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NCI, DCPC

Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN: INDOLE-3-CARBINOL

DRUG IDENTIFICATION

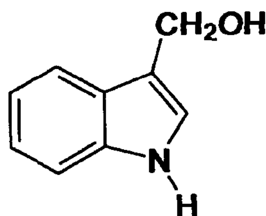
CAS Registry No.: 700-06-1

CAS Name (9CI): 1*H*-Indole-3-methanol

Synonyms: Indole-3-methanol

Molecular Wt.: 147.17

Structure:



EXECUTIVE SUMMARY

Indole-3-carbinol (I3C) is a non-nutritive component of cruciferous vegetables [1]; consumption of these vegetables has been associated with decreased risk for cancer in humans [2]. When administered orally to experimental animals, I3C is an effective chemopreventive agent against a wide variety of carcinogens. Much of I3C's chemopreventive activity stems from its capacity to induce both phase I and II enzymes involved in carcinogen metabolism [*e.g.,* 3–7]. Indeed, the drug is especially efficacious when administered before or during carcinogen administration [8,9] and has been shown to decrease carcinogen binding to DNA [*e.g.,* 9,10]. Although many agents, including I3C, induce several phase II enzymes [11], the indole's capacity to induce multiple families of cytochrome P450-dependent isozymes is relatively unique. I3C induces TCDD-type (CYP1A family), phenobarbital-type (CYP2B family) and dexamethasone-type (CYP3A family) isozymes [5,6,12]. Grubbs and coworkers report that even after 15 weeks of exposure, the livers of Sprague-Dawley rats continue to respond to I3C by demonstrating high levels of phase I and II enzyme induction [12]. Many of these enzyme-inducing effects are not due to the parent compound, but to condensation products produced upon contact with gastric acid [13– 15]. Certain of these oligomers have been shown to interact with the Ah receptor and presumably this is the pathway involved in induction of the CYP1A family and certain other drug metabolizing enzymes. In addition some of the oligomers formed also demonstrate chemopreventive activity [8,16]. Formation of numerous acid condensation products with differential enzyme-inducing activities [15,17] may explain, at least in part, the pleiotropic effects of I3C.

I3C also prevents spontaneous tumorigenesis and tumor-induction by direct-acting carcinogens in estrogen-responsive tissues [18,19]. Increased estrogen conjugation and excretion via induction of Phase II enzymes could contribute to these effects [20]. The I3C acid condensation product indolo-[3,2*b*]carbazole (ICZ) decreases estrogen receptor levels in breast cancer cells in culture [21]. Although ICZ is

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also a weak estrogen and binds to the estrogen receptor, the action of this Ah receptor agonist is primarily antiestrogenic in human breast cancer cells. An additional mechanism for the chemopreventive effects of I3C in estrogen-responsive tissues has been attributed to modulation of cytochrome P450-dependent estradiol metabolism. Estradiol is metabolized via two mutually exclusive pathways. Hydroxylation at C-2 yields 2-hydroxyestrone; hydroxylation at C-16α yields 16α-hydroxyestrone which is reduced to form estriol. 16α-hydroxyestrone covalently binds the estrogen receptor, decreases its degradation and has estrogenic effects similar to estradiol. Increased estradiol-16α-hydroxylation has been associated with increased risk for breast cancer in women [22] and mice [23], and 16α-hydroxyestrone has been reported to be genotoxic to mammary cells [24]. Because attempts to directly decrease estradiol 16α-hydroxylation have been unsuccessful, attention has focused on increasing the alternate 2-hydroxylation pathway [25]. In rodents, I3C increases estradiol 2-hydroxylation under the same experimental conditions in which it reduces mammary [18] and endometrial [19] tumor development. The drug also enhances estradiol 2-hydroxylation in humans [25] apparently through CYP1A family by oligomers of I3C.

I3C is an effective chemopreventive agent in several animal cancer models, including rat mammary gland, liver, tongue, and endometrium and mouse mammary gland, colon, lung, and forestomach. These studies are adequate to support its clinical development. Additional studies in a mouse lung model are in progress. Based on the ability of I3C to both inhibit DMBA-induced mammary cancer in rats, and enhance estradiol 2–hydroxylation, the target organ of highest clinical interest is the breast.

