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NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN: VITAMIN A

DRUG IDENTIFICATION

CAS Registry No.: 68-26-8

CAS Name (9CI): Retinol

Synonyms:
Axerophthol
3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonate-traen-1-ol
NSC-329481
all-*trans*-Retinol
Vitamin A
Vitamin A_1
Vitamin A Alcohol

Other Retinoids:

3,4-Didehydroretinol (CAS No. 79-80-1)
- 3-Dehydroretinol
- (all-E)-3,7-Dimethyl-9-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2,4,6,8-nonatetraen-1-ol
- Vitamin A_2

Retinal (CAS No. 116-31-1)
- Vitamin A Aldehyde
- all-*trans*-Retinaldehyde

all-*trans*-Retinoic Acid (CAS No. 302-79-4)
- (all-E)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic Acid
- Renova (Ortho Pharmaceutical)
- Retin-A® (Ortho-McNeil)
- β-*trans*-Retinoic Acid
- Ro 1-5488
- Tretinoin (LF) (Genzyme Corp.)
- Vesanoid (Hoffman-LaRoche)
- Vitamin A Acid

9-*cis*-Retinoic Acid (CAS No. 5300-03-8)

13-*cis*-Retinoic Acid (CAS No. 4759-48-2)
- Isotretinoin
- Accutane®

Retinyl Acetate (CAS No. 127-47-9)
- all-*trans*-Retinyl Acetate
- Vitamin A Acetate

Retinyl Palmitate (CAS No. 79-81-2)
all-*trans*-Retinyl Palmitate
Retinol, Hexadecanoate

Vitamin A Mixture (50:50 Retinyl Acetate:Retinyl Palmitate)

Molecular Wt.: 286.5 (Retinol)
300.4 (all-*trans*-Retinoic Acid)
328.5 (Retinyl Acetate)
524.9 (Retinyl Palmitate)

EXECUTIVE SUMMARY

Vitamin A refers to a group of nutrients (*e.g.*, retinol and its esters, 3,4-didehydroretinol, retinal) that are required for growth and bone development, vision, reproduction, epithelial differentiation, immune system integrity, and biochemical reactions such as mucopolysaccharide synthesis, cholesterol synthesis and hydroxysteroid metabolism [1]. Since vitamin A cannot be synthesized by humans, it must be supplied via diet or supplements. Preformed retinol (vitamin A_1) is present as retinyl esters in animal foods such as eggs, whole milk, butter, meat, and fish liver oils; 3,4-didehydroretinol (*i.e.*, vitamin A_2) predominates in fresh-water fish [1,2]. Another dietary source of retinol is through intestinal conversion of provitamin A carotenoids (*e.g.*, α-carotene, β-carotene, cryptoxanthin) found in green and yellow vegetables and fruits to retinal [3]. The recommended daily allowance (RDA) for vitamin A from all dietary sources is 1,000 retinol equivalents (RE); since 1 RE is defined as 1 μg retinol and 1 IU=0.3 μg retinol, the RDA is ≈3,333 IU retinol [4].

As shown in Figure 1, other naturally occurring retinol analogs (retinoids) are produced during metabolism of retinol, *i.e.*, all-*trans*-retinoic acid (all-*trans*-RA), 9-*cis*-retinoic acid (9-*cis*-RA) and 13-*cis*-retinoic acid (13-*cis*-RA). All these retinoids actually vary qualitatively and quantitatively in vitamin A activity [5,6]. For example, 3,4-didehydroretinol (vitamin A_2) has only 30–40% of the biological activity of retinol [1,2], and all-*trans*-RA does not prevent visual or reproductive dysfunction, since the acid cannot be converted back to retinal or retinol [6].

Retinol and its analogs also vary quantitatively and qualitatively in chemopreventive activity. In general, they act at the promotion phase of carcinogenesis by inhibiting proliferation [7] and inducing differentiation [*e.g.*, 8,9]. The basis for these actions may be binding to retinoid receptors (RARα, -β, -γ and RXRα, -β, -γ) belonging to the superfamily of steroid and hormone receptors [10]. All these receptors function as transcription factors that regulate the expression of specific genes. Changes in transcription of specific genes are reflected in the following observed retinoid chemopreventive functions: modulation of intercellular (connexin) [11,12] and intracellular signalling (PKC, ODC); growth factor (TGFβ, TGFα, EGF, IGF-I), receptor (EGFR) [10] and oncogene expression [*e.g.*, 13]; modulation of hormones (progesterone) and immune response [14]; altered extracellular matrix (collagen, angiogenesis) and proteolytic enzymes [10]; and inhibition of viral replication [10]. The mechanistic differences result from differences in binding affinities to receptors. The known retinoid receptors have little affinity for retinol, but specific subfamilies bind 9-*cis*-RA (RARs, RXRs), all-*trans*-RA (RARs), and 13-*cis*-RA (RARs). For example, all-*trans*-RA is a more potent ligand for RARγ than the other two isoforms, and has been shown specifically to induce secretion of TGFβ in normal and HPV-immortalized cells [15] and to decrease expression of c-*myc* and c-*erb*B [13]. However, effects *in vivo* are also related to differences in receptor distribution between tissues and the pharmacokinetics of the retinoid.

Some retinoids have other antipromotion and antiinitiating properties. Antioxidation and free radical scavenging can inhibit either phase of carcinogenesis [16]. Effects on metabolism can inhibit procarcinogen-induced initiation. Retinol (B(*a*)P, aflatoxin B_1 (AFB_1), IQ, MeIQx, MeIQ, MCA, cyclophosphamide), retinyl esters (AFB_1), and all-*trans*-RA (AFB_1) inhibited mutagenicity in the Ames *Salmonella* assay [17–22], DNA binding [22,23] and metabolism [8,21,22] of these carcinogens.

At the organism level, several types of information suggest that vitamin A inhibits tumorigenesis. Experiments in the 1920s demonstrated that many epithelial tissues (respiratory, gastrointestinal (GI),

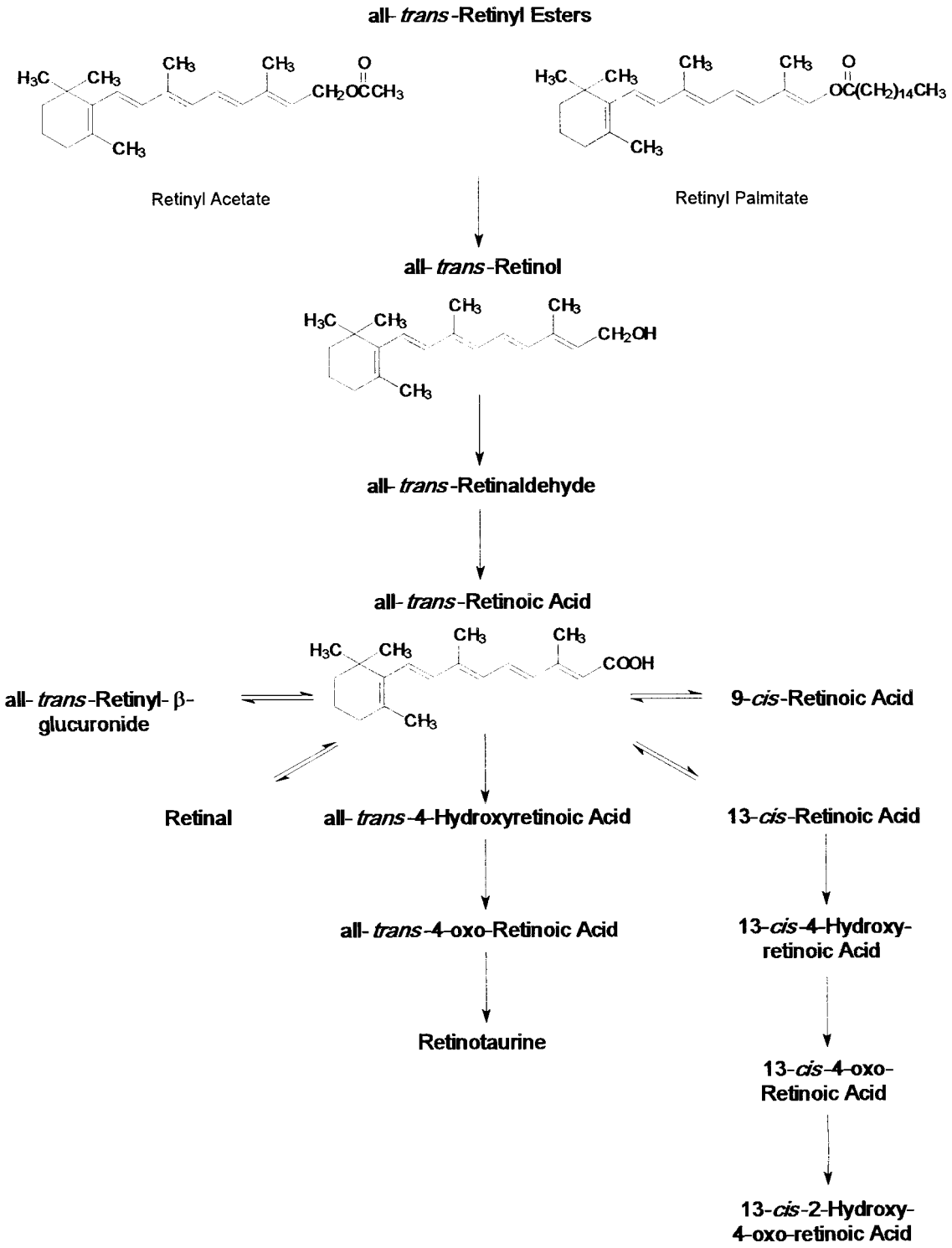

Figure 1. Metabolic Pathway of Retinol

genitourinary) in animals fed vitamin A-deficient diets displayed histological similarities (squamous metaplasia, hyperkeratinization) with neoplastic tissue, as well as carcinomas [24,25]. Epidemiologic studies to test this effect showed the most consistent relationship between low dietary and serum β-carotene (provitamin A) and development of cancers of the lung, cervix, ovary, esophagus, larynx, oral cavity and nasopharynx [3,26,27]. In contrast, there is a lack of consistent association between retinol and cancer incidence for many organs; in fact, positive associations have been suggested for esophageal, pharyngeal, laryngeal, pancreatic and prostate cancer in dietary studies [28, 29]. However, if only those studies measuring retinol and carotenoids separately are considered, significantly lower dietary intake of preformed retinol has been associated with increased risk for breast [30] and colon cancer [31] in prospective studies and with lung [32–34] and prostate cancer [35] in retrospective studies. Serum retinol has been inversely associated with risk for GI cancer [36,37] in prospective studies and with sarcoma [38] and lung [39], prostate [40], bladder [41] and ovarian [42] cancer cases in retrospective studies. The lack of consistency between these types of epidemiologic assessments may result from the fact that serum retinol does not reflect dietary/supplement intake in generally well-nourished populations [43–45], since liver stores buffer over a wide range [28,46]. Serum values are also not always reliable, since only a single sample may be taken and retinol can degrade in storage. Finally, in retrospective studies, the association could be a result of the disease rather than a cause.

Another type of evidence is efficacy in preclinical models of carcinogenesis, which also show differences between retinoids. In published studies, retinoids in general inhibit tumorigenesis in skin, respiratory tract, bladder, mammary glands and GI tract. In the rat mammary gland, the order of potency is 9-*cis*-RA > retinyl acetate >4-HPR; however, retinyl acetate accumulates in the liver and may be accompanied by hepatotoxicity. all-*trans*-RA inhibited mouse skin tumorigenesis when administered orally, but 13-*cis*-RA is more effective and less toxic. Retinyl palmitate appears to be the most potent when given topically in oral cavity studies. Although 13-*cis*-RA was the most effective overall in rat bladder, retinyl acetate also had activity. Finally, it is unclear from preclinical studies if pharmacological rather than physiological doses of retinoids inhibit tumorigenesis in lung.

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. Combining results from published and Chemoprevention Branch studies, retinol and its analogs have inhibited the development of histological intermediate biomarkers such as colonic aberrant crypt foci (retinol) and adenoma (retinyl acetate), papilloma in skin (all-*trans*-RA, retinyl esters, retinol), forestomach (retinyl acetate), bladder (retinyl acetate) and cheek pouch (retinyl esters), CIS in cheek pouch (retinyl acetate), hepatic enzyme-altered foci (retinyl palmitate) and nodules (retinyl acetate), and pancreatic acidophilic foci and ductular proliferation (vitamin A mixture). Although several retinoids inhibited colon aberrant crypt focus development, all-*trans*-RA was the most potent. Retinol, the retinyl esters and all-*trans*-RA have inhibited proliferative biomarkers (*i.e.*, ODC induction) in mouse skin cancer models.

