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NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN: *l*-PERILLYL ALCOHOL

DRUG IDENTIFICATION

CAS Registry No.: 536-59-4

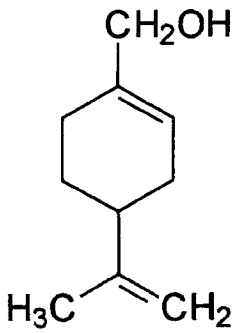
CAS Name (9CI): 4-(1-Methylethenyl)-1-cyclohexene-1-methanol

Synonyms:
Dihydrocuminyl Alcohol
p-Mentha-1,8-dien-7-ol
NSC 641066
Perilla Alcohol
Perillic Alcohol
Perillol

Related Compounds:
d-Limonene
Carvene
1-Methyl-4-(1-methylethenyl)cyclohexene
Perillic Acid
Dihydroperillic Acid

Molecular Wt.: 152.2

Structure:



EXECUTIVE SUMMARY

Perillyl alcohol is a cyclic monoterpene occurring in numerous species of plants, including mints (*Mentha piperita* and *M. spicata*), lavender, perilla (*Perilla frutescens*), *Cymbopogon*, citrus, and cranberries [1–3]. The monoterpene was given "Generally Recognized as Safe" (GRAS) status as a flavoring agent by FEMA in 1965, and is approved as a food additive by the FDA and the Council of Europe [3]. Perillyl alcohol is also used as a fragrance in perfumes, soaps, detergents, lotions, and creams.

Perillyl alcohol is a hydroxylated derivative of *d*-limonene. Both monoterpenes have demonstrated preclinical chemopreventive and chemotherapeutic activity possibly through metabolism to perillic and dihydroperillic acids [4–6]. Both of these metabolites selectively inhibit isoprenylation of small (21–26 kDa) guanine nucleotide-binding proteins [7,8], either by interaction with protein:prenyl transferases [9,10] or by selectively decreasing *ras* levels [11].

Some of these proteins are potentially oncogenic [7], such as the *ras* product p21. $p21^{ras}$ is post-translationally modified from a soluble cytoplasmic protein to a more hydrophobic product by addition of farnesyl [12]. This allows association of p21 with the plasma membrane, which is essential for *ras* transforming activity. Perillyl alcohol also modulates the mevalonate-cholesterol pathway at other points distal to HMGCoA reductase, *i.e.*, synthesis of ubiquinone (CoQ) and conversion of lathosterol to cholesterol [13]. The former may inhibit proliferation of tumor cells, which often rely on glycolysis for ATP production. Additional chemopreventive activities attributed to perillyl alcohol include inhibition of tumor cell proliferation [13–16] (possibly through G_1 cell cycle block [17]), induction of differentiation [18], and enhancement of M6P/IGF-II and TGFβ receptor-activated apoptosis [19–21] and TGFβ levels [22]. Finally, the monoterpene has the potential to induce cytochrome P450, glutathione-*S*-transferase (GST) and glucuronyl transferase activities, since other hydroxylated analogs of *d*-limonene have been found to do so [23].

Preclinical chemopreventive and chemotherapeutic [7,18,24] data are available for both perillyl alcohol and *d*-limonene. In an NCI, Chemoprevention Branch-sponsored rat study, perillyl alcohol significantly inhibited AOM-induced colon and small intestine tumor development. In published chemoprevention studies, perillyl alcohol inhibited rat liver and hamster pancreatic tumor development; in chemotherapy studies, it significantly reduced growth of established hamster pancreatic tumors, caused regression of established rat mammary gland tumors, and retarded growth of a prostate tumor cell xenograft in athymic nude mice. The parent compound *d*-limonene has been shown to inhibit the growth of mouse lung [25] and skin tumors [26], rat mammary gland tumors induced by MNU, DMBA, and direct *in situ* transfer of v-Ha-*ras* [27–30], and rat liver tumorigenesis [23,31], as well as regress established mammary tumors [32,33]. However, clinical development of *d*-limonene is restricted by the large doses needed to achieve an effect [22,34]; for example, the minimum dietary dose for rat mammary tumor prevention is 1–5%, which is equivalent to 35–175 g daily for a 70 kg human. In comparison, perillyl alcohol is *ca.* 5 times more potent than *d*-limonene in *in vitro* assays [7,8] and 5–10 times more potent in chemotherapy studies *in vivo* [35], suggesting that smaller human doses might be as effective. Because of its higher potency than *d*-limonene in regression of mammary gland tumors and its chemopreventive efficacy in rat colon, perillyl alcohol is being considered for further development as a cancer chemopreventive agent by NCI, Chemoprevention Branch and as a cancer chemotherapeutic drug by NCI, Division of Cancer Treatment, Diagnosis and Centers (DCTDC).

