ($t_{1/2}$=30–64 min) rapidly with first-order elimination. More importantly, the pharmacokinetics differed from that of the all-*trans* isomer, which induces its own metabolism and shows decreased efficacy with extended administration. The NCI, Chemoprevention Branch is examining ADME parameters in conjunction with a rat prostate efficacy study.

Limited published clinical data indicate that the oral MTD for 9-*cis*-RA is 100–140 mg/m^2 (*ca.* 11.5 μmol/kg-bw/day) for four weeks in cancer patients; headache was the main dose-limiting toxicity. Other common adverse effects were hyperlipidemia, dry skin and myalgia/arthralgia. As in rodents, 9-*cis*-RA appears to be absorbed ($t_{max} \leq 2$ hr) and excreted ($t_{1/2} \approx 1.6$ hr) fairly rapidly. Significant amounts of metabolites were excreted in urine; high amounts of presumably unabsorbed parent compound were found in feces. It is unclear if 9-*cis*-RA induces its own metabolism in humans.

Successful development of 9-*cis*-RA as a chemopreventive agent will depend on the results of future preclinical efficacy and safety studies. Chemoprevention Branch-sponsored short-term Phase II clinical trials in breast cancer and CIN II/III patients are planned; studies in head and neck and prostate may be considered in the future. Combinations with an antiestrogen in the breast cohort may also be clinically useful. Further preclinical chemopreventive, mechanistic and metabolic studies may identify additional potential target organs.

A Clinical Trials Agreement has been signed with Ligand Pharmaceuticals, Inc., so no problems with

supply of formulated 9-*cis*-RA are anticipated. For preclinical studies, one kilogram is being synthesized under contract with SRI International.

PRECLINICAL EFFICACY STUDIES

In a 1994 study funded by the Laboratory of Chemoprevention, Division of Cancer Etiology at NCI (Dr. Michael Sporn), chronic feeding of 9-*cis*-RA (60 and 120 mg/kg diet, or *ca.* 10 and 20 μmol/kg-bw/day) dramatically inhibited MNU-induced rat mammary tumorigenesis in a dose-dependent manner; it was much more efficacious than the all-*trans* isomer [4]. In this experiment, the agent also increased the efficacy of suboptimal doses of tamoxifen citrate (1.0 or 0.5 mg/kg diet). The Chemoprevention Branch is currently evaluating the preclinical efficacy of 9-*cis*-RA in rat colon (AOM), mammary gland (MNU, 50- and 100-day old rats) and prostate (CA/TP/MNU/TP) and mouse bladder (OH-BBN), lung (B(*a*)P) and lymphatic (ENU in transgenic *pim*) tumor models. Other agent combinations with 9-*cis*-RA are being investigated as chemopreventive strategies for the mammary gland in 100-day old rats, such as DFMO, DHEA, 4-HPR, vorozole, and tamoxifen.

The effects of 9-*cis*-RA on a histological intermediate biomarker for colon cancer have been evaluated by the Chemoprevention Branch. The drug (195 mg/kg diet, or *ca.* 32 μmol/kg-bw/day) decreased the formation of AOM-induced aberrant crypt foci in rat colon. Also, ALRT reported in an abstract that ig administration of 30 mg 9-*cis*-RA/kg-bw/day (100 μmol/kg-bw/day) decreased DMBA/TPA-induced skin papilloma formation in SENCAR mice; lower doses (3, 10 mg/kg-bw/day, or 10, 33 μmol/kg-bw/day) were not efficacious [14]. The Chemoprevention Branch is currently evaluating modulation of retinoid receptors (RARα, RARγ, RXRα), genomic instability (LOH) and apoptosis in mammary gland, lung and liver tissues of female rats to carcinogen.

In vitro and *in vivo* assays have demonstrated the chemotherapeutic efficacy of 9-*cis*-RA in several human cancer models. In the human promyelocytic leukemia cell line HL-60, the retinoid decreased proliferation and induced differentiation and apoptosis; the latter correlated to an increase in transglutaminase type II activity [15]. 9-*cis*-RA also induced differentiation and inhibited proliferation of fresh cells from acute myelogenous leukemia patients [16]. In estrogen receptor-positive breast cancer cell lines MCF-7 and LY2, 9-*cis*-RA inhibited anchorage-independent growth with concomitant reduction in the expression of estrogen-responsive genes (TGF-α, pS2) [17]. Finally, oral treatment (60 mg/kg-bw/day, or 0.2 mmol/kg-bw/day) of athymic nude mice completely regressed human lip SCC xenografts after six weeks.

