Journal of Cellular Biochemistry 26S:114–126 (1996)

NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN: GENISTEIN

DRUG IDENTIFICATION

CAS Registry No.: 446-72-0

CAS Name (9CI): 5,7-Dihydroxy-3-(4-hydroxyphenyl)-4*H*-benzopyran-4-one

Synonyms:
Differenol A
Genisteol
Genisterin
Prunetol
Sophoricol
4',5,7-Trihydroxyisoflavone

Related Compounds:

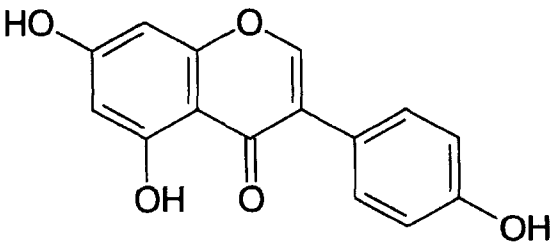
Genistin (CAS No. 529-59-9)
5,4'-Dihydroxy-7-glucosidoisoflavone
Genistein Glucoside

Daidzein (CAS No. 486-66-8)
7-Hydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one

Daidzin
Daidzein Glucoside

Molecular Wt.: 270.2 (Genistein)
432.4 (Genistin)

Structure:



EXECUTIVE SUMMARY

The isoflavone genistein occurs naturally in soy beans [1] and some forage plants [*e.g.*, 2,3] as the glucoside genistin. The latter has been identified in soy food products such as tofu and textured vegetable protein [4–6]; however, most of the isoflavone is unconjugated in fermented soy products such as miso [7]. The other major isoflavones in soybean products are equol, daidzein, and daidzin. Epidemiological studies have shown an inverse association between hormone-dependent cancer incidence/mortality (*e.g.*, breast and prostate) and a traditional soy-rich Asian diet [8–10]. Individuals consuming this diet have 7–110-fold higher plasma [11] and 30-fold higher urinary [12,13] genistein concentrations than individuals consuming a typical Western diet [7]. A causal relationship is suggested by limited studies showing inhibition of carcinogenesis by dietary soy

products in chemical (MNU, DMBA)- [14–17] and radiation-induced [18] rat mammary models and in spontaneous rat [19] and DES-induced mouse prostate models [19]. Genistein itself has inhibited development of DMBA-induced mammary adenocarcinomas in neonatal and prepubertal rats. Conversely, a soy product from which the isoflavones had been extracted had no effect on rat mammary carcinogenesis [19].

Epidemiological evidence for the protective effect of dietary soy is inconsistent for cancers in other organs, such as colorectum, lung and stomach [19]. Results from preclinical efficacy studies using specific soy products are also mixed, which may result from differences in isoflavone content as well as other potentially chemopreventive nutrients and nonnutrients. Preclinical studies with genistein in colon cancer models have also been inconsistent. In an NCI, Chemoprevention Branch-funded study in the AOM-induced rat, dietary genistein (250 ppm, or *ca.* 46.3 μmol/kg-bw/day) increased the size and multiplicity of colon tumors (adenomas, adenocarcinomas). In contrast, lower doses of the isoflavone (75 and 150 mg/kg diet, or *ca.* 13.8 and 27.8 μmol/kg-bw/day) decreased formation of aberrant crypt foci, premalignant lesions in the same model. Finally, genistein inhibited skin tumor multiplicity and increased latency when administered topically during promotion in the two-stage DMBA/TPA mouse model. Thus, target organ efficacy and toxicity may be dose dependent.

