NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

VITAMIN E

DRUG IDENTIFICATION

CAS Registry No.: 59-02-9

CAS Name (9CI): (2R-(2R*(4R*,8R*)))-3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-ol

Synonyms:
α-Tocopherol
d-α-Tocopherol
RRR-α-Tocopherol

Related Compounds:

d,l-α-Tocopherol (CAS No. 10191-41-0)
3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-ol (9CI)
(+)-α-Tocopherol
all-*rac*-α-Tocopherol

d-α-Tocopherol Acetate (CAS No. 58-95-7)
(2R-(2R*(4R*,8R*)))-3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-ol Acetate (9CI)
(+)-α-Tocopherol Acetate
d-α-Tocopheryl Acetate
Tocopheryl Acetate
Vitamin E Acetate

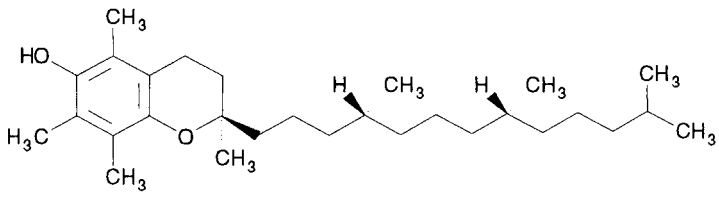
d,l-α-Tocopheryl Acetate (CAS No. 52225-20-4)
(2R*(4R*,8R*))-3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-ol Acetate (9CI)
all-*rac*-α-Tocopheryl Acetate

d-α-Tocopherol Succinate (CAS No. 4345-03-3)
(2R-(2R*(4R*, 8R*)))-Mono(3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-yl)butanedioate (9CI)
d-α-Tocopheryl Succinate
Vitamin E Succinate

d,l-α-Tocopheryl Succinate (CAS No. 17407-37-3)
(3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-yl)butanedioate (9CI)

d-α-Tocopherol Succinate PEG 1,000 (CAS No. 9002-96-4)
(2R-(2R*(4R*,8R*)))-α-(4-((3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-yl)oxy)-1,4-dioxobutyl)-ω-hydroxy-poly(oxy-1,2-ethanediyl (9CI)
Tocofersolano
Vitamin E TPGS

Structure:



d-α-Tocopherol

EXECUTIVE SUMMARY

Vitamin E is widely distributed in foods, with the highest concentrations in the oils of wheat germ, corn, sunflower seeds, rapeseeds and soybeans, as well as in alfalfa and lettuce [1,2]. The lipid-soluble vitamin is generally considered an essential nutrient for higher animals, including humans [3], due to its function as the major antioxidant present in all cell membranes [4]. Eight related naturally occurring substances have vitamin E activity, including α-, β-, δ, and γ-tocopherols (saturated side chains) and tocotrienols (unsaturated side chains) [5,6]. The most active form biologically of these is naturally occurring *d*-α-tocopherol (1.49 IU/mg); in comparison, synthetic, racemic *d,l*-α-tocopherol has lower biopotency (1.1 IU/mg). For drug use, vitamin E is available as *d*- or *d,l*-α-tocopherol, *d*- or *d,l*-α-tocopherol succinate (1.8 and 0.89 IU/mg, respectively), and *d*- or *d,l*-α-tocopherol acetate (1.36 and 1 IU/mg, respectively) [6,7]. In the rest of this document, the enantiomer or conjugate under discussion will be specified, if known (in the literature, the form often is not designated).

Vitamin E reacts with a variety of oxy-radicals and singlet oxygen [8,9]; thus, one of its main antioxidant functions is to prevent the peroxidation of polyunsaturated membrane lipids. Free radicals have been implicated in the etiology of a number of diseases including emphysema and cardiovascular and inflammatory diseases [8], as well as the initiation, promotion and progression of cancer [10–13]. Although animal studies have demonstrated inhibition of carcinogenesis with vitamin E supplementation, antioxidant activity is not a sufficient explanatory mechanism in some models (*e.g.*, DMBA-induced rat mammary glands) [14]. Other properties that may contribute to the observed chemopreventive activity include membrane-related effects (physicochemical stabilization of membranes [15], protection of cytochrome P-450 metabolism [*e.g.*, 16]); stimulation of the immune system [17, 18]; induction of differentiation [19] and gap junctional intercellular communication [20]; and inhibition of proliferation [*e.g.*, 19], arachidonic acid metabolism [reviewed in 21], nitrosamine formation [*e.g.*, 22], and ornithine decarboxylase (ODC) activity [*e.g.*, 23,24]. Although epidemiological studies have shown lower serum vitamin E levels in people who subsequently developed cancer (especially pancreas, stomach, bladder, lung, and other smoking-related tissues) compared with controls [*e.g.*, 25–36], controversy exists as to whether pharmacological doses of vitamin E can be of chemopreventive or therapeutic value. The effect may be limited to increasing deficient or marginally normal serum vitamin levels to normal range. However, vitamin E may potentiate the efficacy of more toxic agents (*e.g.*, selenium, 4-HPR) at lower doses. Several epidemiological studies found that higher risk for cancer development at several sites correlated with the combination of low serum vitamin E with low selenium status [37–39]. Based on these observations and the fact that vitamin E itself has low toxicity, the CB is developing *d*-α-tocopherol (also

as the acetate or succinate) alone and in combination as cancer chemopreventive drugs.