Retinol and its analogs show similar, moderate acute toxicity. Smaller doses are toxic in subchronic studies due to a long biological half-life and bioaccumulation in liver, kidneys, lung and fat. In rats, 10,000 IU/day (*ca.* 34.9 μmol/kg-bw/day) produces hypervitaminosis A, characterized by hair loss, thick skin, fatty changes in liver, heart and kidneys, testicular atrophy, and increased serum lipids and cholesterol. Loss of bone associated with a limping gait appears at 28,000 IU/day; hypertriglyceridemia occurs at 110,000 IU/day.

all-*trans*-RA is not bioaccumulated, and cannot be reduced to form retinol. Instead, it is metabolized rapidly as shown in Figure 1. Since many of the steps are saturable, nonlinear pharmacokinetics are observed. However, all-*trans*-RA is even more chronically toxic than retinol, with hair loss, dermal and mucosal changes, weight loss, and inhibition of spermatogenesis at 16.6 μmol/kg-bw/day. Mice are the most sensitive; similar changes occur at 0.5 μmol/kg-bw/day. all-*trans*-RA is the most potent teratogen of the retinoids.

As with experimental animals, vitamin A has a long half-life and bioaccumulates in human tissues. Pharmacological doses of retinol or its esters (25,000 IU/day, or *ca.* 0.4 μmol/kg-bw/day) have little effect

on plasma retinol; instead, plasma retinyl palmitate levels increase. Daily doses of 300,000 to 600,000 IU vitamin A (*ca.* 4.5–9.0 μmol/kg-bw/day as retinol) for several months are usually required to produce signs of hypervitaminosis A, although intake as low as 50,000 IU/day (*ca.* 0.7 μmol/kg-bw/day) has been reported to be toxic after >18 months. Adverse effects occur in the GI tract (nausea, vomiting, diarrhea, cramping), nervous system (headache and increased intracranial pressure, irritability, paresthesias, vision changes, fatigue), skin (dryness, pruritus, rash, desquamation, alopecia, cheilitis), muscle/joint (myalgia, pain, swelling) and bone (arthralgia, hyperostosis), as well as systemically (hypercalcemia, leukopenia). However, the major concern with chronic administration of retinol is liver toxicity (hepatosplenomegaly, hyperlipidemia, fibrosis), which can present as hepatitis or cirrhosis.

all-*trans*-RA can produce serious side effects, such as intracranial hypertension in children and a "retinoic acid syndrome" in adults. The latter is characterized by fever, pulmonary infiltrates, respiratory distress, kidney failure and coma. At lower doses, the usual retinoid effects of skin and mucosal dryness, headache, and transient increases in triglycerides and transaminases can occur.

Because of early interest in retinoids, many clinical chemoprevention trials involving adjuvant treatment or treatment of a premalignant lesion predated the NCI, Chemoprevention Branch. The earliest studies involved management of skin lesions, some of them premalignant (*e.g.*, actinic keratosis). In two NCI-funded adjuvant trials at the University of Arizona (SKICAP-AK, SKICAP-S/B), oral retinol (25,000 IU, or *ca.* 0.4 μmol/kg-bw/day) had no effect on BCC, although SKICAP-AK found a reduction in SCC incidence. At even higher doses (100,000 IU, or 1.5 μmol/kg-bw/day), adjuvant treatment did not reduce malignant melanoma in a third University of Arizona trial. The NCI has funded two large completed Phase III trials with retinyl palmitate in subjects at high risk for aerodigestive tract cancers: esophagus (Linxian, China) and lung (β-Carotene and Retinol Trial (CARET)). In an area of China with the world's highest mortality from esophageal cancer, no significant effects on cancer incidence or mortality were found after 5¼ years supplementation with retinyl palmitate (5,000 IU, or *ca.* 0.07 μmol/kg-bw/day) plus zinc. CARET was recently stopped 21 months early due to higher incidences of lung cancer and mortality after retinyl palmitate (25,000 IU, or *ca.* 0.4 μmol/kg-bw/day) and 30 mg β-carotene supplementation of participants at high risk for this disease (heavy smokers and asbestos-exposed workers with a history of smoking).

The Chemoprevention Branch has also pursued regression of premalignant lesions or other histological biomarkers as potential surrogate endpoints for cancer, especially in oral cavity, lung, skin and cervix. In many early independent studies, retinoids appeared to regress the clinical lesion oral leukoplakia in tobacco/betel nut chewers. Modulation of dysplasia, the histological intermediate biomarker in this lesion, is being evaluated in an ongoing NCI, Chemoprevention Branch-funded study (Dr. Scott Lippman, University of Texas MD Anderson Cancer Center) of retinyl palmitate plus β-carotene *versus* 13-*cis*-RA. One completed NCI-funded Phase II trial in a lung cohort (Dr. Jerry McLarty, University of Texas at Tyler) found that a lower dose of retinol than that used in the CARET trial (25,000 IU qod, or *ca.* 0.2 μmol/kg-bw/day) in combination with β-carotene had no effect on sputum atypia in asbestos-exposed workers. An additional NCI-funded Phase III trial (Dr. Stephen Lam, British Columbia Cancer Agency) is in progress to evaluate regression of bronchial dysplasia by retinol. One independent study suggested effects in the colon; following removal of colorectal polyps, retinyl palmitate plus vitamins C and E decreased proliferation in normal-appearing tissue. However, the most consistent results have been obtained with cervical intraepithelial neoplasia (CIN), a premalignant lesion. Three completed NCI, Chemoprevention Branch-funded Phase I–III trials (Drs. Earl Surwit, David Alberts and Frank L. Meyskens, Jr., University of Arizona) tested all-*trans*-RA intravaginally in CIN patients and obtained significant regression of CIN I and II; the pilot for a third Phase III trial (Dr. Mack Ruffin, University of Michigan) is in preparation.

Although retinyl acetate has been one of the most effective retinoids in inhibiting mammary carcinogenesis in rat models, it was not chosen for clinical development as a chemopreventive agent for this indication. Because it tends to accumulate in the liver as retinyl esters and has the potential to cause hepatotoxicity, 4-HPR was chosen instead. More recent preclinical data suggest that 9-*cis*-RA is even more potent in a rat mammary carcinogenesis model.

The NCI, Chemoprevention Branch will await the

results of three Phase II and III trials with second primary tumors and intermediate biomarkers as end points to decide if these retinoids should be pursued as lung and head/neck cancer chemopreventive drugs. The Branch will not pursue retinol or its analogs as adjuvant treatments for skin cancer; treatment of premalignant lesions in this tissue will be pursued by independent groups. Because of prospective epidemiological data and favorable effects on proliferation in both human and animal studies, a retinoid may be evaluated in cohorts at high risk for colon cancer; it may be possible to decrease retinoid toxicity by using lower doses in combinations with NSAIDs or calcium. However, it remains unclear whether vitamin A supplementation by retinol or its analogs is chemopreventive in well-nourished populations.

Retinol and its esters are available as nutritional supplements and no supply problems are anticipated. all-*trans*-RA is marketed as a topical treatment for acne vulgaris (*e.g.*, Retin-A 0.025–0.5% gel, cream or liquid) [47] and wrinkles (Renova). Recently, however, it became the first retinoid approved as an oral, second-line treatment for cancer [48]. The indication for all-*trans*-RA (Vesanoid) is remission induction in acute promyelocytic leukemia patients who are refractory to, relapsed from, or contraindicated for, anthracycline chemotherapy.

PRECLINICAL EFFICACY STUDIES

The NCI, Chemoprevention Branch has done only limited efficacy testing with retinol and its analogs (except for skin) due to the availability of published results. Retinoids in general inhibit tumorigenesis in skin, respiratory tract, bladder, mammary glands and GI tract. Early studies at NCI demonstrated that dietary all-*trans*-RA was effective when given during promotion in two-stage mouse skin models. Retinyl acetate is one of the most active retinoids in both the DMBA- and MNU-induced rat mammary carcinogenesis models. Both retinyl acetate and palmitate decreased progression in oral cavity models, although high topical doses can potentiate carcinogenesis.

Identification and validation of intermediate biomarkers as potential surrogate endpoints for cancer is a priority of the Chemoprevention Branch. Retinol and its derivatives have inhibited the development of histological intermediate biomarkers such as colonic aberrant crypt foci (retinol) and adenoma (retinyl acetate), papilloma in skin (all-*trans*-RA, retinol, retinyl esters), forestomach (retinyl acetate), bladder (retinyl acetate) and cheek pouch (retinyl esters), cheek pouch CIS (retinyl acetate), hepatic enzyme-altered foci (retinyl palmitate) and nodules (retinyl acetate), and pancreatic acidophilic foci and ductular proliferation (vitamin A mixture) in some of the same organs. Retinol, the retinyl esters and all-*trans*-RA have inhibited proliferative biomarkers (*i.e.*, ODC induction) in mouse skin cancer models.

Lung: The results of chemopreventive efficacy studies with retinoids in the lung have been contradictory. In the earliest study, Saffiotti found that retinyl palmitate (5 mg 2x/wk, or *ca.* 27.2 μmol/kg-bw/day, ig) administered after intratracheal instillation of B(*a*)P adsorbed onto ferric oxide particles decreased the incidence of respiratory tract lesions (squamous metaplasia, squamous cell papillomas and carcinomas) in Syrian golden hamsters [49]. In contrast, a study with retinyl acetate (1,600 and 2,400 μg/wk in 2 doses/wk, or *ca.* 7.0 and 10.4 μmol/kg-bw/day, ig) by a different group found an increase in benign tumors and no consistent effect on malignant tumors [50]. Possible explanations include the use of a higher B(*a*)P dose in the second study, toxicity from the high retinoid doses, or the housing conditions [51]. An early study in a rat model found that retinyl acetate (1,740 μg 2x/wk, or *ca.* 5.0 μmol/kg-bw/day, ig) reduced the number and size of metaplastic nodules and carcinomas induced by intratracheal MCA [52].

The NCI, Chemoprevention Branch has tested retinol in combination with other agents in the DEN-induced hamster model. Dietary co-administration of retinol (0.032 mg/kg diet, or *ca.* 0.01 μmol/kg-bw/day) with β-carotene (2,147 mg/kg diet) at doses that were ineffective alone significantly reduced lung dysplasia and carcinoma [53,54]. In a second study, a significant decrease in carcinomas was obtained at doses of retinol (4.6 and 9.2 mg/kg diet, or *ca.* 1.9 and 3.9 μmol/kg-bw/day) and β-carotene (1.5 and 3 mg sc, respectively) that were ineffective alone. The distribution pattern of β-carotene appeared to be altered in the presence of vitamin A.

Skin: The first published study on retinoids and skin carcinogenesis in 1967 found that mice exposed to topical DMBA and fed a diet supplemented with "vitamin A" had fewer papillomas/animal than those on a deficient diet [51]. Subsequent studies by Bollag found that oral administration of all-*trans*-RA (200 mg/kg-bw, 1x/2 wk, or *ca.* 47.6 μmol/kg-bw/day, ig) during promotion in the DMBA/croton oil model

decreased papilloma and carcinoma multiplicity and carcinoma incidence. Later studies by a group at NCI's Laboratory of Cellular Carcinogenesis and Tumor Promotion demonstrated that dietary all-*trans*-RA (30 mg/kg, or *ca.* 12.8 μmol/kg-bw/day) inhibited papilloma and/or carcinoma development in initiation/promotion and complete protocols in SENCAR mice: DMBA/TPA [55,56], repeat DMBA [57], and DMBA/mezerein [56].

In an NCI, Chemoprevention Branch-funded study, the chemopreventive efficacy of all-*trans*-RA was evaluated in the transgenic Ha-*ras*/keratin K1 mouse model. Following dietary treatment (10 and 20 mg/kg, or *ca.* 4.3 and 8.6 μmol/kg-bw/day) of 5–6 week old female mice, the incidence of squamous cell papillomas was inhibited significantly (41%) at the low dose, but not the high dose (30%), after 26 weeks. However, body weight gain was also significantly decreased at both doses (37–41%), which may have resulted in the lack of dose response.

Many additional studies with topical all-*trans*-RA administered during promotion have demonstrated inhibition of papilloma and carcinoma development in initiation/promotion (DMBA/TPA, DMBA/anthralin) [*e.g.*, 58–61] and various initiation/promotion/progression (DMBA/TPA/mezerin, DMBA/TPA/benzoyl peroxide, DMBA/TPA/AAPH) models in the mouse [60,62]. The antipromotional effects of topical all-*trans*-RA correlated to the degree of inhibition of ODC induction by tumor promotors TPA [*e.g.*, 63,64] and anthralin [61]. ODC induction is considered to be an intermediate biomarker of proliferation. In one study, all-*trans*-RA was more potent than retinol, retinyl palmitate and retinyl acetate in mouse epidermis in TPA-induced ODC activity [65].