Twenty-eight and 90-day toxicity studies in rats and dogs have been completed. No NOEL was defined in the 28-day rat or dog study or in the 90-day dog study (down to and including 4 mg/kg-bw/day, the lowest dose level tested). In the 90-day rat study, 4 mg/kg-bw/day (*ca.* 0.03 μmol/kg-bw/day) was a NOEL. A three-month Phase I trial in the breast is planned; however, additional preclinical toxicity studies may be needed prior to initiation of clinical trials. No pharmacokinetic data on I3C have been developed. In the stomach, the drug is rapidly converted to oligomers. Development of methods to measure plasma levels of drug metabolite(s) is being undertaken preparatory to initiation of clinical studies.

Some studies have found the drug to be unstable in specific media commonly used to deliver test articles to experimental animals; dosing conditions may also influence outcome. Development of a stable formulation and standardized dosing conditions for further preclinical and clinical studies may be needed.

Bulk I3C is available from a variety of sources including Gallard Schlesinger, Sigma, and Aldrich. It is anticipated that the investigational drug supply will consist of hard gelatin capsules in appropriate strengths.

PRECLINICAL EFFICACY STUDIES

I3C has demonstrated chemopreventive activity in several different animal models of carcinogenesis; these data are sufficient to support clinical development of the agent. In studies sponsored by the Chemoprevention Branch, the drug (50 and 100 mg/day ig, or *ca.* 1.13 and 2.26 mmol/kg-bw/day) was highly effective against DMBA-induced rat mammary tumors when administered either during initiation or during both initiation and promotion. I3C was also active against MNU-induced mammary tumors at doses of 50 or 100 mg/day by gavage (*ca.* 1.13 or 2.26 mmol/kg-bw/day) when administered throughout the course of the experiment, from one week prior to carcinogen to sacrifice at six months post-carcinogen. In both the DMBA and MNU studies, latency was increased [12]. I3C also inhibited MNU-induced mammary tumors when given three days after initiation [26], although it did not appear to be as effective as when it was administered prior to MNU. Further support for I3C's chemopreventive potential in the breast comes from independent studies of activity against DMBA-induced mammary tumors in rats [8] and spontaneous mammary tumors in mice [18]. Increased estradiol 2-hydroxylation, a putative biomarker of decreased breast cancer risk, has been observed in mice [18] and rats [27] upon oral dosing.

Chemoprevention Branch-sponsored studies have also demonstrated efficacy against MAM acetate-induced mouse colon tumors (1.75 and 3.5 g/kg diet, or *ca.* 1.53 and 3.06 mmol/kg-bw/day) and AOM-induced colonic aberrant crypt foci (ACF), putative biomarkers of colon carcinogenesis. Published studies have shown that I3C inhibits spontaneous endometrial carcinogenesis in rats [19], PhIP-induced

ACF in rats [28], and tumors induced by NQO in rat tongue [29], NNK in mouse lung [9], and B(*a*)P in mouse forestomach [8]. The drug also prevents development of tumors induced by DEN in rat liver [30] and AFB_1 in trout liver [31], as well as DEN- [30,32] and multi-carcinogen-induced [33] premalignant liver lesions in rats. I3C is currently being evaluated by the Chemoprevention Branch for its ability to inhibit cigarette smoke-induced DNA adducts in rats.

PRECLINICAL SAFETY STUDIES

Safety: Acute oral toxicity was determined in a Chemoprevention Branch-sponsored rat study. Following preliminary range finding, the definitive test was conducted with a single dose of 2,250 mg/kg-bw, the highest dose which could be administered due to volume limitations and quality of the suspension. Transient lethargy and rough coat were observed on the day of dosing. Since there was no mortality, the LD_{50} was estimated to be >2,250 mg/kg-bw (>15.3 mmol/kg-bw).

Chemoprevention Branch-sponsored 28-day and 90-day studies in rats and dogs have been completed. A NOEL was not established in rats dosed with 20, 60, 200, 600, or 2,000 mg/kg-bw/day ig (0.14, 0.41, 1.36, 4.08, or 13.6 mmol/kg-bw/day) for 28 days. Toxic effects were observed in the hematopoietic system, liver, hair coat, and testes. The dose-limiting toxicity in females was increased relative spleen weight; in males decreased relative testes weight was observed at all doses tested. Based on this study, doses of 4, 20 and 100 mg/kg-bw/day (0.03, 0.14 and 0.68 mmol/kg-bw/day) were chosen for the 90-day study in rats.