Estrogen is generally considered to enhance hormone-related tumorigenesis, especially in the breast and endometrium [20]. However, in some situations, the potential chemopreventive efficacy of genistein appears to be related to its estrogenic effects. For example, prepubertal administration of estradiol, DES, or genistein decreases the formation of DMBA-induced rat mammary tumors, possibly by accelerating differentiation of terminal end buds to lobules [21,22]. This is accompanied by estrogenic effects such as increased uterine and ovarian weights and mammary gland size, and mammary cell proliferation (PCNA staining) [22]. At the cellular level, genistein competes with estradiol for binding to estrogen receptors [23,24]; the complex translocates to the nucleus [25] and stimulates estrogen-related cellular events, although less effectively [3,26–28]. In human estrogen receptor-positive MCF-7 breast cancer cells *in vitro*, genistein stimulated both cell growth and estrogen-dependent pS2 expression at low concentrations (10^{-8}–10^{-5} M) [29]. Since the affinity of genistein to the estrogen receptor is in the same concentration range and proliferation was not observed in receptor-negative cells, this effect appears to result from direct estrogenic activity.

In contrast, genistein may have estrogen antagonistic effects in other situations. Possible mechanisms include induction of sex hormone-binding globulin (SHBG), which regulates clearance and uptake of estrogen and testosterone [30], inhibition of aromatase [31] and 17β-hydroxysteroid oxidoreductase activities [32], and impairment of the CNS or pituitary response to gonadotropins [33,34]. To illustrate, Asian women consuming traditional high soy diets have lower circulating estrogen levels and longer menstrual cycles. In controlled studies with soy products, premenopausal women receiving genistein (primarily as genistin) and daidzin had shorter menstrual cycles, lower serum 17β-estradiol levels, suppressed FSH and LH surges, and lower luteal phase prostaglandins. A longer follicular phase during which breast cell division is lower has been suggested as the mechanism by which soy products reduce cancer risk. In contrast, postmenopausal women receiving soy foods showed only estrogenic responses—increased superficial cells in the vaginal epithelium and suppressed FSH surge [7,35]. This suggests that the effect of genistein depends on the hormonal milieu; in a setting of low endogenous estrogen (*e.g.*, prepuberty, postmenopause), the weak phytoestrogen has estrogenic effects, and in a setting of higher estrogen (premenopause), it acts as an antiestrogen.

Both the epidemiologic data and the mechanism for prevention of prostate cancer are less clear. Genistein may generally decrease androgen levels through inhibition of 7β-hydroxysteroid oxidoreductase, essential for synthesis of both androgens and estrogens, induction of SHBG, and impairment of the CNS or pituitary response to gonadotropins. More specifically, the agent inhibits 5α-reductase [36], which converts testosterone to 5α-dihydrotestosterone (DHT), the main prostatic androgen. Lower levels of markers of this enzyme's activity (3α,17β-androstenediol glucuronide, androsterone glucuronide) have been reported in Japanese men [37], a group with lower risk for prostate cancer and higher urinary and plasma levels of genistein compared with Western populations.

Genistein may also have hormone-independent effects, depending on its concentration or the assay used. For example, higher (2.5×10^{-5}–10^{-4} M) concentrations of genistein inhibited growth of MCF-7 cells even though pS2 was still expressed [29]. The major competing estrogen-independent mechanism is inhibition of tyrosine-specific protein kinase activity [38–40]; this may in turn inhibit cell proliferation [41,42] and growth factor-stimulated responses (EGFR, IGF-I, PDGF) [38,43,44], oncogene expression (*ras*, c-*fos*, c-*jun*) [45,46] or product activity ($pp60^{v\text{-}src}$, $pp110^{gag\text{-}fes}$, $p210^{c\text{-}abl}$) [38,47], bFGF-induced angiogenesis [48], prostaglandin synthesis [49,50], DNA synthesis [51], ornithine decarboxylase activity [52], and immune responses [53], as well as induce differentiation [54, 55]. Other potentially chemopreventive activities which are estrogen-independent include inhibition of reactive oxygen species formation [56–58], topoisomerase activity [59], cytochrome P450 metabolism [60,61] and mutagenicity/clastogenicity of procarcinogens [61,62], as well as induction of cell cycle arrest [63,64] and apoptosis [65–67]. Because of these activities and evidence of its apparent bioavailability on oral administration [11–13], genistein was considered by NCI for further development as a cancer chemopreventive and chemotherapeutic drug.