In studies on UV irradiation-induced skin carcinogenesis, topical retinoids produced extremely variable results. In mice, enhancement, no effect and inhibition have been reported with all-*trans*-RA [66]. Variables which could affect the outcome include strain of mouse, dose, source and schedule of irradiation, and dose, concentration, vehicle and schedule of retinoid treatment. Interestingly, a few studies have reported efficacy using dietary rather than topical administration of all-*trans*-RA [66] or retinyl palmitate [67].

In published studies, retinol and its esters were also effective against tumor development in one- and two-stage mouse skin models. Topical retinol inhibited papilloma incidence and multiplicity initiated by DMBA and promoted with phenol [68]. Dietary retinyl palmitate (350,000 IU/kg diet, or *ca.* 47.1 μmol/kg-bw/day) was effective in the two-stage DMBA/TPA model [69]. In the DMBA/croton resin model, topical retinyl acetate (0.015%, 5x/wk) administered during promotion inhibited tumor multiplicity (76%) more than incidence (33%); in the DMBA/croton oil model, retinol (76%) was more effective than retinyl acetate (29%) in reducing papilloma multiplicity when administered during promotion (0.009%, 5x/wk) [68].

Mammary Glands: More than 15 publications have demonstrated the efficacy of retinyl acetate against rat mammary gland carcinogenesis. In both the DMBA- [70–77] and MNU-initiated models [75, 78–82], significantly decreased incidence and multiplicity and increased latency were observed. Doses of 124–656 mg/kg diet (*ca.* 18.9–99.9 μmol/kg-bw/day) given 3–28 days after the carcinogen were effective. In addition, carcinoma formation induced by ethinyl estradiol [83], x-ray [84] and B(*a*)P [85] was inhibited. In contrast, low doses of all-*trans*-RA (60 and 120 mg/kg diet, or *ca.* 10 and 20 μmol/kg-bw/day) beginning one week after MNU had no effect on tumor number or size [86].

In the DMBA or MNU-induced rat mammary models, retinoid administration usually begins after the carcinogen, or during the promotional phase. In the first studies with retinyl acetate, administration (380 and 760 μmol/kg diet and 250 mg/kg diet, or *ca.* 19–38 μmol/kg-bw/day) continuing from one week after either carcinogen was effective [*e.g.*, 70–72,78]. If intervention begins 1–12 weeks after DMBA, however, it must be continuous to avoid loss of effect by the end of the experiment [72]. Later studies investigated lengthening time to intervention after the carcinogen. Using the direct carcinogen MNU, retinyl acetate (328 mg/kg diet, or *ca.* 50 μmol/kg-bw/day) could be delayed 4–12 weeks, depending on the carcinogen dose, before the chemopreventive effect was lost [87]. In a model of adjuvant treatment, the same dose (328 mg/kg diet) given after surgical removal of the first MNU-induced palpable tumor reaching 1 cm in diameter significantly inhibited the incidence and multiplicity of subsequent tumors [88]. Since treatment presumably began when premalignant lesions were present, this suggests an effect on malignant progression of histological intermediate biomarkers.

Several studies have investigated the influence of

intervention schedules relative to carcinogen initiation on mammary tumorigenesis. In one study, dietary retinyl acetate (250 ppm, or *ca.* 38 μmol/kg-bw/day) inhibited mammary tumor multiplicity when given either during (from two weeks before to one week after DMBA) or after initiation (continuing from one week after the carcinogen) [72]. However, the retinoid may have unexpected effects when given before initiation. In a second study, retinyl acetate (328 mg/kg diet, or *ca.* 50 μmol/kg-bw/day) was given for two months before DMBA; the incidence of benign tumors (fibromas, adenomas, fibroadenomas) increased, but adenocarcinoma development was unaffected [89]. In the MNU model, mammary adenocarcinomas actually increased 50% with the same schedule of retinyl acetate pretreatment.

As a strategy to increase efficacy and decrease the potential for toxicity, retinyl acetate has been administered in combination with other agents. This approach was suggested by synergistic inhibition of tumor incidence and multiplicity in rats by combining retinyl acetate with ovariectomy [25]. Using 2-bromo-α-ergocryptine to inhibit pituitary prolactin secretion, an 81% further reduction in the incidence of mammary tumors was obtained compared with either agent alone [81]. Other effective two-agent combinations with retinyl acetate include BHT [76], DFMO [90], sodium selenite [73,77], magnesium chloride [77], tamoxifen [91] and vitamin C [77]; magnesium chloride, selenite and vitamin C in three-agent combinations with retinyl acetate (administered ip) were also effective [77].

Head and Neck: Retinoids have shown little effect in preclinical models of head and neck cancer. Early studies with topical retinyl palmitate found a potentiating effect in the cheek pouch of the DMBA-exposed hamster [92–94]. Since the retinoids alone produced histologic changes, local irritation from large doses may account for this result [51]. Smaller topical doses (5,000 IU or 5.2 μmol, 2x/wk) appear to cause regression of premalignant lesions in the same model [95]. Topical retinyl acetate (2% of unknown volume, 3x/wk) significantly increased latency in the DMBA-painted cheek pouch [96]. A second study investigated the effect of retinyl acetate (10 mg or 13 μmol, 3x/wk, top) administered at the time of appearance of leukoplakia and dysplasia, modelling trials treating premalignant lesions [97]. A significant decrease in mean tumor (CIS, carcinoma) mass was obtained.

Retinyl acetate has also been evaluated in a model of rat tongue carcinogenesis induced by NQO in drinking water. Dietary administration of the retinoid (100,000 IU/kg diet, or *ca.* 5.2 μmol/kg-bw/day) during the seven months of carcinogen exposure significantly decreased the incidence (50%) and size (≈75%) of invasive carcinomas. Since the incidence of dysplastic lesions and CIS increased, retinyl acetate appears to affect progression.

Gastrointestinal Tract: In the earliest study on forestomach carcinogenesis, oral retinyl palmitate (0.5 ml 10%, 2x/wk, or *ca.* 2.7 μmol/kg-bw/day) reduced papillomas and carcinomas induced by B(*a*)P or DMBA in Syrian hamsters [98]. Later, Saffiotti found a similar reduction in papillomas with retinyl palmitate (5 mg 2x/wk, or *ca.* 27.2 μmol/kg-bw/day, ig) administered after intratracheal instillation of B(*a*)P/ferric oxide in Syrian golden hamsters [49]. A different group subsequently obtained similar results with retinyl acetate (1,600 and 2,400 μg/wk, or *ca.* 8.7 μmol/kg-bw/day, ig) [50].

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 [51]. Pharmacological doses of 13-*cis*-RA (67 μg/g diet, or *ca.* 11 μmol/kg-bw/day) reportedly decreased the incidence of DMH-induced colon tumors by 60%; however, the details of the study (*e.g.*, composition of basal diet) were unavailable for review [99]. Subsequent published studies evaluated the effect of retinyl acetate on the development of DMH-induced rat colon carcinogenesis. Feeding the retinoid (2 mmol/kg diet, or ca. 100 μmol/kg-bw/day) for eight months after the carcinogen significantly decreased the incidence of adenomas (61%), a premalignant lesion; the reduction in adenocarcinoma incidence (30%) was not statistically significant [100]. Interestingly, the retinyl acetate control group (beadlets) also displayed decreased adenoma (70%) and adenocarcinoma (60%) incidences that were also not significantly lower than the DMH control group. No effect on MNU-induced rat colon carcinogenesis was observed in the same experiment.

An NCI, Chemoprevention Branch study investigated the effects of retinol and all-*trans*-RA on development of colonic aberrant crypt foci, which are considered histological intermediate biomarkers with potential for developing into adenomas in rats [101] and perhaps humans [102]. In the rat model, the

chemopreventive agent is administered for four weeks in the diet beginning two weeks after the last AOM exposure. Retinol (195 and 390 mg/kg diet, or *ca.* 34 and 68 μmol/kg-bw/day) produced significant, dose-related decreases in total aberrant crypt foci per rat (22% and 41%, respectively). At the same dietary doses, all-*trans*-RA (32 and 65 μmol/kg-bw/day) produced a 67% reduction at both doses; however, significant weight loss (17% and 36%, respectively) and other signs of toxicity (weak bones, difficulty in moving) occurred. At lower doses in a published study, all-*trans*-RA (75 and 150 mg/kg diet, or *ca.* 12.5 and 25 μmol/kg-bw/day) for four weeks beginning one week after AOM significantly decreased total foci/colon and multiplicity at both doses [103]. A dose-response was observed only in the decrease in the proportion of foci strongly expressing c-*myc*, a proliferation-related gene; an increase in c-*fos* expression, a differentiation-related gene, occurred concomitantly. A significant decrease in body weight gain (9%) was observed only at the highest dose.

Bladder: Early preclinical studies found that vitamin A deficiency alone led to squamous metaplasia and, with FANFT, potentiated urinary bladder carcinogenesis (primarily SCC) in rats [51]. FANFT exposure in animals receiving a vitamin A-adequate diet produced SCC and TCC. A subsequent study found that pharmacological levels of retinyl palmitate (930–2,500 IU/g diet, or *ca.* 49–131 μmol/kg-bw/day) reduced only squamous metaplasia and SCC in these rats [104]. Since the majority of human bladder tumors are TCC, retinoids were tested in OH-BBN-exposed animals, which display multiple stages to this carcinoma (flat and papillary hyperplasia, squamous metaplasia, atypia, TCC). Retinyl acetate (100 and 200 IU/g diet, or *ca.* 4.6 and 9.1 μmol/kg-bw/day) in this rat model decreased both TCC and papilloma incidence [105]. Finally, an abstract reported that all-*trans*-RA (2.5 and 5 mg/kg diet, or *ca.* 0.4 and 0.8 μmol/kg-bw/day) reduced the incidence of papillary tumors induced by MNU applied directly into surgically exposed rat bladder [106].

Other: Inhibition of pancreatic carcinogenesis has been reported for several retinoids [51]. When retinyl acetate (0.025% in diet, or *ca.* 38 μmol/kg- bw/day) was offered to rats after initiation with azaserine, a decrease in the area of atypical acinar cell nodules and the incidence of adenomas and carcinomas was obtained [107]. A vitamin A mixture (retinyl acetate:retinyl palmitate 50:50, 10,000 IU/kg diet, or *ca.* 1.3 μmol/kg-bw/day) also decreased the number of premalignant lesions (intermediate ductal complex) in hamsters fed a diet with 20% lard and exposed to BOP, and the area of acidophilic foci in rats fed the same diet and exposed to azaserine [108].

Retinyl esters have been evaluated in two models of rat hepatocarcinogenesis. In a resistant hepatocyte model (DEN/AAF/PH), retinyl acetate (10 mg/kg-bw on alternate days, or *ca.* 0.1 mmol/kg-bw/day, ig) decreased nodule number and multiplicity by 42%, but had no effect on development of GGT-positive foci [109]. However, in an initiation/promotion model (DEN/3,3',4,4'-tetrachlorobiphenyl), retinyl palmitate (100,000 IU/kg diet, or *ca.* 5.2 μmol/kg-bw/day) decreased the volume and multiplicity of various altered focus phenotypes (GGT, ATPase or G6Pase) [110].

PRECLINICAL SAFETY STUDIES

Safety: The oral LD_{50} values for retinol and its analogs are similar and show moderate acute toxicity, as shown in Table I [111–113].

Much smaller doses are toxic in subchronic studies due to accumulation [112]. In rats, hypervitaminosis A was produced by retinol doses as low as 3 mg/day (10,000 IU/day, or *ca.* 35 μmol/kg-bw/day). The effects can include hair loss, thickened epithelium, fatty changes in liver, heart and kidney, testicular atrophy, myocardial degeneration, and increased serum lipids and cholesterol. At higher doses (28,000–45,000 IU/day, or *ca.* 98–157 μmol/kg-bw/day), a limping gait associated with loss of bone occurred. Hypertriglyceridemia was produced by doses 10,000 times the nutritional requirement (110,000 IU/day, or *ca.* 384 μmol/kg-bw/day).

Subchronic toxicity of all-*trans*-RA has been studied in rats and dogs at doses of 5 and 50 mg/kg-bw/day (17 and 166 μmol/kg-bw/day) [112]. After 13 weeks at the low dose, rats displayed hair loss, dermal and mucosal alterations, inhibition of spermatogenesis, and weight loss. At the high dose, serum transaminase and alkaline phosphatase activities were elevated. Similar signs were seen in dogs; however, mortality at the high dose was 50%. In mice, doses of 150–250 mg/kg-bw/day (499–832 μmol/kg-bw/day) caused alopecia, weight loss, and skin and membrane changes after five days.

