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NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN: 13-*cis*-RETINOIC ACID

DRUG IDENTIFICATION

CAS Registry No.: 4759-48-2

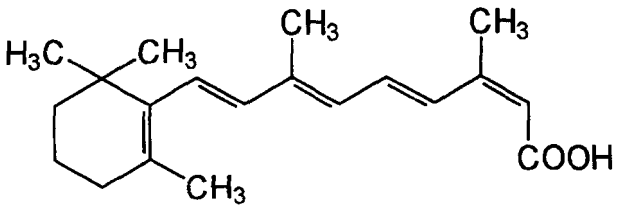
CAS Name (9CI): 13-*cis*-Retinoic Acid

Synonyms:
Accutane® (Hoffman-LaRoche, Inc.)
3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexene-1-yl)2-*cis*-4-*trans*-6-*trans*-8-*trans*-nonatetraenoic Acid
Isotretinoin
NSC-329481
Ro 4-3780

Related Compounds:
all-*trans*-*N*-(4-Hydroxyphenyl)retinamide (CAS No. 65646-68-6)
9-*cis*-Retinoic Acid (CAS No. 5300-03-8)
Retinol (CAS No. 68-26-8)
Retinyl Acetate (CAS No. 127-47-9)
Retinyl Palmitate (CAS No. 79-81-2)
all-*trans*-Retinoic Acid (CAS No. 302-79-4)
Vitamin A Mixture (50:50 Retinyl Acetate:Retinyl Palmitate)

Molecular Wt.: 300.4

Structure:



EXECUTIVE SUMMARY

13-*cis*-Retinoic acid (13-*cis*-RA) is a stereoisomer of all-*trans*-retinoic acid (all-*trans*-RA). It has been marketed as Accutane® (13-*cis*-RA) since 1982 for oral treatment of severe nodular acne unresponsive to conventional therapy [1]. The therapeutic effect is attributed to decreased keratinization and sebaceous gland secretion [1,2]. 13-*cis*-Retinoic acid also occurs naturally as a metabolite of retinol (vitamin A_1) in both humans and animals [3]. It fills some of the nutritional roles of vitamin A [2] with the same potency (1 IU=0.31 μg) [4], except for vision and part of reproduction [2].

Vitamin A appears to be involved in the tumorigenic process, and 13-*cis*-RA shares some of its anti-proliferative [5], differentiation-inducing (moderate) [5] and immune-enhancing effects [6]. 13-*cis*-RA has been specifically shown to inhibit ODC induction [2], downregulate N-*myc* expression [7] and protect against chromosomal damage (bleomy-

cin-induced chromatid breaks in lymphocytes) [8]. Some of the biological effects of retinoids in general result from binding to nuclear retinoic acid receptors belonging to the superfamily of steroid and hormone receptors [9]. All these receptors function as transcription factors that regulate the expression of specific genes. Retinoid receptors are classified into two subfamilies, RARs (RARα, -β, -γ) and RXRs, based on differences in primary structure, sensitivity to synthetic retinoid ligands, and ability to regulate expression of different target genes [10]. For example, all-*trans*-RA binds with high affinity to, and transcriptionally activates, RARs [11]. The RAR transcriptional profile for 13-*cis*-RA was found to be similar to all-*trans*-RA, with potency for RARγ being 10-fold greater than the other two isoforms [12]. However, specific effects *in vivo* are also related to differences in receptor distribution between tissues and the pharmacokinetics of the retinoid. Rat tissues with the highest RARγ expression include trachea, lung, bladder and skin [13]. In humans, most of the RAR receptors in skin are RARγ; significant expression is also found in lung [14,15].

The NCI, Chemoprevention Branch has done only limited efficacy testing with 13-*cis*-RA due to the availability of published results. Retinoids in general inhibit tumorigenesis in skin, respiratory tract, bladder, mammary gland and gastrointestinal (GI) tract. Of these, 13-*cis*-RA is one of the most active retinoids in carcinogen-induced bladder (mouse and rat) and skin (mouse) cancer models. Although it also has activity in lung and oral cavity (hamster) and colon (rat), 13-*cis*-RA was not as effective in mammary glands (rat) as retinyl acetate or 4-HPR. The NCI, Chemoprevention Branch is currently testing 13-*cis*-RA in the hamster cheek pouch model of carcinogenesis.

Because of the availability of published efficacy data, the NCI, Chemoprevention Branch has concentrated on identification of intermediate biomarkers modulated by retinoids. Biomarkers are a priority, since they are potential surrogate endpoints for cancer in clinical trials. In Chemoprevention Branch-funded studies, 13-*cis*-RA inhibited the development of histological biomarkers in rat colon (aberrant crypt foci). In published studies, it inhibited development of histological biomarkers in bladder (atypia), skin (papilloma), pancreas (adenoma), liver (nodules) and lung (adenoma), and proliferative biomarkers in oral cavity and skin (ODC activity).

Preclinical toxicology data on 13-*cis*-RA are available from published studies undertaken by Hoffman-LaRoche and other groups. Dose-related effects (30–170 μmol/kg-bw/day) included decreased body weight gain, erythema, alopecia, mucosal changes, elevated serum alkaline phosphatase, transaminase and triglycerides, increased liver weight, and long bone fractures. In published carcinogenicity studies with 13-*cis*-RA at doses of 6.7–106.5 μmol/kg-bw/day, decreased hemoglobin, increased kidney weights, fibrosis and inflammation of the myocardium, arterial and focal tissue calcification, focal osteolysis of the bone were also observed; an increase in the incidence of pheochromocytoma was considered to be species- and strain-dependent. Similar effects occurred in dogs. The teratogenic potency of 13-*cis*-RA appears to be species specific; rats and mice are insensitive (TD_{Lo}: 250 μmol/kg-bw/day), while monkeys and rabbits are moderately sensitive (TD_{Lo}: 16.6 μmol/kg-bw/day).

The $t_{1/2}$ of 13-*cis*-RA in rodents is much shorter than that in humans. In mice, the value is one hour, which is 10–20 fold less than in humans. The relative AUC values for 13-*cis*-RA are human >> monkey >mouse. The metabolic profile is also different among species [16]. In rodents a considerable portion of the administered dose is glucuronidated to 13-*cis*-retinoyl-β-glucuronide; in monkeys and humans, significant conversion to 4-oxo-13-*cis*-RA occurs. In rats, mice and dogs, biliary excretion and enterohepatic circulation of glucuronide conjugates appear to be important.

Human pharmacokinetic and safety data on 13-*cis*-RA are available from the use of Accutane® as an oral treatment for acne at the recommended doses of 1.7–6.7 μmol/kg-bw/day for 15–20 weeks. The bioavailability of 13-*cis*-RA is approximately 25%. Unlike retinol, 13-*cis*-RA is absorbed directly from the intestines into the portal vein and is transported extensively bound to plasma proteins. There is significant first-pass metabolism, and, as with experimental animals, enterohepatic recirculation occurs, possibly as glucuronide conjugates. The $t_{1/2}$ for the major plasma metabolite 4-oxo-13-*cis*-RA is 20–50 hours, so steady-state levels exceed those of the parent by two to five fold. Approximately 20–30% of the dose is isomerized to all-*trans*-RA. Within 96 hours, 60% of the dose is recovered equally divided between feces and urine.

With approved use of Accutane®, the most fre-

quent adverse event was cheilitis in more than 90% of patients. The durations of both this effect and elevated plasma triglycerides (25% of patients) were related to dose. Increases in the latter over 800 mg/dl have been associated with pancreatitis. Less frequent were decreases in red blood cell parameters (10–20%), increased liver enzymes (10–20%), decreased HDL levels (16%), musculoskeletal complaints (*e.g.*, arthralgia) (16%), rash (10%), GI symptoms (5%), fatigue (5%), headache (5%), corneal opacities, conjunctivitis, decreased night vision and elevated blood sugar. In trials for treatment of disorders of keratinization, higher doses (7.5 μmol/ kg-bw/day) caused skeletal hyperostosis, with symptomatic osteophyte formation and ligament calcification. In the published Phase IIa Italian study in oral leukoplakia patients, adverse effects in patients completing three months of 2.7 μmol/kg-bw/day bid (MTD) were grade I/II hypertriglyceridemia, hypercholesterolemia, conjunctivitis, skin dryness, nausea/ vomiting, headache and increased serum transaminases.

13-*cis*-RA was considered for clinical development as a cancer chemopreventive since it has many of the chemopreventive capabilities of all-*trans*-RA and the retinyl esters, but differs in pharmacokinetics and toxicity. In the 90-day rat study, 3–8 fold higher doses of 13-*cis*-RA were required to produce adverse effects similar to those of all-*trans*-RA and retinol. 13-*cis*-RA is 20 times less potent than all-*trans*-RA as a teratogen. With regard to pharmacokinetics, the duration of efficacy of all-*trans*-RA is hampered by a short $t_{1/2}$ (45 minutes) and induction of its own metabolism on chronic use. In contrast, 13-*cis*-RA has a longer $t_{1/2}$ (five hours), and there is no evidence that ADME changes over time.

13-*cis*-RA is the most widely studied and effective retinoid in cancer chemoprevention trials in head and neck. One of the first randomized, placebo-controlled chemoprevention trials was undertaken independently by Dr. Waun K. Hong and associates (University of Texas MD Anderson Cancer Center) and demonstrated a significant decrease in second primary tumors after high-dose adjuvant 13-*cis*-RA (*ca.* 4.1–8.2 μmol/kg-bw/day). Since toxicity was also significant, two NCI, Chemoprevention Branch-funded trials using lower doses of 13-*cis*-RA in larger cohorts with previous early-stage disease are in progress (Dr. Hong, MD Anderson Cancer Center; Drs. Charlotte Jacobs and Harlon Pinto, ECOG). The other pioneering randomized, placebo-controlled chemoprevention trial in head and neck by Dr. Hong's group demonstrated reversion of dysplastic oral leukoplakia, considered to be a histological intermediate biomarker in the oral cavity, by high-dose 13-*cis*-RA (*ca.* 3.3–6.7 μmol/kg-bw/day). Since more than half of the responders had recurrences after treatment stopped, an NCI, Chemoprevention Branch-funded trial tested and found effective a regimen of high-dose induction (5 μmol/kg-bw/day for three months), followed by low-dose maintenance (1.7 μmol/kg-bw/day for nine months) in those subjects who had stable or responding disease at the end of the first phase. Three NCI, Chemoprevention Branch-funded trials are now in progress at different institutions to further assess the dose or agent combination for treatment of oral leukoplakia and modulation of other intermediate biomarkers (Dr. Scott Lippman, University of Texas MD Anderson Cancer Center; Dr. Samuel Beenken, University of Alabama; Dr. Stimson Schantz, Memorial Sloan-Kettering). The protocol is being finalized for a fourth trial (Dr. Hong, MD Anderson Cancer Center) to evaluate a combination of 13-*cis*-RA, α-interferon and α-tocopherol in treatment of severe dysplasia and CIS and modulation of intermediate biomarkers in the oral cavity and oropharynx.

Chemoprevention in head and neck cancer is considered an excellent model for strategies in the lung, since the concept of field carcinogenesis applies to both regions. An NCI, DCPC-funded multicenter Phase III trial (Dr. Lippman, MD Anderson Cancer Center) in progress is evaluating adjuvant treatment with low-dose 13-*cis*-RA (*ca.* 1.4 μmol/kg-bw/day) in patients with previous Stage I non-small cell lung cancer (NSCLC). Unfortunately, the only completed lung trial (Dr. Hong, MD Anderson Cancer Center) found no effect of 13-*cis*-RA (3.3 μmol/kg-bw/day) on the histological biomarker bronchial metaplasia/dysplasia in smokers. Three additional studies at different sites are evaluating 13-*cis*-RA alone or in combination on histological and other biomarker types in cohorts at high risk for lung cancer (Dr. Hong, MD Anderson Cancer Center; Dr. Joseph Ayoub, Montreal Cancer Institute; Dr. Karen Kelly, University of Colorado Cancer Center).