The NCI, Chemoprevention Branch is now evaluating the preclinical toxicity of genistein preparatory to undertaking a Phase I clinical trial. Ninety-day preclinical toxicity tests with two purified soy isoflavone products containing 90% genistein and 43% genistein in rats (*ca.* 0.03–0.9 mmol genistein/kg-bw/day) and dogs (*ca.* 0.02–0.3 mmol genistein/kg-bw/day) have been undertaken. The dog study was recently completed, and no clinical or histological signs of toxicity were observed.

NCI, DCTDC is no longer developing genistein as a cancer chemotherapeutic drug, but will cooperate with the Chemoprevention Branch in carrying out preclinical efficacy and toxicity testing. Based on the limited chemoprevention data cited above, the epidemiological data associating the high consumption of soy products with low incidences of hormone-dependent cancers, and *in vitro* data indicating growth inhibition of breast and prostate cancer cell lines [*e.g.*, 41,68,69], the Chemoprevention Branch is initially focusing on these sites as targets for clinical drug development. After completion of the preclinical toxicity studies, the Chemoprevention Branch will initiate a Phase I trial with a single-dose portion in normal volunteers and a multidose portion in normal volunteers and inoperable prostate cancer patients to evaluate safety and pharmacokinetics of two purified soy isoflavone products. Following successful completion of this study, the Chemoprevention Branch will consider short-term Phase II trials in presurgical DCIS/breast cancer or prostate cancer patients. The Chemoprevention Branch may be able to initiate these studies before completing the Phase I trial, if sufficient human data are available from NCI, DCTDC studies.

A Clinical Trials Agreement is in place with Protein Technologies International (St. Louis, MO) for supply of two soy isoflavone products—43% and 90% genistein plus small amounts of other isoflavones, fat and carbohydrates. Products with similar isoflavone profiles were used in the Chemoprevention Branch-funded animal toxicity studies. Stable formulations and placebos are being developed for clinical studies of the genistein.

PRECLINICAL EFFICACY STUDIES

One NCI, Chemoprevention Branch-funded chemopreventive efficacy study with genistein has been completed. In the AOM-induced rat, a dietary dose of 250 ppm (*ca.* 46.3 μmol/kg-bw/day) had no effect on colon or small intestine tumor incidence (adenomas, invasive and noninvasive adenocarcinomas), and significantly increased colon tumor multiplicity (primarily noninvasive adenocarcinomas) compared with controls [70]. The higher dose group of 500 ppm (*ca.* 92.5 μmol/kg-bw/day) was terminated early due to significant weight loss.

Published data has shown inhibition of mammary gland tumorigenesis following administration of genistein to immature rats. Genistein at 5 mg sc (*ca.* 1.9 mmol/kg-bw) was given to female neonates on postpartum days two, four and six, followed by DMBA on day 50 [71]. Palpable tumor (primarily adenocarcinoma) latency increased (124 *versus* 87 days in controls) and multiplicity decreased significantly by 42.2%; incidence decreased by only 12%. These effects appeared to be related to accelerated differentiation of mammary terminal end buds to lobules. Proliferation measured as PCNA staining was concomitantly decreased at all levels of development—terminal end buds, terminal ducts, and lobules—compared with DMSO controls. A reduction in DMBA-DNA adducts in mammary gland

tissue has also been demonstrated [72].

A second study evaluated genistein as a chemopreventive agent in prepubertal female rats when given subcutaneously on postpartum days 16, 18, and 20 [21]. Following administration of 500 μg/kg-bw (1.9 mmol/kg-bw) on each of the three days, the multiplicity of tumors induced by DMBA on day 50 decreased by 47.3%, but no significant effects on incidence or latency were found. This chemopreventive effect was apparently a result of the estrogenic activity of genistein, since DES [73] and estrogen [74] produced similar results. Also, the isoflavone caused other estrogenic effects such as early sexual maturity (27 *versus* 37 days), longer estrus cycles (five *versus* four days), and increased numbers of lobules in the mammary gland [21].