Few chronic toxicity studies have been published; most have been undertaken by Hoffman-LaRoche [112]. At 250 times the human

Table I. Oral LD_{50} Values

Retinol/Analog	Species	LD_{50}	
		mg/kg-bw	μmol/kg-bw
Retinol	Mouse	2,570	8,972
Retinyl acetate	Mouse	4,100	12,481
Retinyl palmitate	Mouse Rat	6,060 7,910	11,546 15,070
all-*trans*-RA	Mouse Rat	1,100–4,000 2,000–4,000	3,662–13,315 6,657–13,315
13-*cis*-RA	Mouse Rat	3,389 4,000	11,280 13,314

RDA, retinyl palmitate produced no adverse effects in rats (27.5 mg/kg-bw/day, or 52.4 μmol/kg-bw/day) or dogs (13.8 mg/kg-bw/day, or 26.3 μmol/kg-bw/day) after ten months.

Retinol and its esters are potent and consistent *teratogens* in many species of animals [114,115]. Typical abnormalities include exencephaly in rats, face and mouth deformities in mice, rabbits, guinea pigs and hamsters, and mouth, ear and tail deformities in dogs. However, all-*trans*-RA is the most potent natural teratogenic vitamin A compound in several species—20–40 times greater than retinol—and shows a similar pattern of abnormalities; this metabolite may be primarily responsible for retinol-induced terata [115]. Differences in teratogenicity of retinoids are due to structure, pharmacokinetics and ability to induce RARβ2 mRNA. The mechanism may involve expression of the latter, which in turn induces transcription of a variety of genes at inappropriate time.

ADME: Vitamin A has a long biological half-life and bioaccumulates due to rapid absorption and slow clearance. If fat absorption is normal, retinol is completely absorbed; at larger doses, absorption is incomplete. The esters (retinyl palmitate, retinyl acetate) are hydrolyzed in the GI tract by pancreatic enzymes, allowing absorption of free retinol. In the intestinal cells, retinol is re-esterified with long-chain fatty acids (predominantly palmitate or stearate) which are incorporated into chylomicrons; the latter travel through lymph to the general circulation.

Chylomicron retinyl esters are primarily taken up by the liver, where they are hydrolyzed, re-esterified with palmitate, and stored in the Kupffer cells [1]. The liver contains 50–80% of total body stores of the vitamin [116]; smaller amounts are distributed to kidneys, lungs, adrenal glands, retinas, and intraperitoneal fat [6]. When mobilized, the retinyl esters are hydrolyzed and retinol is released as a complex (1:1:1) with transthyretin and retinol binding protein (RBP). After large doses of retinol, the binding capacity of RBP may be exceeded; the unbound vitamin is carried by lipoproteins and may be responsible for toxic effects on cell membranes resulting from hypervitaminosis A. Circulating retinol-RBP can also be recycled by the liver, leading to homeostatic control of plasma levels.

After uptake by target cells, retinol may then bind to cellular retinol-binding proteins (CRBP) I or II [116]. These proteins serve as nontoxic reservoirs, transfer agents or modulators of metabolism [117]. Retinol appears to be oxidized to retinaldehyde, which is in turn oxidized to all-*trans*-RA; metabolites of the latter include 13-*cis*-RA, 9-*cis*-RA, retinyl-β-glucuronide and 3,4-didehydroretinoic acid.

In rats, tissues such as adipose tissue, kidney, testes, lung and bone marrow take up significant amounts of retinol. Chronic feeding of retinyl acetate increases liver levels of retinyl palmitate and other esters [79]. Six months after supplementation of female rat diet with 328 mg/kg (*ca.* 50 μmol/ kg-bw/day), hepatic retinyl palmitate was 10-fold higher than vehicle-control animals [89].

Retinol is eliminated as a glucuronide conjugate,

which can undergo enterohepatic circulation and oxidation to retinal and all-*trans*-RA. The latter undergoes decarboxylation and conjugation with glucuronic acid, followed by excretion in the feces via the bile. Retinal and retinoic acid are excreted in both the feces and urine; under normal circumstances, retinol is not excreted in the urine.

all-*trans*-RA is primarily derived from endogenous metabolism of retinol. If given orally to rats, it is transported directly via the portal system rather than the lymphatics; circulating all-*trans*-RA is bound to serum albumin rather than RBP [118]. Two-thirds of the absorbed dose is distributed to tissues [119], where specific cellular retinoic acid binding proteins (CRABP I, II) in cell membranes and cytosol function as transfer agents; in the cytosol, they also serve as reservoirs and modulators of metabolism. all-*trans*-RA is not accumulated like retinol, and cannot be reduced back to the retinaldehydes and retinol. Instead, it is cleared rapidly by metabolism to compounds that lack some of the nutritional functions of vitamin A (*e.g.*, 13-*cis*-RA), such as reproductive and vision. Since some of the metabolic steps are saturable, the proportion of metabolites depends upon the administered dose. Nonlinear pharmacokinetics have been observed in rats; however, the terminal half-life tends ultimately to be about 20 minutes. Biliary excretion is the major route of elimination for the metabolites.

The pharmacokinetics of all-*trans*-RA have been described in cynomolgus monkeys [120]. Following ig administration of 10 mg all-*trans*-RA/kg-bw/day (33.3 μmol/kg-bw/day), C_{max}=1,200 ng/ml and AUC=4,607 ng/ml•hr on the first day; these values decreased to a third by the tenth day. Concomitantly, values for the major metabolite all-*trans*-retinyl-β-glucuronide increased, indicating induction of all-*trans*-RA metabolism. 13-*cis*-RA was also detected as a metabolite with slower elimination than all-*trans*-RA, since plasma levels decreased only slightly over the same time period.

CLINICAL SAFETY: PHASE I STUDIES

Because of their use as nutritional supplements, human safety and pharmacokinetics data are available on retinol and its esters. The NCI, Chemoprevention Branch funded two Phase I trials of high retinol doses, one in current or former cancer patients (Drs. Gary Goodman, David Alberts, David Earnest and Frank L. Meyskens, Jr., University of Arizona) and one in normal volunteers (Dr. Alberts, University of Arizona). The safety of chronic low doses is available from Phase II and III studies funded by the Branch, such as CARET. The safety of topical use (intravaginal) of all-*trans*-RA was evaluated in three NCI-funded trials (Drs. Earl Surwit, Alberts and Meyskens, University of Arizona).

Drug Effect Measurement: ODC induction is particularly sensitive to retinoids, especially all-*trans*-RA [118]. This action may take place through suppression of transcription by all-*trans*-RA-bound RARα [121]. Topical all-*trans*-RA inhibited the induction of ODC by cellotape stripping of human skin.

Safety: Daily doses of 300,000 to 600,000 IU vitamin A (*ca.* 4.5–9.0 μmol/kg-bw/day as retinol) for several months are usually required to produce signs of hypervitaminosis A, although intake as low as 50,000 IU/day (*ca.* 0.8 μmol/kg-bw/day) has been reported to be toxic after >18 months [47, 122]. Adverse effects occur in the GI tract (nausea, vomiting, diarrhea, cramping), nervous system (headache and increased intracranial pressure, irritability, paresthesias, vision changes, fatigue), skin (dryness, pruritus, rash, desquamation, alopecia, cheilitis), muscle/joint (myalgia, pain, swelling) and bone (arthralgia, hyperostosis), as well as systemically (hypercalcemia, leukopenia). However, the major concern with chronic administration of retinol is liver toxicity (hepatosplenomegaly, hyperlipidemia, fibrosis), which can present as hepatitis or cirrhosis.

In an NCI, Chemoprevention Branch-sponsored Phase I trial (Drs. Goodman, Alberts, Earnest and Meyskens, University of Arizona), retinol doses of 173,000–500,000 IU qd (*ca.* 2.6–7.5 μmol/kg-bw/day) were evaluated in current or former cancer patients [123]. Two of three patients receiving >467,000 IU qd (>270,000 IU/m^2) for 3–4 months developed hepatomegaly. CNS toxicity characterized by irritability, depression, anxiety and hallucinations occurred in 3/5 patients receiving >415,000 IU qd (>240,000 IU/m^2) for three months.

Interestingly, 300,000 IU retinyl palmitate/day (ca. 4.5 μmol/kg-bw/day) for two years in patients with resected Stage I non-small cell lung cancer did not produce major hepatic toxicity in the completed Italian adjuvant trial [124]. Serum GGT values rose during treatment, but were significantly higher only after two years (149 *versus* 57 IU/l). Serum triglycerides increased 63% over the first year of treatment, and were significantly higher than controls

at 8 and 12 months; however, the levels returned to normal values by two years [125,126]. The majority of adverse events were dermatological (dryness, desquamation, itching).

Information concerning the safety of chronic administration of low doses is available from the CARET studies. No effect on liver function was observed after ≤3.3 years of 25,000 IU retinol/day (*ca.* 0.4 μmol/kg-bw/day) with or without β-carotene in the pilot studies preceding CARET [127, 128]. Only a negligible increase in serum triglyceride levels was observed in the CARET vanguard cohort of 1,845 heavy smokers and asbestos-exposed workers, representing 10,184 person-years of intervention [129]. The study, however, was halted January 10, 1996—21 months early—due to higher incidences of lung cancer (28%), lung cancer deaths (46%) and total deaths (17%) in the supplement group compared with those taking the placebo [130, 131].

The safety of both retinyl acetate and all-*trans*-RA has been evaluated as topical treatments. After treatment of skin with all-*trans*-RA (0.001–0.1% unknown vol) for up to 22 months, the most common adverse events were peeling, erythema and a burning sensation [132,133]. Both retinoids have been investigated as intravaginal treatments for CIN [133]. In a published Phase I/II trial [134], CIN I/II patients applied 3, 6, 9 or 18 mg retinyl acetate (9.1–54.8 μmol) or placebo intravaginally for seven days beginning on day five of three sequential menstrual cycles. Frequent severe adverse events at the highest dose were vulvar irritation and itching; 14% of all treated patients had vaginal burning during the trial. The most common general complaints were fatigue and irritability. NCI, Chemoprevention-Branch funded Phase I/II trials evaluated the safety of all-*trans*-RA applied to a sponge and inserted in a cervical cap or diaphragm. In one trial (Drs. Surwit, Alberts and Meyskens, University of Arizona), doses of 0.05–0.2% (5 ml, or *ca.* 0.008–0.03 μmol/day) for four days commonly produced vaginal irritation, ulceration and discharge, with no evidence of systemic toxicity [135]. A second study by the same group evaluating doses escalating from 0.05% (1 ml, or *ca.* 0.002 μmol/day) for four days identified an MTD of 0.372% (1 ml, or *ca.* 0.01 μmol/day) [136]. A subsequent Phase II study using this dose for induction and maintenance (two days during months three, six and nine) found mild local effects (cervical inflammation, vaginal discharge and itching) more frequent during induction [137]. Mild systemic effects (dry skin, chapped lips, mood change, headache and fever) were also noted, although a previous pharmacokinetic study failed to detect all-*trans*-RA in serum up to 24 hours after a one-day insertion at the same dose [138].

The safety of doses exceeding 6,000 IU vitamin A daily during pregnancy has not been established [47]. Case reports of fetal malformations (CNS, cardiovascular, palate and ear) following ingestion of ≥25,000 IU/day (≥0.4 μmol/kg-bw/day) during pregnancy suggest teratogenicity in humans [1,114]. A recent prospective study found an apparent threshold near 10,000 IU/day in the form of vitamin supplements [139]. Although retinol distributes to milk, the effects of large maternal doses on nursing infants is unknown. Toxicity from large doses is more common in young children than adults. A few hours after a dose of ≈25,000 IU/kg-bw, irritability, drowsiness, dizziness, vomiting, diarrhea, delirium and coma may occur. In infants, increased cranial pressure with bulging fontanelles has been reported.

Because of significant side effects, oral all-*trans*-RA (Vesanoid) has been approved only as second-line treatment for acute promyelocytic leukemia. The maximum tolerated oral dose for this indication is 60 mg/m^2 in children and 150 mg/m^2 (12.3 μmol/kg-bw/day) in adults [140]. In children, intracranial hypertension is dose-limiting. The most serious adverse effects in adults are a rapidly developing hyperleukocytosis, as well as a specific "retinoic acid syndrome" which may or may not include hyperleukocytosis—fever, pulmonary infiltrates, respiratory distress, kidney failure and coma [141,142]. Minor adverse effects of retinoids are also seen at the usual dose of 45 mg/m^2/day (*ca.* 3.7 μmol/kg-bw/day), such as skin and mucosal dryness, headache, and transient increases in triglycerides and transaminases [142,143].

