NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

β-CAROTENE and OTHER CAROTENOIDS

DRUG IDENTIFICATION

CAS Registry No.: 7235-40-7

CAS Name (9CI): β,β-Carotene

Synonyms:
all-*trans*-β-Carotene
β-Carotene (Injectable)
β-Carotene (Soluble)
β-Carotin
(all-E)-1,1'-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17,octadecanonaene,1,18-diyl)bis(2,6,6-trimethylcyclohexene)
trans-β-Carotene (Type I: Crystalline Synthetic, 95% β-Carotene)
trans-β-Carotene (Type III: Crystalline from Carrots, 80–90% β-Carotene, 10–20% α-Carotene, Trace Other Isomers)
Provitamin A
Solatene® (Active Ingredient)

Other Carotenoids:

Astaxanthin (CAS No. 472-61-7)
3,3'-Dihydroxy-(3S,3'S)-β,β-carotene-4,4'-dione

Canthaxanthine (CAS No. 514-78-3)
β,β-Carotene-4,4'-dione
C.I. Food Orange 8
Orobronze
Roxanthin Red 10

α-Carotene (CAS No. 432-70-2)

15-*cis*-β-Carotene (CAS No. 7235-40-7)
15-*cis*-β,β-Carotene

α-Crocetin (CAS No. 27876-94-4)
8,8'-Diapo-ψ,ψ-carotenedioic acid

β-Cryptoxanthin (CAS No. 472-70-8)
(3R)-β,β-Caroten-3-ol

Lutein (CAS No. 127-40-2)
(3R,3'R,6'R)-β,ε-Carotene-3,3'-diol
3,3'-Dihydroxy-α-Carotene

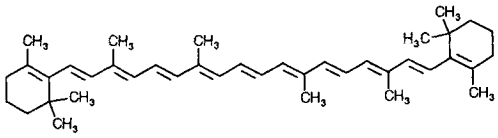
all-*trans*-(+)-Xanthophyll

Lycopene (CAS No. 502-65-8)
ψ,ψ-Carotene
(all-E)-2,6,10,14,19,23,27,31-Octamethyl-
2,6,8,10,12,14,16,18,20,22,24,26,30-dotriacontatridecaene
Rhodopurpurin

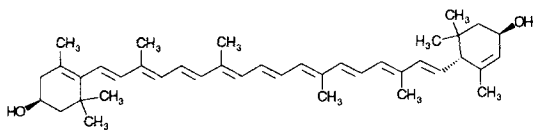
Phytoene (CAS No. 540-04-5)
7,7',8,8',11,11',12,12'-Octahydro-ψ,ψ-carotene

Zeaxanthin (CAS No. 144-68-3)
(3R,3'R)-β,β-carotene-3,3'-diol
all-*trans*-Anchovixanthin
7,7',8,8',11,11,12,12'-Octahydrolycopene

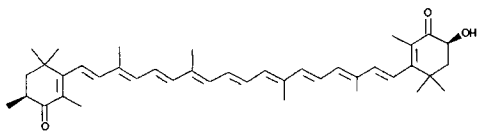
Structures:



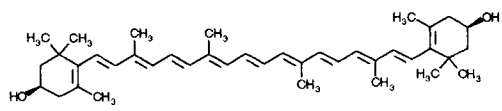
β-Carotene



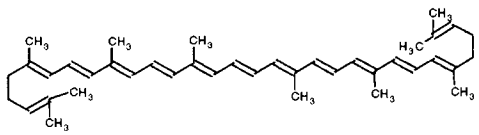
Lutein



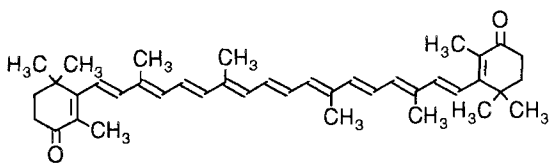
Astaxanthin



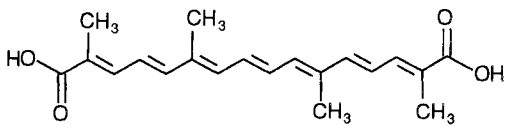
Zeaxanthin



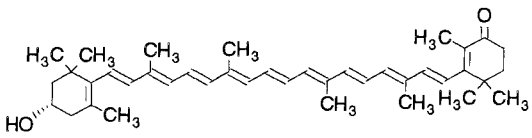
Lycopene



Canthaxanthine



Crocetin



Cryptoxanthin

EXECUTIVE SUMMARY

β-carotene is a naturally occurring carotenoid pigment that is widely distributed in vegetables and fruits [1–4]. Epidemiological studies have associated low dietary and/or plasma levels of carotenoids with higher incidences of certain cancers. Both prospective and retrospective evidence is most consistent for a protective effect of β-carotene against lung cancer [5–13], followed by cancers of the cervix, ovary, esophagus, larynx, oral cavity and nasopharynx [14–25]. The evidence is less consistent for cancers of the breast, prostate, colorectum, and stomach [21,26–30]. Since it is now possible to assess serum levels of specific carotenoids by HPLC, the following inverse epidemiological associations have also been identified: lycopene and CIN, and cancers of the lung and pancreas [31–33]; lutein and lung cancer [34]; and α-carotene and cervical cancer [35].

Part of the mechanism for these inverse epidemiological associations may be metabolism of some carotenoids to vitamin A, *e.g.*, β-carotene, 15-*cis*-β-carotene, α-carotene, and cryptoxanthin [36–38]. Carotenoids from plant sources are considered to account for half of the total vitamin A activity obtained from American diets (0.6 μg β-carotene=1 IU vitamin A) [39,40]. Retinoids in general act at the promotion phase of carcinogenesis by inhibiting proliferation [41] and inducing differentiation [*e.g.*, 42,43]; some retinoids, including vitamin A, have also been shown to have anti-initiating properties [*e.g.*, 42]. However, in several of the epidemiological studies referred to previously, an increase in cancer risk was associated only with low serum or dietary β-carotene, not vitamin A [*e.g.*, 7,11,13,25, 43]. In fact, only 53 of the more than 700 known naturally occurring carotenoids serve as vitamin precursors (Dr. F. Khachick, personal communication), suggesting that they may have chemopreventive activities unrelated to provitamin A activity. Some of these activities include modulation of cytochrome P-450 metabolism (β-carotene, cryptoxanthin, lutein) [44], inhibition of arachidonic acid metabolism (β-carotene) [*e.g.*, 45], antioxidant and free radical/reactive species scavenger (astaxanthin, canthaxanthine, α-carotene, β-carotene, crocetin, cryptoxanthin, lutein, lycopene, zeaxanthin) [*e.g.*, 46–52], immune system modulation (astaxanthin, canthaxanthine, β-carotene) [36,53,54], induction of differentiation and/or gap junction intercellular communication (astaxanthin, canthaxanthine, α-carotene, β-carotene, lutein, lycopene) [*e.g.*, 55–58], inhibition of chromosome damage/instability (canthaxanthine, β-carotene) [reviewed in 59], and antiproliferation, *e.g.*, inhibition of N-*myc* expression (α-carotene, β-carotene) [60], ODC activity (β-carotene) [61], and adenylate and guanylate cyclase activity (β-carotene) [58,62]. Because of the weight of epidemiologic evidence and the relative lack of toxicity, clinical development of β-carotene and other carotenoids as cancer chemopreventive drugs was considered by the CB.

In preclinical efficacy studies, β-carotene and other carotenoids have demonstrated efficacy in many tissues for which epidemiological associations have been reported, except for the lung. β-Carotene and canthaxanthine inhibited tumorigenesis in head/neck and skin models; crocetin, lutein and phytoene were also effective in the skin. β-Carotene and canthaxanthine inhibited mammary gland carcinogenesis in two rat models *in vivo*; β-carotene and lycopene inhibited transformation of mammary organ cultures *in vitro*. In the gastrointestinal tract, β-carotene inhibited colon carcinogenesis, although canthaxanthine was reported to have the opposite effect. β-Carotene and α-carotene were effective in the liver, and canthaxanthine and astaxanthin decreased bladder carcinogenesis.

The inverse epidemiological association between lung cancer risk and serum/dietary carotenoids has not been verified in rodent models. In contrast to humans, these species have low serum carot-

enoids, which are unrelated to dietary intake. One hamster study demonstrated 50% inhibition of lung carcinogenesis with subcutaneous injection of β-carotene, but it was not statistically significant. However, synergistic and additive inhibitory responses have been demonstrated using combinations of β-carotene with retinoids (vitamin A, 4-HPR), oltipraz, and DFMO. In the case of the vitamin A combination, the increased effect has been related to an alteration in the pharmacokinetics of β-carotene.