In the 90-day rat study, the liver was identified as a target organ, manifested as increased absolute and relative liver weight at the mid- and high-dose levels and histopathological changes in high-dose males and females. Hepatic changes were consistent with induction of smooth endoplasmic reticular (SER) enzymes and thus represented an adaptive hepatic response to xenobiotic challenge. The frequency and severity of changes was greater in males than females. The death of one high-dose animal per sex as potentially treatment related. Relative kidney weights were increased in mid- and high-dose males and females, but these findings were not supported by changes in clinical or microscopic pathology. Absolute and relative spleen weights were increased in mid- and high-dose males, but there were no associated clinical or microscopic pathological changes.

In the dose-selection portion of the Chemoprevention Branch-sponsored rat mammary chemoprevention study, six weeks treatment with 200 mg/day, 5 days/week, ig (*ca.* 3.24 mmol/kg-bw/day) caused a 10% reduction in body weight and a 20% increase in liver/body weight ratio; 100 mg/day (*ca.* 2.26 mmol/kg-bw/day) was identified as the MTD in these female Sprague-Dawley rats [12]. During the chemoprevention phase, although body weights were not altered, liver weight/body weight ratios were increased in 33% of rats at 100 mg I3C/day (*ca.* 2.26 mmol/kg-bw/day), 5 days/week, for 15 weeks, compared with vehicle-treated controls. The histological appearance of the livers of these rats was normal.

A NOEL was not established in a Chemoprevention Branch-sponsored 28-day toxicity study in male and female dogs administered 15, 50 or 150 mg/kg-bw/day (0.10, 0.34 or 1.02 mmol/kg-bw/day) of I3C in gelatin capsules. Diarrhea was observed at all doses tested; vomiting, often containing material assumed to be blood, was noted in high-dose groups. Lower body weight and anemia observed in high-dose groups was attributed to these gastrointestinal disturbances. Thymic atrophy was noted in both sexes treated with the high dose and testicular degeneration was observed in high-dose males.

As in the 28-day study, no NOEL was defined in the 90-day dog study with I3C (initiated using dose levels of 4, 20 and 100 mg/kg-bw/day, or 0.03, 0.14 and 0.68 mmol/kg-bw/day gelatin capsules). The gastrointestinal tract and kidney were identified as the target organs. At the 100 mg/kg-bw/day dose level, weight loss and reduced food consumption were noted in both sexes. Severe gastrointestinal effects were observed in the mid- and high-dose groups, and were more severe in males than females. The observed toxicity necessitated cessation of dosing from days 30–37; thereafter, the dose was reduced to 50 mg/kg-bw/day (*ca.* 0.34 mmol/kg-bw/day). After 50% reduction of the high dose, decreased weight gain, occasional dehydration, pale gums, lethargy and emaciation were noted in the high-dose group. These clinical signs were considered to be related anemia and rebound thrombocytosis, but were not associated with any histopathology indicative of gastrointestinal irritation or ulceration. Histopathological changes were seen in high-dose males, including vacuolation of renal tubular epithelium and gastric

parietal cell epithelium, and prostatic and thymic atrophy.

The observed effects on male reproductive organs were considered potentially secondary to test article-related stress. Microscopic vacuolation was considered likely to represent a reversible change, but in the absence of a recovery phase to the study, the nature and safety implications of this could not be evaluated. A number of histopathologic changes were seen at all dose levels tested. A NOEL was not established based on vacuolation of the gall bladder epithelium in both sexes and vacuolation of the adrenal cortical epithelium in females at all treatment levels. The authors noted that cellular vacuolation in the kidney, gallbladder and stomach were consistent with dilation and/or proliferation of SER. Many cytochrome P450 inducers are known to induce proliferation of SER [34].

No two-year carcinogenicity studies have been funded by NCI, Chemoprevention Branch or were identified in the literature. I3C alone was not mutagenic with or without metabolic activation in Ames *Salmonella typhimurium* strains TA98 and TA100, or in CHO cells [35–37]. Because of the limited doses tested in these mutagenicity studies and the potential inadequacy of *in vitro* experiments to reflect the *in vivo* situation, these genotoxicity results are inconclusive.

Some evidence of tumor enhancement by I3C has been reported, especially when the drug was administered subsequent to a carcinogen. These include enhanced DEN- [32] and AFB_1-induced preneoplastic lesions in rat liver [unpublished results cited in 6], DMH-induced cancers in rat colon [38], TPA-induced ODC activity in mouse skin [36], and AFB_1- and DMBA-induced cancers in trout liver [39,40]. None of these studies is sufficient to establish a tumor-enhancing effect of the drug. Synergistic toxicity between I3C and the carcinogens MAM acetate and OH-BBN, (*e.g.*, decreased body weight, high mortality) has been observed in chemoprevention studies conducted by the Chemoprevention Branch.