As single agents, *d*-α-tocopherol or the acetate inhibited tumorigenesis in preclinical oral cavity (hamster buccal pouch and tongue), skin (mouse), mammary gland (rat), liver (rat), colon (mouse) and small intestine (rat) models. Other enantiomers decreased tumor formation in the pancreas (hamster), esophagus (mouse), lung (rat), and ear duct (rat). In combination studies, the three-agent combination of vitamin E acetate with 4-HPR and sodium selenite was most effective in the trachea/lung (hamster) carcinogenesis model; *d*-α-tocopherol acetate alone at the same dose was ineffective. The same three-agent combination and the two-agent combination of *d*-α-tocopherol acetate plus selenite also inhibited mammary gland carcinogenesis (rat).

Two recently completed international Phase III trials were funded in part by NCI. *d,l*-α-Tocopherol acetate decreased the incidence of prostate and colorectal cancer in Finnish male smokers. In the second trial, the combination of vitamin E, selenium and β-carotene reduced the overall mortality rate in Linxian, China; a reduced risk for esophageal cancer was a contributing factor, although it was not statistically significant. An ongoing NCI-funded Phase III trial in collaboration with the National Heart, Lung and Blood Institute is assessing the effect of vitamin E on epithelial cancer incidence in female health professionals. One NCI-funded Phase II trial on prevention of oral cavity and skin cancer by vitamin E is in progress. Finally, two additional completed trials assessed the effect of the vitamin on intermediate biomarkers. A Phase II trial demonstrated primarily clinical modulation of dysplastic oral leukoplakia, and a Phase III study found no effect on the prevention of colonic polyps.

Vitamin E is considered to have low toxicity. The most serious side effect is antagonism of vitamin K activity, which has been demonstrated in both preclinical and clinical studies. Although this requires high doses in both rats and humans (>800 IU), it could be a potentially serious complication in combination with anticoagulant therapy or vitamin K malabsorption syndrome. Potential trial subjects with these conditions or a low platelet count should be excluded.

The pharmacokinetics of vitamin E appear to be similar in rats and humans. As with other fat-soluble vitamins, maximal absorption requires the presence of bile, incorporation into micelles, uptake by the lymph, and transportation to the circulation. In blood, most tocopherol is associated with low-density lipoproteins. It is stored in several organs, especially the adipose tissue, liver and muscle. Vitamin E is metabolized only to a small extent, except for oxidation-reduction. The major route of elimination is in the bile.

The United States Pharmacopeia (USP) monograph [40] defined vitamin E for drug use as *d*- or *d,l*-α-tocopherol, *d*- or *d,l*-α-tocopherol succinate, and *d*- or *d,l*-α-tocopherol acetate [6,7]. *d*- and *d,l*-α-Tocopherol and α-tocopherol acetate are also recognized dietary supplements, and *d*- and *d,l*-α-tocopherol have GRAS status as food additives. Thus, no supply problems are anticipated in development of *d*-α-tocopherol (as the acetate or succinate) as a cancer chemopreventive drug. Water-soluble *d*-α-tocopherol succinate PEG 1,000 will not be developed due to poor absorption in normal individuals; this form also was not efficacious in preclinical cancer models.

Because of the reduction in prostate cancer incidence in the Finnish smokers study, the CB plans to fund a Phase II trial of *d*-α-tocopherol (as the acetate or succinate) in a prostate cohort in 1995. Although the protocol has not been designed, modulation of histological and other biomarkers will be considered due to the preclinical study data. A second Phase II trial is planned in a breast cohort because of both efficacy in preclinical models of mammary carcinogenesis and inhibition of hormone-dependent cancer in the Finnish trial. Future trials may also investigate the combination of vitamin E with selenium in breast or lung cohorts.

PRECLINICAL EFFICACY STUDIES

Published *in vivo* studies have demonstrated the chemopreventive efficacy of *d*-α-tocopherol against hamster buccal pouch and tongue, mouse colon and skin, and rat mammary gland and liver tumorigenesis; the acetate inhibited rat mammary gland, colon and small intestine, and mouse skin tumorigenesis. Closely related compounds (α-tocopherol (NOS), *d,l*-α-tocopherol, α-tocopherol acetate, *d,l*-α-tocopherol acetate) inhibited hamster pancreas and buccal pouch; mouse skin and esophagus; and rat lung, mammary gland and ear duct tumorigenesis. The results, however, tend to be inconsistent, suggesting the presence of modifying factors (*e.g.*, fat, selenium status). In CB-funded studies, the three-agent combination of *d*-α-tocopherol acetate with 4-HPR and sodium selenite

had additive effects against MNU-induced tracheal carcinogenesis in the hamster, although the vitamin alone was ineffective even at higher doses. Other chemopreventive combinations with *d*-α-tocopherol acetate include sodium selenite in the DEN-induced hamster lung and DMBA-induced rat mammary gland models, and the three-agent combination with 4-HPR and sodium selenite in the same models.