PRECLINICAL SAFETY STUDIES

The Chemoprevention Branch has not funded preclinical safety studies on this retinoid; however, some information is available from a mammary gland efficacy study. ALRT has conducted oral subchronic toxicity studies in rats and dogs, teratology studies in mice, and mutagenicity studies.

Safety: In the published NCI-funded chemoprevention study in female rats, no signs of gross toxicity were observed at doses of 60 and 120 mg/kg diet (ca. 10 and 20 μmol/kg-bw/day) for three or 4.5 months; however, "some" weight loss was observed [4]. In a second published study, treatment of nude mice with single oral doses of 10 mg 9-*cis*-RA/kg-bw (33 μmol/kg-bw) decreased plasma retinol levels by 50–60%, which was sustained for at least 48 hours; the decrease was greater after a second drug dose two days later [18]. Because of the important functions of retinol in the body, this finding could have clinical significance.

Retinoids are known teratogens; therefore, the potential effects of 9-*cis*-RA on fetal development are of concern. Reports from two published studies in mice found that 9-*cis*-RA is teratogenic, but is only about half as potent as the all-*trans* isomer. Exposure of mice to single ig doses of 10–100 mg 9-*cis*-RA/kg-bw (33–333 μmol/kg-bw) on day 11 of gestation resulted in a dose-dependent induction of cleft palate at the 25–100 mg/kg-bw doses; limb defects were only observed at the highest dose. Administration of a split dose (2 x 50 mg/kg-bw, two hours apart) significantly increased the drug's teratogenic activity [19]. In a second mouse study, ip administration of 3 mg/kg-bw (10 μmol/kg-bw) on gestation day 7.5 induced a 12% resorption rate in 58 implantation sites. Out of 51 live fetuses, nine cases of microphthalmia and six cases of anophthalmia were observed [20].

Intraamniotic administration of 9-*cis*-RA to cultured whole rat embryos on day 10 of gestation significantly increased branchial arch, somite and cephalic abnormalities; an unusual heart defect was also noted [21].

ADME: Limited published ADME data are available. Preclinical studies in several species have been conducted by the manufacturer, and pharmacokinetic assessment is currently being conducted by the Chemoprevention Branch as part of a preclinical prostate efficacy study in rats. In published studies, a single oral dose of 10 mg/kg-bw (33 μmol/kg-bw) administered to nude mice resulted in peak plasma levels of 3.6 ng/ml (12 nM) 15–30 minutes after administration, which declined rapidly by one hour [18]. A second oral dose administered two days subsequent to the first gave two plasma peaks, one at 30 minutes and the second at three hours; plasma levels were 7.3 and 3.1 ng/ml at the two peak times, respectively. The AUC observed after the two-dose regimen increased to 561 ng/ml/min compared with 364 ng/ml/min after a single dose. Metabolites identified in plasma after administration of 9-*cis*-RA were 4-hydroxy-RA, 4-oxo-RA and all-*trans*-RA; the isomerization state of 4-hydroxy- and 4-oxo-RA could not be distinguished under the experimental conditions employed.

After administration of a higher single dose of 50 mg/kg-bw (166 μmol/kg-bw) to NMRI mice, Cmax of 1,013 ng/ml occurred within 40 minutes [22]. Clearance followed first-order kinetics with a mean $t_{½}$ of 64 minutes. The major plasma retinoid was 9-*cis*-retinoyl-β-glucuronide, with smaller amounts of parent, 9,13-di*cis*-RA and all-*trans*-RA.

ADME studies in pregnant mice have also been published. After a single oral dose (50, 100 or 200 mg/kg-bw, or 166, 333 or 666 μmol/kg-bw) administered on day 11 of gestation, 9-*cis*-RA, all-*trans*-RA, 13-*cis*-RA, and the *trans*- and *cis*-isomers of 4-oxo-RA were found in the embryo. all-*trans*-RA was the major metabolite observed after one hour; C_{max} in the embryo for the all-*trans* isomer was several fold greater than for 9-*cis*-RA [19].

The elimination profile of 9-*cis*-RA in Rhesus monkeys after a single dose of 50 or 100 mg/m^2 (*ca.* 10.2, 20.4 μmol/kg-bw) was first order (harmonic mean $t_{½}$=31 min; Cl=97 ml/min/m^2) and did not appear to be saturable. Pharmacokinetics were linear across the two doses studied [23].