The NCI, Chemoprevention Branch and NCI, DCTDC are coordinating their efforts in the development of perillyl alcohol. Besides the completed rat colon study, the Chemoprevention Branch has preclinical chemoprevention studies ongoing in other models in which *ras* activation is a significant component of tumorigenesis—MNU-induced rat mammary gland, B(*a*)P-induced mouse lung, and BOP-induced hamster pancreas. A major effort in the NCI, Chemoprevention Branch program is the identification of intermediate biomarkers which have potential as surrogate endpoints for cancer incidence in clinical trials. In hamster pancreas, modulation of BOP-induced *ras* mutations in premalignant and malignant tumors is being assessed as a genetic intermediate biomarker and correlated to perillyl alcohol effects on tumor incidence and multiplicity; in female A/J mice, modulation of Ki-*ras* oncogene and RNA expression in NNK-induced lung tumors is being examined. The NCI, DCTDC has provided information on preclinical chemotherapeutic studies in human prostate xenograft models and rat mammary tumor models.

The NCI, Chemoprevention Branch has funded 90-day toxicity studies of perillyl alcohol in rats and dogs. In rats, ig doses of 40, 120, or 400 mg/kg-bw/day (0.3, 0.8, or 2.6 mmol/kg-bw/day) caused no drug-related deaths, abnormal hematology, clinical chemistry, or lesions; however, dose-related hyperexcitement and clear mouth discharge were noted in all treated groups. In dogs, ig doses of 40, 120, or 400 mg/kg-bw/day (0.3, 0.8, or 2.6 mmol/kg-bw/day) had no effect on survival, but all high-dose animals had emesis and mild anemia. The MTD for both rats and dogs was *ca.* 400 mg/kg-bw/day (2.6 mmol/kg-bw/day). The NCI, DCTDC has completed 14- and 28-day preclinical toxicity and ADME studies in rats and dogs, as well as 14-day toxicity tests in nude mice bearing human tumor xenografts. In these studies, dose-limiting toxicities were renal (tubular degeneration) and gastrointestinal in both rats and dogs. Ad-

verse gastrointestinal effects such as emesis and soft/loose stools in dogs and forestomach hyperplasia in rats may be attributable to the direct irritant properties of perillyl alcohol. The 28-day MTDs were reported to be 600 mg/kg-bw/day (*ca.* 3.9 mmol/kg-bw/day) in dogs (as three divided doses) and rats (as two divided doses).

Like its parent *d*-limonene [5,6], perillyl alcohol is rapidly absorbed and metabolized extensively to perillic acid (the putative chemopreventive agent) and dihydroperillic acid. Following a single oral dose of 250 mg perillyl alcohol/kg-bw (1.6 mmol/kg-bw) to a male Beagle dog, the plasma C_{max} for perillic acid reached 1,000 μM at 2.5 hours after dosing and remained over 500 μM for 6 hours. Dihydroperillic acid was also observed at lower levels (30–36 μM) 3–6 hours after dosing. Neither perillyl alcohol nor methyl esters of perillic or dihydroperillic acid were detected in plasma. After 28 days of oral treatment with 600 mg perillyl alcohol/kg-bw/day, the C_{max} for perillic acid in plasma was two-fold higher in dogs than in rats, while the C_{max} for dihydroperillic acid was much lower in dogs.

Breast is the primary target for clinical chemoprevention studies with perillyl alcohol. This choice is based on the affinity of its active metabolites for rat mammary gland tissue [5], its regression of established tumors and prevention of subsequent neoplasms (following a primary tumor) in rat mammary gland, and its close relationship to *d*-limonene, an effective but less potent chemotherapeutic agent in the rat mammary model [*e.g.*, 36,37]. The NCI, DCTDC is sponsoring a Phase I multidose clinical trial of perillyl alcohol in cancer patients escalating from 800 mg/m^2 tid to 1600 mg/m^2 tid, and then 2400 mg/m^2 tid (*ca.* 0.4, 0.8 and 1.2 mmol/kg-bw/day). Escalation to the next highest dose level was dependent on 28 days of treatment without severe toxicity. For comparison, the initial dose is 0.15 MTD in dogs and 0.5 MTD in rats (based on Chemoprevention Branch 90-day toxicity studies) [4]. In addition, the NCI, Chemoprevention Branch-sponsored single-dose pharmacokinetic Phase I trial (Dr. G. Thomas Budd, The Cleveland Clinic Foundation) of perillyl alcohol in normal, healthy women and women at high risk for breast cancer is in progress. This will be followed by a multidose Phase I study in the same population.

It is anticipated that these data will be sufficient to allow the Chemoprevention Branch to initiate a short-term Phase II trial in breast cancer patients with biopsy-proven DCIS or early stage cancer to follow modulation of intermediate biomarkers. The Chemoprevention Branch is also considering a second Phase II trial of perillyl alcohol in prostate cancer patients scheduled for surgery. Justification for this target organ is based on the cytostatic effect of perillyl alcohol on prostate tumor xenografts in athymic nude mice, in addition to its ability to modulate expression of potentially important prostatic growth factors TGFβ and M6P/IGF-II [38]. It is anticipated that these studies will begin in 1997.

The NCI, DCTDC is supplying 250 mg perillyl alcohol (Nippon Terpene Co., >97% pure) with soybean oil (1:1) in soft gelatin capsules for the Chemoprevention Branch-sponsored Phase I clinical trial.