Since the CB has concentrated on agent combinations in preclinical testing, much of the evidence for the efficacy of vitamin E alone has come from published reports. *d*-α-Tocopherol inhibited hamster buccal pouch and tongue (DMBA), rat mammary (daunomycin) and liver (AFB_1), and mouse colon (DMH) carcinogenesis [41–47]. The racemic mixture (*d,l*-α-tocopherol) also inhibited the formation of hamster buccal pouch carcinomas (DMBA) [48–50]; unspecified enantiomers were effective against hamster buccal pouch (DMN, DMBA) and mouse esophagus (MBN) tumors, and rat lung carcinomas (DHPN) [51–54]. In contrast, *d*-α-tocopherol succinate PEG 1,000 was not effective in CB-funded lung/trachea (hamster), mammary gland (rat), or bladder (mouse) studies.

Acetate derivatives of vitamin E have demonstrated chemopreventive activity in several models. *d*-α-Tocopherol acetate was effective against rat mammary gland carcinomas (DMBA, MNU) [55, 56], and mouse skin (DMBA/croton oil) [57] and rat colon (DMH) tumors [58]. α-Tocopherol acetate (NOS) inhibited formation of rat mammary (daunomycin) carcinomas [59]; the racemic acetate was effective against rat ear duct (DMBA) carcinogenesis [60].

Intermediate biomarkers are an important aspect of preclinical efficacy testing. They serve as measures of efficacy in both clinical and preclinical testing, and as potential surrogate endpoints in clinical trials. In published studies, *d*-α-tocopherol decreased the incidence and multiplicity of DMBA-initiated/croton oil-promoted mouse skin papillomas, a histological intermediate biomarker [45,61]. In TPA-induced mouse skin, the vitamin also inhibited induction of ODC activity, a proliferation biomarker [62–64]. α-Tocopherol (NOS) has been extensively tested for prevention of precancerous lesions. The agent decreased the appearance of pancreatic ductular hyperplasia in hamsters (DOPN) [65], papillomas (DMBA/TPA) in mouse skin [23,62,63], atypical tubules in mouse kidney (multiple-carcinogen model) [66] and lung adenomas in rats (DHPN) [54], as well as the putative premalignant lesion, GGT-positive altered foci, in rat liver (DOPN, DEN/partial hepatectomy) [60, 65].

In CB-funded preclinical studies, two combinations with *d*-α-tocopherol acetate have been effective in three models of carcinogenesis: the three-agent combination of the acetate (1 g/kg diet), 4-HPR and selenite; and the two-agent combination of *d*-α-tocopherol acetate and selenite. In the MNU-induced tracheal model in the hamster, the three-agent combination resulted in additive inhibition (30%) of lung carcinomas and papillomas; however, esophageal tumorigenesis was enhanced. Neither *d*-α-tocopherol acetate alone or in two-agent combinations had any substantial chemopreventive effect. In the DEN-induced hamster lung carcinogenesis model, the three-agent combination was most effective. It significantly inhibited lung adenocarcinomas (32%), but not total tumors (carcinomas and papillomas). The combination of *d*-α-tocopherol acetate and selenite significantly decreased both total lung tumors (28%) and papillomas.

In the CB-funded rat mammary gland model, the same two- and three-agent combinations of *d*-α-tocopherol acetate, selenite and 4-HPR decreased the multiplicity and increased the latency of DMBA-induced carcinogenesis. The two-agent combination was not more effective than selenite alone, but decreased weight gain was not observed. A published report has confirmed inhibition of tumor formation in the same model after dietary supplementation with *d*-α-tocopherol (235 mg/kg diet, or *ca.* 0.03 mmol/kg-bw) plus selenite (0.6 mg/kg diet) [67]. Combinations of selenite with *d*-α-tocopherol are being evaluated by the CB because of the complementary actions of the two agents. Vitamin E is the primary membrane antioxidant, while selenium exerts its antioxidant effect as part of the cytosolic enzyme glutathione peroxidase. Thus, protection against both lipid and non-lipid radicals is obtained.

PRECLINICAL SAFETY STUDIES

Safety The CB has funded one-year toxicity studies of water-soluble vitamin E succinate PEG 1,000 at doses of 100, 300 and 1,000 mg/kg-bw/day (ig) in rats and dogs. The in-life phase has been completed, and no abnormalities were reported at necropsy, except for traces of blood in the feces of some dogs. The final report is in preparation.