The results from chemoprevention trials with 13-*cis*-RA in other organ systems have been equivocal. In skin, two large NCI, DCPC-funded trials [Dr. Joseph Tangrea, NCI (ISO-BCC); Dr. Thomas E. Moon, University of Arizona (SKICAP-S/B)] found

no effect on SCC or BCC incidence following low-dose adjuvant treatment of previous skin cancer patients. Other small uncontrolled trials funded by NCI demonstrated prevention of new skin tumors in xeroderma pigmentosum patients; another trial is ongoing. A completed NCI, Chemoprevention Branch-funded trial found no effect of 13-*cis*-RA on dysplastic nevi, which are considered to be precursors to malignant melanoma. In bladder, two independent studies found no effect of 13-*cis*-RA on tumor recurrence rate. In uncontrolled independent and NCI-funded trials, some improvements in hematological parameters were found in myelodysplastic syndromes, which are considered to be precursors to leukemia. However, in two independent controlled trials, an effect was found only in the subgroup of chronic refractory anemia patients. Finally, an abstract on an ongoing independent study has suggested an effect of 13-*cis*-RA + α-interferon in regression of CIN lesions and HPV detection.

13-*cis*-RA has produced significant decreases in second primary tumors and regression of premalignant tumors in three independent and one Chemoprevention Branch-funded randomized placebo-controlled head and neck trials. The Branch will use the results of the four trials in progress to determine if lower doses or agent combinations are strategies to retain efficacy and increase safety in prevention of oral cavity cancer. Because the concept of field carcinogenesis also applies to lung, four ongoing Phase II and III trials with second primary tumors and intermediate biomarkers as endpoints will determine if 13-*cis*-RA should also be pursued a lung cancer chemopreventive. Skin cancer chemoprevention will not be pursued further; however, randomized, placebo-controlled trials may be considered in cohorts at high risk for bladder cancer.

The NCI, DCPC Drug Repository has a supply of 3.75 and 5 mg capsules of drug obtained from BASF. Accutane® is also marketed by Hoffman-LaRoche in dosages of 10, 20, 30 and 40 mg.

PRECLINICAL EFFICACY STUDIES

The NCI, Chemoprevention Branch has done only limited efficacy testing with 13-*cis*-RA due to the availability of published results. Retinoids in general inhibit tumorigenesis in skin, respiratory tract, bladder, mammary gland and GI tract. Of these, 13-*cis*-RA is one of the most active retinoids in carcinogen-induced bladder (mouse and rat) and skin (mouse) cancer models. Although it also has activity in lung and oral cavity (hamster) and colon (rat), 13-*cis*-RA was not as effective in mammary glands (rat) as retinyl acetate or 4-HPR. Identification and validation of intermediate biomarkers as potential surrogate endpoints for cancer is a priority of the Chemoprevention Branch. 13-*cis*-RA inhibited the development of histological (bladder atypia, skin papilloma, lung adenoma) and proliferative (ODC activity in oral cavity and skin) intermediate biomarkers in some of the same organs.

Bladder: The rationale for testing retinoids as chemopreventive agents in the bladder stems from studies showing that vitamin A deficiency led to squamous metaplasia in this organ in rats [17]. Later studies also found that vitamin A-deficient animals had a higher incidence of carcinogen-induced bladder tumors. In the first bladder study with oral 13-*cis*-RA, Sporn *et al.* [18] demonstrated decreased incidence and severity of SCC in rats treated intravesically with MNU. Other investigators obtained similar results [19,20]. Since the majority of human bladder tumors are TCC, the agent was also tested in the OH-BBN-treated rat and mouse models, in which multiple stages are observed (flat and papillary hyperplasia, squamous metaplasia, atypia, TCC). Several studies with these models have shown significant decreased incidence, multiplicity, size and grade of TCC at doses of 200 mg/kg diet in mice (*ca.* 85.5 μmol/kg-bw/day) and 240 mg/kg diet in rats (*ca.* 40 μmol/kg-bw/day) when given after the carcinogen [21–25]. One study also demonstrated reduction in the extent of cellular atypia in both proliferative and malignant lesions [23]; in premalignant lesions, this is considered to be a histological intermediate biomarker.

Skin: In early studies in a two-stage mouse skin carcinogenesis model (DMBA, croton oil), Bollag found that all-*trans*-RA given ig during promotion reduced papilloma and carcinoma multiplicity and carcinoma incidence, as well as induced regression of established papillomas [17]. Subsequent studies with 13-*cis*-RA demonstrated that oral (5–217 mg/kg diet, or *ca.* 2–92.8 μmol/kg-bw/day) or topical administration (17 or 34 nmol 2x/wk) inhibited papilloma development in the DMBA/TPA [4,26,27], TPA [28], B(*a*)P/TPA [29], DMBA/anthralin [30] and MCA [31] models; topical 13-*cis*-RA (3 mg, 2x/wk for three weeks) also decreased carcinoma incidence in the MCA-induced Swiss mouse [31]. Interestingly, these effects correlated to inhibition of both

TPA- [*e.g.*, 27,32,33] and anthralin-promoted [30] ODC activity in mouse skin, which is considered to be a proliferation biomarker.

In a combination study, dietary 13-*cis*-RA plus DFMO in drinking water was compared with each agent alone when administered for 18 weeks beginning two days before promotion in the DMBA-induced/TPA-promoted SENCAR mouse skin model [26]. 13-*cis*-RA alone (100 mg/kg diet, or *ca.* 42.8 μmol/kg-bw/day) decreased papilloma multiplicity by 41% and carcinoma incidence by 52%. DFMO alone (0.25% drinking water) decreased papilloma multiplicity by 81% and carcinoma incidence by 90%; combination treatment was slightly more effective than DFMO alone—88% and 100%, respectively. Unfortunately, the dose of DFMO used was so high that an additive effect with 13-*cis*-RA could not be reliably detected.

Oral Cavity: Several studies have shown 13-*cis*-RA to be effective against DMBA-induced hamster oral carcinogenesis. In an early study, oral administration (10 mg or 9.5 μmol, 2x/wk) in hamsters receiving topical DMBA applications to the cheek pouch delayed development of dysplasia and carcinoma [34]. Three studies obtained similar results using the same dose with tongue carcinoma as the endpoint [35–37]. As with the skin, a concomitant effect on ODC induction was found [38]. The NCI, Chemoprevention Branch has 13-*cis*-RA on test in the DMBA-exposed hamster cheek pouch at a dose of 200 mg/kg diet (*ca.* 80 μmol/kg-bw/day) given both after carcinogen and after the first tumor.

Combining 13-*cis*-RA with other agents as a strategy to increase efficacy has not been successful in this organ system. Offering 13-*cis*-RA (7.5 mg in diet, 2x/wk, or *ca.* 0.9 μmol/kg-bw/day) with 3 ppm selenium (as sodium selenite) in drinking water did not increase the efficacy of 13-*cis*-RA alone in the DMBA-exposed hamster tongue [37]. In a second study, vitamin E did not enhance 13-*cis*-RA inhibition of ODC activity in the DMBA-induced hamster buccal pouch carcinogenesis [38].

Head and Neck: 13-*cis*-RA has shown mixed results in esophageal and tracheal cancer models [17]. Hamsters exposed to MNU followed by 13-*cis*- RA (172 mg/kg diet, or *ca.* 68.7 mmol/kg-bw/day) had significantly elevated risk of mortality from tracheal tumors [39]. An interesting aspect to this study is that the animals had developed squamous metaplasia at the time that intervention began, modelling human studies in which the cohort is at high risk for cancer due to the presence of premalignant lesions. Similarly, a second study demonstrated increases in the incidence of both carcinomas (3x) and total neoplasms (2x) [40].

In vitro, 13-*cis*-RA inhibited cell proliferation in primary cultures of human Barrett's esophagus biopsies ($IC_{50}=10^{-6}$ M) [41]; Barrett's esophagus is a premalignant lesion in which metaplastic columnar epithelium replaces the normal squamous cells. *In vivo*, an early study found that administration of the agent for 64 weeks after MBN protected against mortality from esophageal tumors in the hamster [42]. In contrast, a later study in the same model found no effect on tumor (papilloma, carcinoma) incidence, multiplicity or size when administered in the diet (240 mg/kg, 25 weeks) before, during and after the carcinogen [43]. Since the doses were the same (240 mg/kg diet), the difference in results may have been from the treatment schedule relative to carcinogen or the duration of treatment.

Colon: In the colon, early studies found that vitamin A-deficient rats had higher incidences of AFB_1- and DMH-induced cancers than animals receiving a supplemented diet [17]. A pharmacological dose of 13-*cis*-RA (67 μg/g diet, or *ca.* 11 μmol/kg-bw/day) reportedly decreased the incidence of DMH-induced rat colon tumors by 60%; however, the details (*e.g.*, vitamin A content of diet) of the study were unavailable for review [44]. A subsequent study compared the effect of 13-*cis*-RA against DMH-induced colon carcinogenesis in random bred Sprague-Dawley and inbred Wistar/Furth rats [45]. In Sprague-Dawley rats, adding the agent to the diet (300 mg/ kg, or *ca.* 49.9 μmol/kg-bw/day) beginning one week before initiation significantly increased tumor latency. In Wistar/Furth rats, ig administration of the agent (10 mg/kg-bw or *ca.* 33.3 μmol/kg-bw for 5 days/mo) beginning in the middle of initiation delayed time to tumor onset at a similar level of statistical significance. In other studies, no effect of 67 μg 13-*cis*-RA/g diet (*ca.* 11 μmol/kg-bw/day) on DMH-induced colonic dysplasia or adenocarcinoma [46] or 240 mg 13-*cis*-RA/kg diet (*ca.* 40 μmol/kg-bw/day) on MNU-induced adenocarcinoma development [47] in rats was obtained.

An NCI, Chemoprevention Branch study evaluated the effect of 13-*cis*-RA on growth and expansion of colonic aberrant crypt foci, which are considered histological intermediate biomarkers with potential

for developing into adenomas in rats [48] and perhaps humans [49]. In this model, each rat has a mean of 95 aberrant crypts four weeks after AOM induction, with 70% of the aberrant crypts in single-crypt foci and the remainder in multicrypt foci (≥2 crypts/focus). By week eight, the majority of foci (mean=173/rat) were multicrypt—78.6%. Administration of 13-*cis*-RA (195 and 390 mg/kg diet, or *ca.* 32.5 and 64.9 μmol/kg-bw/day) for four weeks beginning two weeks after the last AOM exposure decreased total foci by 45–51% [50]. The proportion of single- and multicrypt (≥2 crypts) foci decreased similarly—48–52% and 50–55%, respectively.

Lung: The results of efficacy studies with 13-*cis*-RA in the respiratory tract have been very mixed. The agent was effective in an early model in hamsters approximating the incidence of lung cancer in human smokers. SCC incidence in the lung, trachea and larynx was inhibited by 13-*cis*-RA (1.5 or 4.5 mg, 2x/wk, ig, or *ca.* 0.02 and 0.06 μmol/kg-bw/day) administered for life following intratracheal instillation of low-dose B(*a*)P adsorbed onto ferric oxide particles [51]. The high dose reduced carcinoma incidence by 87%, while no SCC occurred at the low dose; however, the carcinogen control incidence of 10% was too low to reliably determine a chemopreventive effect. In a second study, significantly fewer hamsters exposed to 2,6-dimethylnitrosomorpholine had lung adenomas and carcinomas when given 140 mg 13-*cis*-RA/kg diet (*ca.* 56 μmol/kg-bw/day); however, limited data were reported in the abstract [52]. In contrast, 13-*cis*-RA had no effect on lung nodules and carcinomas induced by intratracheal installation of 3-MCA in rats.