A significant effort in the CB program is to identify and validate intermediate biomarkers of cancer as potential surrogate trial endpoints. Preclinical studies have demonstrated modulation of histological intermediate biomarkers, *i.e.*, premalignant lesions, by β-carotene in the oral cavity, skin, colon, forestomach, liver, and pancreas. Crocetin inhibited development of premalignant lesions in the skin and liver, and enhanced hepatic glutathione conjugation.

No toxicological studies of carotenoids have been funded by the CB. Published information demonstrates that β-carotene has very low toxicity in humans and other species. In dogs, the only response noted after doses more than 600-fold higher than the average clinical dose (30 mg qd or *ca.* 0.43 mg/kg-bw) was orange surface lesions and minimal lipid deposition in the liver after two years. In rats at >2,400-fold the human dose for two years, decreased weight gain and discolored feces and hair were observed. No evidence of tumorigenicity or teratogenicity was found; however, there has been one report of embryotoxicity.

The primary effect of high carotenoid intake in humans is carotenodermia, or yellowing of the skin. This effect limits the doses which may be given in blinded clinical trials. Hypervitaminosis A has not been reported after doses up to 300 mg qd, but high doses of canthaxanthine have produced crystalline deposits in the retina which may affect function.

The CB has funded a limited Phase I study of β-carotene which confirmed that intestinal absorption was highly variable between individuals after both single and multiple doses. In published studies, both intestinal absorption and conversion to vitamin A by the intestinal mucosa were highly variable, and could be affected by dietary fat and nutritional status. Carotenoids accumulate in both serum and tissue, including fat, liver, skin, adrenal glands and testes. The seven predominant circulating carotenoids are β-carotene, lycopene, lutein, α-carotene, α-cryptoxanthin, β-cryptoxanthin and zeaxanthin. Elimination occurs slowly, and only by the fecal route.

The NCI has been involved in nine Phase III chemoprevention trials of β-carotene alone or in combination. Two international collaborative trials have been completed in tissues related to smoking (Finland) and in the esophagus (China). In the Finnish α-Tocopherol, β-Carotene Trial (ATBC), 20 mg β-carotene qd significantly increased the incidence of lung cancer (18%) and total mortality (8%). In contrast, a daily combination of 15 mg β-carotene, 30 mg vitamin E and 50 μg selenium reduced total mortality and the risk for esophageal cancer in Linxian, China. The remaining Phase III trials involve prevention of cancer of the breast, colon, head/neck, lung, skin, and multiple sites, plus the six-center Carotene and Retinol Efficacy Trial (CARET) is evaluating prevention of lung cancer in smokers and in asbestos-exposed workers. Four NCI-funded Phase II trials are in progress to investigate modulation of premalignant lesions and other intermediate biomarkers—two in the cervix, one each in the colon and oral cavity. A completed Phase II trial found that 30 mg β-carotene maintained 13-*cis*-retinoic acid-induced improvement in oral leukoplakia lesions, but not as well as a lower dose of 13-*cis*-retinoic acid. Published Phase II trials have investigated modulation of histological biomarkers in colon and cervix; a Phase III trial involved colon polyps.

The crystalline form of β-carotene is an approved color additive for foods, cosmetics and drugs, and a GRAS nutritional supplement [1–4]. It is available in capsule form (Solatene®) from Hoffman-La Roche, and is approved as an orphan drug for treatment of erythropoietic protoporphyria, an inherited photosensitizing skin disorder, at doses of 60–180 mg qd [63,64]. The company will continue to supply β-carotene. Agreements with suppliers of other potentially promising carotenoids (*e.g.*, astaxanthin, α-carotene, lutein, lycopene, zeaxanthin) are being actively negotiated by NCI.

No further clinical trials with β-carotene will be initiated until those in progress have been completed. Trials evaluating efficacy in additional tissues or with different intermediate biomarkers may be considered, as appropriate. Results from the Finnish study in smokers suggest that more investigation into chemopreventive mechanisms or pharmacokinetics may need to be done. For example, attainment of specific plasma or tissue levels may

require different β-carotene doses depending on the absorption capacity of the individual. Finally, other carotenoids which appear to be promising in preclinical testing may also be moved into clinical development.

PRECLINICAL EFFICACY STUDIES

Preclinical chemopreventive efficacy of carotenoids has been demonstrated in many of the tissues which are targets in ongoing clinical trials (see below), except for the lung. (Most of the evidence for chemopreventive activity of β-carotene in the lung has come from an inverse epidemiological association with cancer risk.) Animal efficacy studies using hamster models have not demonstrated significant inhibition of lung tumorigenesis after dietary or parenteral administration [65–67]. As discussed below, hamster serum carotenoid levels are in the ng/ml range and are not related to dietary concentrations [68]; in contrast, human serum levels are in the μg/ml range and reflect dietary intake [69]. However, administration of β-carotene by sc and ip injection produced dose-dependent increases in plasma to μg/ml levels in hamsters during a CB-funded chemoprevention study in the MNU-induced hamster. In an ensuing CB study, subcutaneous injection of β-carotene decreased DEN-induced lung tumorigenesis in the hamster by 50% at two doses (1.5 and 3 mg, 2x/week), but the difference was not statistically significant [70]. However, in the same model, a synergistic response was obtained with the two-agent combination of 1.5 mg injectable β-carotene (0.028 mmol/kg-bw, 2x/week, sc) plus dietary vitamin A (as 4.8 mg retinol/kg diet), and an additive response was seen with 3 mg β-carotene (0.056 mmol/kg-bw, 2x/week, sc) plus 4-HPR (196 mg/kg diet). Interestingly, dietary administration of β-carotene (2,147.4 mg/kg diet or *ca.* 0.48 mmol/kg-bw/day) with vitamin A (9.2 mg retinol/kg diet) also reduced lung tumors [66]. In the latter study, the agent combination demonstrated significantly increased serum β-carotene over that of the ingested carotenoid alone. The distribution pattern of β-carotene may be altered in the presence of vitamin A irrespective of the administration route. Less β-carotene is metabolized and stored in the liver as vitamin A, and so more of the carotenoid remains in circulation and available to other tissues.

Other combinations with injectable β-carotene have shown enhanced inhibition of lung tumorigenesis in the hamster. In CB-funded studies, synergistic combinations with β-carotene in the DEN-induced model included oltipraz and 4-HPR plus oltipraz; additive combinations with β-carotene were DFMO, DFMO plus 4-HPR, and vitamin A plus 4-HPR. Currently, the injectable formulation is on test in the NNK-induced strain A mouse and the MNU-induced hamster lung carcinogenesis models; combinations of injectable β-carotene with fumaric acid and NAC are also being evaluated in the MNU model.

In published studies, only one carotenoid has been shown to inhibit lung carcinogenesis when given as a single agent. In the NQO-initiated/glycerol-promoted mouse, dietary α-carotene inhibited lung tumor development; β-carotene was ineffective [71].

In animal carcinogenesis models relating to the head and neck, published studies have demonstrated efficacy of topical β-carotene in the DMBA-induced hamster buccal pouch with [72] and without benzoyl peroxide promotion [73,74]; dietary β-carotene was also effective against DMN-induced tumors in the same tissue [75] and in DMBA-induced rat salivary glands [76]. Another carotenoid has also had chemopreventive activity in a published study—topical canthaxanthine in the DMBA-induced hamster buccal pouch model [74]. In addition, some work with carotenoids has focused on modulation of intermediate biomarkers in the oral cavity. A significant effort in the CB program is the identification and validation of intermediate biomarkers of cancer and the evaluation of the potential for chemopreventive agents to modulate these markers. Such studies in animals contribute to the development of more efficient screens for identifying new chemopreventive agents, as well as identifying biomarkers to be used as surrogate endpoints in clinical trials [reviewed in 77]. Formation of premalignant papillomas, considered to be histological biomarkers, was decreased by β-carotene in the DMBA-induced hamster buccal pouch [78]. In the CB preclinical testing program, β-carotene modulation of a putative premalignant lesion—GGT-positive altered foci—is being evaluated in the same model.