In published studies, the toxicity of vitamin E in animals is low [reviewed in 7]. Oral LD_{50}s in sev-

eral species were ≥2 g/kg-bw for both *d*-α-tocopherol succinate and *d*-α-tocopherol succinate PEG 1,000 in rats [68,69]. In subchronic studies, *d*-α-tocopherol succinate PEG 1,000 at doses of 0.002, 0.2, and 2% in the diet for 90 days produced no adverse effects on growth, hematology, clinical chemistry, or histology in male and female rats [68]. In two 2-year studies, *d,l*-α-tocopherol acetate was fed to rats at doses up to 2,000 mg/kg-bw/day [70,71]. Growth and survival were unaffected, and inconsistent hepatic changes were observed including alkaline phosphatase and alanine aminotransferase activities. The only carcinogenic effect was a trend to decreased mammary tumors in female rats. Although a few studies have reported enhanced DMH-induced colon tumorigenesis in mice [72–74], these results are in contrast to other studies demonstrating an inhibitory effect [43,56]. Differences in species, dose, carcinogen, form of vitamin E and level of vitamin E in the basal diet could contribute to these disparate results. Finally, it should be noted that *d*-α-tocopherol succinate was nonmutagenic in the Ames/*Salmonella* assay with or without metabolic activation [75].

The most serious toxicity related to vitamin E is antagonism of vitamin K activity. At doses of 2,000 mg α-tocopherol acetate/kg-bw/day for 90 days, longer prothrombin times and decreased hematocrit and hemoglobin were observed in rats; this effect has also been observed in dogs [reviewed in 7]. In one of the two-year rat studies, vitamin K supplementation was necessary to control hemorrhage [71]. This could be a potentially serious complication in humans on anticoagulant therapy or with vitamin K malabsorption syndrome.

Although early reports suggested reproductive problems in rats given high doses of vitamin E, this has not been confirmed in subsequent studies [69]. In one of these, female rats were fed 22.5–2,252 mg α-tocopherol acetate/kg-bw/day during pregnancy and lactation [76]. The pup survival rate, litter size, and pup weight were unaffected. No teratogenic effects were noted, although some eye abnormalities were observed in older offspring from rats fed the highest vitamin dose. A second two-litter reproductive and teratogenic study of *d*-α-tocopherol succinate PEG 1,000 found no adverse effects in rats. Males and females were fed 0.002, 0.02 or 0.2% in the diet for 112 and 175 days, respectively, and their offspring were also fed the compound for 5 weeks after weaning [68]. No differences in reproductive indices, mean gestational period, litter size, sex ratio and mortality of parents or pups were observed. No treatmentrelated morphological changes were found in pups necropsied at 8 weeks of age. Finally, a mouse study of 591 IU *d*-α-tocopherol/animal (*ca.* 13,222 mg/kg-bw/day) on gestation days 7–11 produced only one malformation among 91 fetuses [reviewed in 7].

ADME In rats, absorption varies with bolus and infused administration of vitamin E into the duodenum [77]. For example, 15–20% of a bolus dose of α-tocopherol subsequently appeared in the lymph. In contrast, administration of α-tocopherol acetate as a slow infusion into the duodenum resulted in 65% absorption. Vitamin E is absorbed by passive diffusion from the small intestine to the enterocyte in the rat, but transport through the epithelial cell is not understood. The area of greatest uptake is at the junction between the upper and middle thirds of the small intestine. Reduced absorption has been observed with increasing doses of α-tocopherol.

The vitamin is transported in the blood in association with lipoproteins. In rats, the liver, adipose tissue and skeletal muscle accumulate 90% of α-tocopherol recovered from 10 organs. The adrenal glands have the highest concentration per gram of tissue due to specific binding in this tissue; lung and spleen also have relatively high concentrations. The half-life in rat tissues after chronic feeding of deuterated α-tocopherol acetate ranged from 7.6 days in the lung to 76 days in the spinal cord [reviewed in 78]. As in humans, the major route of excretion is fecal elimination.

CLINICAL SAFETY: PHASE I STUDIES

The CB has completed Phase I trials determining the pharmacokinetics of water-soluble vitamin E succinate PEG 1,000 and lipid-soluble α-tocopherol acetate. The latter appeared to be absorbed much more efficiently. Doses of each derivative up to 1,200 IU qd for 28 days produced no toxicity. Information summarized from published reports confirms that the toxicity of vitamin E (NOS) is very low—up to 2,000 mg daily [reviewed in 7].

Drug Effect Measurement A survey of methods to assess vitamin E status evaluated static measurements (vitamin levels in plasma, erythrocytes, platelets) and functional tests (*e.g.*, peroxidative index, erythrocyte hemolysis or malondialdehyde, breath pentane) [78]. The authors concluded that because of the close association with lipids, plasma α-tocopherol expressed as a ratio to lipid (cholesterol plus triglycerides) was the best

measurement. An alternative is platelet α-tocopherol concentrations, which do not reflect changes in lipid; however, complex procedures are required for platelet isolation.