Mammary Glands: 13-*cis*-RA has not been extensively tested in rodent mammary gland cancer models. In an NCI, Chemoprevention Branch-funded *in vitro* study, the retinoid (10^{-6} M) inhibited DMBA-induced transformation of mouse mammary organ cultures by >60%. In a published study, 1.5 mmol 13-*cis*-RA/kg diet (*ca.* 75 μmol/kg-bw/day) administered beginning one week after DMBA inhibited tumor incidence and multiplicity of rat mammary gland tumors [53]. In contrast, no chemopreventive effect was obtained in the MNU-induced rat model at a dose of 1 mmol/kg diet (*ca.* 50 μmol/kg-bw/day) [54].

Other: Several retinoids have been shown to inhibit development of azaserine-induced pancreatic tumors in rats [reviewed in 17]. However, the effect of 13-*cis*-RA has been equivocal in the BOP-induced hamster. 13-*cis*-RA (0.05–0.2 mmol/kg diet, or *ca.* 2.8–11.1 mol/kg-bw/day [10.5 g diet/day; males 153 g-bw; females 190 g-bw]) reduced adenoma multiplicity in females only; carcinoma development was unaffected [55]. Higher doses of 13-*cis*-RA (0.4 and 0.8 mmol/kg diet, or *ca.* 0.02 and 0.04 mmol/kg-bw/day) with lower doses of BOP had no effect in either sex [56].

13-*cis*-RA (0.02% in diet, or *ca.* 0.03 μmol/kg-bw/day) inhibited hepatocarcinogenesis when offered during the first four weeks of the nine-week exposure to the azo dye 3'-methyl-4-dimethylaminoazobenzene [57]. Endpoints were cirrhosis, nodules and hepatomas.

PRECLINICAL SAFETY STUDIES

The NCI, Chemoprevention Branch has not funded any preclinical toxicity studies with 13-*cis*-RA. Surprisingly little has been published on the toxicity of this agent. Studies by Hoffman-LaRoche found that vitamin A (acetate or palmitate), all-*trans*-RA, retinol, and 13-*cis*-RA had similar oral acute toxicity in mice, rats and rabbits [58]. Hixson and coworkers compared the subchronic toxicity of retinol, all-*trans*-RA and 13-*cis*-RA in mice (3 weeks) and rats (12 and 13 weeks); although the types of effects were similar, 3-8 fold higher doses of 13-*cis*-RA than all-*trans*-RA were required to elicit them [59]. Hoffman-LaRoche described the subchronic and chronic toxicity of 13-*cis*-RA in rats (13 weeks, two years) and dogs (seven weeks, 55 weeks) [1,58].

Safety: The oral LD_{50} for 13-*cis*-RA is 3,389 (11.3 mmol/kg-bw) and >4,000 mg/kg-bw (>13.3 mmol/kg-bw) in mice and rats, respectively, and is ≈1,960 mg/kg-bw (6.5 mmol/kg-bw) in rabbits [1, 58]. In the Hoffman-LaRoche subchronic studies, rats were offered 10–50 mg/kg-bw/day (0.03–0.17 mmol/kg-bw/day) in the diet for 90 days and dogs were administered 120 mg/kg-bw/day (0.4 mmol/kg-bw/day) by capsule for seven weeks. Dose-related effects in both studies included decreased food consumption and body weight gain, erythema, alopecia, mucosal changes, elevated serum alkaline phosphatase and transaminase activities, increased liver weight, and decreased testicular weight [58, 59]. Effects seen only in rats included long bone fractures and elevated serum triglycerides; those seen only in dogs were apparent joint pain and decreased spermatogenesis.

In an independent rat study, similar doses (4–40 mg/kg-bw/day, or 13.3–133 μmol/kg-bw/day) given ig for 12 weeks caused significant dose-related decreases in plasma albumin and increases in hemoglobin and alkaline phosphatase activity. The changes in albumin and hemoglobin were greater in female than male animals. Interestingly, only one bone fracture and no histologic changes occurred [60]. Possible explanations for the differences in the two rat studies are rat strain, and mode and timing of administration (*ad libitum* diet *versus* single intubation).

In mice, the major dose-related effects following administration of 60–400 mg/kg-bw/day ig (200–1331 μmol/kg-bw/day) for 21 days were similar to both rats and dogs in the Hoffman-LaRoche studies: bone fractures, testicular degeneration, reduced spermatogenesis, dermal/epidermal inflammation and hyperkeratosis [61]. Alkaline phosphatase was elevated only at the middle three doses (80, 160, 320 mg/kg-bw/day, or 266, 533, 1065 μmol/kg-bw/day); bone fractures were not always accompanied by elevations in alkaline phosphatase. In comparison, 3–5 fold higher doses of 13-*cis*-RA than all-*trans*-RA were required to produce similar toxicity in mice.

Chronic toxicity study in dogs (55 weeks) and a chronic toxicity/carcinogenicity study in rats (two years) were performed by the manufacturer. Doses of 2, 18, and 32 mg/kg-bw/day (6.7, 59.9 and 106.5 μmol/kg-bw/day) were administered to Fischer 344 rats in the diet following 1 mg/kg-bw/day (3.3 μmol/kg-bw/day) for the first 13 weeks of the study (to avoid fractures in young, growing animals) [1,58,59]. Dose-related effects included increased mortality and decreased food consumption and body weight gain. Decreased hemoglobin and hematocrit and elevated serum triglycerides were observed at the two highest doses. Increased alkaline phosphatase activity and liver and kidney weights were observed in all dose groups. Non-neoplastic histological changes occurring at increased incidences at the two highest doses included fibrosis and inflammation of the myocardium, arterial calcification, focal tissue calcification, and focal osteolysis of the bone. No increase in tumor incidence was obtained after two years, except for pheochromocytoma associated with adrenal medullary hyperplasia at the two highest doses. Pheochromocytoma increases spontaneously with age in this strain, which may be a poor model for humans in which this is a rare tumor. In contrast, decreased incidences of liver adenomas and angiomas and leukemia were noted at the same doses.

In the Hoffman-LaRoche chronic dog study, Beagles were to be treated with 3, 20 and 120 mg/kg-bw/day (1, 67 and 399 μmol/kg-bw/day) by capsule for 55 weeks [1,58,59]. Within four weeks, the high-dose group displayed severe weight loss and debilitation; dosing was resumed eight weeks later on a cycle of 60 mg/kg-bw/day (200 μmol/kg-bw/day) for six weeks and no treatment for two weeks. Clinical toxicity observed during the treatment cycle included severe weight loss, skin lesions, ophthalmic changes (*e.g.*, corneal opacity), decreased hemoglobin and hematocrit, and increased serum alkaline phosphatase activity; most signs diminished during the drug holiday. Microscopic changes included fibrosis and focal calcification in the myocardium and aorta, increased liver weight, testicular atrophy with spermatogenic arrest, and lymph node edema. Although increased liver weight and testicular atrophy with spermatogenic arrest were also observed at the middle dose, no clinical or histological signs of toxicity occurred at the lowest dose.

13-*cis*-RA has species-specific effects on reproduction and development in experimental animals. In Segment I studies performed by the manufacturer, no adverse effects on gonadal function, fertility, conception rate, gestation or parturition were observed in male and female rats at doses up to 32 mg/kg-bw/day (107 μmol/kg-bw/day) [58,59]. However, testicular atrophy and depression of spermatogenesis was noted in dogs administered 20 and 60 mg/kg-bw/day (67 and 200 μmol/kg-bw/day) for 30 weeks in the subchronic dog study discussed above. As with other retinoids, 13-*cis*-RA is a teratogen in experimental animals; however, it is 20 times less potent than all-*trans*-RA [16]. The mechanism is suggested to be elevation of RARβ2 which may mediate expression of a specific group of genes at an inappropriate time. Monkeys and rabbits are moderately sensitive (5 mg/kg/day, or 16.6 μmol/kg-bw/day), while mice and rats are insensitive (75 mg/kg-bw/day, or 250 μmol/kg-bw/day). Humans are the most sensitive (1 mg/kg-bw/day, or 3.3 μmol/kg-bw/day) due to longer plasma $t_{1/2}$ (10–20 fold), interconversion of the 13-*cis*-4-oxo-RA metabolite to all-*trans*-4-oxo-RA, and extensive transplacental distribution.

13-*cis*-RA was not mutagenic in the Ames test (<2x background) in two laboratories. Other tests designed to assess genotoxicity (Chinese hamster cell assay, mouse micronucleus test, *Saccharomyces*

cerevisiae D7 assay, *in vitro* clastogenesis assay in human-derived lymphocytes and unscheduled DNA synthesis assay) were also negative [1].

ADME: The $t_{1/2}$ of 13-*cis*-RA in rodents is much shorter than that in humans [16]. In mice, the value is one hour, which is 10–20 fold less than in humans. The relative AUC values for 13-*cis*-RA are human >> monkey > mouse [62]. The metabolic profile is also different among species [16]. In rodents a considerable portion of the administered dose is glucuronidated to 13-*cis*-retinoyl-β-glucuronide; in monkeys and humans, significant conversion to 4-oxo-13-*cis*-RA occurs. In cynomolgus monkeys, the plasma concentration and AUC values for 4-oxo-13-*cis*-RA were about 40% of parent after administration of 2 mg/kg-bw/day (6.7 μmol/kg-bw/day, ig) for ten days; the corresponding values for all-*trans*-RA (2–3%) and 13-*cis*-retinoyl-β-glucuronide (5–10%) were much lower [62].

Tissue distribution of [^{14}C]-13-*cis*-RA in rats after oral dosing revealed high concentrations of radioactivity in many tissues after 15 minutes, with a maximum in one hour, and declining to nondetectable levels by 24 hours in most tissues [16]. After seven days, however, low levels of radioactivity were detected in the liver, ureter, adrenal, ovary and lacrimal gland.

Biliary excretion and enterohepatic circulation appear to be important in rats, mice and dogs [6]. Glucuronide conjugates of 13-*cis*-RA, all-*trans*-RA and 4-oxo-RA have been detected in the feces and biliary tract. After secretion in the bile, significant reabsorption can occur. For example, the apparent bioavailability of 13-*cis*-RA was 54% in the intact dog and only 14% in the bile-cannulated animal.

CLINICAL SAFETY: PHASE I STUDIES

Safety and pharmacokinetic data on 13-*cis*-RA are available from marketing Accutane® as an oral treatment (1.7–6.7 μmol/kg-bw/day bid for 15–20 weeks) for acne. The NCI, Chemoprevention Branch funded a single Phase I trial evaluating chronic use of lower doses (*ca.* 0.5 μmol/kg-bw/day for nine months). The Phase III ISO-BCC trial (Dr. Joseph Tangrea, NCI, DCPC) funded by NCI, DCPC contributed limited safety data on approximately the same dose for three years. The most common complaints were those reported during acne treatment, although headaches, menstrual changes and bone abnormalities and pain were also seen. The safety of doses higher than those used to treat acne was addressed in an NCI, DCPC-sponsored Phase I/IIa trial (Dr. Bernard Greenberg, University of Arizona) and an NCI, DCT-funded Phase I trial in patients with myelodysplastic syndromes. Unexpectedly, the dose-limiting toxicity (thrombocytopenia *versus* hepatotoxicity) differed between these two studies, possibly due to dosing regimens (bid *versus* qd) or disease subtype.

Drug Effect Measurements: In head and neck and lung, RARβ expression decreases as tissues progress from premalignant to malignant lesions [reviewed in 63]; high-dose 13-*cis*-RA treatment of oral leukoplakia appears to upregulate the receptor [64,65]. This measurement, however, requires tissue sampling. An alternative might be measurement of the major metabolite of 13-*cis*-RA, 4-oxo-13-*cis*-RA, which has a long half-life and is present at levels two-five fold greater than the parent.