In a published report, I3C was not teratogenic in rats treated sc on gestation days eight and nine at doses of 200 or 300 mg/kg-bw (*ca.* 4.53 or 6.79 mmol/kg-bw). A significant decrease in fetal weight was observed at the lower, but not the higher dose. No significant effects on the number of live fetuses/dam or the number of fetuses resorbed were observed [41].

ADME: An NCI, Chemoprevention Branch-sponsored rat pharmacokinetic study was conducted, but the samples have not been analyzed. I3C is unstable in acidic medium; this has hindered the analysis of plasma drug levels. In published reports, the acid condensation products 3,3'-diindolylmethane, 2,3-bis[3-indolylmethyl]indole, indolo[3,2-*b*]-carbazole, 2-(indol-3-ylmethyl)-3,3'-diindolylmethane, and 5,6,11,12,17,18-hexahydrocyclonona[1,2-*b*:4,5-*b*':7,8-*b*'']triindole were found in the stomach and/or small intestinal contents or tissues of rats treated with I3C by gavage; no parent compound was detected [14,17]. De Kruif *et al.* also identified 3,3'-diindolylmethane and 2,3-bis[3-indolylmethyl]indole in rat liver [17]. Low levels of indolo[3,2-*b*]carbazole (0.001% of a dose of 73.5 mg/kg-bw, or *ca.* 0.002 mmol/kg-bw) were found in feces 20 hours after treatment [14]. In model studies, the formation of oligomers was highly pH dependent [17].

Disposition and excretion of I3C equivalents after oral administration of radiolabeled I3C to male Fischer 344 rats have been studied by Stresser and colleagues [42]. The test article was incorporated in diet at 2,000 ppm or administered by gavage for seven days. The authors report that both regimens provided a comparable daily dose of *ca.* 1 mmol/kg-bw, a dose known to be anticarcinogenic in the rat. In the diet, radiochemical purity decline from *ca.* 85 to 40% over 24 hours at ambient temperature. In any dietary exposure to I3C, the degree of polymerization would be influenced by diet, pH, water activity, subject age, and storage temperature for the test chemical.

At least 24 different I3C-derived compounds were found in the ethyl acetate extracts of liver homogenates (<20% of I3C equivalents extractable) after ig dosing. Two of the three predominant peaks represented a dimer and a trimer; no parent compound was detected. A novel metabolite was identified as 1-(3-hydroxymethyl)-indolyl-3-indolymethane (HI-IM). The highest levels of identified metabolites and three unidentified oligomers (*ca.* 1–13 μM) were measured 1.5–6 hours post-dosing. Peak levels in liver (2.45% of delivered dose) and blood were measured six hours post-dosing. With *ca.* 50% of delivered dose accounted for by measured levels in tissues, urine, and gastrointestinal tract contents, 50% was considered as remaining in the carcass.

In the dietary study, steady-state urinary excretion was attained after 40 hours, and fecal excretion after 112 hours. Urinary and fecal excretion accounted for

ca. 75% of the administered dose, with fecal excretion predominating (77%). It was not determined whether the remaining 25% of delivered I3C was not absorbed from the gastrointestinal tract, or whether biliary excretion is a major pathway of elimination. After seven days on labeled diet, liver levels of I3C equivalents (1,154 µM) were *ca.* 3.6 times blood levels. The authors point out that the individual rat hepatic concentrations of three Ah receptor agonists, following delivery of an anticarcinogenic dose of I3C, fail to account for observed CYP1A induction *in vivo*. That is, neither the dimer, the trimer, nor ICZ alone reach sufficient concentrations in liver to account for CYP1A induction by dietary I3C, or to inhibit bioactivation of aflatoxin B_1, but the combination of I3C derivatives in the liver may be sufficient to account for both these activities.

CLINICAL SAFETY: PHASE I STUDIES

No clinical studies have been conducted by the NCI, Chemoprevention Branch. A three-month Phase I trial in women at high risk for breast cancer is planned. In the published Phase II trial discussed below [25], no obvious changes in hematologic or clinical chemistry parameters were observed during the three-month trial; unfortunately, statistical analyses were not performed. Patient complaints were reportedly limited to increased gastrointestinal motility.