Importantly, in both mice [18] and monkeys [23], the pharmacokinetics observed after similar dosing with the all-*trans* isomer, which induces its own oxidative metabolism, were substantially different than with the 9-*cis* isomer [see Pharmacodynamics Issues below].

CLINICAL SAFETY: PHASE I STUDIES

No Chemoprevention Branch-funded clinical studies of 9-*cis*-RA have been conducted. Pharmacokinetic and safety results from two small Phase I/IIa trials in cancer patients partially funded by NCI, DCTDC were recently published. One trial involved 34 advanced cancer patients [24], and the other was in seven acute promyelocytic leukemia (APL) patients [25]. A third Phase I/IIa trial in 22 patients with solid tumors was sponsored by Hoffman-LaRoche [26]. Finally, a fourth independent trial in six healthy volunteers investigated metabolism and excretion [27].

Safety: Published results from the two NCI trials in cancer patients indicate that headache was the most common dose-limiting toxicity after oral administration of 9-*cis*-RA doses ranging from 5–230 mg/m^2 qd (*ca.* 0.4–18.9 μmol/kg-bw/day) for up to 64 days [24,25]. Hyperlipidemia (mild hypercholesterolemia, moderate to marked hypertriglyceridemia) also occurred in most patients, and severity appeared to be both dose- and time-related. Other reactions included facial flushing, dry skin, myalgia/arthralgia, dyspnea (only in lung cancer patients), and hypercalcemia. An MTD of 140 mg/m^2 qd (*ca.* 11.5 μmol/kg-bw/day) for four weeks was reported [24]. Additional safety data are available from ALRT and further studies are being conducted.

In the Hoffman-LaRoche trial, patients with solid tumors were treated with 20–150 mg 9-*cis*-RA/m^2/day (*ca.* 1.6–12.3 μmol/kg-bw/day) in two divided doses for at least four weeks [26]. The dose-limiting effects were headaches as in the previous trials, as well as diarrhea (only in colon cancer patients). Other common, mild to moderate reactions included hypertriglyceridemia, cheilitis and dry skin, myalgia/arthralgia, conjuctivitis and elevated serum alkaline phosphatase. The dose recommended for further Phase II studies was 100 mg 9-*cis*-RA/m^2/day bid (*ca.* 8.2 μmol/kg-bw/day).

ADME: Following a single dose of 5–230 mg 9-*cis*-RA/m^2 (*ca.* 0.4–18.9 μmol/kg-bw) in cancer patients, the 24-hour AUC (74–828 ng•hr/ml) increased proportionally with dose, except that the value at the highest dose was larger than expected (982 ng•hr/ml) [24]. C_{max} also increased with dose (26–1,003 ng/ml), but t_{max} (1.3–2.0 hr) and $t_{½}$ (1.5–1.8 hr) values remained fairly constant. With multiple doses (qd) up to the MTD, the AUC changed little; at higher doses, AUC values were lower than day one. It is unknown if 9-*cis*-RA can induce its own meta-

bolism in humans. Additional ADME data are available from ALRT.

Following 45–150 mg 9-*cis*-RA/m^2/day (*ca.* 3.7–12.3 μmol/kg-bw/day) in two divided doses, C_{max} (161.1–409 ng/ml) and AUC (415.5–1,483.8 ng•hr/ml) on day one appeared to increase proportionally with dose; however, intersubject variability was high [26]. With repeated dosing, C_{max} and AUC decreased from day one to day 22, again suggesting induction of metabolism as for all-*trans*-RA.

Metabolism and excretion of 9-*cis*-RA was investigated in a small study of six healthy men after 28 daily doses of 20 mg [27]. Two hours after the last dose, the major plasma retinoids were the parent compound (29.1 ng/ml), 4-oxo-RA (16.3 ng/ml) and 9,13-di*cis*-RA (1.0 ng/ml); 13-*cis*-RA and all-*trans*-RA were within endogenous range. In contrast to rodents, 9-*cis*-retinoyl-β-glucuronide was not a major plasma metabolite. Within 12–13 hours after the last dose, all plasma retinoid levels were within the normal range, suggesting much more rapid elimination than for 13-*cis*-RA. Significant amounts of metabolites were excreted into the urine, but not feces. In urine, three glucuronides predominated; of these, only 9-*cis*-oxo-retinoyl-β-glucuronide could be identified. In feces, very high concentrations of the parent were detected (up to 89 μg/g), presumably from unabsorbed drug; other retinoids were present at <4% of the parent concentration.