Safety: With approved use of Accutane® (1.7–6.7 μmol/kg-bw/day bid for 15–20 weeks), the most frequent adverse event was cheilitis in more than 90% of patients [1]. The duration of both this effect and elevated plasma triglycerides (25% of patients) was related to dose. Increases in the latter over 800 mg/dl have been associated with pancreatitis. Less frequent were decreases in red blood cell parameters (10–20%), increased liver enzymes (10–20%), decreased HDL levels (16%) and conjunctivitis (40%). Skeletal hyperostosis, with symptomatic osteophyte formation and ligament calcification, has also been observed in trials of disorders of keratinization at mean doses of 2.24 mg/kg-bw/day (7.5 μmol/kg-bw/day) [1,66]; in treatment of acne patients at recommended doses, only minimal hyperostosis was found. Other adverse reactions include musculoskeletal complaints (*e.g.*, arthralgia) (16%), rash (10%), GI symptoms (5%), fatigue (5%), headache (5%), corneal opacities, decreased night vision and elevated blood sugar [1].

Two NCI, DCPC-funded studies evaluated the chronic effects of 13-*cis*-RA at doses at or below those for acne treatment [67]. In a Phase I trial (Drs. Libby Edwards and Norman Levine, University of Arizona), ten healthy volunteers (5/sex) received 10 mg 13-*cis*-RA daily (*ca.* 0.5 μmol/kg-bw/day) for nine months. Two subjects discontinued due to toxicity—one had bone and joint pain and the other discomfort from multiple effects. The most common complaints were those reported for treatment of severe acne, *e.g.*, xerosis and cheilitis; however, the

incidence of mild headaches (70%), and menstrual changes (100% females) were higher than expected. Other complaints were myalgia/arthralgia (30%), dry/burning eyes (30%), photosensitivity, epistaxis (20%) and desquamation (20%). In the Phase III trial (Dr. Tangrea, NCI, DCPC) evaluating low-dose 13-*cis*-RA against recurrence of BCC, 0.14 mg/kg-bw/day (0.5 μmol/kg-bw/day) for three years significantly increased progression of existing hyperostotic abnormalities compared with placebo (40% *versus* 18%); however, the incidence of new skeletal changes was unaffected [68].

A published Phase IIa study in Italian oral leukoplakia patients (n=16) evaluated 13-*cis*-RA at doses (bid) escalating from 0.2 mg/kg-bw/day (0.7 μmol/kg-bw/day) in 0.2 mg/kg-bw/day increments every three months [69]. Adverse effects in patients completing treatment at the MTD of 0.8 mg/kg-bw/day (2.7 μmol/kg-bw/day) were grade I/II hypertriglyceridemia, hypercholesterolemia, conjunctivitis, skin dryness, nausea/vomiting, headache and increased serum transaminases; all were reversible. Only two patients finished the maximum dose (1 mg/kg-bw/day, or 3.3 μmol/kg-bw/day, for three months).

Two NCI studies evaluated the toxicity of even higher doses of 13-*cis*-RA in patients with myelodysplastic syndromes. An NCI, DCPC-sponsored Phase I/IIa trial (Dr. Greenberg, University of Arizona) studied doses (bid) escalating from 1 mg/kg-bw/day at monthly increments of 0.5 mg/kg-bw/day [70]. The highest dose achieved was 2 mg/kg-bw/day (6.7 μmol/kg-bw/day) due to significant toxicity. The most frequent dose-limiting toxicity was unexpected thrombocytopenia occurring in four patients with low pretreatment platelet counts. The most frequent expected adverse reaction was elevated serum triglycerides. Other expected severe adverse events included severe dry skin, cheilitis, conjunctivitis, headache, fatigue, anorexia, and epistaxis. In contrast, an NCI, DCTDC-sponsored Phase I study of 20–125 mg/m^2 qd (*ca.* 1.6–10.3 μmol/kg-bw/day) for ≥21 days in the same population (n=19) found hepatotoxicity (hyperbilirubinemia and elevated SGOT) to be dose limiting (2/2 at the highest dose) [71]. Serum lipids were elevated at doses ≥80 mg/m^2 qd (*ca.* 6.6 μmol/kg-bw/day). The most common adverse effects were hyperkeratosis, cheilosis, and hematological responses. The difference in toxicity profiles between the two studies may have resulted from the dosing regimens (qd *versus* bid) or the sub groups of myelodysplastic syndrome represented in the studies.

Because of pharmacokinetics, humans are more sensitive to the teratogenic effects of 13-*cis*-RA than experimental animals [16]. The major metabolite 13-*cis*-4-oxo-RA can be interconverted to all-*trans*-4-oxo-RA, which has teratogenic potency similar to all-*trans*-RA. Accutane® is marketed in the US with the contraindication that it is not to be used in pregnant women. Infants born to inadvertently exposed women show CNS (hydrocephalus, cerebellar hypoplasia, cerebral cortex abnormalities), craniofacial (cleft palate, eye and external ear abnormalities) and heart defects, and thymus abnormalities.

ADME: Some pharmacokinetic studies with 13-*cis*-RA have found considerable variation in parameters, probably due to differences in formulation and feeding status; food approximately doubles absorption of 13-*cis*-RA [2,6]. After oral administration of a single dose of 80, 160, 240 or 320 mg (*ca.* 3.8–15.2 μmol/kg-bw/day) to healthy volunteers, both C_{max} and AUC were dose-related up to 240 mg, with no further increases at the highest dose. No significant changes in pharmacokinetics occurred with repeat dosing of 80 mg/day (bid) for 25 days. Following the initial 80 mg dose, C_{max}=262 ng/ml (range: 98– 535 ng/ml), AUC=2.8 mg•hr/l and t_{max}=2–4 hr; $t_{½}$ ranged from 7.9 to 36.5 hr. Steady state was reached within 5–7 days, with C_{max}=310 ng/ml, t_{max}=2–4 hr, $t_{½}$=5.9–22.4 hr,and AUC=3.1 mg•hr/l. Pharmacokinetics are similar in cancer patients and patients with dermatological disorders.

The bioavailability of 13-*cis*-RA is approximately 25% [72]. Unlike retinol, 13-*cis*-RA is absorbed directly via the intestinal mucosa into the portal vein. Since 13-*cis*-RA is highly lipophilic, it is transported in blood extensively (99.9%) bound to plasma proteins, primarily albumin; in contrast, retinol circulates complexed specifically with retinol binding protein [6]. In the liver, there is significant first-pass metabolism of 13-*cis*-RA (75% of dose), and enterohepatic circulation occurs [73]. The major metabolite identified in plasma is 4-oxo-13-*cis*-RA [2,6]. The elimination $t_{½}$ of this metabolite is 20–50 hours, so steady-state levels exceed those of the parent by two- to five-fold. Also, approximately 20–30% of a 13-*cis*-RA dose is isomerized to all-*trans*-RA, and minor amounts of 4-hydroxy-13-*cis*-RA have also been identified. Studies with radiolabeled drug show that

significant amounts of unidentified metabolites also occur; limited data suggest that these may include glucuronide conjugates as in experimental animals [62].

Few distribution data are available in humans; however, it does not appear that 13-*cis*-RA accumulates in serum or skin [74]. Within 96 hours, 60% of the dose is recovered, divided equally between feces and urine [75]. Since bioavailability is low, some of the dose in feces may represent unabsorbed drug; however, approximately 20% is eliminated via the bile over the same time period. The major biliary metabolites appear to be glucuronide conjugates of 4-oxo-13-*cis*-RA and 16-hydroxy-13-*cis*-RA; minor metabolites are glucuronide conjugates of 13-*cis*-RA and 18-hydroxy-13-*cis*-RA.

CLINICAL EFFICACY: PHASE II/III STUDIES

Prior to establishment of the NCI, Chemoprevention Branch, Dr. Waun K. Hong and associates (University of Texas MD Anderson Cancer Center) pioneered testing of 13-*cis*-RA in clinical trials. They began the first two randomized, placebo-controlled, double-blind chemoprevention trials in 1982 [76], both of which used high-dose 13-*cis*-RA (>1 mg/kg-bw/day). The trials demonstrated efficacy in regression of oral leukoplakia [77] and as adjuvant treatment of patients with previous head and neck cancer [78]. Three head and neck trials with low-dose 13-*cis*-RA (≤1 mg/kg-bw/day) at MD Anderson Cancer Center were subsequently funded by NCI, Chemoprevention Branch during the period 1988–1992. One of these studies is finished, demonstrating the efficacy of high-dose induction with low-dose maintenance in regression of oral leukoplakia [79]. The two trials in progress are evaluating long-term low-dose 13-*cis*-RA in previous head/neck cancer patients and very low doses of 13-*cis*-RA (0.5 mg/kg-bw/day, or 1.7 μmol/kg-bw/day, for one year, then 0.25 mg/kg-bw/day, or 0.8 μmol/kg-bw/day, for two years) *versus* β-carotene + retinyl palmitate in oral leukoplakia treatment. Recently, an MD Anderson Cancer Center trial evaluating 13-*cis*-RA in combination with α-interferon and α-tocopherol in treatment of premalignant head/neck lesions was also funded by the NCI, Chemoprevention Branch. Finally, three trials have been funded by the Chemoprevention Branch at three other sites. Two studies (Dr. Samuel Beenken, University of Alabama; Dr. Stimson Schantz, Memorial Sloan-Kettering) are evaluating modulation of intermediate biomarkers in oral leukoplakia and an ECOG trial (Drs. Charlotte Jacobs and Harlon Pinto, Stanford University) is using chronic low-dose 13-*cis*-RA in prevention of second primary head/neck tumors.

Because of the success with low-dose 13-*cis*-RA in head and neck, NCI has funded five chemoprevention studies in lung, three of them at MD Anderson Cancer Center. However, in a study begun in 1987, Hong and associates found no change in bronchial metaplasia or dysplasia in smokers after low-dose 13-*cis*-RA [80,81]. A second similarly designed trial evaluating the combination of 13-*cis*-RA with vita min E began recently. Two trials at other sites (Dr. Joseph Ayoub, Montreal Cancer Institute; Dr. Karen Kelly, University of Colorado Cancer Center) are identifying biomarkers modulated by 13-*cis*-RA in smokers. Finally, an MD Anderson Cancer Center trial nearing the accrual goal is evaluating second primary tumor incidence in patients with previously resected lung tumors.

Due to efficacy in early small chemotherapy studies by the NCI, Dermatology Branch, the first NCI-funded chemoprevention trials with 13-*cis*-RA began in 1983 and involved adjuvant treatment of skin cancer patients (ISO-BCC and SKICAP-S/B). These trials and a small trial of low-dose 13-*cis*-RA in dysplastic nevus syndrome found no effect. Two completed and one ongoing uncontrolled trials tested the retinoid in a cohort at very high risk for skin cancer—xeroderma pigmentosum patients.

Head and Neck: 13-*cis*-RA is the most widely studied retinoid in head and neck for prevention of second primary cancers [82]. The lifetime risk for developing a second primary tumor (*e.g.*, lung, head and neck, esophagus) following head and neck cancer (oral cavity, oropharynx, hypopharynx) is 20–40%, probably due to exposure of the entire epithelium to the same carcinogenic insults; genetic predisposition may also play a role [83]. The first randomized placebo-controlled trial that suggested efficacy of 13-*cis*-RA against second primary tumors was undertaken independently by Dr. Hong and associates at MD Anderson Cancer Center. It was actually designed to assess the agent as an adjuvant to radiotherapy or surgery in 103 head and neck cancer patients (half were Stage III or IV) [78]. After 50–100 mg/m^2 qd (*ca.* 4.1–8.2 μmol/kg-bw/day) for one year beginning within 16 weeks of primary treatment, no effect on recurrence of primary cancer was obtained. However, the second primary tumor rate was signifi-

cantly lower in the treatment group (4% *versus* 24%) after a median follow-up of 32 months. At 55 months, the difference was still significant (14% *versus* 31%) [84].