ADME: The general pharmacokinetics of retinol at physiologic doses have been characterized in PRECLINICAL SAFETY STUDIES; only data specifically relating to humans will be discussed here. If fat absorption is normal, retinol is completely absorbed; at larger doses or in patients with fat malabsorption, low protein intake, or hepatic or pancreatic disease, absorption is incomplete. Peak plasma concentrations of retinol occur about 4–5 hours after administration in oil. Normal serum retinol concentrations are 300–700 ng/ml in adults (1–2.4 μM). Retinoid analogs present in nanomolar concentrations in nor-

mal human plasma are all-*trans*-RA (4.4 nM), 13-*cis*-RA (5.4 nM), 13-*cis*-oxoretinoic acid (11.7 nM) and all-*trans*-retinyl-β-glucuronide [119]. With physiologic or pharmacologic doses of retinyl palmitate, plasma all-*trans*-RA and 13-*cis*-RA increase transiently, but return to initial levels with continued dosing.

In general, vitamin A intake as retinol or retinyl palmitate up to 7.5 times the RDA (25,000 IU/day, or *ca.* 0.4 μmol/kg-bw/day) does not appear to substantially affect plasma retinol levels due to physiological controls. In several studies, no more than a 13% increase in plasma retinol occurred after up to 65 months of supplementation [43,131,144,145]. Even in CARET, daily administration of 25,000 IU retinyl palmitate (plus a dose of β-carotene without effect on plasma retinol) for a mean of four years produced only a 10% increase ($p<0.01$) in serum retinol compared with placebo; in contrast, plasma β-carotene was more than 1,100% higher than the placebo group (2,100 *versus* 170 ng/ml) [131]. Only the high dose of 300,000 IU retinyl palmitate/day in the Phase III Italian adjuvant trial in lung cancer patients increased serum retinol by 28% (98 *versus* 72 μg/dl) and RBP by 59% after one year ($p<0.05$) [126].

Pharmacological doses of retinol or retinyl palmitate appear to have more of an effect on plasma retinyl palmitate levels. In an NCI, Chemoprevention Branch-funded Phase I trial (Dr. Alberts, University of Arizona) with daily administration of 25,000 IU retinol (*ca.* 0.4 μmol/kg-bw/day), significant increases in peak plasma levels (80.5%) and AUC (62%) were obtained for retinyl palmitate after three months [144]. The plasma half-life ranged from 16.1 to 14.8 hour over the nine months of the study. Also, in a subset (n=93) of the SKICAP-AK trial, 48–65 months of 25,000 IU retinol qd caused a 334% increase in plasma retinyl palmitate, but only a 13% increase in retinol [145]. In skin, the increases were also higher for retinyl palmitate (114%) than retinol (15%).

There is very little all-*trans*-RA in the human diet and the pharmacokinetics of physiological amounts of the retinoid have not been well-characterized. It is an endogenous metabolite of retinol [5,146]; normal circulating levels vary from 2–5 ng/ml (6.7–16.6 nM) [118,132]. Much of the pharmacokinetic data on pharmacological doses of all-*trans*-RA are from APL and other cancer patients [147]. The oral bioavailability of all-*trans*-RA is *ca.* 50% [148] and values for C_{max} are extremely variable. After doses of 45–80 mg/m^2/day (*ca.* 3.7–6.6 μmol/kg-bw/day) in APL patients, C_{max} (0.03–2.5 μg/ml, or 0.1– 8.3 μM) increased in a nonlinear fashion and occurred 60–120 min after ingestion [141,148].

all-*trans*-RA isomerizes to 9-*cis*-, 11-*cis*- and 13-*cis*-RA in the liver, with subsequent oxidation to the 4-hydroxy and 4-oxo metabolites by cytochrome P450 as shown in Figure 1 [148]. The latter are eliminated in the bile and urine as glucuronide conjugates. However, after daily treatment of APL patients for 2–6 weeks, a 20–50% decrease in plasma concentration and AUC occurs, with concomitant disease relapse and resistance. This appears to result from induction of metabolism, since a ten-fold increase in urinary 4-oxo-retinoic acid metabolites was observed. Treatment with ketoconazole, an inhibitor of cytochrome P450 metabolism, one hour prior to the retinoid increased all-*trans*-RA AUC by >300 ng•hr/ml [147].

After a single dose of radiolabeled all-*trans*-RA, 30% and 60% of the radioactivity were excreted in the urine and feces, respectively. Elimination appears to be dose-dependent and saturable; the terminal $t_{1/2}$ after a single 45 mg/m^2 dose (*ca.* 3.7 μmol/kg-bw) was 39–58 minutes. After doses of up to 80 mg/m^2 (*ca.* 6.6 μmol/kg-bw) in APL patients, the apparent plasma $t_{1/2}$ was 16.8–77.4 minutes [141].

Topical all-*trans*-RA has limited percutaneous absorption (2%), with most remaining in the stratum corneum [132]. This would not affect the normal plasma level of all-*trans*-RA, 2–5 ng/ml (6.7– 16.6 nM).

CLINICAL EFFICACY: PHASE II/III STUDIES

Because of early interest in the retinoids, many Phase II trials evaluating treatment of premalignant lesions and other intermediate biomarkers or in subjects at high risk for a first or second primary tumor predated the NCI, Chemoprevention Branch. The NCI has funded two large completed Phase III trials with retinyl palmitate in subjects at high-risk for esophageal (Linxian, China) and lung cancer (CARET). One additional Phase II trial using retinol in a cohort similar to CARET is also finished. Three Phase III trials with retinol as adjuvant treatment in melanoma and non-melanoma skin cancer are complete. The Chemoprevention Branch has also pursued regression of premalignant lesions or other histological biomarkers as endpoints in additional trials. Three

completed Phase I–III trials tested all-*trans*-RA in CIN patients, and a Phase III trial is in progress. Two additional Phase III trials are in progress: regression of bronchial dysplasia by retinol and a comparison of retinyl palmitate plus β-carotene *versus* 13-*cis*-RA in oral leukoplakia patients.

Head and Neck: The NCI (Dr. Philip Taylor, Cancer Prevention Studies Branch) has collaborated with the Cancer Institute of the Chinese Academy of Medical Sciences to evaluate the effect of vitamin A in combination with other agents in a Phase III trial in Linxian, China, the area with the world's highest mortality rate from esophageal/gastric cardia cancer [149,150]. Participants (n=29,584) from the general population were treated daily with 5,000 IU retinyl palmitate (*ca.* 0.08 μmol/kg-bw/day) and 22.5 mg zinc, three other vitamin/mineral combinations or placebo in a 2x4 design (8 arms). After 5¼ years, there were no overall effects on cancer incidence or mortality. Although a 62% lower gastric cancer prevalence (OR: 0.38 *versus* 0.58) was found in 391 subjects undergoing a repeat endoscopy at the end of the trial, it was not significantly different from placebo (p=0.09) [151]. In a second trial, subjects from the same region who had grade 1–2 esophageal dysplasia were randomized to a multivitamin supplement containing 10,000 IU retinyl acetate (*ca.* 0.15 μmol/kg-bw/day) or placebo daily [152]. After six years, no effect on esophageal/gastric cardia cancer was obtained; however, in those undergoing repeat endoscopy, odds of reversion to nondysplastic cytology was significantly higher in the supplement group.

Retinoids alone and in combinations with other agents have been evaluated as a treatment for oral leukoplakia as well as a modulator of potential intermediate biomarkers (*e.g.*, dysplasia, micronucleated cell frequency) in studies of tobacco/betel nut chewers and reverse smokers in India. In a placebo-controlled study of vitamin A (NOS), 200,000 IU/wk (*ca.* 0.4 μmol/kg-bw/day) for six months produced complete clinical remission of existing lesions in more than half of the treated group (57%) compared to only 3% in the placebo group [153]. Upon biopsy, polarity of the basal cell layer nuclei and condensed chromatin in the epidermal layer—markers of dysplasia—also improved. Development of new leukoplakia was totally suppressed by the vitamin (100% *versus* 21%). The protective effect could be maintained for an additional eight months by low doses of vitamin A [154]. A second study comparing a lower dose of vitamin A (NOS) (50,000 IU, 2x/wk, or *ca.* 0.2 μmol/kg-bw/day) plus β-carotene with the carotenoid alone or placebo found clinical remission was significantly higher only in the combination group after six months [155]. No effect on the development of new leukoplakia was observed. In contrast, micronucleated cell frequency was significantly decreased to similar levels in both treatment regimens compared with placebo.

A placebo-controlled trial in India evaluated the effect of a combination of retinol acetate (25,000 IU 2x/wk, or *ca.* 0.1 μmol/kg-bw/day for months 1–4 and 9–12; 10,000 IU 2x/wk, or *ca.* 0.04 μmol/kg-bw/day for months 5–8), riboflavin, zinc sulphate and selenomethionine on oral intermediate biomarkers in reverse smokers [156,157]. The endpoints were clinical response of lesions (red or white leukoplakia, if present) and DNA adducts and micronuclei frequency in exfoliated buccal mucosa cells. After one year, the treatment group (n=150) had significantly higher rates of clinical regression (57% *versus* 47%) and lower rates of new lesion development (12% *versus* 38%) than the placebo control group (n=148) [156]. At baseline, subjects with lesions had significantly higher DNA adducts and micronucleated cell frequency than those without lesions. However, after the treatment period, both subjects with and without lesions had significantly fewer DNA adducts and micronucleated cells compared with the relevant subjects on placebo [157].

In a series of small, published intervention studies, vitamin A (NOS) with or without β-carotene was shown to decrease the incidence of micronuclei in exfoliated oral mucosal cells in populations at high risk for oral cancer due to chewing of betel quid and/or tobacco [155,158–160]. Micronucleus formation is a marker of the extent of chromosome breakage in dividing cells, but improvement in this genetic endpoint does not always correlate to histological regression of the lesion. However, both betel nut and tobacco chewing are risk factors for the development of oral cancer, and are also strong inducers of micronucleus formation.

An ongoing NCI, Chemoprevention Branch-funded study (Dr. Scott Lippman, MD Anderson Cancer Center) is comparing the efficacy of 25,000 IU retinyl palmitate (*ca.* 0.4 μmol/kg-bw/day) + 50 mg β-carotene qd for three yrs *versus* induction with 13-cis-RA (0.5 mg/kg-bw/day) for one year and maintenance with a lower dose of 13-*cis*-RA (0.25

mg/kg-bw/day) for two yrs [161]. After a two-year follow-up period, the incidence and duration of clinical and histological response of dysplastic oral leukoplakia/erythroplakia will be evaluated.

Lung: Both adjuvant treatment and modulation of intermediate biomarkers have been investigated with retinol and derivatives in the lung. 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 uncontrolled trials [162].

The NCI-funded Phase III (Dr. Gilbert Omenn, University of Washington) CARET was designed to determine the effect of 25,000 IU retinyl palmitate (*ca.* 0.4 μmol/kg-bw/day) plus 30 mg β-carotene daily on lung cancer incidence in heavy smokers (n=14,254) and asbestos-exposed workers with a history of smoking (n=4,060) [131,163,164]. CARET was preceded by two placebo-controlled pilot studies initiated in 1985 [127,128,165]. The pilot studies demonstrated tolerability and treatment compliance in two high-risk groups: 1,029 smokers receiving 25,000 IU retinol qd (*ca.* 0.4 μmol/kg-bw/day) with and without 30 mg β-carotene qd or placebo for a median of 1.5 years, and 816 workers occupationally exposed to asbestos or those with radiographic evidence of asbestos-related lung diseases treated daily with 15 mg β-carotene plus 25,000 IU retinyl palmitate (*ca.* 0.4 μmol/kg-bw/day) or placebo for 1.5–3.3 years [127, 128,166, 167]. The efficacy phase was funded with the original study participants continuing as a vanguard population; however, all agent treatment arms were combined to receive the Phase III daily intervention regimen to determine an effect on lung cancer incidence, as well as incidences of mesothelioma, other cancers, coronary heart disease, and overall mortality, after six years. Accrual into CARET by investigators at six sites (see Table II) began in 1989, with follow-up to conclude in April 1998 [161,163,165, 167]. As discussed previously, however, active intervention was halted 21 months early due to higher incidences of lung cancer and mortality in the supplement group [130,131]. Follow-up will continue for five years post-intervention.

A completed independent Phase III adjuvant trial in Italy investigated the effect of retinyl palmitate (300,000 IU/day, or *ca.* 4.5 μmol/kg-bw/day for one year) on the rate of second primary and recurrent tumors in patients (n=307) with resected Stage I non-small cell lung cancer within the prior 2 months [124,125]. After a median follow-up of 46 months, the incidence of cancers was reduced 24% (37% *versus* 48%), and latency was significantly longer ($p<0.05$) compared with an observation-only group.

The Phase III EUROSCAN trial of oral retinyl palmitate (300,000 IU/day, or *ca.* 4.5 μmol/kg-bw/day for the first year and 150,000 IU/day or *ca.* 2.3 μmol/kg-bw/day during the second year) with and without *N*-acetyl-*l*-cysteine sponsored by the EORTC (European Organization for Research and Treatment of Cancer) uses a 2x2 factorial design. The study is accruing patients (n=2,600) curatively treated for laryngeal, oral cavity, and NSCLC from 14 countries to examine the effect of intervention on survival, rate of recurrence of primary cancers or metastasis, and occurrence rate of second primaries [168,169]. The trial began in June 1988, and the end of intervention is expected in September 1996 [169]. Adverse events in the first 1,115 patients enrolled, most common in the retinoid groups, consisted mainly of mucocutaneous complaints, headaches and dyspepsia [170].