Safety Relatively few side effects have been reported in double-blind studies of daily vitamin E supplementation as high as 3,200 IU [reviewed in 7]. In healthy female subjects receiving 600 IU *d,l*-α-tocopherol acetate daily (*ca.* 600 mg) for 4 weeks, serum triglycerides were significantly increased [79]. In both males and females, serum thyroid hormone levels (T-3, T-4) were significantly decreased; however, this was not observed in women using oral contraceptives. In another study in adult-onset diabetics given daily 2,000 IU all-*rac*-α-tocopherol acetate supplements (2,000 mg) for 6 weeks, thyroid levels were not altered [80]. Angina pectoris patients receiving 3,200 IU *d*-α-tocopherol succinate daily (1,778 mg) reported a low incidence of intestinal cramps and diarrhea [81]. In a survey of trials and case reports, some symptoms have been inconsistently reported [69]. These include breast symptoms, altered creatinine metabolism, fatigue and/or muscular weakness, depressed leukocyte function, hyperthermia, and thrombophlebitis. However, most of these cases were not observed in double-blinded, controlled trials.

A significant concern after prolonged daily intake of >800 IU vitamin E is bleeding in vitamin K-deficient patients [6,82]. Subjects with decreased blood coagulation capacity secondary to vitamin K malabsorption or anticoagulant therapy should be excluded from clinical trials.

ADME According to published information, absorption of vitamin E from the gastrointestinal (GI) tract is dependent on the ability to digest and absorb fat. As with other fat-soluble vitamins, maximal absorption requires the presence of bile and incorporation of the vitamin into micelles [4,6]. After dietary administration, 25% absorption occurs when measured in the lymph and 35–85% when determined as fecal excretion [1,83]. When given as esters, α-tocopherol is hydrolyzed during absorption. At high daily intake (>400 IU, or >268 mg *d*-α-tocopherol) this process appears to be rate-limiting, since higher plasma levels were obtained with free tocopherol [77]. After lymph uptake, tocopherol is transported to the circulation in chylomicrons, which equilibrate with other plasma proteins. No specific carrier or binding protein has been identified; most tocopherol is associated with low-density lipoproteins [1,77]. The normal range for plasma tocopherol is 6–14 mg/L [6]. Vitamin E is stored in several organs; the highest absolute amounts occur in the adipose tissue, liver and muscle [1,77]. With continued daily dosing, adipose concentration increases, but the content of most tissues increases very slowly or not at all [1, 77,84].

Vitamin E is metabolized only to a small extent, except for oxidation-reduction. In liver, the primary metabolites are glucuronides of tocopheronic acid and its γ-lactone [6]. Quinone metabolites have also been identified. Fecal elimination via the bile is the major route of excretion (some enterohepatic circulation may occur); the rest appears as glucuronide conjugates in the urine [6,77].

The limited results from the Phase I trial of fat-soluble α-tocopherol acetate confirms the published information. After a single 400, 800 or 1,200 IU dose, the acetate was absorbed well, but interindividual variation was evident. C_{max} did not differ significantly between doses; 800 IU approximately doubled the baseline plasma tocopherol level to 20 mg/L. For all doses, t_{max}=12–15 hr and baseline plasma levels were reached by 36–48 hours post-administration. With daily dosing for 28 days, a steady state was maintained. In contrast, the water-soluble succinate derivative was poorly absorbed after 1 or 28 daily doses, and produced minimal changes in plasma tocopherol levels.

With longer administration of vitamin E, serum tocopherol levels appear to be maintained at the higher level. In a published randomized, double-blind trial, plasma levels approximately doubled to 20.3 mg/L after ingestion of 800 IU *d,l*-α-tocopherol (728 mg) for 8–16 weeks [85]. In the recently completed Phase III chemoprevention trial (Dr. E.R. Greenberg, Dartmouth College) of 400 mg *d*-α-tocopherol + 1 g vitamin C qd or 400 mg *d*-α-tocopherol + 1 g vitamin C + 25 mg β-carotene qd, serum levels increased from 14 to 20 mg/L, which was maintained to the end of the four treatment years [86].

CLINICAL EFFICACY: PHASE II/III STUDIES

Two international Phase III trials partially funded by NCI have recently been completed—the combination of vitamin E, selenium and β-carotene in esophageal cancer in China and the effect of *d,l*-α-tocopherol acetate alone and in combination with β-carotene on Finnish male smokers. An ongoing Phase III trial in collaboration with the National Heart, Lung and Blood Institute is assessing the effect of vitamin E on epithelial cancer incidence in female health professionals.

One NCI-funded Phase II trial on prevention of oral cavity and skin cancer by vitamin E is in progress. Finally, two additional completed trials have assessed the effects of the vitamin on intermediate biomarkers, *i.e.*, a Phase II trial with modulation of dysplastic oral leukoplakia as the endpoint, and a Phase III study on prevention of colonic polyps.