However, in the previous study, the frequency of severe adverse events in the first 44 patients prompted reduction of the dose from 100 to 50 mg/m^2/day (*ca.* 8.2 to 4.1 μmol/kg-bw/day) [78,84]. One-third of the 13-*cis*-RA group did not finish intervention due to adverse events or noncompliance. Two NCI, DCPC-funded multicenter Phase III trials are now in progress to validate these results with lower chronic doses in larger cohorts; also, the patients are limited to previous early-stage (Stage I/II) head/neck cancer to decrease the competing risk of initial tumor recurrence or metastasis (see Table I). The first trial (Dr. Hong, MD Anderson Cancer Center) is evaluating the effect of 30 mg 13-*cis*-RA qd (*ca.* 1.4 μmol/kg-bw/day) for three years on incidence and latency of second primary tumors in 1,080 patients. The second trial (Drs. Jacobs and Harlon Pinto, Stanford University) in patients with a previous SCC is evaluating incidence and latency of recurrent tumors, second primary tumors, and dysplasia after administration of 10 mg qd (*ca.* 0.5 μmol/kg-bw/day) to 275 patients for two years.

13-*cis*-RA is also the most widely studied retinoid in head and neck for regression of premalignant lesions (*e.g.*, oral leukoplakia, laryngeal papillomatosis, Barrett's esophagus) [82]. Oral leukoplakia is defined clinically as a white plaque on the surface of the oral mucosa [85]; those lesions that are dysplastic on histological examination are considered precancerous [86]. Several early uncontrolled studies demonstrated that 13-*cis*-RA could regress both the clinical and histological lesion [87–89]. This led to the first randomized, placebo-controlled, double-blind chemoprevention trial, which was conducted independently by Dr. Hong and associates at MD Anderson Cancer Center. Subjects with biopsy-proven hyperplastic or dysplastic oral leukoplakia were randomized to 1 or 2 mg 13-*cis*-RA/kg-bw (3.3 or 6.7 μmol/kg-bw/day) or placebo daily for three months, with a six-month follow-up [77]. During treatment, clinical responses were observed more frequently in the 13-*cis*-RA groups (67% *versus* 10%); more importantly, dysplasia reversed in 54% of treated patients compared with 10% receiving placebo. However, 2–3 months after treatment stopped, more than 50% of responders recurred or developed new lesions. Also, adverse reactions (skin dryness, erythema and peeling, conjunctivitis, hypertriglyceridemia) occurred more frequently at 2 mg/kg-bw/day (6.7 μmol/kg-bw/day) than at the lower dose. At the higher dose, 47% of patients required dose reduction due to adverse effects.

The efficacy of a lower maintenance dose (0.5 mg/kg-bw/day, or 1.7 μmol/kg-bw/day) was also demonstrated after high-dose induction. A completed NCI-sponsored Phase II trial (Drs. Scott Lippman and Hong, MD Anderson Cancer Center) compared low-dose 13-*cis*-RA with β-carotene as maintenance therapy [79]. Patients with clinically evident oral leukoplakia received effective but toxic treatment with 13-*cis*-RA (1.5 mg/kg-bw or 5.0 μmol/kg-bw qd) for three months during the induction phase. Those with responding (55%) or stable lesions (35%) were stratified histologically and randomized to a daily maintenance dose of either 0.5 mg 13-*cis*-RA/kg-bw (1.7 μmol/kg-bw/day) or 30 mg β-carotene for an additional nine months. 13-*cis*-RA maintenance produced further response in 92% of patients compared with 42% improved or stabilized with β-carotene. The authors suggest that the difference in response may result from selection of those responding specifically to retinoids during the induction phase.

In a published Phase IIa study of Italian oral leukoplakia patients (n=16), 36% (5/14) had ≥50% reduction in lesion size at divided oral doses of 0.2–0.6 mg 13-*cis*-RA/kg-bw/ day (0.7–2.0 μmol/ kg-bw/day); an additional 36% of evaluable patients had minor responses [69]. However, during the 12-month follow-up, two patients showed regression of a positive response.

Three NCI, Chemoprevention Branch-funded trials are in progress evaluating 13-*cis*-RA in oral leukoplakia patients. An ongoing Phase III trial (Dr. Lippman, MD Anderson Cancer Center) is comparing the efficacy of long-term low-dose 13-*cis*-RA (induction: 0.5 mg/ kg-bw/day or 1.7 μmol/kg-bw/day for one year; maintenance: 0.25 mg/kg-bw/day or 0.8 μmol/kg-bw/day for two years) *versus* 50 mg β- carotene + 25,000 IU retinyl palmitate for three years [90]. After a two-year follow-up period, the incidence and duration of clinical and histological response of dysplastic oral leukoplakia/erythroplakia will be evaluated. The three other studies will be identifying other intermediate biomarkers in oral cavity modulated by 13-*cis*-RA and correlating these

with histological/clinical regression of the lesion. A pilot study (Dr. Beenken, University of Alabama) near completion used induction with 1 mg 13-*cis*-RA/kg-bw/day (3.3 μmol/kg-bw/day) for 2–3 months, followed by one-half to one-quarter of the dose for various durations. Interim results show that TGFα expression was significantly reduced by the agent, but it did not correlate to lesion response. The protocol for a subsequent placebo-controlled trial is being finalized to determine the effect of a similar induction and maintenance regime on ploidy, nuclear cytomorphometry, TGFα and other proliferation biomarkers (PCNA, MIB-1, EGFR, c-*erb*B-2 expression). Finally, the protocol for a Phase II trial (Dr. Schantz, Memorial-Sloan Kettering) evaluating modulation of oral cavity biomarkers (native cellular fluorescence, cytokeratins, blood group antigens, lectins, PCNA, ploidy, nuclear polymorphism index) by 1 mg 13-*cis*-RA/kg-bw/day compared with placebo is also being finalized.

The protocol for a fourth NCI, Chemoprevention Branch-funded trial (Dr. Hong, MD Anderson Cancer Center) evaluating modulation of intermediate biomarkers in patients with severe dysplasia or CIS of the oral cavity and oropharynx is being finalized. A combination of 100 mg 13-*cis*-RA/m^2 qd (8.2 μmol/ kg-bw/day) with interferon and α-tocopherol for six months is being used; dose escalation may occur after one month. The biomarkers include chromosomal changes and genetic instability, p53, PCNA, EGFR, and RAR.

Respiratory (larynx, trachea, lung) papillomatosis is characterized by growth of metaplastic lesions on vocal cords, requiring surgical intervention to maintain a patent airway. The malignant potential is generally low (2–3%) unless accompanied by heavy tobacco use. In an NCI, DCPC-funded pilot study (Dr. D. Alberts, University of Arizona), high-dose 13-*cis*-RA (0.5–2 mg/kg-bw/day or 1.7–6.7 μmol/kg- bw/day for 5–20 months) reduced laryngeal papilloma size and number in 3/5 patients [91]. An independent placebo-controlled study attempted to test 13-*cis*-RA as adjuvant therapy by randomizing (n=36) to two years of treatment (100 mg/m^2, or *ca.* 8.2 μmol/kg-bw/day qd) or placebo after surgery for respiratory papillomatosis [92]. The study was stopped after the first nine patients due to severe toxicity; only one-third of the treated patients (2/6) completed therapy at half-dose.

Barrett's esophagus is the replacement of the normal squamous lining of the esophagus with metaplastic columnar epithelium. It is considered a precursor to adenocarcinoma. An independent Phase II trial of 1 mg 13-*cis*-RA/kg-bw/day (3.3 μmol/kg- bw/day) in 16 Barrett's esophagus patients was undertaken to evaluate the effect of the retinoid in reversing the condition [93]. Only 11 patients completed six weeks of treatment, and no clinical response was obtained. Adverse dermatological and serum lipid effects were frequent; in addition, two cases of esophageal ulcers not usually associated with retinoid toxicity were observed.

Lung: Chemoprevention in head and neck cancer is considered an excellent model for strategies in the lung, since the concept of field carcinogenesis [94] applies to both regions. An NCI, DCPC-funded multicenter Phase III trial (Dr. Lippman, MD Anderson Cancer Center), of low-dose 13-*cis*-RA in prevention of lung cancer in progress was based on the successful trial in head and neck cancer patients discussed previously [78]. The placebo-controlled, double-blind trial will determine if 30 mg 13-*cis*-RA qd (*ca.* 1.4 μmol/kg-bw/day) for three years will prevent the development of second primary tumors in patients with a previous Stage I NSCLC [95]. Accrual is near completion.

Metaplasia and dysplasia are considered to be premalignant lesions in this organ. Regression of bronchial metaplasia was reported following treatment with another retinoid (etretinate) in an uncontrolled trial [reviewed in 96]. The first small, uncontrolled trial with 13-*cis*-RA (0.5–3 mg/kg-bw/day or 1.7–10.0 μmol/kg-bw/day for a minimum of 28 days) found no effect on squamous metaplasia/dysplasia in cells obtained from sputum samples [97], which may be due to the interindividual variability in the endpoint and the lack of quantitative cytological analysis. A placebo-controlled Phase II trial (Dr. Hong, MD Anderson Cancer Center) funded by NCI, DCPC evaluated modulation of the same endpoint in endoscopic biopsies from chronic smokers. After six months of treatment with 1 mg 13-*cis*-RA/kg-bw/day (3.3 μmol/kg-bw/day) and follow-up for one year, the same rate and magnitude (≈55%) of histological response was obtained in both groups [80,81]. An ongoing NCI, DCPC-funded Phase III trial (Dr. Hong, MD Anderson Cancer Center) is evaluating the effect of the same 13-*cis*-RA dose in combination with vitamin E on bronchial metaplasia/dysplasia in smokers with a previous lung

or laryngeal cancer [98].

Another NCI, DCPC-sponsored Phase II trial in progress is investigating modulation of regulatory intermediate biomarkers in bronchial brushings—decreased RARβ2 expression and increased RARβ4/β2 ratio. This trial (Dr. Ayoub, Montreal Cancer Institute) is determining normalization of these biomarkers in smokers with chronic obstructive lung disease and low baseline RAR values. Preliminary results suggest that RARβ2 expression increased over baseline following the treatment period; correlation of this effect to 13-*cis*-RA must wait until the study is unblinded [99]. However, studies at the MD Anderson Cancer Center suggest that RARβ expression is decreased in both oral leukoplakia and dysplastic lesions in head and neck SCC patients, and 13-*cis*-RA treatment (1.5 mg/kg-bw/day, or 5 μmol/kg-bw/day) for three months significantly increased the incidence of leukoplakia cases expressing the receptor from 39% to 91% [65].

Finally, an NCI-funded Phase III study (Dr. Kelly, University of Colorado Cancer Center) is evaluating the effect of 50 mg 13-*cis*-RA/day (2.4 μmol/kg-bw/day) with and without α-tocopherol for one year on biomarkers in former smokers or previous head/neck cancer patients with atypia, dysplasia or CIS. The biomarkers include Ki-67, p53, HER-2/*neu*, c-*fos*, EGFR, TGFα, Rb, RAR, and transferrin receptor.