Several trials have evaluated modulation of histological intermediate biomarkers by retinol and its derivatives. In the first study, regression of bronchial metaplasia was reported following treatment with another retinoid (etretinate) in uncontrolled trials [162]. However, a recent placebo-controlled trial found no difference in improvement of sputum atypia between the etretinate and placebo groups [171]. Other trials are evaluating the combination of retinol and β-carotene in former asbestos workers [172]. The endpoint in a completed NCI-funded Phase II trial (Dr. Jerry McLarty, University of Texas) was sputum atypia, which is prevalent in this population; moderate or severe atypia in sputum specimens is a risk factor for lung cancer. Administration (n=755) of 25,000 IU retinol qod (*ca.* 0.2 μmol/kg-bw/day) plus 50 mg β-carotene qd for a median of five years produced no change in atypia incidence compared to placebo. An ongoing NCI-funded Phase II study (Dr. Carrie Redlich, Yale University) is assessing the effect of the CARET regimen of retinyl palmitate and β-carotene on intermediate biomarkers in asbestos-exposed workers [173]. Six months after randomization to treatment or placebo, subjects (n=50) will be assessed for effects on squamous metaplasia, inflammation, cytokines, and growth factors in biopsied tissue. The latter may be less variable than sputum cells. Finally, an ongoing NCI-funded Phase III trial

(Dr. Stephen Lam, British Columbia Cancer Agency) is investigating the effect of 50,000 IU retinol alone (*ca.* 0.75 μmol/kg-bw/day) on bronchial dysplasia and other biomarkers (loss of heterozygosity, quantitative nuclear morphometry, telomerase) in heavy smokers.

Skin: Some of the earliest studies involved management of skin lesions because retinoids accumulate in this tissue, and induction of epidermal differentiation is a well-known response. Three NCI studies have evaluated the efficacy of orally administered retinoids *in* preventing the development of second primary tumors in two different populations—patients with previous SCC/BCC and patients with previous malignant melanoma. In the SKICAP-AK trial (Dr. Thomas Moon, University of Arizona and the Southwest Skin Cancer Prevention Study Group), subjects (n=2800) with a history of >10 actinic keratoses and ≤2 previous SCC/BCC were randomized to 25,000 IU retinol/day (*ca.* 0.4 μmol/kg-bw/day) or placebo for five years [174,175]. Retinoid-treated participants had a significantly lower incidence of new SCC, but no change in BCC. In contrast, the three-arm SKICAP-S/B trial (Drs. Meyskens and Alberts, University of Arizona) found no reduction in the incidence of new skin cancers in patients (n=525) with prior multiple SCC or BCC after three years of treatment with retinol (25,000 IU/day, or *ca.* 0.4 μmol/kg-bw/day), 13-*cis*-RA or placebo [174–177].

A SWOG study (Dr. Meyskens, University of Arizona) investigated adjuvant treatment with oral retinol then retinyl palmitate in patients (n=248) with resected primary malignant melanoma [178]. Following treatment with 100,000 IU/day (*ca.* 1.5 μmol/kg-bw/day) for 18 months with a median of eight years of follow-up, no difference in overall or disease-free survival was found compared with observation only. Of those receiving the retinoids, 12% had grade 3–4 toxicities.

In contrast, dysplastic nevi, precursors of melanoma and considered histological intermediate biomarkers, have responded to topical all-*trans*-RA treatment [179,180]. For example, a small independent placebo-controlled study of 21 patients (six nevi/patient) demonstrated significant improvement in both clinical and histological parameters; 47% (7/15) of nevi treated with 0.05% all-*trans*-RA of unknown volume daily for four months reverted to benign nevi or disappeared completely [181].

Topical all-*trans*-RA has been used to treat actinic keratoses since 1960 [132,182]. The latter are premalignant skin lesions (histological intermediate biomarker) that increase risk for squamous cell carcinoma [183]. A number of small published trials have shown a decrease in lesion number after application of 0.05–0.1% all-*trans*-RA cream for up to 15 months [*e.g.*, 182,184].

Cervix: Because of epidemiological data and decreased HPV expression in response to all-*trans*-RA, retinoids were chosen for evaluation in women at risk for cervical cancer. Regression of CIN was chosen as the endpoint due to a report of decreasing CRBP with increasing CIN grade [185]. In four NCI, Chemoprevention Branch-funded studies, all-*trans*-RA applied to a sponge and inserted in a cervical cap or diaphragm was tested in women with CIN. In the first Phase I/II study (Drs. Surwit, Alberts and Meyskens, University of Arizona), 0.05–0.2% all-*trans*-RA (5 ml, or *ca.* 0.008–0.03 μmol/day) was applied to a cervical sponge and inserted in a diaphragm daily for four days [135]. One-third of CIN I/II patients had ≥25% regression in lesion size. In the second study by the same group, doses escalating from 0.05% all-*trans*-RA (1 ml, or *ca.* 0.002 μmol/day) applied to a sponge inserted in a cervical cap were evaluated in a similar population [136, 186]. After four days, complete regression was obtained in 45% of women treated with 0.16– 0.48%. In the Phase II study (Drs. Surwit, Sheldon Weiner and Meyskens, University of Arizona), four-day induction at the MTD (1 ml 0.37%, or *ca.* 0.01 μmol/day) identified in the previous study was followed by maintenance at the same dose for two days during months three, six and nine [137]. At 12 months, total lesion regression was obtained in 50% (10/20), with equal distribution between mild and moderate dysplasia; half the nonresponders had improvement in the severity of their lesion. Finally, a Phase III placebo-controlled trial (Dr. Surwit, Southern Arizona Surgical Oncology, Inc.) used a regimen similar to the Phase II trial, except maintenance only occurred at months three and six [187]. At follow-up biopsy (range 9–27 months), CIN II regression rate was 59% higher in the treatment group compared with placebo (p=0.04); no treatment effect was observed with CIN III.

An additional NCI, Chemoprevention Branch-funded pilot study (Dr. Mack Ruffin, University of Michigan) in preparation for a Phase III trial is in the final planning stages. Women with CIN II or III will be randomized to three intravaginal doses of all-

trans-RA (1 ml 0.16–0.37%, or 0.005–0.01 μmol/day) by CIN grade and HPV type (low *versus* high risk). Twelve weeks after four daily applications, modulation of cervical intermediate biomarkers (HPV copy/cell, HPV E6/E7 expression) will be evaluated. The Phase III trial will correlate CIN regression to normal mucosa with biomarker response at the highest effective dose from the pilot study.

Hematopoietic System: all-*trans*-RA has been approved as second-line treatment for acute promyelocytic leukemia (APL). APL, a subtype (10%) of acute myeloblastic or nonlymphoblastic leukemia, is characterized by failure of promyelocytes to undergo differentiation into mature granulocytes [141,148]. Cytogenetically, the disease is identifiable by a translocation [t(15;17)(q21;q22)] which fuses the RARα gene on chromosome 17 and the PML (promyelocytic leukemia) gene on chromosome 15. Early deaths are usually due to severe hemorrhage, which is exacerbated by conventional chemotherapy. Clinical trials indicate that the common dose of 45 mg/m^2/day (*ca.* 3.7 μmol/kg-bw/day) induces remission in 64–100% of patients [148] characterized by blast differentiation, expansion of normal metaphases, and appearance of a normal karyotype. The significance of this finding is that terminal differentiation is an alternative mechanism to cytotoxicity for treatment of malignant diseases.

Because of the success in APL, all-*trans*-RA was also evaluated as a treatment for myelodysplatic syndromes, a group of five hematopoietic disorders considered to be precursors to leukemia [188]. They occur primarily in the elderly and are characterized by ineffective hematopoiesis leading to anemia and a relatively indolent course, although death may occur from infection, hemorrhage or progression to malignant disease. Unfortunately, most trials of all-*trans*-RA in myelodysplastic syndromes have shown only temporary small effects [*e.g.*, 189,190]. For example, 26% (6/23) of patients receiving 45 mg all-*trans*-RA/m^2/day (*ca.* 3.7 μmol/kg-bw/day) showed some improvement in peripheral blood counts or reduced bone marrow blasts, which did not last for more than five weeks [191]. The difference in response between APL and myelodysplasia may be from the type of genetic lesion, different disease process (blocked maturation in APL *versus* impaired differentiation in myelodysplasia), or the pharmacokinetics of the retinoid.

Colon: In an independent Italian Phase II study, the effect of a combination of retinol palmitate (30,000 IU qd, or *ca.* 0.5 μmol/kg-bw/day) with vitamins C and E on proliferation was evaluated in normal-appearing rectal mucosa taken from patients (n=42) after removal of colorectal polyps [192]. After six months, the proliferative compartment (ratio of labeled cells in the upper 40% of the crypt to the total labeled cells per crypt) in biopsies from the supplement group was significantly lower (45%) than baseline, primarily due to decreases in compartments 4 and 5. In contrast, no effect on total thymidine labeling index was found in the supplement group. No changes in either proliferative measurement were found in the placebo group. Follow-up six months later demonstrated that the labeling values were no longer statistically different from baseline. Expansion of the proliferative compartment is considered to be a proliferative intermediate biomarker in the DMH-induced mouse colon carcinogenesis model and in patients with colorectal polyps [*e.g.*, 193,194]. Increased total thymidine labeling is not always observed in the same crypts [195].

PHARMACODYNAMICS

Vitamin A has a long biological half-life and bioaccumulates in liver, kidney, lung, and fat. Hypervitaminosis A can occur in rats at retinol doses as low as 34.9 μmol/kg-bw/day. In preclinical efficacy studies in mouse skin and rat colon models, similar doses inhibited the appearance of premalignant lesions. In other models such as rat liver and bladder, doses of esters as low as 5.2 μmol/kg-bw/day were effective. In bladder models, however, 13-*cis*-RA affected more endpoints (TCC incidence, multiplicity, size and grade) [196] and appears to have less chronic toxicity than retinol and its esters and all-*trans*-RA.

all-*trans*-RA produced decreased body weight, dermal and mucosal changes, hair loss and inhibition of spermatogenesis in rats at 16.6 μmol/kg-bw/day. At a slightly lower oral dose (12.5 μmol/kg-bw/day), the retinoid inhibited development of aberrant crypt foci in rat colon and papillomas and carcinomas in mouse skin. However, 13-*cis*-RA is 3– 5 times less toxic than all-*trans*-RA and was more potent in mouse skin models (0.2 μmol/kg-bw/day).

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

The drug effect measurements that have been sug-

gested are ODC induction and RAR expression. These, however, require tissue samples and may not be applicable in all organ systems. In plasma, retinol levels are controlled, but retinyl palmitate has been shown to increase significantly after oral administration of 25,000 IU retinol (*ca.* 0.4 μmol/kg-bw/day) for three months. Inhibition of ODC induction has been demonstrated with retinol and its esters and all-*trans*-RA in mouse skin cancer models. 13-*cis*-RA had the same effect in skin and oral cavity. It is unknown if this measurement is modulated in other tissues or in humans. The dose relationship of this response needs to be characterized before it could be used as a drug effect measurement.

Safety Issues

Chronic use of retinoids is limited by their accumulation and resulting toxicity. Retinol intake can produce CNS, GI, skin and mucosa, eye, bone and metabolic effects; however, liver toxicity is the major concern at doses used for cancer chemoprevention. all-*trans*-RA can cause CNS, GI, skin, mucosa, liver and lipid abnormalities. During all-*trans*-RA treatment of APL, an often severe retinoic acid syndrome occurs. Even at lower doses in chemoprevention or adjuvant trials, these effects can alter quality of life and informed consent about such potential effects can limit accrual.

Even with topical treatment, all-*trans*-RA has the potential for producing systemic effects. In two trials treating CIN by intravaginal application, fatigue, irritability, dry skin and lips, headache and fever were noted. Interestingly, a pharmacokinetics study failed to detect the retinoid in serum up to 24 hours after a one-day insertion. More research is needed to clarify the potential for vaginal uptake and systemic toxicity.

