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NCI, DCPC

Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN: CURCUMIN

DRUG IDENTIFICATION

CAS Registry No.: 458-37-7

CAS Name (9CI): (E,E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione

Synonyms:
C.I. 75300
C.I. Natural Yellow 3
Curcuma
Diferuloyl Methane
Turmeric Yellow

Related Compounds:

Curcuma longa, Aqueous Extract
Curcuma longa, Acetone/Methanol Extract
Curcuma longa, Volatile Oil

Bisdemethoxycurcumin (CAS No. 33171-05-0)
1,7-Bis(4-hydroxyphenyl)-1,6-heptanediene-3,5,-dione
Bis(4-hydroxycinnamoyl)methane

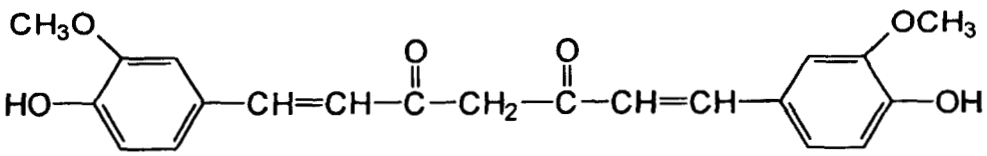
Demethoxycurcumin (CAS Nos. 24939-17-1, 22608-11-3)
1-(4-Hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptanediene-3,5,-dione
4-Hydroxycinnamoyl(feruloyl)methane

Dihydroferulic Acid (CAS No. 1135-23-5)
4-Hydroxy-3-methoxybenzenepropanoic Acid

Tetrahydrocurcumin (CAS No. 36062-04-1)
1,7-Bis(4-hydroxy-3-methoxyphenyl)-3,5-heptanedione
Turmeric (Powdered Rhizome of *Curcuma longa*)
Turmeric Oleoresin (CAS No. 8024-37-1)

Molecular Wt.: 368.4

Structure:



EXECUTIVE SUMMARY

Curcumin is a spice used extensively in curries and mustards as a coloring and flavoring agent. It is the major yellow pigment extracted from turmeric, the powdered rhizome of the perennial herb *Curcuma longa* [1]. Turmeric is also used as a coloring and flavoring agent in foods such as gelatins and puddings, condiments, soups, meats, and pickles [2], and is generally recognized as safe (GRAS) for these uses [3,4] as either the powder (1–5% curcumin) [1] or the oleoresin (organic extract containing 40–85% curcumin) [5]. In contrast, curcumin itself is not on the GRAS list, and has been given a temporary acceptable daily intake (ADI) level of 0.1 mg/kg-bw (0.27 μmol/kg-bw) by the Joint FAO/WHO Expert Committee on Food Additives pending the completion of carcinogenicity and reproductive toxicity studies [6,7].

In India and Southeast Asia, curcumin is used extensively in food and also as a treatment (as turmeric) for inflammation [8–10], skin wounds [10] and tumors [8,11], and other complaints [10]. One uncontrolled trial reported a response (reduced lesion size, pain, exudate, itching) in 65% of oral cavity carcinoma cases [11]. Preclinical studies have also demonstrated tumor prevention in carcinogen-induced colon, duodenum, forestomach, mammary gland, oral cavity (tongue), and skin models. All these biological effects may reflect the primary pharmacological actions of curcumin as both an antioxidant [*e.g.*, 12] and an antiinflammatory [*e.g.*, 13, 14]. As an antioxidant, it scavenges active oxygen species such as hydroxyl radical [9,15–18], superoxide anion [9,19], and singlet oxygen [20]; it also interferes with lipid peroxidation [12,18,21–28], xanthine oxidase activity [29] and nitrite/nitrogen oxide production [30,31]. The antiinflammatory activity of curcumin may result from decreased arachidonic acid release and metabolism via diminished activities of phospholipases A_2 (through phosphatidylcholine) and Cγl (through phosphatidylinositol 4,5-biphosphate),Δ^5-desaturase, cyclooxygenase and lipoxygenase [32–38]. Oxidant stress [39,40] and prostaglandin biosynthesis [41] have been linked to both the initiation and postinitiation stages of carcinogenesis. Consistent with its antioxidant and antiinflammatory activity, curcumin has demonstrated chemopreventive activity during both stages of cancer formation. It has been specifically shown to inhibit mutagenesis [42–45], clastogenesis [46–48] and DNA-carcinogen adduct formation [49–53]; decrease expression of c-*jun* [54,55], c-*fos* [55] and c-*myc* oncogenes [55], possibly through inhibition of protein kinases [35,56]; inhibit ODC activity [1,57,58] and EGFR function [59,60]; modify cytochrome P450 [52,61,62]; and enhance glutathione-*S*-transferase activity [27,62,63] and DNA repair [cited in 64]. Finally, curcumin may have hormonal [10,65] and antiviral effects [66].

The NCI, Chemoprevention Branch already has FDA-approved NSAIDs under clinical development which specifically inhibit the cyclooxygenase enzyme (aspirin, ibuprofen, piroxicam, sulindac) and may be more potent antiinflammatories in animal studies (*e.g.*, 20 mg ibuprofen/kg-bw equivalent to 200 mg curcumin/kg-bw [67]). However, curcumin also affects arachidonic acid mobilization and, as a result, both the cyclooxygenase and lipoxygenase pathways, and displays additional antiinitiation and antipromotional activities. In this regard, its chemopreventive activities are similar to glycyrrhetinic acid, but without the adverse glucocorticoid effects. Due to these factors and its perceived human safety following centuries of use in food and medicine, curcumin is being considered for clinical development by the Chemoprevention Branch.

Curcumin appears to be relatively safe in preclinical studies when administered orally. Subchronic studies (up to 90 days) in rats, dogs and monkeys generally showed limited adverse effects. In Chemoprevention Branch-funded studies of commercial grade curcumin, minor changes in body weights in rats and hematological values in rats and dogs were not considered biologically significant. Consistent with prostaglandin synthesis inhibition, curcumin caused gastric ulcerations in mice, but only at doses double the median effective anti-inflammatory dose (0.15 mmol/kg-bw/day for 6 days). The agent is reported to be noncarcinogenic in rats, but the study details were not available for review. However, turmeric oleoresin containing 79–85% curcumin produced equivocal responses after two-year bioassays in rats and mice. Increased incidences of clitoral gland adenomas in female rats, hepatocellular adenomas in female and male mice, and small intestinal carcinomas in male mice were not dose-related.

Early preclinical pharmacokinetics studies suggested that curcumin was poorly absorbed from the gastrointestinal tract. In later studies with radiolabeled pigment, *ca.* 60% of an oral dose was absorbed,

and appeared to be transported by the bile, metabolized and conjugated, and re-excreted into the gut. Detectable tissue levels were obtained with doses of ≥400 mg in rats (3.6 mmol/kg-bw as pure curcumin).

Because of its long history of food use, curcumin is perceived to be safe for human intake; however, few actual data exist. Small efficacy trials suggest that ingested doses up to ≈2,000 mg/day for 18 weeks lack adverse effect. Since pharmacokinetics information was also unavailable in the literature, a Phase I trial (Dr. Dean Brenner, University of Michigan) of pure curcumin has been funded by the Chemoprevention Branch to define both safety and ADME of single doses escalating from 50 mg in normal, healthy subjects and of multiple doses (3 months) in subjects at high risk for epithelial cancer. Arachidonic acid products and enzyme activities will be evaluated in rectal epithelium as drug effect measurements.

The NCI, Chemoprevention Branch is considering a Phase II trial of curcumin in dysplastic oral leukoplakia patients. Topical administration is a possibility, since doses (0.01–2 mmol) similar to the ADI (0.02 mmol) were effective when applied topically in mouse skin and rat oral cavity tumor models. A Phase II trial in a cohort at high risk for colon cancer may also be considered, depending on results of the Phase I trial.