Skin: Although 13-*cis*-RA is used in the treatment of acne, it has not been proven to reduce new skin cancers in high-risk cohorts or as treatment for premalignant disorders. Early small chemotherapy studies by the NCI, Dermatology Branch [100–102] suggested a reduction in new skin cancers in BCC patients using 13-*cis*-RA. These studies led to the strategy of testing chemopreventive efficacy in a very high-risk population—xeroderma pigmentosum patients [reviewed in 103]. This rare disorder is characterized by a deficiency in repair of UV-damaged DNA; patients develop skin cancers at a frequency 1,000 times greater than the general population. In the first intramural study (Drs. Kenneth Kraemer, John DiGiovanna, Alan Moshell and Gary Peck, NCI), seven patients were treated with high-dose 13-*cis*-RA (2 mg/kg-bw/day, or 6.7 μmol/kg-bw/day) for two years and then observed for ≥one year. Five patients tolerated intervention [103–105]. In the four who responded, the annual tumor frequency decreased 70–93%; however, this increased 8.5-fold during the post-treatment period. Due to severe adverse reactions, the second intramural trial treated the same seven patients with low-dose 13-*cis*-RA (0.5 mg/kg-bw/day or 1.7 μmol/kg-bw/day) for 8–26 months [103,105,106]. Five patients responded, but only one had a similar response to that at the high dose; four had intermediate responses. Patients with inadequate results are continuing at an intermediate dose (1 mg/kg-bw/day or 3.3 μmol/kg-bw/day) [103]. An uncontrolled Phase II trial by the NCI, National Institute of Arthritis and Musculoskeletal and Skin Diseases (Dr. DiGiovanna) is continuing to use a similar high-risk cohort—subjects with clinically diagnosed xeroderma pigmentosum (XP) or nevoid basal cell carcinoma syndrome (NBCCS) who have had two treated skin cancers/year over the previous two years. Over the two-year treatment period, either 0.5 mg 13-*cis*-RA/kg-bw/day (1.7 μmol/kg-bw/day) will be administered for six months, increasing by 0.5 mg/kg-bw/day every six months if not previously treated with the agent, or, if previously treated with 13-*cis*-RA, that dose is administered. After follow-up for one year, the lowest dose reducing skin cancer incidence will be identified.

Two NCI, DCPC-funded trials evaluated the efficacy of even lower doses of 13-*cis*-RA in reducing new BCC and/or SCC in patients with previously resected lesions—the Phase II SKICAP-S/B trial and the Phase III Isotretinoin Basal Cell Carcinoma (ISO-BCC) Prevention Trial (see Table I). In the three-arm SKICAP-S/B trial (Dr. Thomas E. Moon, University of Arizona), patients (n=525) with prior multiple SCC or BCC were randomized to 5–10 mg 13-*cis*-RA (*ca.* 0.2–0.5 μmol/kg-bw/day), retinol or placebo for three years [6,107–109]. No reduction in the incidence of new skin cancers was observed in either treatment group. In the ISO-BCC trial at eight cancer centers (Dr. Tangrea, NCI, DCPC), patients with a BCC in the previous five years (n=981) were randomized to 10 mg 13-*cis*-RA (*ca.* 0.5 μmol/kg-bw/day) or placebo daily for three years, with follow-up for two years [110– 113]. The retinoid had no effect on the incidence or multiplicity of new BCC; additionally, adverse reactions such as elevated serum triglycerides, mucutaneous reactions and hyperostosis were significantly more frequent.

Dysplastic nevi are cutaneous lesions containing proliferating atypical melanocytes that retain pigment in their cytoplasm [114]. They are considered to be both markers of increased risk for melanoma

and precursors of some melanomas. An NCI-sponsored Phase II randomized trial (Drs. Levine and Frank L. Meysken, Jr., University of Arizona) of oral 13-*cis*-RA (40 mg bid, or *ca.* 3.8 μmol/kg-bw/day for four months) in patients with multiple dysplastic nevi (n=11) found no difference in pre- and post-treatment biopsies [115]. However, no new dysplastic nevi or melanoma were observed during intervention, suggesting a chemopreventive effect.

Hematopoietic and Lymphoid Systems: Myelodysplastic syndromes are a group of five hematopoietic disorders considered to be precursors to leukemia [116]. They occur primarily in the elderly and are characterized by impaired maturation and ineffective hematopoiesis leading to anemia and a relatively indolent course, although death may occur from infection, hemorrhage or progression to malignant disease. Several small uncontrolled studies, including one NCI, DCPC-funded Phase I/IIa trial [70], have shown improvements in hematological parameters in these patients following use of 13-*cis*-RA [reviewed in 117]. Later independent controlled trials found equivocal results. In a double-blind, placebo-controlled trial, patients (n=68) were randomized to receive 100 mg 13-*cis*-RA/m^2 (*ca.* 8.2 μmol/kg-bw/day) or placebo qd [118]. After six months, no differences in bone marrow biopsies or blood counts were observed between groups. A second randomized trial found an effect only in the subgroup of chronic refractory anemia patients [117], suggesting that it may be necessary to analyze each myelodysplastic subgroup separately.

Mycosis fungoides are T-cell lymphomas characterized by involvement of the skin; with progressive disease, lymph node and visceral involvement are observed [119]. An NCI, DCPC-funded Phase II trial (Dr. John Kessler, University of Arizona Cancer Center) evaluated the clinical and histological response of biopsy-proven tumors to 1–2 mg 13-*cis*-RA/kg-bw/day (3.3–6.7 μmol/kg-bw/day, qd or bid). Clinical responses were obtained in 44% (11/25) of subjects, with six additional minor responses [120,121].

Bladder: Although the epidemiologic evidence for an etiologic role for vitamin A in bladder cancer is inconclusive [122], 13-*cis*-RA was effective in suppressing or preventing bladder tumor growth in rats and mice [*e.g.*, 18,19,22,123,124]. Only two clinical intervention studies were identified in the literature—both negative. A small study (n=19) by the National Bladder Cancer Collaborative Group A compared historical recurrence data with 0.5 mg 13-*cis*-RA/kg-bw/day tid (5 μmol/kg-bw/day) for four weeks, escalating to 1 mg/kg-bw/day tid (10 μmol/kg-bw/day) for 20 weeks, in patients with previous recurrent Ta,T1 tumors [123,125]. Of the seven patients completing treatment, only one did not show recurrence; thus, the recurrence rate was actually higher (86%) than the historical rate of 57– 70%. A second study randomized patients (n=25) to four arms: 20 mg of 13-*cis*-RA (1 μmol/kg-bw/day), etretinate or vitamin A *versus* placebo. After 25 months, no differences in recurrence rate were observed [described in 122].

Cervix: Several studies have shown efficacy of topical all-*trans*-RA in regression of CIN lesions [*e.g.*, 126, Vitamin A Clinical Development Plan in this supplement]. A single study of oral 13-*cis*-RA in this cohort was identified in the literature. Due to possible synergism with interferon, CIN II and III patients were treated with 13-*cis*-RA (0.5–1 mg/kg-bw/day or 1.7–3.3 μmol/kg-bw/day) and IFNα2a (3 mIU/day, im) for eight weeks [127]. As reported in an abstract on 17 evaluable patients, histological responses were obtained in 53%; detection of HPV decreased in 62% of patients [127].

PHARMACODYNAMICS

Since the toxicity of 13-*cis*-RA was 3–8 fold less than retinol or all-*trans*-RA in preclinical studies, it was hoped that chemopreventive doses without safety concerns were possible. The recommended dose for acne treatment is 0.5–2 mg/kg-bw/day (1.7–6.7 μmol/kg-bw/day) for 15–20 weeks. However, the estimated MTD for extended intake (12 months) in humans is 0.8 mg/kg-bw/day (2.7 μmol/kg-bw/day) based on a small Italian study in oral leukoplakia patients. In head and neck chemoprevention trials, the first effective high doses were greater than the MTD, *i.e.*, 4.1–8.2 μmol/kg-bw/day. Significantly more adverse events occurred at these doses, such as skin dryness, erythema and peeling, conjunctivitis and hypertriglyceridemia, requiring dose reduction in many patients. A lower maintenance dose (1.7 μmol/kg-bw/day) for nine months following a three-month high-dose induction period (5 μmol/kg-bw/day) has been effective in treatment of oral leukoplakia. Thus, one of the major problems is identifying an effective 13-*cis*-RA dose that does not produce unacceptable adverse effects in healthy populations. Another problem is loss of the response following discontinuation of the drug. Several trials are ongoing or near initiation to establish the efficacy

and safety of even lower doses and long-term maintenance. For example the ongoing Phase III oral leukoplakia trial (Dr. Lippman, MD Anderson Cancer Center) is determining the efficacy of a regimen involving induction with 1.7 μmol/kg-bw/day for one year, followed by maintenance with 0.8 μmol/kg-bw/day for two years; the second arm is receiving 50 mg β-carotene with 25,000 IU retinyl palmitate as a comparison. Other trials are evaluating combinations with α-interferon and vitamin E.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

The drug effect measurements that have been suggested are ODC induction and RAR expression. These, however, require tissue samples and may not be applicable in all organ systems. In head and neck and lung, RARβ expression decreases as tissues progress from premalignant to malignant lesions [63]; 13-*cis*-RA treatment of oral leukoplakia appears to upregulate the receptor.

Safety Issues

As discussed above, the major problem in clinical development of 13-*cis*-RA is identifying an effective dose that does not produce unacceptable adverse effects in healthy populations. Effective doses 1 mg/kg-bw/day (3.3 μmol/kg-bw/day) are considered high and are associated with significant adverse effects. Lower maintenance doses (0.5 mg/kg-bw/day, or 1.7 μmol/kg-bw/day) were shown to be effective in a single oral leukoplakia study after nine months. It is unknown if this or even lower doses would be effective in all situations. In the Phase III ISO-BCC trial, 0.14 mg/kg-bw/day (0.5 μmol/kg-bw/day) for three years significantly increased the incidence of progression of existing hyperostotic abnormalities, elevated serum triglycerides and mucocutaneous reactions compared with placebo; however, the incidence of new skeletal changes was unaffected. Unfortunately, this dose had no effect on the recurrence of BCC.

Pharmacodynamics Issues

One of the major problems with retinoids is that the chemopreventive actions are reversible on drug withdrawal, requiring maintenance treatment after induction of the response. This is not surprising, since the major action appears to be slowing of progression. Chronic administration of 13-*cis*-RA in healthy populations requires identification of a low dose that lacks adverse effects, but retains efficacy.

Regulatory Issues

No regulatory issues have been identified.

Intermediate Biomarker Issues

In preclinical studies, 13-*cis*-RA inhibited the development of histological biomarkers in rat colon (aberrant crypt foci), bladder (atypia), skin (papilloma), pancreas (adenoma), liver (nodules) and lung (adenoma) and proliferative biomarkers in cheek pouch and skin (ODC activity). In clinical trials, regression of various premalignant conditions has also been demonstrated, such as oral dysplastic leukoplakia, laryngeal papillomas, mycosis fungoides and, possibly, myelodysplastic syndromes. Other types of intermediate biomarkers, such as TGFα and RARβ expression, have also been modulated in oral leukoplakia. Since 13-*cis*-RA primarily interferes with progression, other types of intermediate biomarkers are under evaluation as surrogate endpoints for cancer incidence and as functional endpoints correlating with premalignant lesion regression. In three ongoing NCI, Chemoprevention Branch-funded oral leukoplakia studies, modulation of various genetic (chromosomal changes, genetic instability, ploidy, *c-erb*B-2 expression, p53), proliferation (PCNA, MIB-1, EGFR, TGFα), differentiation (cytokeratins, blood group antigens, lectins), histological (nuclear polymorphism), and other (native cellular fluorescence) biomarkers by 13-*cis*-RA is being evaluated. These endpoints could aid in identification of an effective, but safe, dose.

As more is discovered about retinoid receptors, expression of genes modulated by specific receptors should be evaluated in cancer chemoprevention studies. These could include genes involved in differentiation, proliferation, and biochemical pathways, as well as oncogenes and tumor suppressors.

Supply and Formulation Issues

The NCI, DCPC Drug Repository has a supply of 3.75 and 5 mg capsules of bulk drug obtained from BASF. Accutane® is marketed by Hoffman-LaRoche; no future supply problems are anticipated.