A strategy to increase efficacy and decrease toxicity has been to combine chemopreventive agents. Recently, however, CARET was stopped 21 months early due to higher incidences of lung cancer (28%), lung cancer deaths (46%) and total deaths (17%) following supplementation of subjects at high risk for lung cancer with 25,000 IU retinyl palmitate (*ca.* 0.4 μmol/kg-bw/day) plus 30 mg β-carotene compared with those taking the placebo [130,131]. This result is difficult to explain in view of the weight of epidemiological evidence suggesting an inverse relationship between lung cancer and dietary or serum β-carotene and possibly retinol. The impact of supplementation is unknown, although antioxidants have the potential to serve as prooxidants [197, 198]. Follow-up of CARET participants will continue for five years to determine if the effect is sustained. In addition, an ongoing Phase II trial (Dr. Redich, Yale University) may provide information on the effect of the same regimen on early biomarkers of lung cancer and perhaps understanding of the mechanism for the CARET result.

Pharmacodynamics Issues

One of the major problems with retinoids is that the chemopreventive actions are reversible on drug withdrawal. This requires maintenance treatment after induction of the response. This is not surprising, since the major action appears to be slowing of progression. As discussed above, chronic administration can produce toxicity due to accumulation of the retinoid, especially with retinol and its esters.Human use of all-*trans*-RA is limited by the rapid development of drug resistance and the occurrence of the often severe retinoic acid syndrome. Resistance and relapse of APL appear to be due to induction of cytochrome P450 metabolism of all-*trans*-RA and decreased AUC and plasma levels. Two suggestions for dealing with this are concomitant treatment with an inhibitor of metabolism, such as ketoconazole or liarozole [199–201], or intermittent dosing [202].

Regulatory Issues

Since retinol and its esters can be considered nutrients, regulatory requirements for use in clinical trials can be uncertain. The practice of the NCI, Chemoprevention Branch has been to obtain approval under the IND mechanism for conducting trials with these agents at doses above the RDA. all-*trans*-RA, however, is a metabolite of retinol and is not obtained from the diet. Although INDs have been obtained by the investigator or the NCI as sponsor previously for this retinoid, the planned pilot study (Dr. Ruffin, University of Michigan) administering all-*trans*-RA intravaginally to CIN patients was given exempt status by the FDA. The reason may be that the study design and all-*trans*-RA dose are similar to the Phase III trial in CIN I and II patients undertaken by Dr. Meyskens.

Intermediate Biomarker Issues

Retinol and its analogs have inhibited the development or increased regression of histological biomarkers in preclinical carcinogen-induced models in

several organ systems, *e.g.*, rat colon aberrant crypt foci and adenomas, mouse skin papilloma, and hamster cheek pouch papilloma and CIS. The agents were most effective when given after the carcinogen or as an adjuvant treatment. However, unexpected results were obtained in some models when the retinoid was administered before or during initiation. In the NQO-exposed rat tongue, dietary administration of retinyl acetate during extended carcinogen exposure increased the incidence of dysplasia and CIS, but significantly decreased adenocarcinoma incidence [203]. This result might be expected if retinyl acetate inhibits progression of premalignant lesions. However, in a second published study, dietary retinyl acetate offered for two months before initiation with DMBA increased the incidence of rat mammary fibromas, adenomas and fibroadenomas and had no effect on invasive tumors [89]. In clinical studies, the most appropriate use of retinoids may be in preventing progression of existing premalignant lesions, rather than during carcinogen exposure. The results of CARET in individuals at high risk from cigarette smoke and/or asbestos exposure may be evidence of this caveat. Successful regression of premalignant lesions has been suggested by a few small controlled clinical trials involving dysplastic oral leukoplakia, CIN, and dysplastic nevi and actinic keratosis in skin.

The proliferation biomarker ODC induction has been modulated by retinol and its analogs in two-stage mouse skin carcinogenesis models. Also, the proportion of foci expressing *myc*, a proliferation-related oncogene, decreased following treatment of AOM-induced rats with all-*trans*-RA [103]. These effects may result from regulation of the expression of specific genes by retinoid receptor subfamilies or isoforms. A human study has also suggested effects on other indices of proliferation. In a published Italian Phase II trial, retinyl palmitate combined with vitamins C and E regressed expansion of the proliferative compartment in normal-appearing colon mucosa taken from patients during removal of colon polyps [192]. 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.

all-*trans*-RA has been specifically shown to decrease HPV expression, a risk biomarker in the cervix. Since some types of HPV confer higher risk of progression to CIN, regression of these lesions by all-*trans*-RA will be correlated with HPV copy/cell and HPV expression after stratification by HPV type (low *versus* high risk) in an NCI, Chemoprevention Branch-funded Phase III trial (Dr. Ruffin, University of Michigan).

Supply and Formulation Issues

Retinol and the esters are available as dietary supplements and oral all-*trans*-RA is marketed for APL treatment. No supply issues are anticipated.

Clinical Studies Issues

Although retinyl acetate has been one of the most effective retinoids in inhibiting mammary carcinogenesis in rat models, it has not undergone clinical development as a chemopreventive agent for this indication. Since it is metabolized similarly to retinol, retinyl acetate tends to accumulate in the liver as retinyl esters and has the potential to cause hepatotoxicity [51]. In contrast, 4-HPR is effective in both mouse and rat models and is metabolized and stored in the mammary tissue of both rodents and humans. Thus, 4-HPR was selected for clinical evaluation as a breast cancer chemopreventive. More recent preclinical data suggest that 9-*cis*-RA is even more potent in a rat mammary carcinogenesis model.

Three NCI-funded trials have shown regression of CIN by all-*trans*-RA. As discussed in Intermediate Biomarker Issues, a pilot study preceding a Phase III trial is in the final planning stages to determine the effect of all-*trans*-RA on HPV expression and copy number after stratification by HPV type (low *versus* high risk). Since all-*trans*-RA appears to produce both local and systemic side effects after intravaginal use, other strategies also may be considered. A combination of an RXR- and an RAR-specific ligand recently showed more than additive effects in inhibiting proliferation of human cervical carcinoma ME180 cells *in vitro* [204]. Since 9-*cis*-RA binds both receptor types, the NCI, Chemoprevention Branch is considering a short-term Phase II trial in presurgical CIN II patients with intermediate marker modulation as endpoints.

A large volume of epidemiologic data have suggested that β-carotene is inversely associated with lung cancer risk. In studies that measured carotenoids and retinoids separately, four found an inverse association between serum or dietary preformed retinol and lung cancer cases in retrospective studies. A

review of controlled clinical trials found only one in which retinyl palmitate (300,000 IU/day, or *ca.* 4.5 μmol/kg-bw/day) had a significant effect on second primary and recurrent lung cancers— increased latency [125]. At this dose, significant increases in hepatic enzymes and dermatological complaints occurred over the two-year administration [124]. In contrast, 13-*cis*-RA has produced significant decreases in second primary tumors and regression of premalignant tumors in controlled head and neck trials. Since the concept of field carcinogenesis also applies to lung, the NCI, Chemoprevention Branch will await the results of three Phase II and III trials with second primary tumors and intermediate biomarkers as endpoints to decide if these retinoids should be pursued in these organ systems.

The only other trials in which retinol or its analogs have shown efficacy are one each in skin and colon. Retinol (25,000 IU/day, or *ca.* 0.4 μmol/kg-bw/day) decreased the incidence of SCC in subjects with a history of ≥10 actinic keratoses and ≤2 SCC/BCC. Several small published trials with topical all-*trans*-RA have shown regression of actinic keratoses or dysplastic nevi, precursors to SCC and melanoma, respectively. Since two other studies evaluating adjuvant treatment were negative, NCI will not pursue this indication. Treatment of premalignant lesions will be pursued by independent groups.

Prospective epidemiological data from one study measuring dietary preformed retinol and two studies with serum retinol have suggested an inverse relationship with colon and GI cancer. Also, preclinical efficacy studies found that retinyl acetate and all-*trans*-RA decreased development of AOM-induced aberrant crypt foci and altered expression of oncogenes in preclinical studies. Finally, decreased proliferative compartment expansion in normal-appearing mucosa from subjects with previous polyps and receiving retinyl palmitate (30,000 IU/day, or *ca.* 0.4 μmol/kg-bw/day) and vitamins C and E was observed in an Italian trial. It may be possible to decrease retinoid toxicity but retain the efficacy in the colon by using lower doses in combinations with NSAIDs or calcium. However, it remains unclear if vitamin A supplementation by retinol or its analogs is chemopreventive in well-nourished populations.

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Table II. Clinical Trials of Vitamin A 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 and ADME)					
PO1-CA-27502 Pharmacokinetics and Metabolism of Retinol Administered at a Chemopreventive Level to Normal Subjects (Dr. David Alberts, University of Arizona) 3/85–10/86	---	Normal, healthy volunteers 13 subjects	25,000 IU retinol qd for 9 mo	Pharmacokinetics: Blood, skin, subcutaneous fat Safety: Liver function tests, liver scan, serum lipids	Study completed. Significant increase in serum retinyl palmitate C_{max}, AUC. Skin levels were not different from age-matched controls Published report: [144]
PO1-CA-27502 Phase I Trial of Retinol in Cancer Patients (Drs. Gary Goodman, David Alberts, David Earnest, and Frank Meyskens, Jr., University of Arizona) 11/79–1/82	---	Advanced cancer patients who refused or failed other treatment: 8 Patients with previously resected malignant melanoma from pilot study for adjuvant trial: 5	Cancer patients: 100,000–200,000 IU retinol/m^2 qd (173,000–396,000 IU qd) for 2–12 mo Melanoma patients: 500,000 IU retinol qd (240,000–350,000 IU/m^2 qd) for 2–4 mo	Safety: Hematology, liver/spleen scans, renal and hepatic function tests, blood chemistry	Study completed. Hepatomegaly developed in two patients receiving >270,000 IU/m^2 qd after ≥3 months. CNS toxicity (irritability, depression, anxiety, hallucinations) occurred in 3/5 at doses >240,000 IU/m^2 qd for 3 months. Elevated serum triglycerides, headaches, dry skin, cheilosis at ≥200,000 IU/m^2 Published report: [123]
PO1-CA-27502 Evaluation of Topically Applied *trans*-Retinoic Acid in the Treatment of Cervical Intra-epithelial Lesions (Drs. Earl A Surwit, David Alberts, and Frank L. Meyskens, Jr., University of Arizona)	Cervix	Women with CIN II or III 18 women	Topical 5 ml 0.05%, 0.1% and 0.2% all-*trans*-RA in alcohol and 0.1% in cream applied to collagen sponge in standard diaphragm inserted daily for 4 days	Safety: Colposcopic and clinical examinations on days 2, 3, 4, and 7; liver function tests on day 4; plasma drug levels Efficacy: Lesion response	Study completed. Vaginal irritation, discharge and ulceration most common toxicity. No evidence of systemic absorption or hepatoxicity. At end of treatment, 33% (6/18) had ≥25% regression in surface area of lesions; complete regression obtained in 1 patient Published report: [135]

Table II. Clinical Trials of Vitamin A 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 and ADME) (*cont.*)					
UO1-CA-27502 Phase I Trial of β-all-*trans*-Retinoic Acid Delivered via a Collagen Sponge and Cervical Cap (Drs. Frank L. Meyskens, Jr., David S. Alberts and Earl A. Surwit, University of Arizona)	Cervix	Women with biopsy-proven CIN I or II 35 women	Topical 1 ml 0.05% all-*trans*-RA cream applied to cervical sponge in cervical cap inserted daily for 4 days; doses escalated by modified Fibonacci scale	Safety: Colposcopic and clinical evaluations on days 1, 2, 3, 8 and 30; liver function on day 4 Efficacy: Lesion response by cytology, colposcopy and biopsy at 3-month intervals up to 15 months	Study completed. No systemic effects; unacceptable vaginal toxicity (burning, discharge, bleeding) at 0.484%. Most frequent effect was mild cervical inflammation. Although cervical tissue uptake of [^{3}H]-all-*trans*-RA 4 hrs after the first 1-day insertion was significantly higher at 0.375% than 0.05%, there was no difference after 24 hrs. Blood sampling indicated no systemic absorption Complete regression obtained in 14% of women treated with 0.05–0.117% and in 45% of women treated with 0.158–0.484%. A concentration of 0.372% (MTD) chosen for Phase II trial in a similar cohort Published reports: [136,138,186]

Table II. Clinical Trials of Vitamin A 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 II (Dose-titration, efficacy, intermediate biomarkers)					
RO1-CA-27502, CA-40889 Phase II Trial of β-all-*trans*-Retinoic Acid for Cervical Intraepithelial Neoplasia via Collagen Sponge and Cervical Cap (Drs. Earl Surwit, Sheldon Weiner and Frank L. Meyskens, Jr., University of Arizona Health Sciences Center)	Cervix	Women with biopsy-proven CIN	Topical 1 ml 0.372% all-*trans*-RA cream applied to cervical sponge in cervical cap inserted daily for 4 days; maintenance with daily insertion for 2 days at 3, 6 and 9 mo	Efficacy: Clinical and histological regression by Pap smears and colposcopy at 3, 6, 9 and 12 mo Safety: Clinical examination and colposcopy	Study completed. Total lesion regression in 50% (10/20), with equal distribution between mild and moderate dysplasia Most frequent local side effects were cervical inflammation and vaginal discharge and itching during induction. Systemic effects included dry skin, chapped lips, mood change, headache, fever and abdominal cramping Published report: [137]
RO1-CA-68148-02 Biomarkers of Disease and Vitamin A in Asbestos Workers (Dr. Carrie A. Redlich, Yale University) 8/95–7/97	Lung	Asbestos-exposed workers	25,000 IU retinyl palmitate + 30 mg β-carotene qd for 6 mo	Intermediate Biomarkers: Cytokines, p53, growth factors Efficacy: Normalization of squamous metaplasia (if present)	Study in progress; 21 subjects had completed the treatment as of May, 1996 Published report: [173]