A Clinical Trial Agreement between the Chemoprevention Branch and Gene Print, Inc. (Bala Cynwyd, PA) has been signed. Gene Print, Inc. is providing a pure curcumin formulation with a defined particle size which will be administered mixed into orange juice in the Phase I trial.

PRECLINICAL EFFICACY STUDIES

Curcumin inhibited tumor formation in mouse skin, forestomach, duodenum and colon and in rat tongue, colon, mammary glands and sebaceous glands. In one- and two-stage mouse skin models, efficacy was demonstrated by topical application during initiation, promotion, and both phases of carcinogenesis. In mouse colon and rat tongue, curcumin had greater effect when offered during activation of procarcinogens (AOM, NQO) than during promotion. In combination studies, curcumin plus 4-HPR appeared to be more effective than either agent alone against rat mammary tumorigenesis. Curcumin has also inhibited intermediate biomarkers in many of these tissues, especially formation of histological biomarkers such as aberrant crypt foci (colon), dysplasia (colon, tongue), papillomas (skin, forestomach), and adenomas (colon, duodenum). Modulation of other types of biomarkers has been demonstrated in the tongue (proliferation), colon (genetic, proliferation) and skin (proliferation). Currently, the Chemoprevention Branch is testing curcumin in a mouse lung model.

The earliest data demonstrating the chemopreventive efficacy and antiinflammatory activities of curcumin are from published studies on mouse skin [14]. Using topical administration, tumor formation was inhibited in both one- (20-methylcholanthrene or DMBA) and two-stage (DMBA-induced/croton oil-promoted or B(*a*)P-induced/TPA-promoted) models [51,57,68–71]. More recently, two alcohol extracts of turmeric (curcumin I and III) containing yellow phenolic compounds (NOS) inhibited DMBA-induced papillomas with and without TPA promotion in mouse skin [62]. Finally, in a similar tissue, dietary curcumin inhibited squamous cell carcinomas in the oral cavity (primarily tongue) of NQO-treated rats [72].

As with other antiinflammatories such as aspirin [*e.g.*, 73,74] and sulindac [*e.g.*, 75–77], curcumin inhibited colon tumorigenesis in several preclinical models [32,78]. In the AOM-exposed rat, a Chemoprevention Branch-funded study of 8 and 16 g curcumin/kg diet (*ca.* 1.1 and 2.2 mmol/kg-bw/day) demonstrated decreased colon adenomas and adenocarcinomas at the highest dose; however, a significant decrease in weight gain (9.6%) was observed in both the curcumin only and the AOM plus curcumin groups compared with the relevant controls [79]. In a study from the same laboratory, an eight-fold lower dose (2,000 ppm, or *ca.* 0.3 mmol/kg- bw/day) was effective without toxicity in the same model [32]. In a second Chemoprevention Branch study in mouse colon, 25 and 50 g/kg diet (*ca.* 8.7 and 17.4 mmol/kg-bw/day) significantly inhibited MAM acetate-induced adenomas and adenocarcinomas. A published study of the AOM-induced mouse colon model produced similar results [78].

In other Chemoprevention Branch studies, curcumin was effective in the MNU-induced rat mammary carcinogenesis model at a dose of 16 g/kg diet (*ca.* 2.2 mmol/kg-bw/day) administered over the entire course of the experiment. Lower doses of curcumin alone and in two-agent combinations with 4-HPR and tamoxifen citrate were also tested, but 120-day old rather than 50-day old rats were used to better model

human carcinogenesis. Tumor incidence was significantly inhibited (70%) at the low dose of 10 g/kg diet (*ca.* 1.4 mmol/kg-bw/day), but not at the high dose (54.5%) of 20 g/kg diet (*ca.* 2.7 mmol/kg-bw/day). Combining high-dose curcumin with 4-HPR (2 mmol/kg diet, or *ca.* 0.1 mmol/kg-bw/day) completely inhibited tumor development; it should be noted, however, that 4-HPR alone was also very effective (85%) and liver weights increased significantly (24%) in the combination group. The combinations with tamoxifen were less effective than tamoxifen alone. Although interpretation of these results is complicated by the low tumor incidence in the carcinogen control groups (23–33%), they do suggest that curcumin and 4-HPR should be further evaluated as a chemopreventive agent combination.

Since curcumin inhibited transformation of DMBA-induced/TPA-promoted mouse mammary organ cultures [80], the Chemoprevention Branch also tested it against DMBA-induced mammary gland tumorigenesis in the rat. Following doses of 10 and 20 g/kg diet (*ca.* 1.4 and 2.7 mmol/kg-bw/day) over the entire experiment, tumor incidence and multiplicity were not affected significantly; the high dose increased tumor latency [79].

Finally, published studies have demonstrated inhibition of forestomach papillomas [48,78] and carcinomas [78] in the B(*a*)P-exposed mouse. An alcohol extract of turmeric (curcumin I) was also effective in this model [62]. For this reason, curcumin is also under evaluation by the Chemoprevention Branch in the B(*a*)P-induced mouse lung assay. In a second model, food-grade curcumin decreased duodenal adenomas in the ENNG-treated mouse [78].

The efficacy of curcumin has been shown to vary with time of treatment in several models, especially with carcinogens requiring metabolic activation (procarcinogens). As an antioxidant and antiinflammatory, it can inhibit cancer formation when given during either initiation [*e.g.*, 51,71] or promotion [*e.g.*, 57,68,70] as illustrated in mouse skin models. For example, formation of DMBA-induced/croton oil-promoted papillomas was inhibited by topical curcumin applied only during initiation; however, treatment during both phases doubled the effect [71]. In other models, such as rat tongue (NQO) [72] and mouse colon (AOM) [78], curcumin was more effective when given during initiation with carcinogens requiring metabolic activation than during promotion. This is consistent with its specific effects on phase I xenobiotic metabolism [*e.g.*, 61, 62]. For example, in a published AOM-induced rat carcinogenesis study [78], 2% food-grade curcumin in the diet during either only initiation or initiation plus postinititiation was equally effective in decreasing tumor multiplicity; the same dose offered during postinitiation only was half as effective. Curcumin also inhibited procarcinogen-induced mutagenicity (DMBA, B(*a*)P) in the Ames *Salmonella* assay [43, 44], clastogenicity (B(*a*)P) in the mouse bone marrow micronucleus assay [47,48], and DNA adduct formation (B(*a*)P) in an *in vitro* assay [49] and in rat liver [50] and mouse skin [51] *in vivo*.

The relative chemopreventive efficacy of the curcuminoids varies with assay system; however, food-grade curcumin, pure curcumin and demethoxycurcumin may be generally equivalent. Food-grade curcumin contains three similar compounds when measured by HPLC—curcumin (69–77%), demethoxycurcumin (17%) and bisdemethoxycurcumin (3–6%) [78]. One published chemoprevention study using the AOM-induced colon model in mice reported that pure curcumin (>98%) was as effective as the commercial grade containing 97% curcuminoids (77% curcumin, 17% demethoxycurcumin, 3% bisdemethoxycurcumin) [78]. A second study in DMBA-initiated/TPA-promoted mouse skin found that topically applied commercial curcumin, pure curcumin and demethoxycurcumin were equivalent in inhibiting tumor incidence and multiplicity; bisdemethoxycurcumin and tetrahydrocurcumin were less active [81]. In contrast, bisdemethoxycurcumin increased the life span (13.3 days) more than pure curcumin (9.7 days) or demethoxycurcumin (11.7 days) of mice bearing Ehrlich ascites tumors; however, demethoxycurcumin reduced tumor volume most effectively [82]. In mechanistic studies, the relative antiinflammatory activities of these compounds measured as inhibition of TPA-induced mouse ear edema were: commercial curcumin, 93%; demethoxycurcumin, 91%; pure curcumin, 85%; bisdemethoxycurcumin, 79%; and tetrahydrocurcumin, 51%. *In vitro* antioxidant effects of the individual curcuminoids were similar, except bisdemethoxycurcumin was 2–3 times more potent in some systems [82,83].