Based on preliminary data, genistein was not effective in the MNU-induced rat mammary model. In a study described in an abstract, daily intraperioneal injections of 0.8 mg (*ca.* 9.9 μmol/kg-bw) were administered to rats beginning at 35 days of age; after 180 days, the observed reductions in tumor incidence and multiplicity were not statistically significant ($p<0.09$) [75]. The tested dose was much lower than those found to be effective in immature rats given DMBA or the estimated dose from dietary soybean protein in the same model as discussed below [*i.e.*, 15].

In a published report on the DMBA-induced/TPA-promoted mouse skin model, topically administered genistein (1 and 5 μmol, 2x/wk) significantly decreased tumor multiplicity (36% and 46%, respectively) and increased latency (*ca.* 3 weeks) after 1–7 weeks [76]. Although tumor incidence also decreased, it was not statistically significant.

Induction of differentiation and/or inhibition of growth using genistein have been demonstrated *in vitro* in various types of cancer cells, including rat and human prostate [77], and human melanoma [42], leukemia [55,78], neuroblastoma [79], and gastrointestinal [66]. However, genistein (0.07– 0.28 mg/kg-bw/day, or 0.26–1.0 μmol/kg-bw/day) administered *in vivo*, in drinking water failed to inhibit the growth of rat prostate cancer MAT-Lylu cells subcutaneously implanted into male rats [77]. Tumor weight reduction (9–26%) obtained with intraperitoneal administration of 0.14–0.43 mg/kg-bw/day (0.5–1.6 μmol/kg-bw/day) was not significant or dose dependent.

A significant effort in the Chemoprevention Branch program is the identification and validation of intermediate biomarkers of carcinogenesis. In Chemoprevention Branch-funded studies, genistein at 75 and 150 mg/kg diet (*ca.* 13.8 and 27.8 μmol/kg-bw/day) inhibited the number of aberrant crypt foci (ACF), histological intermediate biomarkers in the AOM-induced rat model of colon carcinogenesis [80,81]. The number of aberrant crypts/focus was not reduced. In contrast, dietary genistein (250 ppm, or *ca.* 46.3 μmol/kg-bw/day) increased the size and multiplicity of colon tumors induced by AOM, as discussed previously [70].

Dietary soy bean products are a source of genistein. In published studies, soy products inhibited development of x-ray- [18], DMBA- [*e.g.*, 14, 15,17] and MNU-induced rat mammary tumors [*e.g.*, 15, 16]. Studies using the standard model with MNU exposure at 50 days of age show chemopreventive effects of soy administered to either prepubertal or adult rats. With 20% soybean protein offered in the diet from 25 days of age, tumor multiplicity decreased *ca.* 60% [15]. The genistein content of the diet was 231.8 μg/g diet (*ca.* 42.9 μmol/kg-bw/day); urinary excretion of the isoflavone was 420 μg compared to 0.1 μg in controls receiving AIN-76A diet. Alternatively, administration of soybean protein from 84 days of age reduced tumor incidence and total number *ca.* 50% and increased latency *ca.* 40% [16]. However, addition of methionine equivalent to the casein-containing control diet, attenuated the effects to 20% reduction in incidence and 28% increase in latency.

Limited preclinical data from a spontaneous rat and a DES-induced mouse prostate model [19] suggest that dietary soy bean products inhibit the development of prostate cancer. Finally, in an intermediate biomarker study, dietary soy also prevented the development of "precancerous changes" in a neonatal estrogen-induced mouse prostate model of carcinogenesis; no experimental details were available [described in 13]. It should be noted that none of these studies with soy products conclusively demonstrates that genistein is responsible for the cancer inhibitory effects observed. Besides isoflavones, other substances in soy with chemopreventive potential include polyphenols, protease inhibitors, phytosterols, saponins, and inositol hexaphosphate [82].