Clinical Studies Issues

13-*cis*-RA has produced significant decreases in second primary tumors and regression of premalignant tumors in three independent and one Chemoprevention Branch-funded randomized placebo-controlled head and neck trials. Four additional trials to evaluate the safety and efficacy of lower doses and maintenance doses and to identify modulated intermediate biomarkers in oral leukoplakia have been funded by the Chemoprevention Branch. One trial is evaluating 13-*cis*-RA in combination with vitamin E and the cytokine α-interferon as a strategy to increase efficacy and safety.

Because the concept of field carcinogenesis applies to lung and four retrospective epidemiological studies measuring carotenoids and retinoids separately found an inverse association between serum or dietary preformed retinol and lung cancer cases, the NCI, Chemoprevention Branch pursued clinical development of 13-*cis*-RA in this tissue. The first trial did not find an effect of 13-*cis*-RA on bronchial metaplasia/dysplasia; however, four new Phase II and III trials with second primary tumors and intermediate biomarkers as endpoints will determine if this retinoid should be pursued as a lung cancer chemopreventive.

Although 13-*cis*-RA was one of the most potent chemopreventives (0.2 μmol/kg-bw/day) in murine skin cancer models, results in clinical trials have been equivocal. Two large NCI-funded adjuvant trials (SKICAP-S/B, ISO-BCC) found no effect on new SCC and/or BCC incidence at low 13-*cis*-RA dose (0.2–0.5 μmol/kg-bw/day). Low to high doses (1.7–6.7 μmol/kg-bw/day) in small, uncontrolled studies in xeroderma pigmentosa patients appear to decrease the annual tumor rate. An NCI, NIAMS trial is ongoing in a similar cohort evaluating the lower dose (1.7 μmol/kg-bw/day). The NCI, Chemoprevention Branch will not pursue this indication for 13-*cis*-RA.

Finally, 13-*cis*-RA was the most effective retinoid in preclinical bladder cancer models. However, two small independent clinical trials obtained no reduction in tumor recurrence rate following doses of 1–1.7 μmol/kg-bw/day. It is unknown if pharmacokinetic differences between rodents and humans are responsible. Then NCI, Chemoprevention Branch may consider a future trial in subjects at high risk for bladder cancer, although accrual has been slow in other NCI, Chemoprevention Branch trials with this cohort.

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Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase I (Safety, ADME)					
PO1-CA-27502 Clinical Toxicity of Low-Dose Isotretinoin (Dr. Libby Edwards; Project PI: Dr. Norman Levine, University of Arizona)	---	Healthy male and female volunteers 5 male, 5 female volunteers	10 mg 13-*cis*-RA/day for 9 mo	Safety: Blood chemistry, liver function, serum lipids	Study completed. Two subjects discontinued due to toxicity—one due to bone and joint pain and one due to discomfort from multiple effects. Most common complaints were xerosis, cheilitis, and mild headaches (70%), and menstrual changes (100% females). Published report: [67]
Phase II (Dose-titration, efficacy, intermediate biomarkers)					
PO1-CA-27502 Phase I/II Study of 13-*cis*-Retinoic Acid in Myelodysplastic Syndrome (Dr. Bernard R. Greenberg; Project PI: Frank L. Meyskens, Jr. University of Arizona)	Hemato-poietic	Patients with myelodysplastic syndrome 18 patients	1 mg 13-*cis*-RA/kg-bw/day (bid) for 1 mo, increasing by 0.5 mg/kg-bw/day at monthly intervals to a maximum of 3 mg/kg-bw/day	Efficacy: Red blood cell transfusion requirements, bone marrow cell maturation Safety: Liver and kidney function, serum lipids	Study completed; three partial responses obtained Highest dose achieved was 2 mg/kg-bw/day due to significant toxicity, including severe dry skin, cheilitis, conjuctivitis, headache, fatigue, anorexia, epistaxis, elevated serum triglyceride, and unexpected thrombocytopenia. Published report: [70]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) ***(continued)***					
PO1-CA-27502 Regression of Agressive Laryngeal Papillomatosis with 13-*cis*-Retinoic Acid (Dr. David S. Alberts, University of Arizona Cancer Center)	larynx	Patients with three previous laser surgeries for laryngeal papillomas 5 patients	1–2 mg 13-*cis*-RA/kg-bw/day for 5–20 mo; escalation to 2 and 3 mg/kg-bw/day at 2-month intervals if no response	Efficacy: Clinical and histological remission of lesions Safety: Hematology, clinical chemistry, spinal x-rays	Pilot study completed with two complete and one partial response Published report: [91]
UO1-CA-48369 (DM 87-105) A Randomized Double-blind Study of 13-*cis*-Retinoic Acid (Ro 4-3788) *vs* Placebo in Chronic Smokers with Squamous Metaplasia and/or Dysplasia in Bronchial Biopsies (Dr. Waun K. Hong, University of Texas MD Anderson Cancer Center) 12/87–3/93 IND 21,576	Lung	Active or previous (within 6 months) chronic smokers (≥15 pack-years) with dysplasia and/or metaplasia index ≥15% 67 smokers	1 mg 13-*cis*-RA/kg-bw or placebo qd for 6 months, follow-up for 1 year (cross-over of placebo group who progressed or were stable to 13-*cis*-RA for 6 months, follow-up for 6 months)	Efficacy: Incidence and magnitude of histological response, bleomycin-induced chromatid breakage in peripheral lymphocytes Intermediate biomarkers: EGFR, transglutaminase, involucrin, keratin, sputum cytology and micronuclei Safety: Liver function, serum triglycerides, chest x-ray	Study completed. No effect on lesions—similar rate and magnitude of histological response in both groups (≈55%)—however, a greater reduction in metaplasia index occurred in participants who stopped smoking in the treatment group than in the placebo group Published reports: [80,81, 136]

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate iomarkers) ***(continued)***					
Protocol 94.10 A Feasibility Prospective Randomized Study of Chemoprevention of Lung Cancer with13-*cis*-Retinoic Acid in a High Risk Population with Companion Tumor Marker Evaluation (Dr. Joseph Ayoub, Montreal Cancer Institute) 7/94- IND 21,576	Lung	Smokers with chronic obstructive lung disease and decreased RAR-β2 or increased β4/β2 ratio in bronchial brushings 100 smokers	30 mg 13-*cis*-RA or placebo qd for 6 months, follow-up until death	Efficacy: Lung cancer incidence Intermediate biomarkers: Normalization of RAR-β2 expression or β4/β2 ratio Safety: Serum triglycerides	Study in progress. Accrual at 21 patients, with 8 completing treatment as of April 1996. Preliminary blinded results suggest that RAR-β2 expression increased over baseline after the treatment period Published report: [99]
PO1-CA-27502 Phase II Study of 13-*cis* Retinoic Acid in Mycosis Fungoides (Dr. John Kessler, University of Arizona Cancer Center) 12/83–4/87 IND 21,576	Lymphoid	Biopsy-proven cutaneous T-cell lymphoma (mycosis fungoides) involving 10% skin surface 25 patients	2 mg 13-*cis*-RA/kg-bw/day (qd or bid) in first 16 patients, with subsequent patients started at 1 mg/kg-bw/day until disease progression or toxicity	Efficacy: Clinical and histological response of lesions Drug effect measurement: Immune system response (PHA, T-cell number), prostaglandin synthesis Safety: Liver function tests, CBC, serum lipids, spine x-ray	Study completed with 11 (44%) clinical responses, 3 of which were complete disappearance of lesions. Median response duration was 8 months Dose reductions were required in most patients beginning at the higher dose. Published reports: [120,121]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) ***(continued)***					
UO1-CA-68639-01 (UAB 9435) A Randomized, Double-blind, Placebo-controlled Phase II Clinical Trial of 13-*cis*-Retinoic Acid (13-*cis*-RA) in Oral Dysplastic Leukoplakia (Dr. Samuel W. Beenken, University of Alabama at Birmingham) 7/95–7/98	Oral cavity	Biopsy-proven dysplastic oral leukoplakia 96 patients (32/arm)	1 mg 13-*cis*-RA/kg-bw qd for 3 months, then 0.25 mg/kg-bw qd for 9 months, or placebo for 12 months; after 3 months, nonresponders receiving placebo rerandomized	Efficacy: Clinical and histological regression of lesions Intermediate biomarkers: Ploidy, nuclear cytomorphometry, PCNA, MIB-1, TGFα, p185$^{c\text{-}erbB\text{-}2}$, EGFR	Protocol is being finalized for submission to FDA
NO1-CN-55170 A Phase II Clinical Trial of 13-*cis*-Retinoic Acid Against Oral Leukoplakia (Dr. Stimson P. Schantz, Memorial Sloan-Kettering Institute for Cancer Research) 9/95–9/97	Oral cavity	Biopsy-proven dysplastic oral leukoplakia 30 patients	1 mg 13-*cis*-RA/kg-bw/day (tid) or placebo for 6 months, follow-up for 6 months	Efficacy: Clinical and histological response, follow-up recurrence Intermediate biomarkers: Native cellular fluorescence, cytokeratin 19, blood group antigens (H, Lewis), lectins, PCNA, DNA ploidy, nuclear polymorphism index	Protocol being finalized