A significant effort in the NCI, Chemoprevention Branch program is to identify and validate intermediate biomarkers of carcinogenesis and to evaluate the potential of chemopreventive agents to modulate

these markers. Such studies in animals contribute to identification of biomarkers to be used as surrogate endpoints for cancer in clinical trials. As discussed above, inhibition of histological biomarkers has been demonstrated in several tissues, such as adenomas in the duodenum and papillomas in the forestomach and skin. In a Chemoprevention Branch study, curcumin (16 g/kg diet, or *ca.* 2.2 mmol/kg-bw) demonstrated activity against a histological biomarker of colon cancer in AOM-treated rats—the formation of aberrant crypt foci. A published report from an NCI grant corroborated this result, and also demonstrated modulation of proliferation biomarkers (ODC activity, protein kinase activity) [35]. In mouse colon exposed to the same carcinogen, dietary curcumin inhibited both a histological (dysplasia) and a proliferation biomarker ([^{3}H]-thymidine incorporation) [84]. In a model of oral carcinogenesis, decreases in both histological (dysplasia) and proliferation biomarkers (polyamine levels, BrdU labelling, nucleolar organizer region (AgNOR) counts and area) were observed in NQO-treated rat tongue [72]. In various mouse models of skin tumorigenesis, curcumin also inhibited proliferation-related biomarkers (ODC activity, [^{3}H]-thymidine incorporation) [1,36,57,81]. Finally, modulation of a genetic biomarker (nuclear aberrations) induced by DMH [85] or B(*a*)P [86] in mouse colon was reported.

PRECLINICAL SAFETY STUDIES

Due to limited preclinical toxicity data on curcumin in the literature, the Chemoprevention Branch undertook acute rat and 90-day rat and dog studies. Eight-week rat, 90-day rat and monkey, and three-generation rat studies have been published; however, details are available only for the eight-week study. National Toxicology Program (NTP)-sponsored two-year carcinogenicity bioassays were performed with turmeric oleoresin instead of curcumin.

Safety: In Chemoprevention Branch-funded toxicity studies, commercial curcumin at single ig doses of 1,380–3,500 mg/kg-bw (3.7–9.5 mmol/kg-bw) produced no adverse effects in rats except for colored feces; thus the LD_{50} is >3,500 mg/kg-bw. In published studies, single ig doses up to 5,000 mg/kg-bw (13.6 mmol/kg-bw) had no effect on clinical signs or relative organ weights in male and female rats [87].

In the 90-day rat study funded by the Chemoprevention Branch, administration of curcumin at 1,140, 1,515, 1,995, 2,630, and 3,500 mg/kg-bw/day ig (3.1–9.5 mmol/kg-bw/day) produced clinical signs of colored feces and yellow fur. In males, decreased reticulocyte counts in all but the 1,515 mg/kg-bw/day group and increased mean corpuscular hemoglobin (MCH) at two doses (1,995 and 2,630 mg/kg-bw/day) were not considered biologically significant. Decreases in body weights (7.7%) in all male treatment groups except for the lowest dose and in high-dose females (5.3%) were minor and not statistically significant. The NOEL in rats appears to be ≥3,500 mg/kg-bw/day.

In dogs, doses of 250, 500, and 1,000 mg curcumin/kg-bw/day (0.7–2.7 mmol/kg-bw/day) were administered in a gelatin capsule formulation. Statistically significant elevations in MCH concentration in females at the two highest doses were not considered to be biologically relevant since overt anemia was not detected. The NOEL for curcumin appears to be >1,000 mg/kg-bw/day (>2.7 mmol/kg-bw/day) in male and female dogs.

Studies of curcumin in the literature have also demonstrated a lack of significant toxicity. In rats receiving 0.1–2.0% curcumin in the diet (*ca.* 0.1–2.7 mmol/kg-bw/day) for eight weeks, no effects on food intake, body weight gain, hematology, serum chemistry, or histology of the gastrointestinal tract, liver, spleen or kidney were observed [88]. In a similar study, curcumin was administered to rats at doses up to 1,000 mg/kg/day po for three months and to monkeys at doses up to 800 mg/kg/day po for three months without any evidence of adverse effects on growth, behavioral, biochemical and histopathological parameters [cited in 8,89].

A single report of gastric ulcerations associated with curcumin was found in the literature [90]. At doses used for antiinflammation in mice (ED_{50}= 50 mg/kg-bw/day ig for 6 days), food-grade curcumin appears to protect the stomach from ulcers through decreased gastric juice or increased mucin secretion; at higher doses (100 mg/kg-bw/day, or 0.3 mmol/kg-bw/day), decreased mucin production resulted in ulcerations. In the NTP carcinogenicity bioassay discussed below, dietary turmeric oleoresin (79–85% curcumin) at 50,000 ppm (*ca.* 5.6 mmol curcumin/kg-bw/day) was also associated with increased incidences of ulcers, inflammation and hyperplasia of the forestomach, cecum and colon of male rats [5].

Studies on rats over three generations reportedly failed to show any carcinogenic effect of curcumin [unpublished data, cited in 87]. These data are un-

available for review; however, two-year NTP bioassays of turmeric oleoresin (79–85% curcumin) have been performed in mice and rats [5]. Although there was no evidence of carcinogenesis in male rats administered 2,000–50,000 ppm (*ca.* 0.3–5.6 mmol curcumin/kg-bw/day), equivocal evidence was obtained in female rats and male and female mice. The incidence of clitoral gland adenomas increased in all female rats treated with the oleoresin; however, carcinomas occurred only in the low-dose group. In male mice receiving the same dietary doses, the hepatocellular adenoma incidence increased at the middle dose (*ca.* 1.4 mmol curcumin/kg-bw/day) and small intestinal carcinomas occurred at the two lower doses (*ca.* 0.6, 1.4 mmol curcumin/kg-bw/day). In female mice, only hepatocellular adenomas increased at the middle oleoresin dose (4.4 mmol curcumin/kg-bw/day). Further studies are needed to ascertain whether these adverse effects were random, or related specifically to curcumin or other components of turmeric oleoresin.

Mixed results were obtained in genotoxicity studies. Curcumin was not mutagenic when tested to the limits of solubility in the Ames *Salmonella* assay with and without metabolic activation [91–94] and in the mouse dominant lethal assay [95]. In contrast, positive results have been obtained in some assays of clastogenicity *in vitro* [96] and *in vivo* [97], depending on the dose and length and route of exposure. Although no effect on mouse bone marrow cells was observed at 0.015% in the diet for 12 weeks (*ca.* 0.05 mmol/kg-bw/day) [95], a higher dose of 500 ppm for 9 months (*ca.* 0.2 mmol/kg-bw/day) increased chromosomal breaks/cell; however, the increase was barely over twice background and incidence of aberrant cells was not significantly altered [97]. Although single injections (ip) of 25–200 mg/kg-bw (0.07–0.5 mmol/kg-bw) to Swiss mice increased sister chromatid exchanges (SCEs) significantly in the same cells, no dose achieved twice the background rate.

No reports of reproductive toxicity related to curcumin were found in the literature. Doses of 600 and 1,600 mg/kg administered to rats and rabbits on gestation days 6–15 produced no effects on implantation, resorption, live and dead embryos, or skeletal or visceral abnormalities [cited in 89]. Long-term studies on rats over three generations failed to show any teratogenic effect or impairment of reproductive capacity by curcumin [unpublished data, cited in 87]. In contrast, extracts of turmeric were reported to decrease testosterone production in male rats and implantation in female rats [reviewed in 10]; however, study details were not available for review.