PRECLINICAL EFFICACY STUDIES

The NCI, Chemoprevention Branch has shown preclinical efficacy of perillyl alcohol in the AOM-induced rat colon model. However, doses of 1,000 and 2,000 ppm in the diet had opposite effects on invasive adenocarcinomas in the colon and small intestine, compared with the control group. A dose of 1,000 ppm perillyl alcohol (*ca.* 0.3 mmol/kg-bw/day) suppressed invasive adenocarcinomas in the colon by 70%, but had no effect in the small intestine; however, a dose of 2,000 ppm inhibited adenocarcinoma incidence by 70% in the small intestine, but had no effect on these tumors in the colon. Both doses showed only modest activity against total (invasive + noninvasive) tumor incidence in the colon, but 2,000 ppm reduced total tumor incidence by 73% in the small intestine. Three other efficacy studies are in progress, including: MNU-induced rat mammary gland (2.5 and 5 g/kg diet, or *ca.* 0.8 and 1.6 mmol/kg-bw/day), B(*a*)P-induced mouse lung (2.5 and 5 g/kg diet), and BOP-induced hamster pancreas (3.2 or 6.3 mmol/kg-bw/day)tumor models (4 and 8 g/kg diet, or *ca.* 3.2 and 6.3 mmol/kg-bw/day). In all these targets, activation of *ras* is a significant component of tumorigenesis [39–42]. In studies published in abstracts, perillyl alcohol was reportedly effective during the promotion phase of liver carcinogenesis [19] in rats and against BOP-induced pancreatic tumors in hamsters [43]. Rats administered 2% perillyl alcohol in the diet (*ca.* 6.6 mmol/kg-bw/day) between 2 and 19 weeks after DEN exposure had a 10-fold lower mean liver tumor mass than basal diet controls. This effect correlated to a 5–10-fold increase in apop-

totic indices and two-fold increases in TGFβ type I and M6P/IGFII receptor expression [19]; binding of the latent TGFβ complex to the M6P/IGF-II receptor can result in TGFβ activation and apoptosis. In contrast, no change in tumor cell proliferation was observed. Finally, hamsters exposed to 1 or 2% dietary perillyl alcohol (*ca.* 7.9 or 15.8 mmol/kg-bw/day) developed 27% or 64% fewer pancreatic tumors, respectively, than controls [43].

It has been reported that hydroxylated derivatives are more potent than *d*-limonene in preventing DMBA-induced mammary tumors in rats when given during initiation; however, perillyl alcohol was not cited specifically [37]. Published data confirm that perillyl alcohol causes regression of established mammary tumors and prevents development of subsequent tumors. When offered at 2.5% in diet (*ca.* 8.2 mmol/kg-bw/day) for 10 weeks following the first palpable tumor, 81% (22/27, $p<0.01$) of rats exhibited complete regression of DMBA-induced primary mammary tumors compared with 31% of pair-fed controls, and subsequent tumor development was prevented [35]. Time to regression of the first tumor in the perillyl alcohol-treated group was also shorter by two-thirds compared with the control group. In contrast, a higher dose of *d*-limonene (10% in diet) produced a lower regression rate [7]. Finally, 2.5% perillyl alcohol in the diet completely prevented subsequent tumor development when administered after the appearance of the first DMBA-induced mammary tumor; the multiplicity of subsequent tumors in the positive control group was 1.5 [35].

Since the perillyl alcohol dose (2.5% in diet) used in the mammary tumor regression study above produced lower body weights than pair-fed controls, a dose-response study of 0.5–2% in diet (*ca.* 1.6–6.6 mmol/kg-bw/day) was initiated in rats with advanced DMBA-induced tumors (≥10 mm). Significant ($p<0.01$) complete plus partial regression rates were obtained at all doses (25–75%); however, 1% in the diet (3.3 mmol/kg-bw/day) was required for significant complete regression (35%) [35]. Interestingly, the perillyl alcohol dose (1% of diet, or *ca.* 3.3 mmol/kg-bw/day) that blocked formation of subsequent tumors in rats was 10 times higher than the dose (1,000 ppm in diet, or *ca.* 0.3 mmol/kg-bw/day) that was chemopreventive in the colon. However, in mammary tumor regression studies, treatment was only during the promotion/progression phase, while rats in the colon study were treated during both initiation and promotion.

Two published chemotherapy studies reported perillyl alcohol efficacy against human prostate cancer xenografts in athymic nude mice and pancreatic tumor xenografts in hamsters. In an abstract on the first study, two cycles of 450, 600, or 800 mg/kg-bw twice daily for 15 days slowed growth, but did not cause regression of established human prostate tumors in mice [24]. In the second study, 3% dietary perillyl alcohol (*ca.* 23.6 mmol/kg-bw/day) was fed to hamsters with established hamster pancreatic carcinomas; the tumor growth rate significantly slowed to less than half of the control rate and 16% of the tumors completely regressed compared with none in the control group [43]. *In vitro* experiments with human prostate and human pancreatic tumor cells showed similar inhibitory effects.