The first complete Phase III trial was a collaboration between the Cancer Institute of the Chinese Academy of Medical Sciences and NCI, DCPC (Dr. P. Taylor, CPRP) to determine the effect of a vitamin E (30 mg α-tocopherol qd), selenium (50 μg qd as high selenium yeast) and β-carotene (15 mg qd) combination on esophageal cancer incidence in the population (n=29,584) of Linxian, China [87,88]. This area has the highest mortality rate from esophageal and gastric cardia cancer in the world. After 5.25 years of combination treatment, endoscopy performed on a sample of the subjects found a 42% reduction in risk for esophageal cancer compared with untreated controls; however, this was not statistically significant [87]. In contrast, significantly lower total mortality occurred in the treatment group, which was mainly due to lower cancer rates [87]. It should be noted that this population had subclinical deficiencies of several nutrients including vitamin E.

A second completed Phase III trial of *d,l*-α-tocopherol acetate (50 mg qd) alone and in combination with β-carotene (20 mg qd) was a joint project of NCI, DCPC (Dr. D. Albanes) and the National Public Health Institute of Finland (Dr. O.P. Heinonen) [89]. The cancer incidence in Finnish male smokers aged 50–69 years was determined after a median of 6.1 years on each regimen. In the groups receiving the vitamin, the incidence of lung cancer was unchanged; however, the incidence of prostate and colorectal cancer was reduced 34 and 16%, respectively. A concomitant increase in median serum α-tocopherol levels from 12.4 to 17.3 mg/L was observed. These results are intriguing, since previous observational data suggesting efficacy at these target sites have been equivocal [reviewed in 89,90]. However, the study was designed only to evaluate a treatment effect on lung cancer incidence. No statistics were reported, and the apparent decrease in prostate cancer may have been due to chance in light of the large number of cancer sites analyzed. In addition, although mortality from ischemic heart disease and ischemic stroke decreased in the α-tocopherol group, deaths due to hemorrhagic stroke increased; however, the overall increase in mortality was not significant. The cause may have been the reduction of platelet adhesion by vitamin E [91].

The third Phase III trial (Dr. J.E. Buring, Brigham and Women's Hospital) on prevention of cancer is in progress. This large trial in female health professionals ≥45 years of age, which is similar in design to the Physicians' Health trial, has been funded by the NCI and the National Heart, Lung and Blood Institute [92]. The effect of vitamin E, β-carotene or aspirin on the incidence of epithelial cancers (especially breast, lung, colon) will be assessed. In the vitamin E arm, the endpoints also include effects on cardiovascular events.

Recently, an epidemiological association between vitamin E supplement intake (≈100 IU, or 67–91 mg) and 50% reduction in oral cavity cancer incidence was reported [93]. However, 95% of the subjects also took other multivitamin supplements, which may have had additive effects. The efficacy of vitamin E alone in prevention of oral cavity and skin cancer may be clarified in the NCI Phase II Program Project trial in progress.

Two completed NCI-funded clinical trials have assessed the effects of vitamin E on histological intermediate biomarkers. A Phase II trial under the Cooperative Community Oncology Program (CCOP; Dr. S.E. Benner) investigated modulation of oral leukoplakia and erythroplakia because of the preclinical data on *d,l*-α-tocopherol in the hamster buccal pouch model of carcinogenesis [48,50, 51]. Patients (n=58) were treated with 400 IU *d*-α-tocopherol bid (537 mg daily as the *d*-isomer) for 24 weeks, with clinical and histopathologic response as endpoints. An interim report (n=43) found 46% histologic response and 21% clinical response [94]; however, the clinical response rate increased to 65% if only subjects whose lesion measurements were taken at appropriate times (n=31) were considered [95]. Scrapings of oral mucosa were also taken before and after treatment to evaluate the effect of vitamin E on a putative genetic biomarker, micronucleated cell frequency [96]. Although the frequency of micronucleated cells decreased in both visible lesions and normal mucosa, it did not correlate with the clinical or histological response.

Second, a recently completed Phase III trial (Dr. E.R. Greenberg, Dartmouth College) investigated antioxidant-mediated prevention of new colonic polyps, which are well-accepted precursors of adenocarcinomas [86]. Patients (n=751) with a previously resected polyp (FAP excluded) were randomized to four daily treatment groups: 400 mg vitamin E + 1 g vitamin C; 25 mg β-carotene;

400 mg vitamin E + 1 g vitamin C + 25 mg β-carotene; or placebo. After 4 years, no treatment effects on new polyp incidence, multiplicity, or size were observed, although serum α-tocopherol increased *ca.* 40%. A smaller published study also showed lack of significant effect of 400 mg vitamin E + 400 mg vitamin C daily on colon polyps after two years [97]. In contrast, 70 mg *d,l*-α-tocopherol + 30,000 IU vitamin A + 1,000 mg vitamin C daily for 3–6 months modulated one of two proliferative intermediate biomarkers in patients with colorectal polyps [98]. Abnormal expansion of the proliferative compartment (measured as [^{3}H]-thymidine labeling index in the upper 40% of rectal colon crypts)—one of the earliest changes seen in DMH-induced mouse colon carcinogenesis—was significantly reduced [43]; however, the overall crypt labeling index was unaltered. The reason for the lack of correspondence between the response of the proliferative biomarker and the histological biomarker is unknown, although the Phase III trial may have been too short to demonstrate an effect on polyp recurrence.