ADME: In the Chemoprevention Branch-funded subchronic toxicity studies discussed above, curcumin was not detected in plasma following ig administration of up to 3,500 mg/kg-bw/day (≤9.5 mmol/kg-bw/day) to rats and up to 1,000 mg/kg-bw/day (≤2.7 mmol/kg-bw/day) to dogs. These were the maximum possible doses due to limits of solubility in the formulations. The poor gastrointestinal absorption of curcumin suggested by this result was reported in two earlier publications. When administered orally to rats, 75% of a 1 g/kg-bw dose (2.7 mmol/kg-bw) was excreted in the feces, while negligible amounts appeared in the plasma (*ca.* 0.05 μM) and urine [87]. Additional studies have suggested that, depending on dose, some curcumin may be detectable in tissues. Using 0.6 mg tritiated curcumin ig (*ca.* 0.005 mmol/kg-bw), 89.4% and 6.3% were excreted in the feces and urine, respectively, within 72 hr [98]. However, assessment of tissue radiolabel after a 400 mg dose (*ca.* 3.6 mmol/kg-bw) revealed that detectable levels remained in the blood (13%), liver (5.2%), and kidney (0.5%) after 12 days; 59.1% was excreted in the feces, and only one-third of that was unmetabolized curcumin [99]. This study showed that significantly more of the pigment is absorbed (60–66%) over a range of doses (10–400 mg) than suggested by fecal excretion [99,100]. Following ip or iv injection, active transport by the bile and extensive metabolism by the liver were demonstrated [87,98]. The major biliary metabolites were glucuronides of tetra- and hexahydrocurcumin, with dihydroferulic acid as a minor metabolite [98]. Thus, a portion of orally administered curcumin appears to be absorbed and metabolized, transported by the bile, and re-excreted into the gut by enterohepatic circulation [78].

CLINICAL SAFETY: PHASE I STUDIES

As a food additive, curcumin was given a temporary ADI of 0.1 mg/kg-bw (0.27 μmol/kg-bw) by the Joint FAO/WHO Expert Committee on Food Additives pending the completion of preclinical carcinogenicity and reproductive toxicity tests. A Phase I trial (Dr. Dean Brenner, University of Michigan) has been funded by the Chemoprevention Branch to describe single- and multidose pharmacokinetics and safety of a clinical formulation of pure curcumin; the

protocol is being finalized. A few small Phase II clinical trials have been published to assess efficacy in treatment of inflammation, atherosclerosis and HIV. Limited human dose and safety data are available from these trials.

Drug Effect Measurement: Curcumin diminishes synthesis of phospholipase, lipoxygenase, and cyclooxygenase products, which could serve as drug effect measurements in easily accessible tissues or tissues of interest. A published study on the AOM-induced rat demonstrated decreased colon adenoma and adenocarcinoma formation (2,000 ppm, or *ca.* 0.3 mmol/kg-bw/day) with concomitant reductions in products of phospholipases A_2 (phosphatidylcholine, arachidonic acid) and Cγ1 (inositol 1,4,5-triphosphate, diacylglycerol, arachidonic acid), cyclooxygenase (PGE_2, $PGF_{2\alpha}$) and lipoxygenase (5(*S*)-, 8(*S*)-, 12(*S*)-, 15(*S*)-HETE) in both normal colon mucosa and tumor tissue [32]. The Chemoprevention Branch-sponsored Phase I trial will evaluate cyclooxygenase and lipoxygenase activities and PGE2 and 5(*S*)-HETE levels in rectal epithelium as drug effect measurements for pure curcumin.

Safety: Turmeric is a GRAS spice that has been used in foods at levels ranging from 0.05–760 ppm in diet [2]. In some cultures, dietary turmeric intake is estimated to be 0.1–3.8 g/day, which is *ca.* 0.1–4.4 μmol curcumin/kg-bw/day, assuming turmeric is 3% curcumin [101]. A temporary ADI for curcumin of 0.1 μmg/kg-bw (0.3 μmol/kg-bw) was selected in 1987 by the Joint FAO/WHO Expert Committee on Food Additives pending completion of carcinogenicity and reproductive toxicity tests [7]. In the carcinogenicity bioassays, however, the NTP substituted turmeric oleoresin (79–85% curcumin) because of the unavailability of pure curcumin, and equivocal results with the oleoresin were obtained (see above) [5]. The status of the reproductive toxicity testing is unknown.

The Chemoprevention Branch-sponsored Phase I trial will assess the safety of a clinical formulation of pure curcumin with a defined particle size to increase absorption; food-grade curcumin is heterogeneous in both components and particle size. In the trial, single doses of the formulation will escalate from 50 mg; the three-month multidose portion will determine the safety of doses chosen from the single-dose study. Limited human safety information is available from a few small clinical trials to demonstrate efficacy of curcumin or turmeric in treatment of arthritis and postoperative inflammation [8,10,102]. In one double-blind study of patients with rheumatoid arthritis (n=18), a dose of 1,200 mg curcumin qd (*ca.* 0.05 mmol/kg-bw/day) for two weeks produced no side effects [103]. This study has reportedly been expanded (n=31), and doses of 1,800–2,100 mg qd (*ca.* 0.07–0.08 mmol/kg-bw/day) for 5–6 weeks were without adverse effect; however, the data were not available for review [reviewed in 8].

Since curcumin has antiviral properties relating specifically to the AIDS virus [66], it is being evaluated in these patients. In a preliminary study in HIV-seropositive individuals, no adverse effects were noted at an average dose of 2,000 mg/day for an average of 127 days [104].

One small short-term trial (n=10) evaluated the effectiveness of curcumin in scavenging active oxygen species as a preventive measure against atherosclerosis [105]. A daily dose of 500 mg (*ca.* 0.02 mmol/kg-bw) for seven days significantly decreased serum lipid peroxides and total cholesterol and increased HDL-cholesterol without adverse effects.

ADME: No information was found in the literature. The Chemoprevention Branch-funded Phase I trial will describe single- and multidose pharmacokinetics of a pure curcumin formulation with a defined particle size.

CLINICAL EFFICACY: PHASE II/III STUDIES

No Phase II or III cancer chemoprevention trials of curcumin have been funded by the Chemoprevention Branch or identified in the literature. As discussed above, only small trials investigating the efficacy of the agent in treating arthritis and other inflammatory conditions and HIV were found. One uncontrolled study examined an ethanol extract of turmeric (0.5% curcumin) or curcumin as an external treatment for cancers of various organs, including oral cavity (37), breast (7), vulva (4), and skin (3) [11]. Three topical applications per day (total dose unknown) to the oral cavity produced a response in 65% (24/37) of squamous cell carcinoma cases; response was characterized as reduced lesion size, pain, exudate or itching. The Chemoprevention Branch is considering Phase II trials of curcumin in oral leukoplakia patients and in cohorts at high risk for colon cancer.

Currently, curcumin is in clinical trials in AIDS patients [66]. In a preliminary trial in HIV-seropositive individuals, it significantly increased CD-4 and

CD-8 cell counts after an average dose of 2,000 mg/day for an average of 127 days [104].