As part of the NCI, Chemoprevention Branch program to identify and validate intermediate biomarkers of cancer, two studies are investigating perillyl alcohol modulation of G-protein farnesylation. In the first, the incidence of BOP-induced Ki-*ras* mutations at codon 12 in premalignant (papillary hyperplasia, carcinoma *in situ*) and malignant pancreatic tumors is being assessed in male hamsters; changes in Ki-*ras* mutation incidence by perillyl alcohol will be correlated to effects on tumor incidence and multiplicity. In the second study, perillyl alcohol modulation of Ki-*ras* oncogene and RNA expression in NNK-induced lung tumors in female A/J mice will be measured.

Both perillyl alcohol and *d*-limonene are rapidly absorbed and efficiently metabolized to perillic acid and dihydroperillic acid in rats and dogs [5,44]; a comparison of rat and human metabolites of *d*-limonene suggests that the metabolism is similar [45]. *In vitro*, perillic acid was found to be the most potent inhibitor of isoprenylation in NIH 3T3 cells among a series of *d*-limonene derivatives; it was five-fold more effective than *d*-limonene (*n.b.*, NIH 3T3 cells do not metabolize monoterpenes) [6,8]. Perillyl alcohol was also an effective inhibitor of NIH 3T3 cell growth (IC_{50} = 1.3 mM).

PRECLINICAL SAFETY STUDIES

The NCI, DCTDC has completed 14- and 28-day preclinical toxicity and ADME studies in rats and dogs, as well as 14-day toxicity tests in nude mice preliminary to chemotherapy efficacy studies [4]. NCI, Chemoprevention Branch-funded 90-day tox-

icity studies of perillyl alcohol rats and dogs have also been completed.

Safety: Results are available from the NCI, DCTDC-funded 14-day dose-range finding and 28-day toxicity studies in rats and dogs [4,44]. In the 14-day study, male Fischer 344 rats were administered 75–900 mg perillyl alcohol/kg-bw daily (0.5–6 mmol/kg-bw/day) in three divided doses (q8h, ig in soybean oil). At the highest dose, histopathological lesions included forestomach inflammation with hyperplasia and testicular epithelial degeneration. In the 28-day study in rats, perillyl alcohol doses were 200, 600 and 1,000 mg/kg-bw/day (1.3, 3.9 and 6.6 mmol/kg-bw/day) in soybean oil, administered ig in two divided doses due to its direct irritant properties. Adverse effects in the 600 and 1,000 mg/kg-bw/day dose groups included 12–13% body weight loss on days 4–10, increased ALT (32–79% in females at 600 mg/kg-bw/day and in both sexes at 1,000 mg/kg-bw/day), dose-related forestomach hyperplasia, and testicular atrophy. At the highest dose only, renal tubular degeneration (with rising BUN) and mortality (10% of males, 50% of females) concomitant with splenic atrophy (females only) and hepatocyte cytoplasmic vacuolization were also observed. All effects were reversible except testicular atrophy. The lowest dose, 200 mg/kg-bw/day (1.3 mmol/kg-bw/day), had no clinical or histological effects and the 28-day MTD was 600 mg/kg-bw/day (3.9 mmol/kg-bw/day) in two divided doses.

In the NCI, Chemoprevention Branch 90-day toxicity study in Fischer 344 rats, ig doses of 40, 120, or 400 mg perillyl alcohol/kg-bw/day (0.3, 0.8, or 2.6 mmol/kg-bw/day) in soybean oil caused no drug-related deaths, abnormal hematology/clinical chemistry, or histopathological lesions. Liver, kidney, and lung weights were increased in high-dose females, but the organs were not histologically abnormal. Dose-related hyperexcitement and clear mouth discharge were noted in all treated groups. Despite significant weight loss (10%) in the high-dose group, the MTD for males was ≥400 mg/kg-bw/day (2.6 mmol/kg-bw/day), but ≤400 mg/kg-bw/day for females because of the unknown significance of organ weight increases.

In the 14-day NCI, DCTDC study in Beagle dogs, males and females were administered 60, 300, 600, or 1,200 mg perillyl alcohol/kg-bw/day (0.4–7.9 mmol/kg-bw/day) in three divided doses formulated in soft gelatin capsules containing 250 mg of perillyl alcohol (1:1, w/w in soybean oil) [4,44]. No clinical signs of toxicity were observed during the 14-day dosing period except for dose-related increases in vomiting and soft/loose stools. (Similarly, in the DCTDC pharmacokinetics study described below, both dogs given 500 mg perillyl alcohol/kg-bw vomited, and dogs receiving 250 mg/kg-bw licked their lips excessively, which often precedes vomiting). At the high dose, leukocytosis and thrombocytosis were also observed. In the 28-day Beagle dog study, total doses were 300, 600 and 1,200 mg/kg-bw/day (2.0, 3.9 and 7.9 mmol/kg-bw/day), formulated in soft gelatin capsules and administered in three divided doses. One dog in the 1,200 mg/kg-bw/day group died on day 13 showing signs of gastrointestinal distress following consistent low food consumption; mild bone marrow atrophy and lymph node/tissue atrophy were also found in this dog. Emesis and abnormal stools were noted at all dose levels except at 300 mg/kg/day, with dose-related renal tubular degeneration, thymic atrophy and increased BUN at the two highest dose levels. The 28-day MTD in dogs was reported to be 600 mg/kg-bw/day (3.9 mmol/kg-bw/day) in three doses/day with a NOEL of 300 mg/kg-bw/day.