PHARMACODYNAMICS

Although efficacy in preclinical models of oral cavity and skin carcinogenesis have generally been consistent, results with vitamin E in other target organs have been equivocal. However, more consistent results have been found in animals suffering from vitamin E deficiency, especially in combination with high-fat diets. It is unclear whether nutritional or pharmacological doses of vitamin E alone inhibit cancer in humans, as demonstrated in the Finnish lung cancer study. The plasma levels of α-tocopherol achieved in this study with 50 mg vitamin E were only about one-third over baseline. Standard supplements of vitamin E contain a few hundred milligrams and one or two capsules daily can double blood levels [99]. Additionally, the Finnish cohort was treated for only six years. Thus the results of this study do not preclude the potential for significant chemopreventive activity of higher doses of vitamin E administered for longer periods of time. Although in this study the combination of vitamin E and β-carotene was also not efficacious, several preclinical studies have demonstrated enhanced chemopreventive activity of vitamin E in combination with other antioxidants, most notably sodium selenite and/or retinoids. In this regard, vitamin E is known to interact with other antioxidants: it complements the antioxidant activity of selenium and is also known to have a sparing effect on β-carotene [99]. Indeed, a combination of low levels of these three agents decreased total mortality in the Linxian, China cohort; this decrease was mainly due to lower cancer rates. Thus, the development of combinations of vitamin E with other antioxidants and or retinoids may be the most effective clinical strategy to pursue.

Another consideration is the interindividual variability in serum vitamin E levels after supplementation. Pharmacokinetic studies have shown that absorption is variable, depending on fat digestion and absorption and the presence of bile. In addition, tissue levels increase very slowly or not at all with daily dosing. Vitamin E may only be effective in populations with subnormal vitamin status, such as smokers or in nutritionally deficient populations, such as the Linxian, China cohort.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

A survey of methods to assess vitamin E status evaluated static measurements (vitamin levels in plasma, erythrocytes, platelets) and functional tests (*e.g.*, peroxidative index, erythrocyte hemolysis or malondialdehyde, breath pentane) [78]. The authors concluded that because of the close association with lipids, plasma α-tocopherol expressed as a ratio to lipid (cholesterol plus triglycerides) was the best static measurement. A potentially useful functional test of vitamin E status is breath pentane excretion, which correlates with *in vivo* lipid peroxidation in rats. In humans, it was shown to decrease from baseline after daily supplementation with 100 IU *d*-α-tocopherol acetate qd (73.5 mg) for 10 days. Although the method is sensitive and non-invasive, it may be of limited use as a routine test. An alternative is plasma total radical-trapping antioxidant potential (TRAP), which measures the capacity of antioxidants to resist *in vitro* peroxidation [100]. Daily supplementation with 1 g *d,l*-α-tocopherol for 28 days significantly increased TRAP values in normal subjects; however, the relative contributions of other antioxidants would need to be determined.

Safety Issues

The recommended daily allowance of vitamin E is 10–20 IU (6.7–13.4 mg as *d*-α-tocopherol) [4]. The only toxicology issue is hemorrhage at high doses

from vitamin K deficiency. Potential trial subjects on anticoagulant therapy, or with a vitamin K malabsorption syndrome or low platelet counts should be excluded.

Pharmacodynamics Issues

The positive clinical data generated to date for vitamin E have been in a nutritionally deficient population in China. The chemopreventive activity of the vitamin in healthy, well-nourished populations has not been established. Possible interactions with dietary fat should also be examined.

Although *d*-α-tocopherol succinate PEG 1,000 is well-absorbed in individuals with cholestatic liver disease or other malabsorption syndromes [101, 102], it was poorly absorbed in the Phase I trial involving normal individuals. Because it also lacked efficacy in preclinical studies, there are no plans for further clinical development of the succinate PEG 1,000 derivative of vitamin E.

Regulatory Issues

No regulatory issues are anticipated in future planned clinical studies, assuming that the forms of vitamin E used are those defined in the USP monograph and that chronically administered daily doses do not exceed the accepted levels for other studies. New trials are planned for 1995 in prostate and breast cohorts.

Supply and Formulation Issues

Many commercial vitamin E products are legally marketed according to USP requirements without identification of the actual form included. The majority of commercially marketed formulations in which the vitamin E component is identified are *d*-α-tocopherol succinate and d-α-tocopherol acetate in 100 to 1,000 IU capsules, *d*-α-tocopherol succinate in 100–1,000 IU tablets, or a water-miscible solution of *d,l*-α-tocopherol acetate at 50 IU/ml [6]. The drug supply for a study with placebo would probably require special formulation.