PHARMACODYNAMICS

The dose of commercial curcumin effective in the AOM-induced rat colon model without toxicity was 2,000 ppm (*ca.* 0.27 mmol/kg-bw/day). Concomitant reductions in arachidonic acid, PGE_2, $PGF_{2\alpha}$, and 5(*S*)-HETE in colon mucosa were observed. Using a NOEL of 9.5 mmol/kg-bw/day, the safety margin in rats for oral administration is ≥35. This suggests that a safe chemopreventive dose can be attained in humans. The general strategy for trials of food components is to escalate from several times normal exposure levels (ADI=0.1 mg/kg-bw), resulting in an initial dose of 0.3 mg/kg-bw or ≈25 mg. The latter (*ca.* 1 μmol/kg-bw/day) is much lower than the chemopreventive dose in rat colon; however, the small homogeneous particles in the pure clinical formulation may be better absorbed than the heterogeneous, food-grade spice. The strategy in the Chemoprevention Branch-sponsored Phase I trial will be to escalate from 50 mg to identify the dose of pure curcumin that affects the arachidonic acid cascade (cyclooxygenase and lipoxygenase activities, PGE2 and 5(*S*)-HETE levels) in the rectal epithelium without adverse effects. Based on this dose, five doses will then be selected for the multidose portion of the Phase I trial in subjects at high risk for epithelial cancer (*e.g.*, colon) for further characterization; a Phase II trial in a colon cohort may be considered.

As mentioned above, a Phase II chemoprevention trial in oral leukoplakia patients is also under consideration by the Chemoprevention Branch. The ADI for human consumption of curcumin as a food additive (7 mg, or 0.02 mmol) is similar to the topical dose in the DMBA-induced mouse skin tumorigenesis study (4 mg, or 0.01 mmol) which was effective when given during initiation [71]. In the rat tongue study, 500 mg/kg diet would be expected to directly contact this tissue at a similar dose (0.02 mmol/day). Thus, the equivalent of the daily human food level of curcumin administered topically could be effective, especially in populations with ongoing initiation such as tobacco smokers/chewers.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

PG synthesis (PGE_2 and $PGF_{2\alpha}$), a generally used and well-documented drug effect measurement for NSAIDs, may also be appropriate for curcumin. General analytical methods for these measurements are also well-characterized, although it is critical that procedures for PG measurements are standardized and validated for specific tissues targeted in chemoprevention trials, *i.e.*, oral mucosa, colon. Since curumin does not specifically inhibit the enzyme cyclooxygenase, effects on other portions of the arachidonic acid cascade should be considered as drug effect measurements, such as products of phospholipases A2 and Cγ1 (arachidonic acid, inositol 1,4,5-triphosphate) and lipoxygenase (5(*S*)-, 8(*S*)-, 12(*S*)-, 15(*S*)-HETE). These were reduced by curcumin in AOM-exposed rat colon mucosa. In the Chemoprevention Branch-sponsored Phase I trial, cyclooxygenase and lipoxygenase activities and 5(*S*)- HETE and PGE_2 levels will be evaluated in rectal tissue as human drug effect measurements for pure curcumin.

Safety Issues

Curcumin is presumed to be safe due to a long history in Asia of human use up to an estimated 95 mg/day (*ca.* 3.7 μmol/kg-bw/day) as the food additive turmeric (3.8 g/day); however, there is little evidence from controlled studies. In small clinical efficacy studies, doses of 2,100 mg qd (*ca.* 0.08 mmol/kg-bw/day) for 6 weeks in rheumatoid arthritis patients [reviewed in 8] and at ≈2,000 mg qd (0.08 mmol/kg-bw/day) for 18 weeks in AIDS patients were reportedly safe [104]. A temporary dietary ADI of 0.1 mg curcumin/kg-bw (0.27 μmol/kg-bw/day) was assigned by a joint FAO/WHO committee pending further preclinical carcinogenic and reproductive toxicity studies; however, the NTP carcinogenicity bioassay tested turmeric oleoresin in place of curcumin and obtained equivocal results. Carcinogenicity studies of curcumin may be required for Phase III chemoprevention trials.

Pharmacodynamics Issues

In rats, an oral dose of 400 mg curcumin (*ca.* 3.6 mmol/kg-bw) appears to be required to attain detectable tissue levels [99]. This is more than 13,000 times greater than the ADI for commercial curcumin as a human food additive (0.27 μmol/kg-bw). However, it is not known if the rat is an appropriate model for gastrointestinal absorption and pharmacokinetics of the pigment in humans. The latter will be addressed

in the Phase I trial sponsored by the Chemoprevention Branch.

Regulatory Issues

The single-dose Phase I trial protocol to determine the safety and pharmacokinetics of pure curcumin is being finalized. Since the general regulatory strategy for such agents has been to escalate several fold from the level used in foods (ADI= 0.1 mg/kg-bw, or *ca.* 7 mg), the dosing will escalate from 50 mg.

Intermediate Biomarker Issues

Preclinical studies demonstrated modulation of histological biomarkers by ingested curcumin in the carcinogen-exposed gastrointestinal tract, including dysplasia (colon, tongue), adenomas (forestomach, colon), papillomas (duodenum) and aberrant crypt foci (colon). Formation of papillomas was also inhibited by topical application to skin. This suggests that a Phase II trial with regression/prevention of dysplastic oral leukoplakia as the endpoint would be feasible with either topical or ingested curcumin. Proliferation biomarkers were also modulated in studies of rat tongue—BrdU labelling, polyamine levels, and Ag-NOR counts and area. The same types of markers were affected in models of colon (ODC and protein kinase activity) and skin ([^{3}H]-thymidine incorporation, ODC activity) carcinogenesis. Evaluation of proliferation biomarkers could be included in the proposed oral leukoplakia trial.

Supply and Formulation Issues

Food-grade curcumin actually contains three similar compounds when measured by HPLC—curcumin (69–77%), demethoxycurcumin (17%) and bisdemethoxycurcumin (3–6%) [78]. The total curcuminoid content may be >98% when assayed by fluorescence. One published chemoprevention study has reported that pure curcumin (>98%) is equally as effective as the commercial grade containing 97% curcuminoids (77% curcumin, 17% demethoxycurcumin, 3% bisdemethoxycurcumin) in the AOM-induced rat colon [78]. Additional *in vivo* and *in vitro* studies found that the effects of the individual curcuminoids varied with the assay system. Since commercial curcumin varies in composition and particle size, pure curcumin in a defined, homogeneous particle size will be provided by Gene Print, Inc. The Phase I trial will evaluate the pharmacokinetics and bioequivalence of this formulation when administered at doses escalating from 50 mg by mixing in orange juice.

Clinical Studies Issues

Commercial curcumin inhibits arachidonic acid mobilization and displays antiinitiation and antipromotion activities. Because of this combination of potential chemopreventive mechanisms, demonstrated activity in many preclinical carcinogenesis models, and its perceived human safety, the agent is entering the initial stages of clinical evaluation. A Phase I trial assessing the safety and pharmacokinetics of single and multiple doses of curcumin was recently funded by the Chemoprevention Branch; the single-dose protocol is being finalized. A formulation of pure curcumin with a defined particle size will be provided by Gene Print, Inc. to allow a homogeneous drug product and increase absorption. Since commercial curcumin was shown to decrease rat colon carcinogenesis with concomitant reductions in arachidonic acid metabolites, future Phase II trials might include a cohort with colon polyps or otherwise at high risk for colon cancer. A Phase II chemoprevention trial in oral leukoplakia patients is also under consideration.