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) ***(continued)***					
Pilot Study (UAB 1101) Analysis of the Effect of Oral Administration of 13-*cis*-Retinoic Acid on Proto-oncogene Expression and Amplification in Patients with Leukoplakia (Dr. Samuel W. Beenken, University of Alabama at Birmingham) 11/91– IND 21,576	Oral cavity	Biopsy-proven dysplastic oral leukoplakia 22 patients	1 mg 13-*cis*-RA/kg-bw qd for 2–3 months; those with clinical response maintained with 0.25–0.5 mg 13-*cis*-RA/kg-bw qd for indefinite time	Efficacy: Clinical and histological response of lesion Intermediate biomarkers: EGFR, TGF-α, HER-2/*neu*, *c-myc* and *int*-2 expression/amplification Safety: Chest x-ray, serum triglycerides, liver and renal function tests, CBC	Study near completion; two patients on maintenance. Interim published results show complete resolution obtained in 4/9 (44%) of patients completing induction, but all had recurrence after cessation of treatment Baseline expression of EGFR and TGFα significantly higher in leukoplakia compared with normal tissue in 7 patients analyzed, significantly reduced TGFα expressiont did not correlate with lesion resolution Published reports: [134, 137]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) ***(continued)***					
UO1-CA-46303 A Phase II Study of 13-*cis* Retinoic Acid (Ro 4-3780) with Randomization to 13-*cis* Retinoic Acid or β-Carotene Treatment in Patients with Premalignant Lesions of the Oral Cavity (Dr. Waun K. Hong and Scott Lippman, University of Texas MD Anderson Center) 9/87–6/91 INDs 21,576 and 22,446	Oral cavity	Biopsy-proven dysplastic oral leukoplakia or erythroplakia 66 patients	Induction with 1.5 mg 13-*cis*-RA/kg-bw/day for 3 months, then subjects with responding or stable disease received maintenance with 0.5 mg 13-*cis*-RA/kg-bw/day or 30 mg β-carotene/day for 9 months; follow-up for 28 months (Note: half of the patients received the wrong dose of β-carotene)	Efficacy: Clinical and histological response Intermediate biomarkers: p53 expression, RAR-β, micronucleated cell frequency, involucrin, transglutaminase I, keratin K1 Safety: Serum triglycerides, CBC, liver function tests	Completed study found 55% clinical and 43% histological response during induction. During maintenance, 92% on 13-*cis*-RA continued to respond or improved clinically compared with only 42% on β-carotene Baseline p53 expression increased with lesion grade, and high expression correlated with lack of clinical response to 13-*cis*-RA induction. RARβ expression was lower in dysplastic lesions, but the expression rate increased after 13-*cis*-RA induction (90% *vs.* 40%). Published reports: [79,128, 129,131,135,138]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) ***(continued)***					
Intramural Prevention of Skin Cancer in Xeroderma Pigmentosum with the Use of Oral Isotretinoin (High Dose) (Drs. Kenneth Kraemer, John DiGiovanna, Alan Moshell and Gary Peck, NCI) Investigator IND	Skin	Xeroderma pigmentosum patients with >2 BCC or SCC/year for prior 2 years 7 patients	2 mg 13-*cis*-RA/kg-bw/day bid for two years, follow-up for 1 year	Efficacy: Compare frequency and total number of skin tumors before and after treatment Safety: Liver function, serum lipids, spinal x-rays	Study complete. Four patients (4/5) completing treatment had significantly reduced total tumor numbers compared with the prior two years. In these four, tumor frequency decreased 70–93%; however, it increased by an average of 8.5-fold during follow-up Adverse reactions were cheilitis, dry skin, conjunctivitis, triglyceridemia, spinal hyperostosis. One patient dropped due to triglyceridemia and one due to abnormal liver function tests Published reports: [103–105]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) *(continued)*					
Intramural Prevention of Skin Cancer in Xeroderma Pigmentosum with the Use of Oral Isotretinoin (Low Dose) (Drs. Kenneth Kraemer, John DiGiovanna, and Gary Peck, NCI) Investigator IND	Skin	Xeroderma pigmentosum patients from high-dose study 7 patients	0.5 mg 13-*cis*-RA/kg-bw/day for 1 yr; if no response, sequentially increased to 1 or 1.5 mg/kg-bw/day	Efficacy: Compare frequency and total number of skin tumors before and after treatment Safety: Liver function, serum lipids, spinal x-rays	Study complete. Effective dose varied between the 5 responding patients. The lowest dose was associated with fewer and milder adverse reactions Published reports: [103,105, 106]
NIAMS-91-AR-0161 Use of Isotretinoin for the Prevention of Skin Cancer in Patients with Xeroderma Pigmentosum or Nevoid Basal Cell Carcinoma Syndrome (Dr. John DiGiovanna, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NCI) Investigator IND	Skin	Subjects with clinically diagnosed xeroderma pigmentosum (XP) or nevoid basal cell carcinoma syndrome (NBCCS) and 2 treated skin cancers/yr during prior two years 20 XP, ≤50 NBCCS	If no previous 13-*cis*-RA treatment—0.5 mg 13-CRA /kg-bw/day for 6 months, increasing by 0.5 mg/kg-bw/day every 6 months for a total of two years, follow-up for one year; if previously treated with 13-*cis*-RA—that dose used for two years	Efficacy: Identify lowest dose reducing skin cancer rate Safety	Report: [90]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) *(continued)*					
PO1-CA-27502 Phase II Trial of 13-*cis* Retinoic Acid in the Treatment of Dysplastic Nevus Syndrome (Drs. Norman Levine and Frank L. Meyskens, Jr. Project PI: Dr. Thomas E. Moon, University of Arizona Cancer Center) 12/83–? IND 21,576	Skin	Patients with >10 biopsy-proven dysplastic nevi 11 patients	40 mg 13-*cis*-RA bid for 16 wk	Efficacy: Clinical and histological response of lesions Safety: Serum lipids, liver function tests, CBC, spinal x-ray, urinalysis	Study completed. No significant effect on clinical or histological lesions in 8 patients completing treatment. Published reports: [115, 132]
Phase III (Efficacy, intermediate biomarkers)					
PO1-CA-52051 (RTOG-9115, MDA-CCOP-DM-90094) Chemoprevention Trial with Low-Dose 13-*cis*-Retinoic Acid to Prevent Second Primary Tumors in Head and Neck Cancer Patients (Dr. Waun K. Hong, University of Texas MD Anderson Cancer Center; also RTOG and CCOP) 12/90– Investigator IND	Head/neck	Patients with previous Stage I/II head/neck cancer controlled by surgery or radiotherapy within last 16 wk–three year 1,080 patients	30 mg 13-*cis*-RA/day (tid) or placebo for three years, follow-up for 4 years	Efficacy: Incidence and latency of second primary tumor, survival Intermediate biomarker: Bleomycin-induced chromosome breaks Safety: Liver and kidney function, serum lipids, x-rays, drug levels	Study in progress; multi-institutional accrual (RTOG, CCOP) was 847 as of May 1996 Published reports: [76,90, 130,133,139]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase III (Efficacy, intermediate biomarkers) ***(continued)***					
UO1-CA-68089-01 (ID 94008) Phase III Prevention Clinical Trials Utilizing Intermediate Endpoints and Their Modulation by Chemopreventive Agents. Biochemoprevention for Advanced Premalignant Lesions in Upper Aerodigestive Tract (Dr. W.K. Hong, University of Texas MD Anderson Cancer Center) 7/95–7/98 Investigator IND?	Head/neck	Patients with severe dysplasia or CIS of oral cavity and oropharynx 35 patients	100 mg 13-*cis*-RA/m^2 qd + 3 x 10^6 U α-interferon/m^2 q Mon & Thurs + 1,200 IU α-tocopherol qd for 1 mo with escalation to 6 mo, follow-up for 1 yr	Efficacy: Clinical and histological regression of lesions Intermediate biomarkers: Chromosomal changes, p53 expression/mutation, PCNA, EGFR, nuclear RAR, apoptosis, genetic instability, LOH Safety: Clinical chemistry, serum lipids	Study in progress
EST-C-0590 (Mayo/NCCTG-88-74-51) Double Blind Phase III Trial of Effects of Low-Dose 13-*cis*-Retinoic Acid on Prevention of Second Primaries in Stage I/II Head and Neck Cancer (Drs. Charlotte Jacobs and Harlan Pinto, NCOG, plus ECOG, Mayo) 4/89– IND 32,769	Head/neck	Patients with curatively treated Stage I/II head/neck SCC cancer (surgery or radiotherapy) within previous 180 days 275 patients	10 mg 13-*cis*-RA (≥60 kg-bw) or 7.5 mg (<60 kg-bw) qd, or placebo qd for two years, then follow-up until death	Efficacy: Incidence and latency of recurrence and second primary malignancy or dysplasia, survival Safety: Liver and kidney function, calcium, lipids, x-rays	Study in progress. As of December 1995, 147 eligible cases had been accrued. The most common grade 3 toxicity (>100% baseline) has been increased serum triglycerides (13 patients) Report: [90]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase III (Efficacy, intermediate biomarkers) ***(continued)***					
NCI-P92-0014 Phase III, Double-Blind, Randomized Trial of 13-*cis*-RA *vs.* Placebo to Prevent Second Primary Tumors in Patients with Totally Resected Stage I Non-small Cell Lung Cancer (Dr. Scott Lippman, University of Texas MD Anderson Cancer Center; also CLGB, SWOG, RTOG, NCCTG) 91– Investigator IND	Lung	Patients with resected Stage I NSCLC within prior 3 years 1,134 patients	30 mg 13-*cis*-RA or placebo qd for three years, follow-up for 4 yrs	Efficacy: Incidence of second primary tumors, survival Safety: Liver and kidney function, triglycerides, x-rays	Study in progress. As of May 1996, 1,168 patients had been accrued Report: [90,95]
U19-CA-68437-01 (DM 94-121) A Randomized Double-blinded Chemoprevention Trial in Former Smokers with 13-*cis*-Retinoic Acid and α-Tocopherol *versus* Placebo (Dr. Waun K. Hong, MD Anderson Cancer Center) 7/95–4/00 Investigator IND	Lung	Former smokers (≥20 pack-years) with previous Stage I/II laryngeal or Stage I NSCLC treated by surgery (≥3 mo) and dysplasia and/or metaplasia index >15% 128 patients	1 mg 13-*cis*-RA/kg-bw + 1,200 IU vitamin E *vs.* placebo qd for six months; if at least minor response, treatment for six more months Cross-over of nonresponding or progressing patients on placebo to treatment group for second six months	Efficacy: Histological regression of bronchial metaplasia/dysplasia Intermediate biomarkers: Chromosome sensitivity, RAR, epithelial vitamin A Safety: Liver and kidney function, serum lipids, x-ray	Study in progress; accrual at 6 patients as of April 1996 Published report: [98]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase III (Efficacy, intermediate biomarkers) ***(continued)***					
SPORE NCI-V94-0506 Phase III Study of 13-*cis*-RA with or without Vitamin E for the Chemoprevention of Lung Cancer (Dr. Karen Kelly, University of Colorado Cancer Center) 1/93– IND ?	Lung	Former heavy smokers (40 pack-years) with moderate or severe dysplasia, CIS, or resected head/neck cancer with atypia or dysplasia 60 smokers	50 mg 13-*cis*-RA/day, or 50 mg 13-*cis*-RA + 800 mg α-tocopherol/day, or observation for one year, follow-up for two years	Intermediate biomarkers: Ki-67, p53, HER-2/*neu*, c-*fos*, EGFR, TGFα, Rb, RAR, transferrin receptor, n-cam antigen, 43-9F antigen, folate binding protein Safety: Serum lipids, liver function tests, chest x-ray	Study in progress Report: [90]
PO1-CA-52051 (DM 90-096) Phase III Randomized Chemoprevention Study of Long-Term, Low-Dose 13-*cis*-RA *vs.* β-Carotene/Vitamin A in Patients with Pre-malignant Lesions of the Oral Cavity (Dr. Scott Lippman, University of Texas MD Anderson Cancer Center) 2/93– Investigator IND	Oral cavity	Biopsy-proven dysplastic oral leukoplakia or erythroplakia 120 patients (60/arm)	0.5 mg 13-*cis*-RA/kg-bw/day for one year, then 0.25 mg/kg-bw/day for two years, or 50 mg β-carotene + 25,000 IU retinyl palmitate for 3 yrs, follow-up for 2 yrs	Efficacy: Incidence and duration of clinical and histological response Intermediate biomarkers: Micronucleated cell frequency, retinoid receptors Safety: Liver and kidney function, serum lipids and electrolytes, x-rays	Study in progress Report: [90]

Table I. Clinical Trials of 13-*cis*-Retinoic Acid Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase III (Efficacy, intermediate biomarkers) ***(continued)***					
ZO1-CN-00103-11 Use of Isotretinoin in Prevention of Basal Cell Carcinoma (ISO-BCC) (Dr. Joseph A. Tangrea, NCI, DCPC, CPSB; 8-center study) 2/83–8/91 IND 21,576	Skin	Men and women with ≥2 BCC in prior 5 yrs 981 subjects	10 mg 13-*cis*-RA or placebo qd for three years, follow-up for two years	Efficacy: Reduction in cancer incidence Safety: Serum lipids, liver function, spinal x-rays	Study completed. No effect on cumulative incidence of the first new BCC or the annual rate of BCC tumor formation (multiplicity) Incidence of elevated serum triglycerides (7% *vs.* 2%), hyperostotic axial skeletal changes and mucocutaneous reactions (70% *vs.* 35%) significantly higher in agent-treated group Published reports: [68,110–113]
PO1-CA-27502 (Protocol A83.8) Phase III Chemoprevention of Skin Cancer Program Project (SKICAP-S/B) (Drs. Norman Levine and Frank L. Meyskens, Jr.; Project PI: Dr. Thomas E. Moon, University of Arizona) 12/83–4/92 IND 21,576	Skin	Patients with prior multiple (≥4) SCC or BCC, 1 within last year 719 patients	5 mg (<65 kg bw)–10 mg 13-*cis*-RA or 25,000 IU retinol or placebo qd for 3–4¼ years	Efficacy: Incidence of first and total BCC and SCC Safety: Serum lipids, liver function, dermatologic assessment	Study completed. No reduction in skin cancer incidence in either treatment group Published reports: [6,107–109]

13-*cis*-RETINOIC ACID DEVELOPMENT STATUS