In the NCI, Chemoprevention Branch 90-day Beagle dog toxicity study, po doses (capsules) of 40, 120, or 400 mg perillyl alcohol/kg-bw/day (0.3, 0.8, or 2.6 mmol/kg-bw/day) were administered in soybean oil. Emesis was observed in all high-dose males and females (females were more sensitive than males); no deaths occurred. Mild anemia was observed in the 400 mg/kg-bw groups in week 13; PT, activated PTT, and albumin/globulin ratio were significantly increased in females at week 13, possibly from severe emesis. Gross necropsy revealed testicular atrophy in one high-dose male. The MTD was ≤400 mg/kg-bw/day.

Dose selection in a Chemoprevention Branch-funded intermediate biomarker study with A/J mice suggests that mice may be more sensitive than rats or dogs. Mice injected ip with ≥500 mg perillyl alcohol/kg-bw (3.3 mmol/kg-bw) were dead or comatose five minutes after dosing; mice treated with 100 or 200 mg/kg-bw/day ip (0.7 or 1.3 mmol/kg-bw/day) appeared intoxicated and showed dysplasia of the hind limbs for several hours after dosing. Changing the vehicle from 95% PBS/5% cremophor EL to tricaprylin resulted in a slight improvement. In NCI, DCTDC preclinical chemotherapy studies, nude

mice receiving 1 g perillyl alcohol/kg-bw/dose, ig, twice daily (1:1 in sesame oil; 13.1 mmol/kg-bw/day) lost weight (3 g) by day 10 of treatment and two of the three mice died on day 13 of treatment. In an efficacy study with nude mice bearing HT-29 xenografts, the same dose in soybean oil (1:1) (13.1 mmol/kg-bw/day) was more toxic; 70% (7/10) of the group died after two doses. At 1,800 mg perillyl alcohol/kg-bw/day in two doses, the mice became lethargic after three doses. Finally, in nude mice with PC-3 prostate tumor xenografts, 600 or 800 mg perillyl alcohol/kg-bw (3.9 or 5.3 mmol/kg-bw) caused early deaths (3/10 mice at 600 mg/kg-bw by day four and 6/10 mice at 800 mg/kg-bw after five doses).

ADME: Studies carried out by the NCI, DCTDC [4] indicate that perillyl alcohol, like its parent *d*-limonene [5,6], is rapidly absorbed after oral administration and metabolized extensively to perillic acid and dihydroperillic acid. Following a single ig dose of 250 mg/kg-bw perillyl alcohol (1.6 mmol/kg-bw) to a male Beagle dog, the plasma C_{max} for perillic acid reached 1,000 μM at 2.5 hours after dosing and remained >500 μM for 6 hours. Dihydroperillic acid was also observed at lower levels (30–36 μM) 3–6 hours after dosing. Neither perillyl alcohol nor methyl esters of perillic or dihydroperillic acid were detected in plasma. Low levels of the esters were reportedly found in rat blood after administration of 1 g *d*-limonene/kg-bw (7.3 mmol/kg-bw) [5]; however, the investigators indicated that they could have been formed *in vitro* during processing of blood samples. Perillic acid and dihydroperillic acid had a similar pharmacokinetic profile; the $t_{½}$s were 3.2 hours and 3.4 hours, respectively; however, the AUC for perillic acid was 21 times greater [46].

Multidose plasma pharmacokinetics of perillyl alcohol were evaluated in the NCI, DCTDC 28-day toxicity studies in rats and dogs. The parameters in rats at the MTD (600 mg/kg-bw/day divided into two doses) were: C_{max}=190.3 μM for perillic acid, t_{max}=2 hr, and elimination $t_{½}$=4–7 hr; C_{max}=124.7 μM for dihydroperillic acid, t_{max}=9 hr. For the same total daily dose given to dogs in three divided doses, C_{max}= 543.9 μM (males) and 434.4 μM (females), t_{max}=2 hr, and elimination $t_{½}$=2.2 hr; dihydroperillic acid C_{max}=18.8 and 21.9 μM for males and females, respectively and t_{max}=2 hr. The perillic acid C_{max} increased linearly with dose in both rats and dogs. Plasma levels of dihydroperillic acid were very different in the two species: 40–81% and 3–4% of the corresponding perillic acid values in rats and dogs, respectively.

A published study investigated plasma metabolites in rats following acute and chronic administration of perillyl alcohol [35]. Four hours following a single dose of 1 g/kg-bw ig (6.6 mmol/kg-bw), the total terpene concentration was 1,600 μM, with 1,130 μM as perillic acid and 80 μM as dihydroperillic acid. The methyl esters of these metabolites were also identified—380 μM perillic acid methyl ester and 10 μM dihydroperillic acid methyl ester; however, it is unknown if they were produced during analysis. In comparison, plasma concentration profiles differed after a single dose of 1 g *d*-limonene/kg-bw (7.3 mmol/kg-bw: 670 μM total terpenes, 270 μM perillic acid, 200 μM dihydroperillic acid, and 90 μM *d*-limonene).