β-Carotene has also been effective against tumorigenesis in a related tissue, skin. In published studies, dietary supplementation inhibited development of both squamous cell carcinomas and premalignant papillomas induced in mouse skin by UV, DMBA with croton oil promotion, UV with TPA promotion, and B(*a*)P with UV promotion [79–85]. Topical β-carotene administration was also

effective in the DMBA-initiated/TPA-promoted mouse model [86]. Other carotenoids have been positive in published studies on mouse skin tumorigenesis. Crocetin (ip) inhibited DMBAinitiated/croton oil-promoted squamous cell carcinoma and papilloma development, but was less effective than β-carotene [87,88]. Canthaxanthine (po) reduced tumors in the B(a)P/UV-induced model [79,80], and both canthaxanthine (po) and phytoene (ip) were effective in the UV-induced model [80,81,89]. Finally, it was reported in an abstract that topical lutein suppressed tumor formation when applied during TPA promotion in the DMBA-initiated mouse skin model [90].

Although epidemiological evidence for an inverse association between breast cancer risk and carotenoids is equivocal, preclinical studies have shown efficacy. In CB-funded assays, β-carotene (10 mg/kg-bw/day, or 0.02 mmol/kg-bw/day, sc) was effective against MNU-induced rat mammary carcinogenesis *in vivo*, and both β-carotene (10 μM) and lycopene inhibited formation of hyperplastic alveolar nodules (HAN) in DMBA-exposed mouse mammary organ culture *in vitro*. Published studies demonstrated reduction of tumorigenesis by β-carotene *in vivo* in the DMBA-induced rat [91,92] and the virus-associated mouse mammary models [93]; canthaxanthine (po) inhibited mammary adenocarcinomas in the MNU-induced rat model [94]. Currently, injectable β-carotene is on test by the CB in the DMBA- and MNU-induced rat models, including combinations with fumaric acid and NAC in the latter assay.

Mixed results have been obtained with carotenoids in preclinical models of gastrointestinal tumorigenesis. In published studies, oral β-carotene inhibited colon adenomas and adenocarcinomas in the DMH-induced mouse [95], and topical administration to cheek pouch mucosa decreased forestomach papillomas in the DMBA-induced hamster [78]. In contrast, dietary canthaxanthine was reported to increase tumorigenicity in the DMH-exposed rat colon [96]. Because of the efficacy of dietary β-carotene against premalignant lesions (adenomas, papillomas), 15-*cis*-β-carotene, β-carotene type III, β-carotene type I, and injectable β-carotene are currently on test in the aberrant crypt focus assay in the AOM-induced rat colon model.

Three carotenoids have been effective against tumorigenesis in the liver. In published studies, oral β-carotene decreased hepatocellular carcinomas in aflatoxin B_1-exposed rats [97]. In addition, the carotenoid was effective against premalignant lesions, including nodules (induced by 2-AAF, or DEN/2-AAF/partial hepatectomy/2-AAF) and acidophilic foci (induced by DEN/2-AAF/partial hepatectomy/2-AAF) [98,99]. Crocetin (ig) also inhibited precancerous changes induced by aflatoxin B_1 in rat liver, which correlated with enhanced hepatic glutathione levels and glutathione-*S*-transferase and glutathione peroxidase activities, as well as decreased formation of DNA adducts [100,101]. Finally, spontaneous hepatomas in C_3H/Be mice were decreased by orally administered α-carotene, but not β-carotene [71].

In the bladder, astaxanthin decreased the incidence of transitional cell carcinomas in the OH-BBN-exposed mouse [102]. Finally, oral β-carotene inhibited formation of premalignant lesions in the pancreas. In the BOP- and *l*-azaserine-induced hamster, tubular ductal complexes were decreased [103,104].

PRECLINICAL SAFETY STUDIES

Safety β-carotene has very low toxicity in published rodent and dog studies, even after chronic administration. No toxicity was observed in dogs with daily oral administration of up to 8 g β-carotene/kg-bw (14.9 mmol/kg-bw/day) for 6 days [105–107]. Dietary supplementation up to 250 mg/kg-bw/day (0.46 mmol/kg-bw/day) for 2 years resulted in orange foci on the surface and minimal lipid deposition within the periportal zone of the liver [2]. In mice and rats, no effects related to doses up to 1,000 mg/kg-bw/day (1.8 mmol/kg-bw/day) for 2 years on blood chemistry, urinalysis and organ weights were found. Treated mice had vacuolated liver cells, and rats displayed decreased weight gain at all dose levels. However, β-carotene administered at a level of 1 g/kg diet (0.09 mmol/kg-bw/day) to four successive generations of rats had no adverse effects on growth, food consumption, hematological parameters, or reproduction [107]. Histological examination revealed only vitamin A deposits in the Kupfer cells of the liver. Hamsters displayed only slight increases in hepatic vitamin A after 1,130,000 IU/kg diet (*ca.* 0.28 mmol/kg-bw/day) for 106 days. No evidence of tumorigenicity was found in any chronic studies, and β-carotene was not genotoxic in either *in vitro* or *in vivo* assays [reviewed in 108].

No evidence of embryotoxicity or teratogenicity was observed in rats (to 1,000 mg/kg-bw/day, or

1.86 mmol/kg-bw/day) and rabbits (to 400 mg/kg-bw/day, or 0.74 mmol/kg-bw/day) following oral adminstration of β-carotene to females during organogenesis [2]. No maternal mortality or other signs of toxicity were noted in any dosage group in the rat, but a slight reduction in body weight gain was observed at the highest dose level in rabbits. In contrast, an increased number of resorptions, slightly decreased fetal survival, and severely retarded sternebral ossification were observed in rats at what appeared to be 1,800 mg/kg-bw/day [109], although the actual dose was unclear [108]. The effect of β-carotene on the growth and reproductive performance was also studied in male and female rats administered 100, 250, 500 and 1,000 mg/kg/day in their diet throughout three generations [2]. Mating performance, pregnancy rate, mean duration of gestation, litter size, pup mortality, and mean pup weights were unaffected by β-carotene exposure.

ADME Carotenoids are normal constituents of the blood and tissues of humans, birds, fish, and cattle, but not most rodents [37]. The intestinal mucosa of hamsters and other rodents efficiently converts β-carotene to vitamin A; thus, very little ingested β-carotene is absorbed intact unless it is administered in sufficient quantity to saturate the enzyme system [110]. In hamsters, most of the absorbed β-carotene is stored in the liver [68]. In rats, the uptake and depletion rates are unique; there is a dose-response relationship between the amount ingested and the concentration in each tissue [36,111]. Following administration of 2% β-carotene in the diet (*ca.* 1.83 mmol/kg-bw/day), plasma levels reached steady-state after 3 days, and tissue concentrations were highest in liver, ovary and adrenal glands; tissue half-lives varied from 3 days in plasma to 16 days in lung and adrenal glands [111]. Accumulation of β-carotene as retinol in the liver may explain the toxicity and microscopic hepatic changes in rodents.

Absorption and distribution of [^{14}C]-labelled lycopene and canthaxanthine have been investigated in a published study in rats and monkeys. Following a single oral dose, t_{max} for radioactivity occurred between 4–8 hrs in rats and 8–48 hrs in monkeys [112]. Absorption was more variable in the monkey. Both species accumulated the carotenoids in all organs examined, but the liver contained the largest amounts. In monkeys, the clearance of lycopene from the plasma was much slower than canthaxanthine. No evidence of metabolism of either carotenoid was found in rats or monkeys.

CLINICAL SAFETY: PHASE I STUDIES

The general pharmacokinetics of carotenoids will be outlined from published information, followed by specific data on each carotenoid when available. The CB has funded a Phase I trial, which will be discussed. The carotenoids generally have low toxicity; however, diseases that mimic the major adverse effect—yellowing of the skin—should be ruled out.

Drug Effect Measurement Carotenoid status can be assessed by either plasma or tissue levels or by a functional approach. Plasma levels should not be determined spectrophotometrically, because many components also absorb maximally in the 450 nm region [113]. HPLC techniques are much more accurate and use very small samples, plus specific carotenoids can be assessed [114]. Quality control can be carried out using lyophilized sera from the NIST. Possible functional measurements include determination of carotenoid metabolites, inhibition of oxidative modification of LDL, and measurement of cell/tissue damage or protection of DNA [113].