CLINICAL EFFICACY: PHASE II STUDIES

As discussed above, three Phase I/IIa studies of 9-*cis*-RA in cancer patients have been published. The only patient of the 55 evaluable achieving a complete response was in the APL trial; the patient had received a high dose of 230 mg/m^2/day for 64 days [24–26]. Nine additional patients with solid tumors had disease stabilization for up to five months.

Phase IIb studies of oral 9-*cis*-RA will be conducted by ALRT for treatment of several cancers including non-Hodgkin's lymphoma, hormone-refractory prostate cancer, and APL [28]. Phase III studies of a topical formulation are also being conducted for Kaposi's sarcoma and mycosis fungoides; interim data from a Phase I/II trial indicated a response in 30% (n=43) of Kaposi's patients [29].

Short-term Phase II studies in breast cancer and CIN II/III patients are under consideration by the Chemoprevention Branch; the retinoid would be administered between diagnostic biopsy and surgery.

PHARMACODYNAMICS

In preclinical studies, orally administered 9-*cis*-RA inhibited development of rat mammary tumors at doses of 10 and 20 μmol/kg-bw/day. The lower dose is approximately equivalent to the human MTD of 140 mg/m^2/day (*ca.* 11.5 μmol/kg-bw). Although the lower dose of the drug alone was not as effective as the higher dose in preventing carcinogen-induced rat mammary cancers, it was still highly efficacious ($p=0.002$). Furthermore, in combination with suboptimal doses of tamoxifen, the low dose of 9-*cis*-RA displayed even greater chemo preventive activity ($p<0.001$ compared to untreated controls). Thus, a dose lower than the human MTD, administered in combination with tamofixen and/or other agents, may be clinically useful.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

Very little information is available on biochemical effects of 9-*cis*-RA that could be used as drug effect measurements. *In vitro* incubation of human osteosarcoma cells with 9-cis-RA induced expression of cellular retinoic acid-binding protein (CRABP-II) [30,31]. The applicability of this finding to *in vivo* studies is unknown. Other retinoids decrease plasma IGF-I [32] and induce different tissue TGF-β isoforms [33]. Expression of these growth factors may also be modulated by 9-*cis*-RA. Finally, expression of retinoid receptors should be investigated.

Safety Issues

Like other retinoids, 9-*cis*-RA is teratogenic. This will necessitate stringent birth control procedures if women of child-bearing age are treated with the drug. In mice, 9-*cis*-RA also lowers plasma retinol levels; because of the important function of retinol in the body, this finding could have important clinical significance. Indeed, treatment of breast cancer patients with the retinoid 4-HPR (200 mg/day, three-day drug holiday/month) caused a significant reduction in plasma retinol levels which resulted in diminished night vision. Mild and moderately diminished dark adaptation occurred at plasma 4-HPR levels of 160 and 100 ng/ml, respectively [34, 35].

9-*cis*-RA is a high-affinity ligand for RXRs, which can form heterodimers with the peroxisome prolif-

erator-activated receptor (PPAR) [36]. Chemicals which activate this receptor (*e.g.*, trichlorophenoxyacetic acid herbicides, phthalate ester plasticizers, hypolipidemic drugs) dramatically increase the size and number of peroxisomes in the liver and kidney, and are termed peroxisome proliferators. PPAR activation increases transcription of peroxisomal acyl-CoA oxidase, which degrades long-chain fatty acids and cholesterol through β-oxidation [36,37]; a concomitant effect of most peroxisome proliferators is hypolipidemia [38]. Since peroxisome proliferators have also been shown to induce liver cancer in rodents [39], a tissue in which high levels of PPAR and RXR are coexpressed, this may be a concern for human use of 9-*cis*-RA [1]. However, recent clinical data showing that this retinoid induces hyperlipidemia rather than hypolipidemia, also suggests that peroxisome proliferation and liver cancer would not be a potential adverse effect. Nonetheless, carcinogenicity studies should be initiated before clinical trials greater than one year in duration are started. In contrast, the elevations in plasma triglycerides and cholesterol observed in clinical studies with 9-*cis*-RA are of concern as risk factors for artherosclerosis, thrombosis and cardiac infarction [40]. In addition, published data suggest that 9-*cis*-RA may have a second effect *in vivo*— increased fibrinogen synthesis. Fibrinogen participates in the final phase of the blood coagulation cascade and is an independent risk factor for occlusive coronary thrombosis [41]. 9-*cis*-RA was more potent than RAR-(all-*trans*-RA) and RXR-specific ligands in increasing fibrinogen levels *in vitro*; all-*trans*-RA produced the effect *in vivo* [42], suggesting that 9-*cis*-RA would also. Finally, there is limited human evidence that 9-*cis*-RA decreases plasma levels of HDL-cholesterol and apolipoprotein A-I, the major protein constituent of high-density lipoprotein (HDL) as well as HDL-cholesterol [43]. Decreased HDL-cholesterol is associated with accelerated development of atherosclerotic lesions. The combination of these three effects suggests that 9-*cis*-RA has the potential for significantly increasing cardiovascular disease risk factors. In long-term clinical trials with this agent, subjects should be closely monitored for serum lipids, fibrinogen, and other surrogate markers for cardiovascular disease. A low-dose combination with an antiestrogen or an RXR-selective retinoid (*e.g.*, LGD1069) may also be considered to decrease these potential toxicities.