Following dietary administration of 2% perillyl alcohol (*ca.* 6.6 mmol/kg-bw/day) for 10 weeks (equivalent to the 1 g/kg-bw acute dose), circulating total terpenes and perillic acid decreased to 820 μM and 480 μM, respectively; plasma dihydroperillic acid increased slightly to 230 μM [35]. In comparison, a higher dose of *d*-limonene (10% in diet or 36.7 mmol/kg-bw) resulted in only 270 μM total terpenes, 130 μM perillic acid, and 230 μM dihydroperillic acid after the same time period. If perillic acid is the cancer inhibitory metabolite, chronic dosing of perillyl alcohol in rats is potentially 20.7 times more active than *d*-limonene, since 3.7-fold higher plasma concentrations of perillic acid are obtained from a dose that is 5.6 times lower.

CLINICAL SAFETY: PHASE I STUDIES

NCI, DCTDC is sponsoring a Phase I clinical trial of perillyl alcohol in 30 cancer patients with advanced solid tumors. The regimen consists of one or more 28-day treatment cycles starting at a projected level of 2.4 g perillyl alcohol/m^2 in three divided doses (*ca.* 0.4 mmol/kg-bw/day) with dose escalation in three patient cohorts until dose-limiting toxicity is reached [4]. The objectives are to identify safe doses for a Phase II trial and characterization of human pharmacokinetics. The Chemoprevention Branch recently began a single-dose Phase I pharmacokinetic trial of perillyl alcohol in healthy women and those with a history of breast cancer (Dr. G. Thomas Budd, The Cleveland Clinic Foundation). The single-dose trial includes five dose levels ranging from 0.5–3.5 g/m^2 (0.1–0.6 mmol/kg-bw) with 6 patients/cohort;

blood and urine will be collected and analyzed at 24 and 48 hours, respectively. The second task, a three-month multidose trial, will follow with subjects from the same populations. The Chemoprevention Branch anticipates that results from preclinical and Phase I toxicity studies and ADME data from the NCI, DCTDC in addition to data from its Phase I pharmacokinetic trial will satisfy FDA requirements for initiation of short-term Phase II clinical trials.

CLINICAL EFFICACY: PHASE II/III STUDIES

The NCI, Chemoprevention Branch is considering two short-term Phase II clinical chemoprevention studies for 1997 (see Table I). In the first trial, perillyl alcohol would be administered to breast cancer patients with biopsy-proven DCIS or early stage cancer during the 2–4 week period between diagnosis and surgery. Modulation of intermediate biomarkers, primarily proliferation indices, would be evaluated. The second trial would involve modulation of intermediate biomarkers in prostate cancer patients scheduled for surgery during the 3–8 week period before surgery.

PHARMACODYNAMICS

Perillyl alcohol was >5 times more effective than *d*-limonene at inhibiting isoprenylation of small G-proteins [8] and at inducing tumor regression in rat mammary gland [35]. Regression of mammary tumors was achieved by 5% *d*-limonene in the diet (*ca.* 18.4 mmol/kg-bw/day) with little or no toxicity [27,30]; however, perillyl alcohol was equally effective at 0.5–1% of the diet (*ca.* 1.6–3.3 mmol/kg-bw/day). If MTDs for perillyl alcohol and *d*-limonene are 2.6 mmol/kg-bw/day (according to the Chemoprevention Branch 90-day toxicity study in rats) and 36.7 mmol/kg-bw/day [35], respectively, then the chemotherapeutic ratios would be 1.6 and 2.0, respectively, in this model. However, in the AOM-induced rat colon carcinogenesis study, the Chemoprevention Branch reported significant inhibition of tumors by 1,000 ppm perillyl alcohol (*ca.* 0.3 mmol/kg-bw/day), which results in a chemopreventive ratio of 8. An explanation of the discrepancy between the chemotherapeutic and chemopreventive doses in the mammary and colon studies was suggested by the ability of *d*-limonene to induce hepatic detoxification enzymes [17]. Since perillyl alcohol exposure in the colon study was during initiation with AOM, as well as promotion/progression, carcinogen activation may have been inhibited. In the mammary tumor regression study with the direct-acting carcinogen MNU, the agent was given only during promotion/progression.

Assuming that rat data can be extrapolated to humans, an estimate of effective chemopreventive doses of perillyl alcohol can be calculated. If regression of mammary tumors in rats can be accomplished by 0.5% diet (1.6 mmol/kg-bw/day) and chemoprevention of colon cancer by 1,000 ppm (0.3 mmol/kg-bw/day) in rats, chemoprevention of human breast tumors may occur at doses between 0.1 and 0.25 g/kg-bw/day (0.7 and 1.6 mmol/kg-bw/day). If chemopreventive doses are lower than tumor regression doses, the range of doses planned for the single-dose Phase I clinical trial (0.5–3.5 g/m^2 or 0.1–0.6 mmol/kg-bw) are appropriate and well below the MTD level of 28 g/day (2.6 mmol/kg-bw/day) for men and 25.5 g/day (2.6 mmol/kg-bw/day) for women extrapolated from Chemoprevention Branch preclinical toxicity studies in rats and dogs (*ca.* 400 mg/kg-bw/day or 2.6 mmol/kg-bw/day).