Safety The carotenoids as a group have very low toxicity. The primary adverse effect from high β-carotene intake is yellowing of the skin (carotenodermia) from presence of the lipochrome in keratin and subcutaneous fat [reviewed in 36]. It appears to be observed at total plasma carotenoid levels >4 μg/ml, or ingestion of 12–30 mg β-carotene qd for 25–42 days [115]. The importance of carotenodermia is in ruling out other causative factors, such as diabetes mellitus, jaundice, and hypothyroidism [116]. In NCI-funded pilot studies preceding CARET (see **CLINICAL EFFICACY: PHASE II/III STUDIES**), the only adverse effect seen after two years of 30 mg β-carotene qd was mild yellow skin color; no changes in hepatic enzymes or plasma lipoproteins were observed [117–119]. A small proportion (2/61) of Phase I trial subjects displayed yellowing of the skin after ingesting 45 mg qd for three weeks [120]. A single subject at the same dose level reported blurred vision, occasional double vision, and exacerbation of presbyopia after 18 days; all symptoms subsided within two weeks after discontinuing treatment. This response was unrelated to plasma vitamin A levels, which remained unchanged in all subjects. Vitamin A intoxication has not been noted in NCI-funded or published studies up to doses of 300 mg β-carotene qd, probably due to slow conversion from the carotenoid and close regulation of vitamin

levels [36,121]. Finally, some erythropoietic porphyria patients report gastrointestinal disturbances [64].

Limited toxicity data exist on other carotenoids. Canthaxanthine is an approved food color additive, but it has been used without regulatory approval for attaining a skin color similar to suntan. Excessive intake produces discolored plasma and feces, which probably have no physiological significance; however, crystalline deposits occur in the retina of all subjects ingesting 60 g. A change in retinal function after long-term treatment has been observed in a few individuals with these deposits [122]. In contrast, retinas of individuals with erythropoietic protoporphyria given high doses of β-carotene for up to 10 years showed no crystalline deposits [discussed in 1].

Lycopene is a more intensely colored pigment than β-carotene. High lycopene intake may mimic carotenodermia; however, a deeper orange is usually observed [116]. The importance of this condition is in ruling out other causes, as discussed above.

The dominant carotenoids in human and monkey retina are lutein and zeaxanthin [123,124]. Lutein is dispersed throughout the the entire retina, whereas zeaxanthin is concentrated primarily in the macular pigment. These carotenoids may minimize chromatic aberration as well as provide the central retina with protection against damaging photochemical reactions [125]. Macular carotenoids appear to be selectively accumulated compared with serum, and varied in amount up to four-fold between individuals [124]. It is unclear what potential toxic or protective effects, if any, ingestion of high levels of lutein or zeaxanthin would have in the eye.

ADME Following oral administration in food or as pure compounds, carotenoids are solubilized by the bile acids present in the intestine [reviewed in 37]. They may be absorbed through the plasma membrane of mucosal cells, incorporated into chylomicrons, transported in the lymphatic system, and emptied into the blood. However, a significant portion of β-carotene is cleaved by 15,15'-β-carotenoid dioxygenase in the mucosal cells to two retinal molecules. Retinal is either oxidized to retinoic acid or reduced to form retinol (0.6 μg β-carotene=1 IU vitamin A) [1,36,126,127]. A recent study of two individuals given 1 mg [^{13}C]-β-carotene (>95% label) found that approximately 64% of the absorbed dose entered the plasma as retinyl esters, 21% as retinol, and 14% as β-carotene [128]. It should be noted, however, that the rate of absorption and extent of conversion to vitamin A varies widely between individuals, and may be affected by dietary fat and nutritional status [37]. Both carotenoids and vitamin A are transported in the blood in association with lipoproteins, primarily the low-density fraction (LDL); no specific binding proteins have been identified in humans. Serum carotenoid levels from dietary intake are 1–3 μg/ml [1,129,130]; however, supplements of 180 mg qd (*ca.* 5.0 mmol/kg-bw qd) for 10 months produce levels reaching 6.4–13.6 μg/ml. Serum contains about 10 carotenoids, of which the most prominent are β-carotene (15–30%, or 0.2–0.4 μg/ml), lycopene, lutein, zeaxanthin, β-cryptoxanthin and α-carotene [131]. Carotenoids also accumulate in tissue, including fat, liver, and the epidermal and dermal layers of skin [113]. Excretion occurs slowly by the intestinal route; urinary excretion has not been reported. Approximately 25–75% of carotenoids consumed are not absorbed, and are found unchanged in the feces.

Both published and CB-funded work suggest that only a small fraction of a single oral β-carotene dose (*ca.* 10–30%) is absorbed from the intestine, but it is highly variable between individuals. A limited CB-funded Phase I trial investigated the pharmacokinetics of β-carotene after single doses up to 150 mg [132]. The range of plasma C_{max} was not given; however, values shown for two of the subjects at the highest dose varied almost two-fold (1.31, 0.7 μg/ml). Values for t_{max} ranged from 6 to 24 hrs. The investigators suggested that two populations exist—poor absorbers and good absorbers. Published studies have also shown large inter-individual variability in absorption of oral β-carotene supplements [*e.g.*, 133–135], with one detailed study reporting a coefficient of variation of 48.9% in initial serum levels after single doses of 15–180 mg [136]. Serum levels began to rise at 2–3 hrs postadministration, with t_{max} at 5 hrs if β-carotene was taken with a liquid meal; in fasting subjects, t_{max} was delayed by two hrs. The range of C_{max} after a dose of 15 mg was 0.09–0.49 μg/ml. Serum concentrations remained significantly elevated for 24 hr after all doses, and were unaffected by food intake. The AUC_{0-8} increased linearly with dose and correlated positively with serum triglycerides.

Several published multidose pharmacokinetics studies of 15–60 mg qd indicate that steady-state serum levels are reached over a range of 5–20 days [reviewed in 63]. The CB-funded Phase I study of 15 and 45 mg β-carotene qd (Solatene®) had com-

parable results after 8 weeks (n=61) [120].

The effect of extended β-carotene intake on serum levels has been evaluated in several NCI-funded chemoprevention studies. In the CARET pilot study on cigarette smokers, median serum β-carotene increased from 0.17 to 2.1 μg/ml after doses of 30 mg β-carotene qd for four months [119]. In a published pilot study, a plateau in serum β-carotene at four months (1.8 μg/ml) was maintained throughout the remaining 6 months of treatment with 15 mg qd [137]. After one year of 50 mg β-carotene qd in a Phase III chemoprevention trial in the skin (Dr. E.R. Greenberg, Dartmouth College), the median plasma level increased from 0.18 to 1.7 μg/ml, but the range was –0.17 to +8.6 μg/ml [138]. The variable most closely related to large increases in plasma β-carotene was higher baseline levels, suggesting more efficient intestinal absorption in those individuals.

A published study investigated the effect of daily divided doses of 51 mg, 102 mg and 300 mg on absorption of β-carotene [63,121]. Three times greater absorption was evident in the steady-state plasma values (1.14 *vs* 3.42 μg/ml) after 9 days of 17 mg tid with meals instead of 51 mg qd. As the dose increased to 34 and 102 mg tid, steady-state plasma levels increased (4.1 and 7.1 μg/ml, respectively), but not at the same rate as the amount administered. The time to steady state remained at 9–10 days for all treatment regimens.

The type of diet ingested appeared to alter β-carotene pharmacokinetics. In the CB-funded Phase I trial, a high-fat diet for 5-days resulted in increased absorption of the provitamin compared to low fat, and the plasma concentrations remained high during the remaining 16 days of treatment, although meals were self-selected. The group given a low-fat diet for 5 days also had significantly increased plasma levels over baseline, but not until days 13–15 of treatment. A published study demonstrated no change in plasma levels (0.33 μg/ml) for 200 hrs with a single dose of 51 mg taken after a 12-hour fast and followed by an additional 6 hours of no fat [63]. However, when the same dose was administered with 200 g dietary fat, serum β-carotene peaked at four-fold baseline at 40 hours posttreatment.

β-Carotene is stored primarily in human fat (80–85%), but is found in most tissues including the epidermal and dermal layers of the skin [reviewed in 36,37]. When reported as concentration per g wet tissue, the carotenoid always occurs at higher levels in adrenal glands, liver, and testes than in kidney, ovary, serum, and fat. However, the concentrations within a single tissue varied substantially between individuals, reaching a 50-fold range in the liver [139,140].

Elimination of carotenoids is slow. Following single doses of 45 mg and 90 mg β-carotene, plasma $t_{½}$ could only be estimated at 4–5 days and 5–6 days, respectively, due to the large range in individual values [132].