Pharmacodynamics Issues

Pharmacokinetics, target tissue metabolism, and distribution of cellular RXR and RAR receptors could contribute to the chemopreventive profile of 9-*cis*-RA. Based on preclinical data, the 9-*cis*-isomer does not appear to induce its own metabolism. This finding could be potentially useful in maintaining plasma drug levels. Indeed, increased metabolic capacity is believed to contribute to the ultimate failure of all-*trans*-RA therapy in APL patients [18]. In contrast, clinical data showing that AUC values for 9-*cis*-RA were lower on day 22 than day one suggest that metabolism or excretion may change with multiple dosing. Further studies are needed to clarify these observations. In addition, studies in target tissues on the interconversion of the 9-*cis* and all-*trans* isomers may be necessary to clarify the role of the two isomers in intracellular signaling pathways. Because the lowest dose with demonstrated efficacy in animal chemoprevention studies is close to the human MTD, additional dose-titration studies both alone and in combination with tamoxifen and/or other agents should be conducted to determine the lowest efficacious dose.

Regulatory Issues

Conduct of long-term Phase II studies may necessitate extended preclinical toxicology prior to initiation. It is unknown if 9-*cis*-RA acts as a peroxisome proliferator in humans or experimental animals. Also, preclinical toxicology data on combinations with antiestrogens would be required before initiation of human testing using this strategy.

Intermediate Biomarker Issues

The identification of intermediate biomarkers that can serve as surrogate endpoints for cancer in clinical trials will depend on the target organs selected. Potential biomarkers for use in the proposed short-term Phase II trial in breast cancer patients include ductal carcinoma *in situ* (DCIS) and other histologic markers, DNA ploidy, nuclear morphometry, and MIB-1 [44]. Potential biomarkers in the cervix include ploidy, micronucleated cell frequency, PCNA, EGFR, and keratins.

Supply and Formulation Issues

A Material Transfer Agreement has been signed with ALRT. Other potential suppliers of 9-*cis*-RA include Hoffman-LaRoche and Kuraray. Under

agreement with Hoffman-LaRoche, it may be possible to obtain formulated drug and placebo. Purchase of bulk drug will require additional time for formulation and stability studies. One kilogram is being synthesized under contract with SRI International for use in preclinical studies.

Clinical Studies Issues

Successful development of 9-*cis*-RA as a chemopreventive agent will depend on the results of preclinical efficacy and safety studies. A Clinical Trials Agreement with the supplier must be in place before NCI, Chemoprevention Branch-funded human studies can be initiated. One NCI-sponsored preclinical study demonstrated inhibition of rat mammary carcinogenesis; thus, the major target for clinical chemoprevention studies is the breast. A Phase II trial in breast cancer patients with administration of 9-*cis*-RA between diagnostic biopsy and surgery is under consideration by the Chemoprevention Branch. Possible Phase II studies of 9-*cis*-RA with an antiestrogen in the same cohort would necessitate conducting preclinical toxicity studies using the drug combination.

Trials in other target tissues are also being considered—cervix, prostate and head/neck. The design of the cervix trial would be similar to the breast study, but the cohort would be CIN II/III patients. Topical all-*trans*-RA [45,46] has demonstrated some efficacy in this population. However, a combination of an RXR- and an RAR-specific ligand showed more than additive effects in inhibiting proliferation of human cervical carcinoma ME180 cells *in vitro* [47]. Since 9-*cis*-RA binds both receptor types, it would appear to be the best strategy in this tissue. Pending results of preclinical efficacy studies, a short-term trial in a prostate cancer cohort may also be considered. Since the ratio of estrogen to testosterone increases with age along with the incidence of benign prostatic hyperplasia and prostate cancer, potential suppression of estrogen-related activities by 9-*cis*-RA may be a useful strategy. Finally, studies in head and neck cancer patients may be conducted based on the effectiveness of other retinoids in these target organs.