PRECLINICAL SAFETY STUDIES

Safety: The limited toxicity information available

indicates that genistein is not highly toxic; adverse effects reported in animal studies included decreased food consumption and reduced body weight gain. Conflicting *in vitro* data on genotoxic effects exist; no lifetime bioassays have been performed to determine genistein's carcinogenicity. However, due to its estrogenic potential, significant reproductive effects in both ruminants and non-ruminants may result from genistein ingestion. Different responses to phytoestrogens, including genistein, have been observed in several mouse strains; it is unclear if this also indicates a potential for different responses between species.

In order to proceed with the Phase I studies of genistein, subchronic toxicity studies in two species must be performed. NCI, Chemoprevention Branch-funded 90-day studies in dogs with two purified soy isoflavone products have been completed—5, 25 and 70 mg/kg-bw/day of 90% genistein (0.02, 0.09 and 0.3 mmol genistein/kg-bw/day) and 10, 50 and 140 mg/kg-bw/day of 43% genistein (0.02, 0.08 and 0.2 mmol genistein/kg-bw/day). Following administration in capsules to male and female Beagle dogs, no clinical or histological signs of toxicity were observed. Rat studies at doses of 10–250 mg/kg-bw/day of 90% genistein (0.04–0.9 mmol/kg-bw/day) and 20–500 mg/kg-bw/day of 43% genistein (0.03–0.8 mmol genistein/kg-bw/day) are in progress.

In the Chemoprevention Branch-funded colon efficacy study, AOM-treated male rats receiving 500 ppm genistein in the diet (*ca.* 92.5 μmol/kg-bw/day) exhibited 14% body weight loss after only two weeks, necessitating termination of this dose. However, rats at a lower dose of 250 ppm genistein (*ca.* 46.3 μmol/kg-bw/day) with or without AOM showed no difference in body weight from the respective controls after 25 weeks into the 50-week study. In a published subchronic study, daily administration of 45 mg (*ca.* 4.5 mmol/kg-bw/day) for four weeks to Swiss mice (both sexes) resulted in death (2/8) and significant depression of body and organ weight gain (testes, adrenals, kidneys and spleen), even after adjustment for decreased food consumption [83].

No carcinogenicity studies with genistein were found in the published literature. Although it induced DNA strand breaks in human cells *in vitro* [42,84], genistein was not mutagenic in the Ames *Salmonella* mutagenicity assay [85,86]. Carcinogenicity bioassays will probably be required before initiation of long-term clinical trials.

The estrogenic effects of genistein were discovered following reports of reduced fertility, dystocia, and uterine prolapse in female sheep allowed to graze on subterranean clover (*ca.* 7 g or 26 mmol genistein/day) [87,88]. Pathological changes were evident in the urogenital tract of castrated male sheep. Estrogenic responses were also observed in female and castrated male guinea pigs fed clover [87] and in some strains of male and female mice [3, 26,87,89,90] and female rats [71] fed genistein. A total of 5–20 mg of the isoflavone fed to immature mice (unspecified strain) over 4–6 days produced a dose-related increase in uterine weight of 100–450% [3,26]; however, the potency relative to estrone was only 1/6,900 [3]. In contrast, absolute and relative uterine weights of CD-1 mice did not respond to intubation of a total of 8 mg genistein over four days; the response to DES was also less than with other strains of mice [90].

A 14-day study of the reproductive effects of genistein fed at 2, 10 and 15 mg/day to immature (18 days old), spayed (60 days old) and intact (60 days old) female mice produced persistent vaginal cornification in all groups, plus precocious vaginal opening in immature mice (5.5 day versus 10.8 days in controls) [87]. After a portion of the intact females was continued on the high dose (15 mg/day or *ca.* 1.6 mmol/kg-bw/day, 31–55 days), 80% (8/10) of the females mated with control males, but half terminated in pseudopregnancy or fetal resorption. A second mating produced small litters in all females, and the incidence of full-term stillborn pups increased. A more severe effect on fertility was observed in adult male mice fed the same dose of genistein despite a shorter exposure time (22–25 days). Half (5/10) the males did not mate, and only 2/5 of the matings produced offspring by normal females.