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

Based on published mechanistic and biochemical studies, possible drug effect measurements to be evaluated include products of the mevalonic acid metabolic pathway [13,47], serum M6P/IGF-II receptors, and TGFβ [22].

Safety Issues

The NCI, DCTDC and the Chemoprevention Branch DCPC are cooperating in developing appropriate preclinical toxicity data and Phase I safety data to carry out chemotherapy and chemoprevention efficacy trials. Toxicity studies of 14-, 28- and 90-day duration in rats and dogs are complete. Published studies and data developed by the NCI, DCTDC and Chemoprevention Branch thus far indicate that the agent has low toxicity; the primary adverse effects involve the gastrointestinal tract, kidneys and possible testicular atrophy. In the 90-day Chemoprevention Branch toxicity study which administered 40, 120, and 400 mg/kg-bw/day perillyl alcohol (0.3, 0.8, and 2.6 mmol/kg-bw/day) ig, dose-related hyperexcitement and salivation occurred in rats which may have resulted from direct irritant properties. This was

demonstrated in dogs by emesis in the high-dose group (400 mg/kg-bw/day, or 2.6 mmol/kg-bw/day). The MTDs for female rats and dogs of both sexes were *ca.* 400 mg/kg-bw/day (2.6 mmol/kg-bw/day/day), which is greater than the dietary dose which regressed mammary tumors (1.7 mmol/kg-bw) and the effective chemopreventive dose in the AOM-induced colon cancer model (0.7 mmol/kg-bw/day). Because perillyl alcohol may be effective against both initiation and promotion/progression phases of carcinogenesis, it is anticipated that chemopreventive doses administered on this schedule will be lower than those required for chemotherapy.

In published studies, *d*-limonene, the parent compound of perillyl alcohol, has been reported to induce a hyaline droplet renal nephropathy in adult male rats. The protein responsible for this pathology has been identified and is unique to certain strains. Although the protein is not present in humans, members of the same superfamily have been found; thus it is possible, but not probable that this effect may occur in humans [48]. The studies performed by the NCI, DCTDC and NCI, Chemoprevention Branch suggest that renal nephropathy in both sexes may be induced by doses above 400 mg/kg-bw/day.

Prior to carrying out clinical chemoprevention trials of more than one-year duration, carcinogenicity and reproductive system studies should be initiated.

Pharmacodynamics Issues

Preclinical efficacy data for perillyl alcohol in rat mammary gland are from a chemotherapy study; however, the relationship between chemopreventive and chemotherapeutic doses has not been defined. Significant regression of rat mammary carcinomas was seen at 0.5% (1.6 mmol/kg-bw) in the diet [35] administered during promotion/progression phases only. When the drug was administered during initiation and promotion/progression phases of carcinogenesis in the colon carcinogenesis model, the effective dose was 1,000 ppm (0.3 mmol/kg-bw), suggesting that chemopreventive doses administered by the same schedule may be lower than those required for chemotherapy.

Efficacy in liver and mammary gland appear to be related to tissue disposition of active metabolites and the mechanism of perillyl alcohol itself, suggesting that breast, liver, and lung are primary targets for chemoprevention studies of the agent. The NCI, Chemoprevention Branch is currently funding preclinical efficacy studies of perillyl alcohol in rat mammary glands, mouse lung, hamster pancreas, and mouse skin. The results of these studies may help to define appropriate targets for chemoprevention.

Regulatory Issues

Perillyl alcohol has GRAS status as a flavoring agent and is an approved food additive; however, since the Chemoprevention Branch and DCTDC clinical trials are administering higher doses than food additive intakes, safety trials are required before further evaluation can be undertaken. An IND has been approved for a Phase I single dose clinical trial in females at risk for breast cancer, followed by a multidose trial in the same population.

Supply and Formulation Issues

Perillyl alcohol is available from several commercial sources. The NCI, DCTDC [4] has found a supplier (Nippon Terpene Co., Kobe, Japan) of >97% pure compound that can provide sufficient bulk drug for clinical studies; all DCTDC and Chemoprevention Branch IND-directed studies will be carried out with the same supply of perillyl alcohol. The NCI, DCTDC [4] has developed a soft gelatin capsule formulation containing 250 mg perillyl alcohol diluted 1:1 (w:w) with soybean oil which is available for the Chemoprevention Branch Phase I trial. Soybean oil is the vehicle of choice because it has low susceptibility to oxidation and will provide a long shelf-life for the formulation.

The number of 250 mg capsules required for the higher doses may jeopardize compliance and compromise the digestive system. At 3.5 g/m^2/day, the highest of the planned doses, the subject would have to consume 24 capsules. Consideration should be given to more concentrated capsules or a different mode of administration for the multidose study.