When β-carotene is discontinued, plasma concentrations decline slowly, although large interindividual variation was again noted. Return of plasma β-carotene to baseline took 10–40 days after discontinuance of 8 weeks of 30–45 mg qd in the CB-funded Phase I trial [120]. When 17–102 mg tid (51–306 mg daily) was discontinued, serum β-carotene showed biphasic exponential decay with a $t_{½}$ of 10 days [63]. The early phase was the result of continued release of the provitamin from the small intestine into the blood stream.

Only limited information exists on the pharmacokinetics of carotenoids other than β-carotene. When lycopene is administered as heat-processed tomato juice containing 50–120 mg/L, serum t_{max}= 24–48 hrs and $t_{½}$=2–3 days [141]. As with β-carotene, large interindividual differences in absorption of the carotenoid existed, with increases in serum lycopene over baseline ranging from 0.04–0.19 μg/ml after the same dose. Also, the uptake of lycopene increased with dose, but not linearly. Although absorption was similar to β-carotene, distribution appeared to differ between the two carotenoids. Of the organs assayed, lycopene predominated in the testes, whereas β-carotene was the major carotenoid in other tissues such as liver and spleen [140,141]. In the adrenal glands and pancreas, the major carotenoid varied between lycopene and β-carotene.

Lutein is also one of the five predominating carotenoids in plasma and tissue. Interindividual variability is high due to nonspecific absorption of free lutein following metabolism of the dietary esterified form [142,143]. Oral administration of 10 mg lutein qd for 21 days produced a 4–5-fold increase in plasma levels within a week of treatment [142].

CLINICAL EFFICACY: PHASE II/III STUDIES

NCI has been involved in nine Phase III chemoprevention trials of β-carotene alone or in combination. Of these, the results of two collaborative trials in the esophagus (Linxian, China) and in tis-

sues affected by smoking (Finland) have been published. The largest trial in progress is CARET, which involves seven investigators and six centers (see Table I). The remaining Phase III trials are evaluating prevention of cancer of the colon, breast, head/neck, lung, skin, and multiple sites. In addition, the six-center CARET is evaluating prevention of lung cancer in smokers and in asbestos-exposed workers. Four NCI-funded Phase II trials are in progress investigating the modulation of premalignant lesions and other intermediate biomarkers in the cervix (2), colon, and oral cavity. A completed Phase II trial evaluated a maintenance dose of β-carotene after retinoid-induced response in oral leukoplakia. Published Phase II trials have investigated modulation of histological biomarkers in colon and cervix; a Phase III trial involved colon polyps. In the following discussion, each tissue will be discussed separately, including both NCI and other published trials.

Because of the large number of epidemiological studies showing a consistent inverse association between lung cancer risk and serum/dietary β-carotene, five trials have involved this tissue. A completed Phase III trial—the ATBC trial—was a joint project of NCI (Dr. D. Albanes) and the National Public Health Institute of Finland (Drs. O.P. Heinonen, J.K. Huttenen) [144]. The ATBC trial evaluated the effect of 20 mg β-carotene qd (as water-soluble beadlets) alone and in combination with 50 mg *d,l*-α-tocopherol acetate qd in Finnish male smokers aged 50–69 years. Surprisingly, the lung cancer incidence was 18% higher ($p=0.01$) after 5–8 years of β-carotene supplementation compared with placebo. Total mortality also increased significantly (8%), primarily from lung cancer and ischemic heart disease. No evidence of an interaction between the two agents was found. Interestingly, the incidence of lung cancer in the placebo group was highest in those at the lowest quartile of serum β-carotene, just as in many epidemiological studies. This suggests that stratifying the β-carotene-treatment arm by serum β-carotene might show a dose-response, since some individuals are less efficient absorbers of carotenoids. Another consideration is that several epidemiological studies have shown cigarette smokers to have lower blood carotenoid levels than nonsmokers [*e.g.*, 145–147]. The dose of β-carotene used in this study may not have been high enough to increase blood levels appropriately in this population. However, median serum β-carotene did increase from 0.18 μg/ml to 3.0 μg/ml after 3 years, which is greater than that reported for CARET subjects after 4 months of 30 mg β-carotene qd [144].

CARET, an NCI-funded Phase III trial in progress (Dr. G.S. Omenn, University of Washington), was preceded by two placebo-controlled pilot studies initiated in 1985 [reviewed in 148]. The pilot studies demonstrated tolerability and treatment compliance in two high-risk groups: 1,029 smokers receiving 30 mg β-carotene qd, 25,000 IU vitamin A qd, or both 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 and 25,000 IU vitamin A (as retinyl palmitate) for 1.5–3.3 years [117–119,149]. 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 of 30 mg β-carotene plus 25,000 IU vitamin A (as retinyl palmitate). Accrual by seven investigators at six sites (see Table I) into CARET began in 1989, with a target population of 4,277 workers with occupational exposure to asbestos and history of smoking and 13,629 heavy smokers with a 20 pack-year history [150]. As of April, 1993, accrual was complete at five sites with a total of 4,000 asbestos-exposed males and 11,105 smokers (40% female); the accrual phase should finish in Spring, 1994 [148]. Treatment will be for a mean of 6 years, follow-up concluding in April, 1998, and interim endpoint analyses in Fall, 1994 and 1996 [148–151]. The primary trial endpoint is lung cancer incidence, with incidences of mesothelioma, other cancers, coronary heart disease, and overall mortality as secondary endpoints. It will be interesting to compare the efficacy of this treatment regimen with the lack of β-carotene effect in the Finnish ATBC trial discussed above.

A third NCI-funded Phase III trial in the lung is in progress (Dr. J.W. McLarty, University of Texas, Tyler Lung Cancer Chemoprevention Program). Men occupationally exposed to asbestos are randomized to receive 50 mg β-carotene or 25,000 IU vitamin A qod [152]. The study is examining bronchial epithelial changes monitored by sputum cytology as the primary measure of response. Duration of treatment was to be three years; however, a three-year treatment extension was requested and granted. Trial results should be available this year.

In the final NCI-funded lung study, Kuller and colleagues [153] conducted a pilot study at the University of Pittsburgh among male and female ciga-

rette smokers. Approximately 400 subjects over the age of 55 were randomized and received either 15–30 mg β-carotene daily or a placebo [154]. A Phase II trial has been funded comparing quantitative DNA analysis of sputum epithelial cells with standard sputum cytology as a marker of premalignant abnormalities [152]. A related published trial demonstrated a 27% decrease in micronucleated sputum cells in heavy smokers given 20 mg β-carotene qd for 14 weeks [155].

Consistent inverse epidemiological evidence also shows that dietary intake of fruits and/or vegetables is associated with lower risk of oral and nasopharyngeal cancers, and patients with these cancers have low serum β-carotene levels [*e.g.*, 21,156]. In addition, β-carotene and some of the retinoids have been effective in inhibiting formation of precancerous lesions in the DMBA-induced hamster cheek pouch model [150,157,158]. Although the head and neck area shares etiology with lung cancer, it can be monitored noninvasively; thus, four trials have been initiated by NCI, primarily in the oral cavity. Most of the Phase II trials to be discussed involve regression or inhibition of the clinical lesion, oral leukoplakia, and modulation of other intermediate biomarkers.

A Phase III trial is in progress which is funded by the Cooperative Community Oncology Program at NCI, and is being carried out by the Southwest Oncology Group (Dr. H.S. Garewal, Department of Veterans Affairs Medical Center, Tucson, Arizona) [150]. Preliminary results from a pilot trial indicated that a response rate of 71% was observed in evaluable oral leukoplakia patients after 3–6 months of 30 mg β-carotene qd [158–160]. Changes in lesion size and color observed clinically were confirmed histologically. The Phase III trial was initiated with a vanguard phase to determine adequacy of accrual over 18 months, since the target population is 1,300–1,750 patients with previously treated Stage I/II squamous cell cancer of the oral cavity, pharynx, larynx or paranasal sinuses [150]. The endpoints in the trial will be prevention of a second primary or recurrence of the treated cancer by the same dose taken for 5 years.