REFERENCES

1. Conney, A.H., Lysz, T., Ferraro, T., Abidi, T.F., Manchand, P.S., Laskin, J.D. and Huang, M.-T. Inhibitory effects of curcumin and some related dietary compounds on tumor promotion and arachidonic acid metabolism in mouse skin. *Adv. Enzyme Regul.* 31: 385–396, 1991.
2. HSDB *Hazardous Substances Data Bank.* Available online via National Library of Medicine (NLM). Latest update, Jan., 1995.
3. U.S. Food and Drug Administration. Substances generally recognized as safe. Spices and other natural seasonings and flavorings. *Code of Fed. Reg.*, Title 21, Sect. 182.10. April, 1994.
4. U.S. Food and Drug Administration. Substances generally recognized as safe. Essential oils, oleoresins (solvent-free) and natural extractives (including distillates). *Code of Fed. Reg.* Title 21, Sect. 182.20. April, 1994.
5. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of turmeric oleoresin (CAS No. 8024-37-1) (major component 79%–85% curcumin. CAS No. 458-37-7) in F344/N rats and B6C3F$_1$ mice (feed studies). *NTP Technical Report Series*, No. 427, 1993.
6. Shah, R.G. and Netrawali, M.S. Evaluation of mutagenic activity of turmeric extract containing curcumin, before and after activation with mammalian cecal microbial extract of liver microsomal fraction, in the Ames *Salmonella* test. *Bull. Environ. Contam. Toxicol.* 40: 350–357, 1988.
7. Anon. Expert committee asks information on metals in PES. *Food Chemical News*, March 16, 1992.
8. Srimal, R.C. Curcumin. *Drugs Future* 12: 331–333, 1987.
9. Reddy, A.Ch.P. and Lokesh, B.R. Studies on the inhibitory

effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. *Mol. Cell. Biochem.* 137: 1–8, 1994.

10. Ammon, H.P.T and Wahl, M.A. Pharmacology of curcumin. *Planta Med.* 57: 1–7, 1991.
11. Kuttan, R., Sudheeran, P.C. and Josph, C.D. Turmeric and curcumin as topical agents in cancer therapy. *Tumori* 73: 29–31, 1987.
12. Donatus, I.A., Vermeulen, S. and Vermeulen, N.P.E. Cytotoxic and cytoprotective activities of curcumin. Effects on paracetamol-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. *Biochem. Pharmacol.* 39: 1869–1875, 1990.
13. Srimal, R.C. and Dhawan, B.N. Pharmacology of diferuloyl methane (curcumin), a nonsteroidal anti-inflammatory agent. *J. Pharm. Pharmacol.* 25: 447–452, 1973.
14. Huang, M.-T., Lysz, T., Ferraro, T. and Conney, A.H. Inhibitory effects of curcumin on tumor promotion in mouse epidermis. In: Wattenberg, L., Lipkin, M., Boone, C.W. and Kelloff, G.J. (eds.) *Cancer Chemoprevention*. Boca Raton, FL: CRC Press, pp. 375–391, 1992.
15. Zhao, B., Li, X., He, R., Cheng, S. and Xin, W. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys.* 14: 175–185, 1989.
16. Kunchandy, E. and Rao, M.N. Oxygen radical scavenging activity of curcumin. *Int. J. Pharmaceut.* 58: 237–240, 1990.
17. Tønnesen, H.H. and Greenhill, J.V. Studies on curcumin and curcuminoids. XXII: Curcumin as a reducing agent and as a radical scavenger. *Int. J. Pharmaceut.* 87: 79–87, 1992.
18. Shih, C.-A. and Lin, J.-K. Inhibition of 8-hydroxydeoxyguanosine formation by curcumin in mouse fibroblast cells. *Carcinogenesis* 14: 709–712, 1993.
19. Srivastava, R. Inhibition of neutrophil response by curcumin. *Agents Actions* 28: 298–303, 1989.
20. Subramanian, M., Sreejayan, Rao, M.N.A., Devasagayam, T.P.A. and Singh, B.B. Diminution of singlet oxygen-induced DNA damage by curcumin and related antioxidants. *Mutat. Res.* 311: 249–255, 1994.
21. Sharma, S.C., Muhktar, H., Skarun, S.K. and Murti, C.R.K. Lipid peroxide formation in experimental inflammation. *Biochem. Pharmacol.* 21: 1210–1214, 1972.
22. Shalini, V.K. and Srinivas, L. Lipid peroxide induced DNA damage: Protection by turmeric (*Curcuma longa*). *Mol. Cell. Biochem.* 77: 3–10, 1987.
23. Soudamini, K.K., Unnikrishnan, M.C., Soni, K.B. and Kuttan, R. Inhibition of lipid peroxidation and cholesterol levels in mice by curcumin. *Indian J. Physiol. Pharmacol.* 36: 239–43, 1992.
24. Rao, S. and Rao, M.N.A. Curcumin inhibits iron-dependent lipid peroxidation. *Int. J. Pharmaceut.* 100: 93–97, 1993.
25. Reddy, A.Ch.P. and Lokesh, B.R. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol. Cell. Biochem.* 111: 117–124, 1992.
26. Rao, S. and Rao, M.N.A. Curcuminoids as potent inhibitors of lipid peroxidation. *J. Pharm. Pharmacol.* 46: 1013–1016, 1994.
27. Reddy, A.Ch.P. and Lokesh, B.R. Alterations in lipid peroxides in rat liver by dietary *n*-3 fatty acids: Modulation of antioxidant enzymes by curcumin, eugenol, and vitamin E. *J. Nutr. Biochem.* 5: 181–188, 1994.
28. Rajakumar, D.V. and Rao, M.N.A. Antioxidant proper ties of dehydrozingerone and curcumin in rat brain homogenates. *Mol. Cell. Biochem.* 140: 73–79, 1994.
29. Lin, J.K., Shih, C.A. and Lin-shiau, S.Y., Inhibition of xanthine dehydrogenase/oxidase by curcumin in PMA induced NIH3T3 cells. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 167, abstract no. 993, 1995.
30. Chan, M.M.-Y., Ho, C.-T. and Huang, H.-I. Effects of three dietary phytochemicals from tea, rosemary and turmeric on inflammation-induced nitrite production. *Cancer Lett.* 96: 23–29, 1995.
31. Unnikrishnan, M.K. and Rao, M.N.A. Curcumin inhibits nitrogen dioxide induced oxidation of hemoglobin. *Mol. Cell. Biochem.* 146: 35–37, 1995.
32. Rao, C.V., Rivenson, A., Simi, B. and Reddy, B.S. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. *Cancer Res.* 55: 259–266, 1995.
33. Ammon, H.P.T., Safayhi, H., Mack, T. and Sabieraj, J. Mechanism of antiinflammatory actions of curcumine and boswellic acids. *J. Ethnopharmacol.* 38: 113–119, 1993.
34. Flynn, D.L. and Rafferty, M.F. Inhibition of 5-hydroxy eicosatetraenoic acid (5-HETE) formation in intact human neutrophils by naturally-occurring diarylheptanoids: Inhibitory activities of curcuminoids and yakuchinones. *Prostaglandins Leukot. Med.* 22: 357–360, 1986.
35. Rao, C.V., Simi, B. and Reddy, B.S. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. *Carcinogenesis* 14: 2219–2225, 1993.
36. Huang, M.-T., Lysz, T., Ferraro, T., Abidi, T.F., Laskin, J.D. and Conney, A.H. Inhibitory effects of curcumin on *in vitro* lipoxygenase and cyclooxygenase activities in mouse epidermis. *Cancer Res.* 51: 813–819, 1991.
37. Shimizu, S., Jareonkitmongkol, S., Kawashima, H., Akimoto, K. and Yamada, H. Inhibitory effect of curcumin on fatty acid desaturation in *Mortierella alpina* 1S-4 and rat liver microsomes. *Lipids* 27: 509–512, 1992.
38. Srivastava, K.C., Bordia, A. and Verma, S.K. Curcumin, a major component of food spice turmeric (*Curcuma longa*) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. *Prostaglandins Leukot. Essent. Fatty Acids* 52: 223–227, 1995.
39. Kensler, T.W., Trush, M.A. and Guyton, K.Z. Free radicals as targets for cancer chemoprotection: Prospects and problems. In: Steele, V.E., Stoner, G.D., Boone, C.W. and Kelloff, G.J. (eds.) *Cellular and Molecular Targets for Chemoprevention*. Boca Raton, FL: CRC Press, pp. 173–191, 1992.
40. Chan, M.M.-Y. Inhibition of tumor necrosis factor by curcumin, a phytochemical. *Biochem. Pharmacol.* 49: 1551–1556, 1995.
41. Marnett, L.J. Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res.* 52: 5575–5589, 1992.
42. Nagabhushan, M. and Bhide, S.V. Nonmutagenicity of curcumin and its antimutagenic action *versus* chili and capsaicin. *Nutr. Cancer* 8: 201–210, 1986.
43. Mulky, N., Amonkar, A.J. and Bhide, S.V. Antimutagenicity of curcumins and related compounds: The structural requirement for the antimutagenicity of curcumins. *Indian*

Drugs 25: 91–95, 1987.