Intermediate Biomarker Issues

Intermediate biomarkers are an important aspect of preclinical efficacy testing. They serve as measures of efficacy in both clinical and preclinical testing, and as potential surrogate endpoints in clinical trials. In preclinical testing, vitamin E has inhibited formation of histological biomarkers (premalignant lesions) in skin, pancreas, liver, kidney and lung, as well as induction of ODC activity, a proliferation biomarker, in skin. Because of these data, modulation of histological and other biomarkers will be considered as endpoints in the Phase II prostate trial under consideration (see below).

Clinical Studies Issues

Because of the reduction in prostate cancer incidence in the Finnish smokers study, the CB plans to fund a Phase II trial of *d*-α-tocopherol (as the acetate or succinate) in a prostate cohort in 1995. Although the protocol has not been designed, modulation of histological and other biomarkers will be considered due to the preclinical study data. A second Phase II trial is planned in a breast cohort because of both efficacy in preclinical models of mammary carcinogenesis and inhibition of hormone-dependent cancers in the Finnish trial.

Combinations of agents with α-tocopherol are a priority for clinical chemoprevention development. In the recently completed study in Linxian, China, α-tocopherol combined with selenium (as high selenium yeast) and β-carotene decreased esophageal cancer by 42%. Although this was not statistically significant, the doses were very low—30 mg vitamin E, 15 mg β-carotene and 15 mg selenium. This is a nutrient-deficient population; it is unknown if higher doses would be effective in a U.S. cohort. This should be addressed in future Phase II or II trials.

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Table I. Clinical Trials of Vitamin E 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-85104-02 Phase I and Pharmacokinetic Studies of Vitamin E (Dr. Nikolay V. Dimitrov, Michigan State University) 6/91–12/92 IND 39,263 (Acetate) IND 40,722 (Succinate PEG 1,000)	----	Normal subjects 9 subjects	400, 800, and 1,200 IU *d*-α-tocopherol acetate qd for 1 or 28 days 400, 800, and 1,200 IU *d*-α-tocopherol succinate PEG 1,000 qd for 1 or 28 days	Single dose and multidose pharmacokinetics; toxicity	Study completed Single dose: Acetate t_{max}=12– 15 hours for all doses; C_{max} did not differ between doses. Baseline plasma levels reached by 36–48 hours post-administration Succinate poorly absorbed. Multidose: Acetate doubled baseline serum tocopherol; succinate produced only minimal elevations No toxicity observed

Table I. Clinical Trials of Vitamin E 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)					
Planned Study 1995–	Breast		50 mg *d*-α-tocopherol qd		Study not designed
UO1-CA-45809-04 CCOP Study Pilot Study of the Feasibility of Performing a Chemoprevention Trial through Community Clinical Oncology Programs (CCOPs) Using Vitamin E to Treat Oral Leukoplakia (Dr. S.E. Benner, North Western University Cancer Center) Investigator IND	Oral Cavity	Patients with clinical oral leukoplakia or dysplasia 58 patients	400 IU α-tocopherol bid for 24 weeks	Efficacy: clinical and histological response Toxicity; plasma levels Feasibility of chemoprevention trial at 7 CCOP sites	Study near completion Interim report on 43 patients completing treatment found 46% clinical responses and 21% histological responses; however, response did not correlate with the decrease in micronucleated cell frequency Plasma α-tocopherol doubled, and mild toxicity was reported (vertigo, aches, fatigue, fluid retention) Published reports: [94,95]
PO1-CA-27502 Phase II Chemoprevention of Skin Cancer Program Project: Skin, Oral Cavity 7/80–11/97 Investigator IND	Oral Cavity	Oral cavity cancer patients			Study in progress

Table I. Clinical Trials of Vitamin E 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)*					
Planned Study Phase II Chemoprevention of Prostate Cancer 1995–	Prostate		50 mg *d*-α-tocopherol qd		Study not designed
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 patient (FAP excluded) 751 patients	400 mg vitamin E + 1 g vitamin C qd; or 25 mg β-carotene 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: [86]
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) Investigator IND	Esophagus	Commune residents 40–69 years of age in high-risk area 29,584 residents	30 mg vitamin E+ 15 mg β-carotene + 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: [87,88].

Table I. Clinical Trials of Vitamin E 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) Investigator IND	Epithelial cell origin (breast, lung, colon)	Female health professionals, age ≥45 years 62,600 women	600 IU vitamin E qod; or 100 mg aspirin qod; or 30 mg β-carotene qod (and all 8 possible combination) for 4 years	For vitamin E, incidence of total epithelial cancer; vascular events	Study in progress
NO1-CN-45165 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	Male smokers, age 50–69 years 29,133 smokers (*ca.* 7,280/arm)	20 mg *d,l*-α-tocopherol acetate qd; or 50 mg β-carotene mg qd; or both for 5–8 yr (median, 6.1 yr) 8 years	Efficacy: Incidence of lung or other cancers in male smokers	Study completed No effect either alone or in combination on lung cancer incidence. The significance of the 34% decrease in prostate cancer and the 16% decrease in colon cancer is unclear. Published report: [89]

VITAMIN E DEVELOPMENT STATUS