REFERENCES

1. Mangelsdorf, D.J., Umesono, K. and Evans, R.M. The retinoid receptors. In: Sporn, M.B., Roberts, A.B. and Goodman, D.S. (eds.) *The Retinoids: Biology, Chemistry, and Medicine*, 2nd Edition. New York: Raven Press, pp. 319–349, 1994.
2. Hill, D.L. and Grubbs, C.J. Retinoids and cancer prevention. *Annu. Rev. Nutr.* 12: 161–181, 1992.
3. 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.
4. Anzano, M.A, Byers, S.W., Smith, J.M., Peer, C.W., Mullen, L.T., Brown, C.C., Roberts, A.B. and Sporn, M.B. Prevention of breast cancer in the rat with 9-*cis*-Retinoic acid as a single agent and in combination with tamoxifen. *Cancer Res.* 54: 4614–4617, 1994.
5. Tate, B.F., Levin, A.A. and Grippo, J.F. The discovery of 9-*cis*-Retinoic acid. A hormone that binds the retinoid-X receptor. *Trends Endocrinol. Metab.* 5: 189–194, 1994.
6. Kizaki, M., Ikeda, Y., Tanosaki, R., Nakajima, H., Morikawa, M., Sakashita, A. and Koeffler, H.P. Effects of novel retinoic acid compound, 9-*cis*-retinoic acid, on proliferation, differentiation, and expression of retinoic acid receptor-α and retinoid X receptor-α RNA by HL-60 cells. *Blood* 82: 3592–3599, 1993.
7. Majewski, S., Szmurlo, A., Marczak, M., Jablonska, S. and Bollag, W. Synergistic effect of retinoids and interferon alpha on tumor-induced angiogenesis: Anti-angiogenic effect of HPV-harboring tumor-cell lines. *Int. J. Cancer* 57: 81–85, 1994.
8. Kurie, J.M., Buck, J., Eppinger, T.M., Moy, D. and Dmitrovsky, E. 9-*cis*- and all-*trans*-Retinoic acid induce a similar phenotype in human teratocarcinoma cells. *Differentiation* 54: 123–129, 1993.
9. Han G., Chang, B. and Sidell, N. Comparison of effects of all-*trans* retinoic acid and 9-*cis* retinoic acid in inducing differentiation of human neuroblastoma cells. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 35: 37, abstract no. 219, 1994.
10. Nagy, L., Thomazy, V.A., Shipley, G.L., Fesus, L., Lamph, W., Heyman, R.A., Chandraratna, R.A.S. and Davies, P.J.A. Activation of retinoid X receptors induces apoptosis in HL-60 cell lines. *Mol. Cell. Biol.* 15: 3540–3551, 1995.
11. Cesario, R.M., McClurg, M.R., Wong, G.L., Sicurello, J.P., Heyman, R.A. and Lamph, W.W. Growth inhibition of multiple myeloma and squamous cell carcinoma by 9-*cis*-Retinoic acid. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 35: 275, abstract no. 1645, 1994.
12. Rubin, M., Fenig, E., Rosenauer, A., Menendez-Botet, C., Achkar, C., Bentel, J.M., Yahalom, J., Mendelsohn, J. and Miller, W.H., Jr. 9-*cis*-Retinoic acid inhibits growth of breast cancer cells and down-regulates estrogen receptor RNA and protein. *Cancer Res.* 54: 6549–6556, 1994.
13. Schippers, I.J., Kloppenburg, M., Snippe, L. and Ab, G. 9-*cis*-Retinoic acid represses estrogen-induced expression of the very low density apolipoprotein II gene. *Mol. Cell. Endocrinol.* 105: 175–182, 1994.
14. Shalinsky, D.R., Gregory, M.L., Bischoff, E.D., Shirley, M.A., Ulm, E.H., Spath, K.L., Dixon, S.A., Gottardis, M.M. and Hayes, J.S. Inhibition of papilloma formation by LGD1057 (9-*cis*-Retinoic acid (RA)) and related RA isomers in the SENCAR mouse two-stage model of skin carcinogenesis. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 35: 139, abstract no. 831, 1994.
15. Gottardis, M.M., Lamph, W.W., Shalinsky, D.R., Wellstein, A. and Heyman, R.A. The efficacy of 9-*cis*-Retinoic acid in experimental models of cancer. *Breast Cancer Res. Treat.* 38: 85–96, 1996.
16. Sakashita, A., Kizaki, M., Pakkala, S., Schiller, G., Tsuruoka,

N., Tomosaki, R., Cameron, J.F., Dawson, M.I. and Koeffler, H.P. 9-*cis*-Retinoic acid: Effects on normal and leukemic hematopoiesis *in vitro*. *Blood* 81: 1009–1016, 1993.