Greater reproductive effects from genistein were observed in female rats treated neonatally (postpartum days two, four and six) than those treated closer to puberty (postpartum days 16, 18 and 20). Following neonatal administration of genistein (0.5 mg/kg-bw, or 0.2 mmol/kg-bw), 50-day old rats had significantly longer estrus cycles and reduced circulating progesterone, as well as fewer corpora lutea [71,91]. Administration of the same dose to prepubertal female rats resulted in slightly longer estrus cycles, but no effect on circulating progesterone or ovarian follicular development as in 50-day old rats [21,91]. Increased mammary cell proliferation meas-

ured by PCNA staining was observed in rats treated on days 23–29 [22], but not in rats treated on days 16–20 when measured by BrdU staining [21].

Effects of prenatal exposure on sexual differentiation were investigated by exposure of pregnant rats on gestation days 16–20 to 5,000 or 25,000 μg genistein/day sc, estradiol, or DES [92]. Genistein had no adverse effects on pregnancy, delivery or survival, although mean birth weights were significantly lower in the estradiol and high-dose genistein groups. In contrast, estradiol and DES significantly decreased perinatal survival. The mean anogenital distance was significantly shorter in the estradiol and low-dose genistein groups. Age at vaginal opening was significantly later only in the low-dose genistein; effects on estrus cycles were observed only in the DES group. This study illustrates the complex effects of genistein, which are not always related to estrogen.

ADME: Only limited preclinical pharmacokinetic data are available on genistein. Unlike many flavonoids, genistein appears to be absorbed from the gastrointestinal tract of both ruminant and non-ruminant species after oral administration [*e.g.*, 87]. One study in rats has shown that only 9–17% of isoflavones consumed in diets containing 20% soy protein appears in the urine [17]. Although this suggests that only a fraction of isoflavonoids is absorbed, a study in rats with biliary cannulas demonstrated that 43% of duodenally administered genistein is recovered in the bile within four hours. Thus, genistein, in common with other flavonoids, appears to be retained in enterohepatic circulation as a result of gastrointestinal absorption, conjugation in the liver, excretion in the bile, and deconjugation by microflora in the gut [93].

Following a dose of 20 mg genistein/kg-bw (0.07 mmol/kg-bw) in rats, the plasma concentration was 11 μM after two hours [94]. A comparable dose of the glycone in soy extract produced a lower plasma level of 4.9 μM at two hours, but there were no significant differences at eight hours and later. This suggests that the extent of genistein and genistein absorption are similar.

Following single genistein doses of 54 and 200 mg/kg-bw ig (0.2 and 0.7 mmol/kg-bw) to mice, the C_{max} was 1 μg/ml (3.7 μM) at 3–5 minutes; plasma levels decreased to 0.1–0.2 μg/ml (0.4–0.7 μM) by eight hours [95]. In dogs, the average plasma genistein concentration up to six hours after ig administration of 28 mg/kg-bw (0.1 μmol/kg-bw) was 0.055 μg/ml (0.2 μM).

Metabolites of genistein and genistin identified in rats have primarily been conjugates. Oral administration of genistein resulted in eight urinary metabolites, three of which were identified as genistein 4'-*O*-sulfate, genistein 7-*O*-β-D-glucuronide, and genistein 4'-*O*-sulfate-7-*O*-β-D-glucuronide; the last conjugate is a major metabolite [96]. Biliary metabolites included the glucuronide and the sulfate/glucuronide conjugate. In contrast, sheep metabolize genistein to *p*-ethylphenol, a nonestrogenic metabolite [97].