CLINICAL EFFICACY: PHASE II STUDIES

In two published studies, Bradlow and coworkers examined the ability of oral I3C to enhance estradiol 2-hydroxylation, a proposed intermediate endpoint for breast cancer prevention. In the first uncontrolled study, a small group (n=12) of men and women were administered 6–7 mg I3C/kg-bw qd (*ca.* 0.6–0.7 µmol/kg-bw/day) for seven days [43]. The second study was a randomized clinical trial and utilized placebo and cellulose control groups. This trial was also controlled for phase of the menstrual cycle, which has been shown to influence estrogen hydroxylation patterns [44]. Women (n=20) were administered 400 mg I3C qd in capsules (*ca.* 0.04 mmol/kg-bw/day) for three months [25]. Significant increases in estradiol 2-hydroxylation (as evidenced by increased urinary 2-hydroxyestrone/estriol levels) were observed in both studies. In the second study, this increase was evident by the first time point (one month) and maintained over the course of the three-month trial. In 3/20 (15%) subjects, no changes were observed; these subjects were considered non-responders. It should be noted that in the second study, baseline estradiol levels (although showing very large variations) were substantially higher in the I3C treatment group than in the placebo and cellulose-treated control groups. Although these levels decreased to those seen in the control groups by the end of the study, this potential confounding factor was not addressed by the investigators. Although no changes in the hematology or serum chemistry parameters were obvious upon review of the published data, the investigator reported that cholesterol values were slightly elevated in the women receiving I3C. As noted above, no statistical analyses were performed.

PHARMACODYNAMICS

In preclinical studies, I3C inhibited carcinogenesis in rat mammary glands at doses of 1.13 and 2.26 mmol/kg-bw/day. Results from the 90-day toxicology studies indicate that a NOEL was not achieved at doses as low as 0.03 mmol/kg-bw/day in dogs; a NOEL was established at or above the low dose (0.03 mmol/kg-bw/day) in rats. In humans, increased estradiol 2-hydroxylation was achieved at similar levels (0.04 mmol/kg-bw/day); at this dose no significant toxicities were reported. Comparisons between species are difficult because of the limited pharmacokinetic information available at this time. Preclinical efficacy and modulation of estradiol 2-hydroxylation may need to be established at lower doses.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

To further the clinical development of I3C, valid drug effect measurements should be developed. The most obvious are activities of phase I or II enzymes induced by the drug. Especially dramatic effects have been observed on induction of CYP1A in several tissues; ethoxyresorufin *O*-deethylase activity has been employed to measure the inductive effects of I3C on this isozyme in rodents. Differential effects have been observed in different tissues [6,15]. For use in a clinical setting, a non-invasively accessible tissue in which the drug effect could be measured should be determined. Another potential drug effect measure is enhancement of the 2-hydroxylation of estradiol, which can be measured in urine. Methods used to

measure production of hydroxylated estrogen metabolites would need to be standardized.

Safety Issues

Subchronic preclinical toxicology studies in dogs have identified significant toxicities associated with oral administration of I3C; furthermore a NOEL has not been established. The gastrointestinal effects seen in dogs were also noted in a published clinical study; however, the severity in humans appears to be less. Additional genetic toxicity testing may be required. The possible enhancing effects of I3C on chemical carcinogenesis and toxicity should be investigated, especially in light of the wide variety of enzymes induced by the drug. Both inhibitory and enhancing effects on tumorigenesis, dependent on time relative to carcinogen administration, are well-known for other phase I inducers (*e.g.*, TCDD [45,46] and phenobarbital [47]). The fact that the I3C acid condensation product ICZ has both antiestrogenic and estrogenic activities also may have safety implications [21].

Pharmacodynamics Issues

Acid conditions in the stomach cause polymerization of I3C. Metabolites that can be measured in serum or urine must be identified and reliable methods for their quantitation developed. Specific pH conditions can substantially influence the formation of acid condensation products. Because gastric contents are known to influence pH, variations in gastric contents could substantially affect the formation of I3C oligomers. Different acid condensation products have been shown to have different effects on the induction of various enzymes. Thus, standardized conditions for administration of I3C should be developed. Indeed, the dramatic chemopreventive activity observed in Chemoprevention Branch-sponsored rat mammary gland studies was theorized to result from the specific conditions used for drug administration. In that study, I3C was administered ig under special conditions because it was unstable in the feed (see Supply and Formulation Issues, below).

These observations also suggest careful interpretation of *in vitro* studies where acid conditions do not mimic those encountered in the stomach, or in which acid condensation products are not employed. In this regard, it should be noted that *in vitro* studies of I3C stability indicate that it is partially converted to oligomeric products, even under conditions of near neutral pH [15]. However, it is clear that effects observed after intraperitoneal administration of the parent compound are substantially different than after oral administration [13]. Characterization of I3C polymerization products under various culture conditions and comparison with products formed under *in vivo* conditions may be needed to facilitate mechanistic studies.