Table II. Clinical Trials of Vitamin A 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 III (Efficacy, intermediate biomarkers)					
RO1-CA-40889 Phase III Intervention Trial for Cervical Dysplasia (Dr. Earl A. Surwit, Southern Arizona Surgical Oncology, Ltd.) 9/85–4/93 Investigator IND (Dr. Frank Meyskens)	Cervix	Women with biopsy-proven CIN II or III 301 women	Topical 1 ml 0.372% all-*trans*-RA or placebo applied to cervical sponge in cervical cap qd for 4 days, then maintenance qd for 2 days at 3 and 6 mo	Efficacy: CIN regression during 6-month induction/maintenance and 21-month follow-up Safety: Colposcopic and clinical evaluations	Study completed. CIN II regression rate increased by 59% ($p=0.04$) compared to placebo in 141 women available for analysis; no treatment effect was observed in 99 CIN III women Mild local adverse events were recorded during or just after the 4-day induction period; the only significantly greater events (incidence) were vulval burning, itching and irritation Published report: [187]
UO1-CA-68291-01 Phase III Prevention Intervention Trials Utilizing Intermediate Endpoints and Their Modulation by Chemopreventive Agents: Retinoids and Intermediate Biomarkers for CIN II and III (Dr. Mack T. Ruffin IV, University of Michigan) 9/95–9/00 Exempt	Cervix	Women with CIN II or III Pilot study: 160 women (14/dose/CIN grade/HPV type) Phase III: 342 women (57/arm/CIN grade/HPV type)	Pilot study: Topical 1 ml 0.1583%, 0.28% or 0.327% all-*trans*-RA (w/v) via cervical cap qd for 4 days; follow-up at 12 wk Phase III: Dose chosen from pilot study for 4 days, with 2-day administration at 3, 6, 9 mo; follow-up at 12 mo	Phase III efficacy: CIN regression to normal; correlate with intermediate biomarker modulation Pilot and Phase III intermediate biomarkers: HPV copy/cell, HPV E6/E7 expression	Study in final planning stages

Table II. Clinical Trials of Vitamin A 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 III (Efficacy, intermediate biomarkers)					
ZO1-CN-00112-10 Study of Effect of Nutritional Intervention on Esophageal Cancer in Linxian, People's Republic of China (Dr. Philip Taylor, NCI, DCPC, W. Blot, NCI, DCE, and Linxian Nutrition Intervention Study Group) 5/85–5/91	Esophagus	Linxian, China commune residents 40–69 years of age in high-risk area for esophageal/gastric cardia cancer General population trial: 29,584 residents Dysplasia trial: 3,318 residents with grade 1–2 dysplasia	General population trial: 5,000 IU retinyl palmitate + 22.5 mg zinc oxide qd *vs.* riboflavin + niacin *vs.* vitamin C + molybdenum *vs.* β-carotene + vitamin E + selenium *vs.* placebo (8 arms) for 5¼ yrs Dysplasia trial: multivitamin/mineral supplement (containing 10,000 IU retinyl acetate) or placebo for 6 yrs	General population trial: Esophageal/gastric cardia cancer incidence, cancer mortality, total mortality Dysplasia trial: Esophageal/gastric cardia cancer incidence, mortality, intermediate biomarkers (severity of dysplasia, [^{3}H]-thymidine labelling)	General population trial: No overall effect on cancer incidence or mortality. In 391 undergoing repeat endoscopy, lower gastric cancer incidence (62%) not significant (p=0.09) Dysplasia trial: No overall effect on cancer incidence and mortality. In 396 undergoing repeat endoscopy, odds of reversion to non-dysplastic cytology significantly higher in treated group (1.23); also, significantly lower (28.5%) proliferating cell fraction in upper compartment of nonsmoker's epithelium Published reports: [149–152,207,208]

Table II. Clinical Trials of Vitamin A 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 III (Efficacy, intermediate biomarkers) ***(continued)***					
UO1-CA-34290 β-Carotene and Lung Cancer Chemoprevention (Dr. Jerry W. McLarty, Univ. of Texas) 9/84–5/93 IND 24,404	Lung	Men and women occupationally exposed to asbestos 755 subjects	50 mg β-carotene qd + 25,000 IU retinol A qod or placebo for median of 5 yrs (range, 3–6 yrs)	Efficacy: Incidence of sputum atypia Safety: Physical exam, liver function, serum lipids, sputum collection, chest x-ray ADME: Serum retinol and β-carotene	Intervention completed; follow-up for cancer incidence and mortality continues. No difference in incidence or severity of sputum atypia observed between groups Statistically significant increase in serum triacylglycerol in treated group resolved with discontinuance of retinol. Higher incidence of skin yellowing with intervention Published reports: [170,205,206]
CARET PO1-CA-34847 Phase III Chemoprevention of Lung Cancer with Retinoids and β-Carotene (Dr. Gary Goodman, University of Washington) 7/83–1/96 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Current or former (≤6 years) male and female smokers with a ≥20 pack-year history Pilot study: 1,029 smokers Total CARET: 14,254 smokers	Pilot: 30 mg β-carotene qd, 25,000 IU retinol qd, 30 mg β-carotene + 25,000 IU retinol qd, or placebo for 3 yrs Phase III: 30 mg β-carotene + 25,000 IU retinyl palmitate qd or placebo for 5 yrs	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Pilot completed. Phase III trial stopped 21 months early due to increased incidences of lung cancer (28%) and death (17%) in the treatment group Published reports: [127,129,131,165]

Table II. Clinical Trials of Vitamin A 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 III (Efficacy, intermediate biomarkers) ***(continued)***					
CARET PO1-CA-34847 Phase III Cancer Prevention with Retinol and β-Carotene in Persons with Asbestosis (Dr. Gilbert S. Omenn, Univ. of Washington) 7/83–1/96 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Men with asbestosis or occupationally exposed to asbestos for ≥5 yrs Pilot: 816 men Total CARET: 4,060 asbestos-exposed men	Pilot study: 15 mg β-carotene + 25,000 IU retinol qd or placebo for 3 yrs Phase III: 30 mg β-carotene + 25,000 IU retinyl palmitate qd or placebo for 5 yrs 10 years	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Pilot completed. Phase III trial stopped 21 months early due to increased incidences of lung cancer and death in the treatment group Published reports: [128,129,131,165]
CARET RO1-CA-47989 Phase III Chemoprevention Trial of β-Carotene and Retinol (Dr. John R. Balmes, University of California, San Francisco) 9/88–1/96 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Asbestos-exposed smokers Total CARET: 4,060 asbestos-exposed male smokers	30 mg β-carotene + 25,000 IU retinyl palmitate qd or placebo for 5 yrs	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Phase III trial stopped 21 months early due to increased incidences of lung cancer and death in the treatment group Published reports: [131,165]

Table II. Clinical Trials of Vitamin A 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 III (Efficacy, intermediate biomarkers) ***(continued)***					
CARET RO1-CA-48196 Chemoprevention Trial of β-Carotene and Retinol (Dr. James P. Keogh, University of Maryland, Baltimore) 9/88–1/96 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Men exposed to asbestos or with asbestosis Total CARET: 4,060 asbestos-exposed men	30 mg β-carotene + 25,000 IU retinyl palmitate qd or placebo for 5 yrs	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Phase III trial stopped 21 months early due to increased incidences of lung cancer and death in the treatment group Published reports: [131,165]
CARET RO1-CA-48200 β-Carotene and Retinol Chemoprevention Trial in Asbestos (Dr. Mark R. Cullen, Yale University, New Haven Hospital) 9/88–1/96 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Asbestos-exposed workers Total CARET: 4,060 asbestos-exposed men	30 mg β-carotene + 25,000 IU retinyl palmitate qd or placebo for 5 yrs	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Phase III trial stopped 21 months early due to increased incidences of lung cancer and death in the treatment group Published reports: [131,165]

Table II. Clinical Trials of Vitamin A 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 III (Efficacy, intermediate biomarkers) ***(continued)***					
CARET RO1-CA-48203 Phase III Chemoprevention of Lung Cancer (Dr. Barbara G. Valanis, Kaiser Foundation Research Institute) 8/88–1/96 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Cigarette smokers, men exposed to asbestos or with asbestosis Total CARET: 14,254 smokers, 4,060 asbestos-exposed men	30 mg β-carotene + 25,000 IU retinyl palmitate qd or placebo for 5 yrs	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Phase III trial stopped 21 months early due to increased incidences of lung cancer and death in the treatment group Published report: [131,165]
CARET RO1-CA-52596 Phase III Chemoprevention Efficacy Trial of β-Carotene and Retinol (Dr. Frank L. Meyskens, Univ. of Calif., Irvine Cancer Center) 8/91–1/96 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Heavy smokers Total CARET: 14,254 smokers	30 mg β-carotene + 25,000 IU retinyl palmitate qd or placebo for 5 yrs	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Phase III trial stopped 21 months early due to increased incidences of lung cancer and death in the treatment group Published report: [165]

Table II. Clinical Trials of Vitamin A 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 III (Efficacy, intermediate biomarkers) ***(continued)***					
UO1-CA-68381-01 Prevention Clinical Trials Utilizing Intermediate Endpoints and Modulation by Chemopreventive Agents: Lung Cancer Prevention with Biomarker Evaluation (Dr. Stephen C. Lam, British Columbia Cancer Agency) 7/95–7/98 Investigator IND	Lung	Heavy smokers with biopsy-proven bronchial dysplasia 80 smokers	50,000 IU retinol qd for 6 months; if bronchial dysplasia persists, 25 mg sialor tid for 6 months	Efficacy: Dysplasia regression Intermediate biomarkers: RAR-β, LOH (3p, 5q, 9p, p53, Rb), telomerase expression, quantitative morphometry (DNA content, nuclear texture)	Study in progress
PO1-CA-52051 (DM 90-096) Phase III Randomized Chemoprevention Study of Long-Term, Low-Dose 13-CRA *vs.* β-Carotene/Vitamin A in Patients with Premalignant 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)	25,000 IU retinyl palmitate + 50 mg β-carotene for 3 yrs *vs.* 0.5 mg 13-*cis*-RA/kg-bw/day for 1 yr, then 0.25 mg 13-*cis*-RA/kg-bw/day for 2 yr; 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: [161]

Table I. Clinical Trials of Retinol and Analogs 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 III (Efficacy, intermediate biomarkers) ***(continued)***					
SWOG 8049 Randomized Trial of Vitamin A *vs.* Observation as Adjuvant Therapy in High-risk Primary Malignant Melanoma: A Southwest Oncology Group Study (Dr. Frank L. Meyskens, Jr., University of Arizona and SWOG) 1981–	Skin	Patients with previous primary malignant melanoma >0.75 mm thick 248 patients	100,000 IU retinol/day for 9 mo, then 100,000 IU retinyl palmitate for 9 mo; follow-up for median of >8 yr	Efficacy: Survival, disease-free survival Safety: Hematology, clinical chemistry, chest x-ray	Study completed. No treatment effect was observed Published report: [178]
PO1-CA-27502 Phase III Chemoprevention of Skin Cancer Program Project (SKICAPS/B) (Dr. Frank Meyskens, 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 yr 719 patients	5 mg (<65 kg bw)–10 mg 13-*cis*-RA or 25,000 IU retinol or placebo qd for 3–4¼ yrs	Efficacy: Incidence of first and total BCC and SCC Safety: Serum lipids, liver function, dermatological assessment	Study completed. No reduction in skin cancer incidence in either treatment group Published reports: [174–177]
UO1-CA-34256 Phase III Chemoprevention of Skin Cancer by Vitamin A (SKICAP-AK) (Dr. Thomas E. Moon, University of Arizona, and Southwest Skin Cancer Prevention Study Group) 6/84–93	Skin	Patients with >10 actinic keratoses and ≤2 prior BCC or SCC 2,297 patients	25,000 IU retinol or placebo qd for 5 yrs	Efficacy: First occurrence and total number of BCC and SCC Safety: Skin, liver function	Study completed. Significant (32%) reduction in SCC incidence only Published reports: [145,174,175]

VITAMIN A DEVELOPMENT STATUS