An NCI-funded Phase II trial (Drs. H.S. Garewal and D.S. Alberts, University of Arizona) also began as a result of the pilot study discussed above [150,158]. Patients with clinically and histologically proven oral leukoplakia/erythroplakia receive a moderately high dose—60 mg β-carotene qd for 6 months. At the end of the treatment period, responding patients (n=80) are randomized to continue β-carotene or placebo for an additional year to determine the efficacy of maintenance therapy in preventing recurrence. Preliminary results found 56% of patients responding after the 6-month treatment period (n=39), with a 22-fold increase in β-carotene levels in exfoliated mucosal cells [161,162]. A recent published study on oral mucosa punch biopsies also demonstrated significant increases in tissue β-carotene, which correlated with serum levels above 2.4 μg/ml, after 6 months of supplementation with 30 mg β-carotene qd [163]. This suggests that correlation of remission/regression with exfoliated cell β-carotene levels at the end of the trial may lead to more defined treatment regimens. Other endpoints in the trial include immune response, and modulation of intermediate biomarkers such as cytologic abnormalities and micronucleus fre-quency [150,158].

A completed Phase II trial (Dr. S. Lippman, University of Texas, M.D. Anderson Cancer Center) compared β-carotene with low-dose 13-*cis*-retinoic acid as maintenance therapy [164]. Patients with clinically evident oral leukoplakia received effective but toxic treatment with 13-*cis*-retinoic acid (1.5 mg/kg-bw qd) for three months during the induction phase. Those with responding (55%) or stable lesions (35%) were stratified histologically and randomized to a daily maintenance dose of either 0.5 mg 13-*cis*-retinoic acid/kg-bw or 30 mg β-carotene qd for an additional 9 months. 13-*cis*-Retinoic acid maintenance produced further response in 92% of patients; however, 42% were improved or stabilized with β-carotene. It should be noted that half of those on β-carotene received only half of the correct dose. The authors suggest that the difference in response may result from selection of those responding specifically to retinoids during the induction phase.

Published studies also suggest that β-carotene should be effective in regressing oral leukoplakia. In one study, 44% of existing lesions in 18 patients responded to a dose of 90 mg β-carotene qd [reviewed in 158]. In a second publication, the same proportion responded to a daily dose of 30 mg β-carotene [165].

Several NCI-funded and published trials are evaluating combinations of other agents with β-carotene to modulate oral leukoplakia and other intermediate biomarkers. A Program Project trial (Dr. S. Lippman) is comparing 50 mg β-carotene qd plus 25,000 IU vitamin A qd for 3 years with 0.5 mg 13-*cis*-retinoic acid qd for 1 year and then

0.25 mg qd for 2 years [150,166]. The endpoints are prevention of oral leukoplakia, modulation of other intermediate biomarkers, and toxicity. In published trials, a combination of β-carotene plus vitamins E and C produced 60% regression of oral leukoplakia [167]. A Phase II trial (Dr. O. Kucuk, Cancer Research Center of Hawaii) is evaluating time to recurrence, genetic intermediate biomarkers, and toxicity in patients surgically treated for Stage I/II squamous cell cancer of the head/neck and receiving a daily combination of 45 mg β-carotene qd plus 1,200 mg vitamin E qd for 1 year [150]. Finally, a three-agent combination of β-carotene, vitamin A, and vitamin E significantly decreased the risk of developing oral leukoplakia (OR=0.62) in Uzbekistan, an area of high oral cancer incidence [168].

The NCI (Dr. P. Taylor, Cancer Prevention Research Program) has collaborated with the Cancer Institute of the Chinese Academy of Medical Sciences to evaluate the effect of β-carotene 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 [169,170]. Participants (n=29,584) from the general population were treated daily with 15 mg β-carotene plus 30 mg vitamin E (α-tocopherol) and 50 mg selenium (as high selenium yeast) or placebo for 5¼ years. Endoscopy performed on a sample of the subjects found a 42% reduction in risk for esophageal cancer in the supplement group; however, this was not significantly different from placebo [170]. However, significantly lower (p=0.03) total mortality occurred with treatment, which was mainly due to lower total cancer rates [169].

In a series of small, published intervention studies, β-carotene alone and in combination with vitamin A 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 [171–174]. 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.

The NCI sponsored a clinical trial employing β-carotene against non-melanoma skin cancer. The multicenter Phase III Skin Cancer Prevention Study (Dr. E.R. Greenberg, Dartmouth College) began enrollment in 1983 at four sites in California, Minnesota, and New Hampshire [175]. The primary aim was to determine whether 50 mg β-carotene qd increased the time to occurrence of a second primary basal or squamous cell carcinoma in 1,805 patients with a similar, recently resected skin carcinoma. Although serum β-carotene levels increased 8-fold, no increase in skin cancer latency was observed after treatment for 4–5 years when compared with the placebo arm [176].

Three studies specifically addressing colon premalignancy and malignancy include an NCI-funded Phase III and a Phase II trial, and an independent Norwegian trial. The NCI trials are evaluating the effect of β-carotene on colon polyps. A recently completed Phase III trial (Dr. E.R. Greenberg) investigated antioxidant-mediated prevention of new colonic polyps, which are well-accepted precursors of adenocarcinomas [177]. Patients (n=751) with a previously resected polyp (FAP excluded) were randomized to four treatment groups: 25 mg β-carotene qd; 400 mg vitamin E plus 1 g vitamin C and 25 mg β-carotene qd; 400 mg vitamin E plus 1 g vitamin C qd; or placebo. After 4 years, no treatment effects on new polyp incidence, multiplicity, or size were observed, although serum β-carotene increased 2.7-fold (0.21 to 0.57 μg/ml).

The NCI-funded Phase II trial (Dr. S. Mobarhan, Loyola University Medical Center) is investigating other intermediate biomarkers in patients at high risk for colon cancer. The effect of a daily 30 mg β-carotene supplement on modulation of proliferative biomarkers is being evaluated in normal subjects and in patients with a previously resected colon cancer or polyp [150]. After 3 months, those treated patients showing a reduction in ODC activity will be followed for an additional 3 months without carotenoid treatment. Those who do not show this response will receive 180 mg β-carotene qd for three additional months. A published intermediate biomarker study has evaluated the effect of 9 mg β-carotene qd on polyp proliferation when measured as BrdU immunohistochemical labelling in biopsy specimens [178]. After one month, cell proliferation was significantly reduced only at the base of the crypts, but the size of the proliferative compartment was unaffected.

The independent double-blind, placebo-controlled colon trial in Norway (Norwegian Cancer Society) of a daily combination of 15 mg β-carotene, 1,600 mg Ca^{+2} (NOS), 101 μg selenium (NOS),

75 mg vitamin E, and 150 mg vitamin C for 3 years began in July 1989 [179]. Over 18 months, a total of 116 patients with colon polyps were accrued and allocated to separate groups based on the size of the largest polyp (<5 mm, 5–9 mm, >9 mm); each group was then randomized to a placebo or treatment arm. The endpoints are modulation of histological biomarkers (*e.g.*, changes in polyp diameter, number of polyps reaching 5 or 10 mm, new polyp incidence) and colon cancer incidence.

Some epidemiological studies have associated increased risk for premalignant lesions and cancer in the cervix with low serum levels and/or dietary intake of β-carotene [reviewed in 180]. Interestingly, preformed vitamin A did not show the same relationship. Several clinical studies have investigated the efficacy of β-carotene in prevention or regression of premalignant cervical lesions. Two NCI-funded Phase II trials are in progress and one study in the Netherlands has been completed.

One NCI-funded Phase II trial (Dr. S.L. Romney, Albert Einstein College of Medicine) is determining the regression of CIN I or II after 9 months of treatment with 30 mg β-carotene qd versus placebo. The response will be correlated with plasma and tissue levels [150,181]. In a preliminary report, β-carotene levels in exfoliated cervico-vaginal cells and plasma of women with CIN or cervical cancer were significantly less (2- to 4-fold) than controls [181]. After β-carotene supplementation for 6 weeks, both β-carotene levels (cellular and plasma) were markedly higher in almost 80% of a small subset of patients. A second Phase II trial (Dr. M.L. Berman, University of California, Irvine) is currently evaluating the effect of the same β-carotene dose on CIN II or III regression, correlating the histological effect with HPV genotype [150].

A published randomized, placebo-controlled trial in the Netherlands (n=287) examined the effect of a daily 10 mg β-carotene supplement on regression and progression rates of CIN after three months [182]. The treatment did not regress CIN compared with placebo, and the number of patients showing progression was too small to analyze. The authors suggest that the initial biopsy may have increased regression in both groups or that the intervention was too short.