44. Nagabhushan, M., Amonkar, A.J. and Bhide, S.V. *In vitro* antimutagenicity of curcumin against environmental mutagens. *Food Chem. Toxicol.* 25: 545–547, 1987.
45. Oda, Y. Inhibitory effect of curcumin on SOS functions induced by UV irradiation. *Mutat. Res.* 348: 67–73, 1995.
46. Abraham, S.K., Sarma, L. and Kesavan, P.C. Protective effects of chlorogenic acid, curcumin and β-carotene against γ-radiation-induced *in vivo* chromosomal damage. *Mutat. Res.* 303: 109–112, 1993.
47. Lahiri, M., Maru, G.B. and Bhide, S.V. Effect of plant phenols, β-carotene and α-tocopherol on benzo[*a*]pyrene-induced DNA damage in the mouse forestomach mucosa (target organ) and bone marrow polychromatic erythrocytes (non-target organ). *Mutat. Res.* 303: 97–100, 1993.
48. Azuine, M.A., Kayal, J.J. and Bhide, S.V. Protective role of aqueous turmeric extract against mutagenicity of direct-acting carcinogens as well as benzo[*a*]pyrene-induced genotoxicity and carcinogenicity. *J. Cancer Res. Clin. Oncol.* 118: 447–452, 1992.
49. Lahiri, M. and Bhide, S.V. Effect of four plant phenols, β-carotene and α-tocopherol on ^{3}(H)benzopyrene-DNA interaction *in vitro* in the presence of rat and mouse liver postmitochondrial fraction. *Cancer Lett.* 73: 35–39, 1993.
50. Mukundan, M.A., Chacko, M.C., Annapurna, V.V. and Krishnaswamy, K. Effect of tumeric and curcumin on BP-DNA adducts. *Carcinogenesis* 14: 493–496, 1993.
51. Huang, M.-T., Wang, Z.Y., Georgiadis, C.A., Laskin, J.D. and Conney, A.H. Inhibitory effects of curcumin on tumor initiation by benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene. *Carcinogenesis* 13: 2183– 2186, 1992.
52. Deshpande, S.S. and Maru, G.B. Effects of curcumin on the formation of benzo[*a*]pyrene derived DNA adducts *in vitro*. *Cancer Lett.* 96: 71–80, 1995.
53. Smith, W.A., Devanaboyina, U. and Gupta, R.C. Use of microsomal activation system as a potential screening method for cancer chemopreventive agent. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 597, abstract no. 3555, 1995.
54. Huang, T.S., Lee, S.C. and Lin, J.K. Suppression of c-*jun*/AP-1 activation by an inhibitor of tumor promotion in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA* 88: 5292–5296, 1991.
55. Kakar, S.S. and Roy, D. Curcumin inhibits TPA induced expression of c-*fos*, c-*jun* and c-*myc* proto-oncogenes messenger RNAs in mouse skin. *Cancer Lett.* 87: 85–89, 1994.
56. Liu, J.-Y., Lin, S.-J. and Lin, J.-K. Inhibitory effects of curcumin on protein kinase C activity induced by 12-*O*-tetradecanoyl-phorbol-13-acetate in NIH 3T3 cells. *Carcinogenesis* 14: 857–861, 1993.
57. Huang, M.-T., Smart, R.C., Wong, C.-Q. and Conney, A.H. Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-*O*-tetradecanoylphorbol-13-acetate. *Cancer Res.* 48: 5941–5946, 1988.
58. Lu, Y.-P., Chang, R.L., Huang, M.-T. and Conney, A.H. Inhibitory effect of curcumin on 12-*O*-tetradecanoylphorbol-13-acetate-induced increase in ornithine decarboxylase mRNA in mouse epidermis. *Carcinogenesis* 14: 293–297, 1993.
59. Kortula, L. and Kumar, R. Inhibitory effect of curcumin on epidermal growth factor receptor kinase activity in A431 cells. *Biochim. Biophys. Acta* 24: 597–600, 1994.
60. Korutla, L., Cheung, J.Y., Mendelsohn, J. and Kumar, R. Inhibition of ligand-induced activation of epidermal growth factor receptor tyrosine phosphorylation by curcumin. *Carcinogenesis* 16: 1741–1745, 1995.
61. Sambaiah, K. and Srinivasan, K. Influence of spices and spice principles on hepatic mixed function oxygenase system in rats. *Indian J. Biochem. Biophys.* 26: 254–258, 1989.
62. Nagabhushan, M. and Bhide, S.V. Curcumin as an inhibitor of cancer. *J. Am. Coll. Nutr.* 11: 192–198, 1992.
63. Susan, M. and Rao, M.N.A. Induction of glutathione *S*-transferase activity by curcumin in mice. *Arzneimittelforschung* 42-2: 962–964, 1992.
64. Stoner, G.D. and Mukhtar, H. Polyphenols as cancer chemopreventive agents. *J. Cell. Biochem.* 22 (Suppl.): 169–180, 1995.
65. Sathyamoorthy, N., Wang, T.T.Y., Do, L.D. and Phang, J.M. Biological activity of diet-derived flavonoids: Effects on pS2 expression and cellular proliferation. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 596, abstract no. 3548, 1995.
66. Mazumder, A., Raghavan, K., Weinstein, J., Kohn, K.W. and Pommier, Y. Inhibition of human immunodeficiency virus type-1 integrase by curcumin. *Bio chem. Pharmacol.* 49: 1165–1170, 1995.
67. Srivastava, R. and Srimal, R.C. Modification of certain inflammation-induced biochemical changes by curcumin. *Ind. J. Med. Res.* 81: 215–223, 1985.
68. Nishino, H., Nishino, A., Takayasu, J. and Hasegawa, T. Antitumor-promoting activity of curcumin, a major constituent of the food additive "turmeric yellow." *J. Kyoto Pref. Univ. Med.* 96: 725–728, 1987.
69. Takayasu, J., Nishino, H. and Iwashima, A. Antitumor-promoting activity of curcumin. *Proc. Annu. Meet. Jpn. Cancer Assoc.* 46: 60, 1987.
70. Verma, A.K. and Erickson, D. Inhibition of protein kinase C (PKC)-mediated phosphorylation of a novel, low molecular weight compound, and several endogenous polypeptides by palmitoylcarnitine (PC). *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 28: 173, abstract no. 687, 1987.
71. Soudamini, K.K. and Kuttan, R. Inhibition of chemical carcinogenesis by curcumin. *J. Ethnopharmacol.* 27: 227–233, 1989.
72. Tanaka, T., Makita, H., Ohnishi, M., Hirose, Y., Wang, A., Mori, H., Satoh, K., Hara, A. and Ogawa, H. Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis by dietary curcumin and hesperidin: Comparison with the protective effect of β-carotene. *Cancer Res.* 54: 4653–4659, 1994.
73. Reddy, B.S., Rao, C.V., Rivenson, A. and Kelloff, G. Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. *Carcinogenesis* 14: 1493–1497, 1993.
74. Pence, B.C., Dunn, D.M., Zhao, A., Landers, M. and Wargovich, M.J. Experimental chemoprevention of colon carcinogenesis by combined calcium and aspirin. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 35: 624, abstract no. 3719, 1994.
75. Moorghen, M., Ince, P., Finney, K.J., Sunter, J.P., Appleton, D.R. and Watson, A.J. A protective effect of sulindac against chemically-induced primary colonic tumours in mice. *J. Pathol.* 156: 341–347, 1988.