In the NCI, Chemoprevention Branch-funded 90-day toxicity study in dogs, plasma genistein levels varied somewhat with the source. Following deconjugation of plasma samples, concentrations of genistein were 0.17–0.39 μg/ml (0.6–1.4 μM) in dogs receiving 0.02, 0.09 and 0.3 mmol genistein/kg-bw/day as purified soy isoflavone product containing 90% genistein and 7% daidzein and 0.24–0.83 μg/ml (0.9–3.1 μM) in dogs receiving 0.02, 0.08 and 0.2 mmol genistein/kg-bw/day as the isoflavone product containing 43% genistein and 21% daidzein.

CLINICAL SAFETY: PHASE I STUDIES

The protocol for an NCI, Chemoprevention Branch-sponsored Phase I trial of two purified soy isoflavone products containing 43% and 90% genistein is being finalized. The pharmacokinetics and safety will be investigated in normal volunteers in the single-dose and in the multidose pharmacokinetic normal volunteers and Stage C and D prostate cancer patients. At this time, the available human safety and pharmacokinetics information is from epidemiological and controlled studies of dietary soy products.

Drug Effect Measurement: No systematic studies of the effects of genistein on potential drug effect measurements were found. Possibilities include measures of estrogenic activity, such as FSH, LH, 17β-estradiol, progesterone and SHBG levels, and vaginal cytology in premenopausal women [7,82,98], and LH and FSH levels and vaginal cytology in postmenopausal women [7,35]. In men, decreases in FSH, total cholesterol, and testosterone have been reported following intake of soy products or linseed [7]. Inhibition of tyrosine kinase may be relevant in some tissues [38–40].

Safety: No human safety data are available specifically for genistein. Because of the estrogenic responses obtained with dietary soy products,

prolongation of menstrual cycles, suppression of the mid-cycle gonadotrophin, LH and FSH surges, delay in peak progesterone concentrations and changes in vaginal cytology would be expected [7,98]. It is unknown if more serious estrogen-agonistic or -antagonist effects such as those attributed to tamoxifen would be observed, including thromboembolic disease and endometrial proliferation/neoplasia. Some evidence suggests that Japanese women have a high prevalence of endometriosis [7]. Finally, in the presence of advanced liver disease and/or insufficient metabolism of nonsteroidal estrogens, significant accumulation of genistein might occur in plasma [5].

ADME: Genistin from dietary soy is metabolized to the aglycone genistein by gastrointestinal flora. Genistein appears to be absorbed in humans, since individuals on a traditional soy-rich Japanese diet have much higher plasma (7–110-fold) [11] and urinary genistein concentrations (30-fold) than individuals consuming a Western diet [12,13]. For example, in one limited study, higher levels of genistein were detected in plasma of Japanese men who consume high amounts of soy products than in Finnish men who do not; the average concentration in Japanese men was 276 nM compared with 6.3 nM in the Finnish men [11]. Irrespective of diet, most circulating genistein occurred as the glucuronide, which is considered to be biologically inactive [99].

Several short-term, controlled pharmacokinetics studies with soy products have found detectable plasma and urine genistein levels [100–102]. Single doses of 19.3, 36.2, and 55.7 mg genistein (*ca.* 1.1, 2.1, and 3.3 μmol/kg-bw) as soybean milk powder to individuals receiving a controlled liquid diet produced plasma levels of 0.7, 1.1 and 2.1 μM at 6.5 hours, decreasing almost to zero at 24 hours [100]. Twenty-four hour urine recovery was *ca.* 5% at the lowest dose, increasing two-fold at the higher doses; very little was excreted in the feces. Preliminary results from a second study of soy milk administered twice within 26 hours suggest greater absorption, since 15–60% of ingested genistein (dose unknown) was excreted as the glucuronide within 20 hours [101]. The urinary conjugates may include both monoglucuronides (53–76%) and diglucuronides (12–26%) [103].