Two NCI-funded Phase III studies are evaluating the efficacy of β-carotene on cancers at multiple sites. Since 1982, Dr. C.H. Hennekens (Harvard Medical School) and colleagues have been conducting the Physicians' Health Study on the effect of 50 mg β-carotene qd on cancer incidence and cardiovascular disease in 22,071 male U.S. physicians between the ages of 40 and 84 [183–186]. The results were initially expected in 1986, but the study was extended to 1994 due to an insufficient number of cancer cases to determine any meaningful difference among the arms [185].

The second Phase III trial (Dr. J.E. Buring, Brigham and Woman's Hospital) is similar in design to the Physicians' Health Study, and has been funded by the NCI and the National Heart, Lung and Blood Institute. It involves the effect of 50 mg β-carotene, 600 IU vitamin E, or 100 mg aspirin qod, as well as all possible two- and three-agent combinations, on the incidence of epithelial cancers, especially lung, colon and breast, in female health professionals ≥45 years of age [150]. A second endpoint is alteration of risk for vascular events, such as nonfatal myocardial infarction, nonfatal stroke, and total cardiovascular mortality.

PHARMACODYNAMICS

In the NCI-funded CARET pilot study on cigarette smokers, median serum β-carotene increased from 0.17 to 2.1 μg/ml after four months of 30 mg β-carotene qd [119]. This greatly exceeds the serum levels associated with decreased lung cancer incidence in epidemiological studies (≤0.4 ug/ml). If this is a causal association, CARET and other lung trials may demonstrate efficacy. However, the relationship is not simple, as shown by the increase in lung cancer incidence in male Finnish smokers receiving 20 mg β-carotene daily for 6 years; concomitantly, the plasma β-carotene levels increased to 3.0 ug/ml.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

Carotenoid status can be assessed by either plasma or tissue levels or by a functional approach. Plasma levels should not be determined spectrophotometrically, because many components also absorb maximally in the 450 nm region [113]. HPLC techniques are much more accurate and use very small samples, plus specific carotenoids can be assessed [114]. Twenty-one carotenoids can now be identified in human serum; 14 are of dietary origin and 7 may be metabolites [114,187,188].

Quality control can be carried out using lyophilized sera from NIST.

Possible functional measurements include determination of carotenoid metabolites, inhibition of oxidative modificaion of LDL, and measurement of cell/tissue damage or protection of DNA [113].

Safety Issues

The potential for embryotoxicity or teratogenicity of β-carotene has not been adequately studied in humans. Some fetotoxicity was observed in rats at doses 300–400 times the maximum daily dose (300 mg) recommended by the FDA for treatment of erythropoietic protoporphyria [189]. Although no adverse effects were observed in rat fetuses following doses 75-fold higher than the maximum human dose, β-carotene should be used in pregnancy only if the benefit justifies the risk.

Pharmacodynamics Issues

The doses used in clinical trials seem to be sufficient to exceed the serum levels associated with decreased cancer risk in epidemiological studies. However, large interindividual variability in intestinal absorption has been observed, both before and after β-carotene supplementation. This suggests that tissue concentrations in organs of interest would also vary widely. Correlation of efficacy or intermediate biomarker modulation in some Phase II trials with tissue levels may reveal a biological dose-response. It may be necessary to adjust the ingested β-carotene dose in individual subjects depending on their absorption capacity.

Regulatory Issues

At this time, additional toxicity studies would not appear to be required for Phase II and III studies of doses up to the highest dosage in documented clinical trials (100 mg qd) [190]. In spite of the recent results of the Finnish smokers' trial, the weight of animal and human evidence does not support carcinogenicity of β-carotene, especially in the lung. In addition, rodent studies may not be appropriate for modeling human toxicity due to the difference in absorption and metabolism. No published chronic studies in rodents found evidence of tumorigenicity; however, comparable studies have not been undertaken in a nonrodent species to define the carcinogenic risk.

Supply and Formulation Issues

β-Carotene is available from Hoffman-La Roche as 30 mg Solatene® capsules. No supply problems are anticipated for this formulation or placebo. For strengths other than 30 mg, it would be necessary to formulate a drug product and a placebo. Patents have been issued for astaxanthin (15 patents), canthaxanthine (10), α-carotene (15), 15-*cis*-β-carotene (70), crocetin (6), cryptoxanthin (6), lutein (8), lycopene (12), phytoene (4), and zeaxanthin (4). Most of these relate to production methods. It is anticipated that formulations of these carotenoids would require manufacture.

Intermediate Biomarker Issues

Intermediate biomarkers must be expressed differently in high-risk tissue compared with normal tissue, closely linked to the neoplastic process, modulated by the chemopreventive agent, and correlated to altered risk. Sampling for the intermediate biomarker should be as non-invasive as possible, and the assay for the biomarker should be reproducible, sensitive and specific. Special care and consideration must be given to the standardization and quality control of all analytical methods proposed to satisfy regulatory review and to support drug development. Such work must precede sample acquisition.

Clinical Studies Issues

Further trials evaluating efficacy in additional tissues or with different intermediate biomarkers may be considered, as appropriate. Results from the Finnish study in smokers suggests that more investigation into chemopreventive mechanisms or pharmacokinetics may need to be done. For example, attainment of specific plasma or tissue levels may require different β-carotene doses depending on the absorption capacity of the individual.

Other carotenoids which appear promising in preclinical trials may also be considered for future clinical development. It is possible that the inverse association between cancer risk and intake of fruits and vegetables may be due, at least in part, to the protective effect of these carotenoids.

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Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase I (Safety, ADME)					
NO1-CN-45167 Phase I Bioavailability of β-Carotene in Humans (Dr. Nikolay V. Dimitrov, Michigan State University Investigator IND	----	Healthy males and females 61 subjects	Single dose: 15–150 mg β-carotene Multidose: 15, 45 mg β-carotene qd for 8 weeks	Pharmacokinetics and bioavailability; safety	Completed studies found significant interindividual variation in C_{max} and t_{max} Single dose: C_{max} highly variable, t_{max}=6–24 hrs. Multidose: Plasma steady-state reached after 7 days of 15 mg qd and after 2–4 days of 45 mg qd Published single-dose report: [132]; published multidose report: [120]
ZO1-CN-00101-11-CPSB (Intramural Project) Human Studies of Diet and Nutrition (Dr. Philip R. Taylor, NCI, DCPC, CPSB) Investigator IND	----	Normal, healthy subjects	Oral; single and chronic doses of capsules compared to dietary intake	ADME, safety	

Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers)					
RO1-CA-50364 Chemoprevention of Cervical Cancer with β-Carotene (Dr. Michael L. Berman, Univ. of California, Irvine) 9/91–8/96 IND 36,641	Cervix	Women with cervical dysplasia (CIN II, III) 120 women (60/arm)	30 mg β-carotene qd for 6 months, then CIN II non-responders continue for 2 years 5 years	Progression or regression of CIN; correlation with HPV genotype	Study in progress Published report: [150]
UO1-CA-53818 Phase II β-Carotene Clinical Trial Monitoring Cervical Dysplasia (Dr. Seymour L. Romney, Albert Einstein College of Medicine) 2/91–1/95 IND 36,641	Cervix	Women with cervical dysplasia (CIN II) 138 women	30 mg β-carotene qd for 9 months	Efficacy: Regression of CIN; correlation of response with plasma and tissue β-carotene	Study in progress. Preliminary report shows increase in plasma and exfoliated cervical cell β-carotene concentrations after 6 weeks. Baseline levels were lowest in women with CIN or cancer compared with normal subjects Published report: [181]
UO1-CA-53799 Effects of β-Carotene on Colonic Cell Proliferation (Dr. Sohrab Mobarhan, Loyola University of Chicago) 3/91–1/95 IND 36,731	Colon	Previous colon cancer or polyp patients (Duke's A1, B1) 100 patients (40 polyps, 40 cancer, 20 normal)	30 mg β-carotene qd for 3 months; nonresponders based on ODC receive 180 mg qd for 3 more months	Efficacy: Proliferation biomarkers modulation (ODC, BrdU, PCNA, polyamines, Ki-67)	Study in progress Report: [150]

Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) *(continued)*					
PO1-CA-27502 Chemoprevention of Skin Cancer Program Project IIb–1: Chemoprevention Trials in Oral Pre-cancers (Dr. H.S. Garewal, Project PI: Dr. David S. Alberts, University of Arizona) 7/80–11/97 IND 21,576	Oral Cavity	Oral leukplakia, erythro-plakia patients 80 randomized patients	60 mg β-carotene qd for 6 months, then responders randomized to 60 mg β-carotene or placebo qd for 1 year 4 years	Efficacy: Oral leukoplakia modulation, micronuclei, immune response Toxicity	Study in progress; accrual completed 3/93. Preliminary report shows 60% responding after 6 months Published reports: [150,158,161]
UO1-CA-46303 Chemoprevention of Human Premalignant Oral Lesions (Dr. Scott Lippman, University of Texas, M.D. Anderson Cancer Center) 9/87-2/91 IND 21,576	Oral Cavity	Oral leukoplakia patients 70 patients	Induction: 1.5 mg 13-*cis*-retinoic acid/kg-bw qd for 3 months Maintenance: 0.5 mg 13-*cis*-retinoic acid/kg-bw qd or 30 mg β-carotene/kg-bw qd for 9 months 5 years	Efficacy: Remission and recurrence of leukoplakia or erthyroplakia, histo-pathology, micronuclei	Completed study found 42% stabilized or responded with β-carotene maintenance; however, 92% were maintained with 13-*cis*-retinoic acid Published report: [164]

Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase III (Efficacy, intermediate biomarkers)					
UO1-CA-37287 Nutritional Prevention of Polyps in the Large Bowel (Dr. E. Robert Greenberg, Dartmouth College) 9/84–8/93 IND 22,447	Colon	Previous resected colon polyp (FAP excluded) 751 patients (β-carotene arm: 184)	25 mg β-carotene qd; or 400 mg vitamin E + 1 g vitamin C qd; or all three antioxidants for 4 years 9 years	Efficacy: New polyp incidence after 1 or 4 years of treatment, polyps/patient, size of largest polyp	Study completed None of the treatments had an effect on new polyp incidence, multiplicity, or size Published report: [177]
ZO1-CN-00112-10-CPSB Study of Effect of Nutritional Intervention on Esophageal Cancer in Linxian, People's Republic of China (Philip Taylor, NCI, DCPC, W. Blot, NCI, DCE, and Linxian Nutrition Intervention Study Group)	Esophagus	Commune residents 40–69 years of age in high-risk area 29,584 residents	15 mg β-carotene + 30 mg vitamin E + 15 μg selenium (as yeast) qd for 5¼ years	Efficacy: Esophageal cancer incidence, mortality	Study completed. Reduction in mortality significant, but 42% reduction in esophageal cancer risk was not. Published report: [170]
SWOG-9043 Phase III Randomized Placebo-controlled Trial of β-Carotene for Chemoprevention of Second Primaries in Patients with Stage I/II Head and Neck Cancer	Head/ Neck	Head/neck cancer patients Feasibility: 195 patients Phase III: 1,300–1,750 patients	Feasibility: 30 mg β-carotene qd for 18 months Phase III: 30 mg β-carotene qd for 5 years	Feasibility/accrual, toxicity, efficacy as disease-free and overall survival time, and latency to recurrence or second primary	Study in progress Report: [150]

Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	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 exposed to asbestos 755 subjects	50 mg β-carotene qod or 25,000 IU vitamin A qod for 6 years 10 yrs	Efficacy: Sputum cytology	Study extended from 3 to 6 years of treatment; results expected 1994 Report: [152]
CARET PO1-CA-34847 Phase III Chemoprevention of Lung Cancer with Retinoids and β-Carotene (Dr. Gary Goodman, Univ. of Washington) 7/83–6/93 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Cigarette smokers Pilot study: 1,029 smokers Total CARET: 13,629 smokers	Pilot: 30 mg β-carotene qd and/or 25,000 IU vitamin A qd for 3 years Phase III: Same doses of β-carotene + vit. A for 5 years 10 yrs	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Pilot completed. Phase III in progress; accrual at 11,105 (40% female) Published report: [148]

Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	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) 7/83–6/93 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Men with asbestosis or occupationally exposed to asbestos Pilot: 816 men Total CARET: 4,277 men	Pilot study: 15 mg β-carotene qd + 25,000 IU vitamin A qd Phase III: 30 mg β-carotene qd + 25,000 IU vitamin A qd 10 years	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Pilot completed; Phase III in progress with accrual at 4,000 Published report: [148]
CARET RO1-CA-47989 Phase III Chemoprevention Trial of β-Carotene and Retinol (Dr. John R. Balmes, Dr. James E. Cone–previous PI, University of California, San Francisco) 9/88–6/93 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Heavy smokers Total CARET: 13,629 smokers	30 mg β-carotene qd + 25,000 IU vitamin A qd for 5 years 5 years	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Study in progress Published report: [148]

Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	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) 9/88–6/93 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Men exposed to asbestos or with asbestosis Total CARET: 4,277	30 mg β-carotene qd + 25,000 IU vitamin A qd for 5 years	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Study in progress Published report: [148]
CARET RO1-CA-48200 β-Carotene and Retinol Chemoprevention Trial in Asbestos (Dr. Mark R. Cullen, Yale University New Haven Hospital) 9/88–6/93 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Asbestos-exposed workers Total CARET: 4,277	30 mg β-carotene qd + 25,000 IU vitamin A qd for 5 years	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Study in progress Published report: [148]

Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	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) 8/88–6/93 Investigator IND (Dr. Gilbert S. Omenn)	Lung	Cigarette smokers, asbestos-exposed (men only), asbestosis Total CARET: 13,629 smokers, 4,277 asbestos	30 mg β-carotene qd + 25,000 IU vitamin A qd for 5 years	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Study in progress Published report: [148]
CARET RO1-CA-52596 Phase III Chemoprevention Efficacy Trial of β-Carotene and Retinol (Dr. Frank L. Meyskens, Univ. of Calif, Irvine Cancer Center) 8/91–6/95 Investigator IND	Lung	Heavy smokers Total CARET: 13,629 smokers	30 mg β-carotene qd + 25,000 IU vitamin A qd for 5 years 7 years	Efficacy: Lung cancer incidence, mesothelioma incidence, other cancers, coronary heart disease, overall mortality	Study in progress Published report: [148]
UO1-CA-32934 Phase III Chemoprevention Trial of β-Carotene in Skin Cancer (Dr. E. Robert Greenberg, Dartmouth College) 9/82–8/93 IND 22,446	Skin	Previous basal or squamous cell skin cancer 1,805 patients	50 mg β-carotene qd	Efficacy: Latency to recurrence of skin cancer	Study completed. No increase in latency observed Published Report: [176]

Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase III (Efficacy, intermediate biomarkers) ***(continued)***					
RO1-CA-47988 Randomized, Placebo-controlled Study of β-Carotene, Vitamin E and Aspirin for Chemoprevention of Cancer and Cardiovascular Disease in Women (Dr. Julie E. Buring, Brigham and Women's Hospital) 2/91–1/96 Investigator IND	Epithelial cell (primarily breast, lung, colon)	Female health professionals, age ≥45 years 62,600 women	50 mg β-carotene qod, or 100 mg aspirin qod or 600 IU vitamin E qod for 4 years or in all possible combinations of 2 or 3 agents (total 8 arms) for 4 years	Incidence of epithelial cancer, cardiovascular, events and mortality	Study in progress; 41,600 patients randomized Report: [150]
NO1-CN-45165 Phase III Vitamin E and β-Carotene in Cancer Prevention Study (Finland) (Dr. Olli P. Heinonen, National Public Health Institute, Helsinki, Finland and Dr. Demetrius Albanes, The α-Tocopherol, β-Carotene Cancer Prevention Study Group) 1985–1993 Investigator IND	Multiple	Males smokers, 50–69 years of age in southwest Finland 29,133 smokers (*ca.* 7,282/arm)	20 mg β-carotene qd and/or 50 mg vitamin E acetate qd for 5–8 yrs (median: 6.1 years) 8 years	Efficacy: Cancer incidence and mortality	Study completed. Increase in lung cancer incidence (18%) and overall mortality in β-carotene arm Published report: [144]

Table I. Clinical Trials of β-Carotene Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase III (Efficacy, intermediate biomarkers) *(continued)*					
RO1-CA-40360 A Randomized Trial of Aspirin and β-Carotene in U.S. MDs (Dr. Charles H. Hennekens, Harvard Medical School) 12/81–11/96 Investigator IND	Multiple	Healthy male physicians 22,071 physicians (5,515+/arm)	50 mg β-carotene qod and/or 325 mg aspirin qod for 5 years 14 years	Efficacy: Epithelial cancer, cardiovascular disease and mortality Safety	β-Carotene component extended from 1986 to 1994 because insufficient cancer cases to determine treatment effect. Published Report: [186]

BETA-CAROTENE DEVELOPMENT STATUS