17. Fontana, J.A., Nervi, C., Shao, Z.-M. and Jetten, A.M. Retinoid antagonism of estrogen-responsive transforming growth factor α and *pS2* gene expression in breast carcinoma cells. *Cancer Res.* 52: 3938–3945, 1992.
18. Achkar, C.C., Bentel, J.M., Boylan, J.F., Scher, H.I., Gudas, L.J. and Miller, W.H., Jr. 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.
19. Kochhar, D.M., Jiang, H., Penner, J.D. and Heyman, R.A. Placental transfer and developmental effects of 9-*cis* retinoic acid in mice. *Teratology* 51: 257–265, 1995.
20. Collins, M.D., Schreiner, C.M. and Scott, W.J. Comparative potency of all-*trans*- and 9-*cis*-Retinoic acid for the induction of murine exencephaly. *Teratology* 47: 385–386, 1993.
21. Kraft, J.C. and Juchau, M.R. 9-*cis*-Retinoic acid: A direct-acting dysmorphogen. *Biochem. Pharmacol.* 46: 709–716, 1993.
22. Tzimas, G., Sass, J.O., Wittfoht, W., Elmazar, M.M.A., Ehlers, K. and Nau, H. Identification of 9,13-di*cis*- Retinoic acid as a major plasma metabolite of 9-*cis*- Retinoic acid and limited transfer of 9-*cis*-Retinoic acid and 9,13-di*cis*-retinoic acid to the mouse and rat embryos. *Drug Metab. Dispos.* 22: 928–936, 1994.
23. 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.
24. Miller, V.A., Rigas, J.R., Benedetti, F.M., Verret, A.L., Tong, W.P., Kris, M.G., Gill, G.M., Loewen, G.R., Truglia, J.A., Ulm, E.H. and Warrell, R.P., Jr. Initial clinical trial of the retinoid receptor *pan* agonist 9-*cis*- Retinoic acid. *Clin. Cancer Res.* 2: 471–475, 1996.
25. 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 acquired retinoid resistance in acute promyelocytic leukemia. *Blood* 85: 3021–3027, 1995.
26. Kurie, J.M., Lee, J.S., Griffin, T., Lippman, S.M., Drum, P., Thomas, M.P., Weber, C., Bader, M., Massimini, G. and Hong, W.K. Phase I trial of 9-*cis*- Retinoic acid in adults with solid tumors. *Clin. Cancer Res.* 2: 287–293, 1996.
27. Sass, J.O., Masgrau, E., Saurat, J.-H. and Nau, H. Metabolism of oral 9-*cis*-Retinoic acid in the human. Identification of 9-*cis*-retinoyl-β-glucuronide and 9-*cis*- 4-oxo-retinoyl-β-glucuronide as urinary metabolites. *Drug Metab. Dispos.* 23: 887–891, 1995.
28. F-D-C Reports. Ligand/Allergan initiate 10 ALRT1057 *phase 11b* trials. *FDC Reports (The Pink Sheet)* 57: TG6–TG7, 1995.
29. Editorial. Clinical trials update. *Genet. Eng. News* 16: 24, 1996.
30. Durand, B., Saunders, M. Leroy, P., Leid, M. and Chambon, P. all-*trans*- and 9-*cis*-Retinoic acid induction of CRABPII transcription is mediated by RAR-RXR heterodimers bound to DR1 and DR2 repeated motifs. *Cell* 71: 73–85, 1992.
31. Melhus, H., Gobl, A. and Ljunghall, S. Competitive PCR demonstrates that 9-*cis*-Retinoic acid induces cellular retinoic acid-binding protein-II more efficiently than all-*trans* retinoic acid in human osteosarcoma cells. *Biochem. Biophys. Res. Commun.* 200: 1125–1129, 1994.
32. Torrisi, R., Pensa, F., Orengo, M.A., Catsafados, E., Ponzani, P., Boccardo, F., Costa, A. and Decensi, A. The synthetic retinoid fenretinide lowers plasma insulin-like growth factor I levels in breast cancer patients. *Cancer Res.* 53: 4769–4771, 1993.
33. Glick, A.B., McCune, B.K., Abdulkarem, N., Flanders, K.C., Lumadue, J.A., Smith, J.M. and Sporn, M.B. Complex regulation of TGFβ expression by retinoic acid in the vitamin A-deficient rat. *Development* 111: 1081–1086, 1991.