Intermediate Biomarker Issues

Perillyl alcohol has been shown to inhibit tumor cell proliferation and *ras* activity and to induce differentiation and apoptosis *in vitro*. In the DMBA-induced rat mammary gland model, regression of established tumors by the agent was related to induction of M6P/IGF-II and TGFβII receptors. Since *ras* activation is not detected at significant levels in human breast and prostate cancers, the hypothesis that induction of M6P/IGF-II and TGFβ receptors may be

downstream effects of terpene-induced alterations in prenylated signalling molecules is being investigated [10]. M6P/IGF-II and TGFβ proliferation markers would be useful endpoints in short-term Phase II clinical trials in patients diagnosed with these diseases. Apoptosis may also be evaluated using lipocortin-1 and neuroligin-1 induction as markers [21]. Differentiation biomarkers may also be useful study endpoints. Rat mammary tumors regressed by perillyl alcohol are predominantly stromal tissue, rather than the glandular, dysplastic tumors with little stroma seen in the carcinogen controls. Histological observations suggest that regression is accomplished by redifferentiation from carcinoma to fibroadenoma [23]. Perillyl alcohol inhibited growth of human pancreas and colon carcinoma cells *in vitro*. Development of tumors in these tissues has been related in part to *ras* activation [reviewed in 48,49]. In possible future chemoprevention trials in high-risk colon and pancreatic cancer cohorts, *ras* activation should be evaluated as a genetic biomarker.

Clinical Studies Issues

The difference in activity between perillyl alcohol and *d*-limonene may be explained in part by metabolite pharmacokinetics. Although both terpenes are rapidly metabolized to similar compounds, the plasma levels of perillic acid and dihydroperillic acid (the putative active metabolites) are higher for perillyl alcohol-fed rats. Rat ADME data [5] indicate that orally administered *d*-limonene and its metabolites (*e.g.*, perillic acid) are well-distributed to liver, kidney, lung, and, because of lipophilicity, primarily to adipose and mammary tissue. *d*-Limonene is also reported to be similarly metabolized by rats and humans [45]. Therefore, by analogy to *d*-limonene, the same tissues may represent likely targets for chemopreventive activity of perillyl alcohol in humans. Because of the demonstrated chemopreventive efficacy of *d*-limonene in rat mammary gland and lung and the potential for concentration of metabolites in these tissues, breast and lung are important sites for chemoprevention clinical trials of perillyl alcohol. The cytostatic effect of perillyl alcohol on xenografted human prostate cancer cells in athymic nude mice in addition to its ability to modulate important prostatic receptors M6P/IGF-II and TGFβ, suggest that the prostate should also be considered [38]. The NCI, Chemoprevention Branch preclinical studies of perillyl alcohol have demonstrated chemopreventive efficacy in the colon and are evaluating lung, pancreas and skin, other sites where *ras* isoprenylation may be a dominant factor.

It is expected that NCI, DCTDC and Chemoprevention Branch Phase I studies of perillyl alcohol will provide sufficient support for short-term Phase II chemoprevention clinical trials evaluating modulation of intermediate biomarkers. The Chemoprevention Branch is considering such trials in presurgical DCIS or early breast cancer (treated for 2–4 weeks prior to surgery) and prostate cancer patients (treated for 3–8 weeks prior to surgery) (Table I). Potential proliferation biomarkers in these trials include MIB-1, S-phase fraction, TGFβ, and apoptotic index. Based on the preclinical chemopreventive activity of *d*-limonene, lung, colon and pancreas may also be appropriate sites for future chemoprevention trials.

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Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase I (Safety, ADME)					
NO1-CN-55131 Single Dose Pharmacokinetics of Perillyl Alcohol in Healthy Subjects with a History of Breast Cancer (Dr. G. Thomas Budd, The Cleveland Clinic Foundation) 6/95–6/97 IND 51,202	....	Single dose: Healthy females at high risk for breast cancer or Stage I–IIIa breast cancer in remission ≥2 years and no treatments for at least 3 months 30 subjects	Single dose: 0.5, 1.0, 1.67, 2.5 and 3.5 g/m^2 Multidose: Dose chosen from single dose for 3 months	Safety, pharmacokinetics	Study in progress
Planned Study Perillyl Alcohol in Breast Neoplasia: Administration During the Period Between Diagnostic Core Biopsy and Definitive Surgery	Breast	Women with DCIS or mammogram **suspicious for early stage breast cancer** 100 patients (50/arm)	Dose selected from Phase I for 2–4 week period between diagnostic biopsy and surgery 2–4 weeks	Intermediate biomarkers (*e.g.* DCIS grade, nuclear poly-morphism, ploidy, PCNA, MIB-1, S-phase fraction,)	Study not yet designed
Planned Study Perillyl Alcohol in Patients with Prostate Cancer in the Period Prior to Radical Prostatectomy (Presurgical Period): Modulation of Surrogate Endpoint Biomarkers	Prostate	Prostate cancer patients scheduled for radical prostatectomy 100 patients (50/arm)	Dose selected from Phase I for 3–8 week period between diagnostic biopsy and surgery	Intermediate biomarkers (*e.g.*, PIN grade, nuclear poly-morphism, ploidy, PCNA, MIB-1)	Study not yet designed

PERILLYL ALCOHOL DEVELOPMENT STATUS