Regulatory Issues

An IND will be filed by the Chemoprevention Branch. Anticipated concerns include specifications for the clinical trial drug supply that will reflect expected final product specifications and the specifications for the test article used in pivotal preclinical toxicology studies, dose selection for Phase I and II trials, development of methods to measure blood levels of what are determined to be the biologically active moieties of concern, correlation of toxicities in preclinical studies to blood levels, and the potential for synergistic toxicity with other chemicals.

Intermediate Biomarker Issues

All available evidence does not support the hypothesis that increased estradiol 2-hydroxylation is protective for breast cancer [*e.g.*, 48,49]. Critical examination and validation of this hypothesis should be undertaken. Methods used to measure production of hydroxylated estrogen metabolites must be standardized; different methods, (*e.g.*, radiometric *versus* GC-MS) have given significantly different results [50]. Confounding factors, such as timing of sample collection during menstrual cycle [44] and diet [25] must be controlled.

Supply and Formulation Issues

Bulk I3C is available from a variety of sources including Gallard Schlesinger, Sigma, and Aldrich. It is anticipated that the investigational drug supply for clinical trials would consist of hard gelatin capsules in appropriate strengths (initially, 100 mg capsules).

In the Chemoprevention Branch-funded rat mammary study, I3C mixed in the diet, or in corn oil for ig administration, was found to decompose within hours. Therefore it was administered ig after dissolving in 100% degassed ethanol and then mixing in corn oil just prior to dosing. In toxicology studies, good 24-hour stability was reported for 50 mg I3C/ml in carboxymethyl cellulose suspension. I3C is known to

be unstable in acidic media and variations in pH change the nature of the acid condensation products formed. Different acid condensation products have been shown to have different biological activities. Thus, standard formulations and conditions to be used in preclinical efficacy and toxicity testing must be developed. Additionally, these formulations and methods should be appropriate for clinical studies, as the test material used for preclinical toxicology must be representative of clinical drug supplies and adequately meet the specifications for the eventual marketed product.

Clinical Studies Issues

Initiation of clinical trials is, in part, contingent upon availability of an analytical method or methods suitable for measuring plasma levels of the active moieties (parent and/or metabolites), in terms both of efficacy and safety. Results from the 90-day study in dogs indicated that a NOEL was not achieved in that species. Toxicity studies at lower doses may be needed. The potential for synergistic effects with other chemicals should be addressed.

The Chemoprevention Branch is currently planning single and multiple dose Phase I safety and pharmacokinetic clinical trials. The study will be conducted by Dr. Aryeh Hurwitz of the University of Kansas Medical Center, with analytical support provided by Midwest Research Institute. A pilot study at a single dose (probably 400 mg, or *ca.* 0.04 mmol/kg-bw/day) will be completed, and the findings used to modify the protocol for the subsequent ascending single dose phase I safety and pharmacokinetic clinical study. It is anticipated that four women at high risk for breast cancer (eligibility criteria to be defined) will be treated. Blood and urine will be collected at three timepoints for pharmacokinetic analysis. Subjects will be monitored for toxicity. Because of I3C's potent and pleiotropic enzyme inducing capacity, the potential for synergistic toxicities with other agents should be investigated.

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Table I. Clinical Trials of I3C Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoint(s)	Remarks
Phase I (Safety and ADME)					
N01-CN-55121 Phase I Single and Multiple Dose Safety and Pharmacokinetic Clinical Study of Indole-3-Carbinol (Dr. Aryeh Hurwitz, University of Kansas) 7/95–7/97	---	Part 1a: Healthy adult women, 4 subjects Part 1b: Healthy women at high risk for breast cancer, ≈24 subjects Part 2: Healthy women at high risk for breast cancer, ≈24 subjects	Part 1a: Single dose Part 1b: Ascending single dose Part 2: Multiple doses	Part 1a, 1b: Drug absorption, plasma concentration-time profiles, distribution, excretion, toxicity Part 2: Safety, toxicity, steady-state pharmacokinetics; pharmacological and/or biomarker modulation may be undertaken (hormone levels, xenobiotic induction indicators)	Pilot studies are being conducted in rats to develop and validate assays for measurements of indole-3-carbinol and its metabolites in plasma

INDOLE-3-CARBINOL DEVELOPMENT STATUS