34. Decensi, A., Formelli, F., Torrisi, R. and Costa, A. Breast cancer chemoprevention: Studies with 4-HPR alone and in combination with tamoxifen using circulating growth factors as potential surrogate endpoints. *J. Cell. Biochem.* 17G: 226–233, 1993.
35. Decensi, A., Torrisi, R., Polizzi, A., Gesi, R., Brezzo, V., Rolando, M., Rondanina, G., Orengo, M.A., Formelli, F. and Costa, A. Effect of the synthetic retinoid fenretinide on dark adaptation and the ocular surface. *J. Natl. Cancer Inst.* 86: 105–110, 1994.
36. Kliewer, S.A., Umesono, K., Noonan, D.J., Heyman, R.A. and Evans, R.M. Convergence of 9-*cis*-Retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature* 358: 771–774, 1992.
37. Keller, H., Mahfoudi, A., Dreyer, C., Hihi, A.K., Medin, J., Ozato, K. and Wahli, W. Peroxisome proliferator-activated receptors and lipid metabolism. *Ann. N.Y. Acad. Sci.* 684: 157–173, 1993.
38. Moody, D.E., Gibson, G.G., Grant, D.F., Magdalou, J. and Rao, M.S. Peroxisome proliferators, a unique set of drug-metabolizing enzyme inducers: Commentary on a symposium. *Drug Metab. Dispos.* 20: 779–791, 1992.
39. Moody, D.E., Reddy, J.K., Lake, B.G., Popp, J.A. and Reese, D.H. Peroxisome proliferation and nongenotoxic carcinogenesis: Commentary on a symposium. *Fundam. Appl. Toxicol.* 16: 233–248, 1991.
40. Brown, M.S. and Goldstein, J.L. Drugs used in the treatment of hyperlipoproteinemias. In: Gilman, A.G., Rall, T.W., Nies, A.S. and Taylor, P. (eds.) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed. New York: Pergamon Press, pp. 874–896, 1990.
41. Heinrich, J., Balleisen, L., Schulte, H., Assmann, G. and van de Loo, J. Fibrinogen and factor VII in the prediction of coronary risk. Results from the PROCAM study in healthy men. *Arterioscler. Thromb.* 14: 54–59, 1994.
42. Nicodeme, E., Nicaud, M. and Issandou, M. Retinoids stimulate fibrinogen production both *in vitro* (hepato cytes) and *in vivo*. Induction requires activation of the retinoid X receptor. *Arterioscler. Thromb.* 15: 1660– 1667, 1995.
43. 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. Annu. Meet. Am. Assoc. Cancer Res.* 36: 506, abstract no. 3012, 1995.
44. Lagios, M.D. Evaluation of surrogate endpoint biomar kers for ductal carcinoma *in situ*. *J. Cell. Biochem.* 19: 186–188, 1994.
45. Graham, V., Surwit, E.S., Weiner, S. and Meyskens, F.L., Jr. Phase II trial of β-all-*trans*-retinoic acid for cervical intraepithelial neoplasia delivered via a collagen sponge and

cervical cap. *West. J. Med.* 145: 192–195, 1986.

46. Meyskens, F.L., Jr., Surwit, E., Moon, T.E., Childers, J.M., Davis, J.R., Dorr, R.T., Johnson, C.S. and Alberts, D.S. Enhancement of regression of cervical intraepithelial neoplasia II (moderated dysplasia) with topically applied all-*trans*-retinoic acid: A randomized trial. *J. Natl. Cancer Inst.* 86: 539–543, 1994.

47. 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.

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoints(s)	Remarks
Planned Phase II Trial in Presurgical Breast Cohort	Breast	Breast cancer patients between diagnostic biopsy and surgery		Intermediate biomarkers	Study not designed
Planned Phase II Trial in Presurgical Cervix Cohort	Cervix	CIN II/III patients between diagnostic biopsy and surgery		Intermediate biomarkers	Study not designed

9-*cis*-RETINOIC ACID DEVELOPMENT STATUS