76. Skinner, S.A., Penney, A.G. and O'Brien, P.E. Sulindac inhibits the rate of growth and appearance of colon tumors in the rat. *Arch. Surg.* 126: 1094–1096, 1991.
77. Pereira, M.A., Barnes, L.H., Rassman, V.L., Kelloff, G.J. and Steele, V.E. Use of azoxymethane-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents. *Carcinogenesis* 15: 1049–1054, 1994.
78. Huang, M.-T., Lou, Y.-R., Ma, W., Newmark, H.L., Reuhl, K.R. and Conney, A.H. Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. *Cancer Res.* 54: 5841–5847, 1994.
79. Pereira, M.A., Grubbs, C.J., Barnes, L.H., Olson, G.R., Eto, I., Juliana, M., Whitaker, L.M., Kelloff, G.J., Steele, V.E. and Lubet, R.A. Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz(*a*)anthracene-induced mammary cancer in rats. *Carcinogenesis* 17: 1305–1312, 1996.
80. Mehta, R.G. and Moon, R.C. Characterization of effective chemopreventive agents in mammary gland *in vitro* using an initiation-promotion protocol. *Anti cancer Res.* 11: 593–596, 1991.
81. Huang, M.-T., Ma, W., Lu, Y.-P., Chang, R.L., Fisher, C., Manch, P.S., Newmark, H.L. and Conney, A.H. Effects of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin on 12-*O*- tetradecanoylphorbol-13-acetate-induced tumor promotion. *Carcinogenesis* 16: 2493–2497, 1995.
82. Ruby, A.J., Kuttan, G., Babu, K.D., Rajasekharan, K.N. and Kuttan, R. Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer Lett.* 94: 79–83, 1995.
83. Osawa, T., Sugiyama, Y., Inayoshi, M. and Kawakishi, S. Antioxidative activity of tetrahydrocurcuminoids. *Biosci. Biotechnol. Biochem.* 59: 1609–1612, 1995.
84. Huang, M.T., Deschner, E.E., Newmark, H.L., Wang, Z.Y., Ferraro, T.A. and Conney, A.H. Effect of dietary curcumin and ascorbyl palmitate on azoxymethanol-induced colonic epithelial cell proliferation and focal areas of dysplasia. *Cancer Lett.* 64: 117–121, 1992.
85. Wargovich, M.J., Eng, V.W.S. and Newmark, H.L. Inhibition by plant phenols of benzo[*a*]pyrene-induced nuclear aberrations in mammalian intestinal cells: A rapid *in vivo* assessment method. *Food Chem. Toxicol.* 23: 47–49, 1985.
86. Wargovich, M.J. and Newmark, H.L. Inability of several mutagen-blocking agents to inhibit 1,2-dimethylhydrazine-induced DNA-damaging activity in colonic epithelium. *Mutat. Res.* 121: 77–80, 1983.
87. Wahlström, B. and Blennow, G. A study on the fate of curcumin in the rat. *Acta Pharmacol. Toxicol.* 43: 86–92, 1978.
88. Sambaiah, K., Ratankumar, S., Kamanna, V.S., Satyanarayana, M.N. and Rao, M.V.L. Influence of turmeric and curcumin on growth, blood constituents and serum enzymes in rats. *J. Food Sci. Technol.* 19: 187–190, 1982.
89. Govindarajan, V.S. Turmeric—chemistry, technology, and quality. *Crit. Rev. Food Sci. Nutr.* 12: 199–299, 1980.
90. Gupta, B., Kulshrestha, V.K., Srivastava, R.K. and Prasad, D.N. Mechanisms of curcumin induced gastric ulcer in rats. *Indian J. Med. Res.* 71: 806–814, 1980.
91. Ishidate, M., Jr., Sofuni, T. and Yoshikawa, K. Chromosomal aberration tests *in vitro* as a primary screening tool for environmental mutagens and/or carcinogens. *Gann Monogr. Cancer Res.* 27: 95–108, 1981.
92. Ishidate, M., Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M. and Matsuoka, A. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol.* 22: 623–636, 1984.
93. Jensen, N.J. Lack of mutagenic effect of turmeric oleoresin and curcumin in the *Salmonella*/mammalian microsome test. *Mutat. Res.* 105: 393–396, 1982.
94. Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B. and Zeiger, E. *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutagen.* 8 (Suppl. 7): 1–119, 1986.
95. Vijayalaxmi. Genetic effects of turmeric and *curcumin in* mice and rats. *Mutat. Res.* 79: 125–132, 1980.
96. Ishidate, M., Jr., Harnois, M.C. and Sofuni, T. A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.* 195: 151–213, 1988.
97. Giri, A.K., Das, S.K., Talukder, G. and Sharma, A. Sister chromatid exchange and chromosome aberrations induced by curcumin and tartrazine on mammalian cells *in vivo*. *Cytobios* 62: 111–117, 1990.
98. Holder, G.M., Plummer, J.L. and Ryan, A.J. The metabolism and excretion of curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) in the rat. *Xenobiotica* 8: 761–768, 1978.
99. Ravindranath, F. and Chandrasekhara, N. Metabolism of curcumin—studies with [^{3}H]curcumin. *Toxicology* 22: 337–344, 1981.
100. Ravindranath, F. and Chandrasekhara, N. Absorption and tissue distribution of curcumin in rats. *Toxicology* 16: 259–265, 1980.
101. Shankar, T.N.B., Shantha, N.V., Ramesh, H.P., Murthy, I.A.S. and Murthy, V.S. Toxicity studies on turmeric (*Curcuma longa*): Acute toxicity studies in rats, guinea pigs & monkeys. *Ind. J. Exp. Biol.* 18: 73–75, 1980.
102. Satoskar, R.R., Shah, S.J. and Shenoy, S.G. Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. *Int. J. Clin. Pharmacol. Ther. Toxicol.* 24: 651–654, 1986.
103. Deodhar, S.D., Sethi, R. and Srimal, R.C. Preliminary study on antirheumatic activity of curcumin (diferuloyl methane). *Indian J. Med. Res.* 71: 632–634, 1980.
104. Copeland, R., Baker, D. and Wilson, H. Curcumin therapy in HIV-infected patients initially increased CD-4 and CD-8 cell counts. *Int. Conf. AIDS* 10: 216, abstract no. PB0876, 1994.
105. Soni, K.B. and Kuttan, R. Effect of oral curcumin administration on serum peroxides and cholesterol levels in human volunteers. *Ind. J. Physiol. Pharmacol.* 36: 273–275, 1992.

Table I. Clinical Trials of Curcumin Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase I (Safety and ADME)					
NO1-CN-55124 Phase I Single and Multiple Dose Safety and Pharmacokinetic Clinical Study of Curcumin (Dr. Dean E. Brenner, University of Michigan) 6/95–	---	Single-dose study: Normal, healthy men and women Single-dose study: 18 subjects Multidose study: Subjects at high risk for epithelial cancer Multidose study: 30 subjects	Single dose: 50 mg–1 g Multidose: Dose from single dose portion for 3 months	Pharmacokinetics: Single- and multidose ADME for curcumin, tetrahydroxycurcumin, dihydroxyferulic acid Safety: Hematology, clinical chemistry, questionnaire Drug effect measurements: Cyclooxygenase and lipoxygenase activities and 5(*S*)-HETE and PGE_2 levels Intermediate biomarker: Apoptosis	Protocol being finalized
Phase II (Dose-titration, efficacy intermediate biomarkers)					
Proposed Curcumin in Dysplastic Oral Leukoplakia	Oral Cavity	Oral leukoplakia patients			Study not designed

CURCUMIN DEVELOPMENT STATUS