Metabolism of genistein has been suggested by the appearance of dihydrogenistein in the urine of volunteers receiving soy flour on two consecutive days (*ca.* 2.1 μmol genistein/kg-bw/day) [102]. Although bacteria metabolize genistein in the gastrointestinal tract of sheep, it is unknown if this is the source of the metabolite in humans.

CLINICAL EFFICACY: PHASE II STUDIES

After successful completion of the Phase I trial, NCI, Chemoprevention Branch will consider short-term Phase II trials in which genistein will be administered during the period between diagnostic biopsy and definitive surgery in prostate or breast cancer patients. Intermediate biomarkers in prostate tissue would be evaluated as potential surrogate endpoints for cancer. In women with mammograms suspicious for DCIS or early stage breast cancer, modulation of intermediate biomarkers in adenocarcinoma, DCIS and other premalignant lesions would be endpoints. Longer term Phase II trials on prevention of colon or prostate cancer may also be considered. No published reports of clinical trials with genistein were found.

In Asian countries with lower risk for breast cancer, women have lower circulating estrogen levels and longer menstrual cycles [7,98]. Consumption of soy products in controlled studies has shown effects on the hypothalamic-pituitary-gonadal axis to down-regulate ovarian estrogen synthesis [*e.g.*, 7,98,104]. In one U.S. study, premenopausal women ingested soy milk with each meal for one month, resulting in daily genistein intake of *ca.* 200 mg isoflavones (*ca.* 100 mg each genistein, mostly as genistin (*ca.* 3.7 μmol/kg-bw/day), and daidzin) [98]. The average menstrual cycle during the same month increased from 28.3 to 31.8 days, increasing further to 32.7 days one cycle after termination of dosing; however, these changes were not statistically significant. Serum 17β-estradiol levels on days 5–7, 12–14 and 20–22 decreased significantly by 31%, 81% and 49%, respectively. These persisted two to three cycles after soy feeding. Luteal phase progesterone decreased 35%, and DHEA sulfate decreased progressively by 14–30%. Other studies have shown suppressed mid-cycle surges of FSH and LH [7]. These results provide a biological basis for epidemiological observations that soy consumption is inversely related to breast cancer risk.

PHARMACODYNAMICS

In human tumor cell lines, inhibition of growth and induction of differentiation occurred at IC_{50}s of 5–40 μM [19]. These levels are not achieved in Asian populations consuming soy-rich diets (0.3 μM) [11]

or in volunteers receiving single doses of 55.7 mg genistein (2.1 μM) [84]. From the limited data available, mice also did not attain the required plasma levels; the C_{max} after an oral dose of 200 mg genistein/kg-bw (0.7 mmol/kg-bw) was 1 μg/ml (3.7 μM) [95]. However, if inhibition of mammary and prostate cancer by soy products or genistein is the result of effects on the hypothalamic-pituitary-gonadal axis, the required dose may be lower. In women given 200 mg conjugated isoflavones (*ca.* 0.7 μmol/kg-bw/day), serum estradiol and progesterone decreased concomitantly with increased cycle length.

In rats, approximately 60% of orally administered genistein is not absorbed, and much of the absorbed isoflavone may enter enterohepatic circulation. The lowest effective dose against ACF formation in rat colon was 13.8 μmol/kg-bw/day. The estimated total isoflavone intake from consumption of one soy entreé/day is 200 mg [82]; if two-thirds is assumed to be genistein [19], the daily human intake of this isoflavonoid would be 7 μmol/kg-bw. Thus, oral intake of the effective genistein dose would appear to be safe in clinical prevention trials for colon cancer.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

As noted above, no systematic studies pertinent to evaluating drug effects of genistein have been found. A likely potential measurement is inhibition of tyrosine kinase [38,39, 40].

Safety Issues

Estrogenic and reproductive effects are major safety concerns for genistein and should be fully characterized in preclinical toxicity and clinical safety studies. Preclinical reproductive toxicity studies may be undertaken early in clinical development of genistein. Possible species variations in endocrinology should also be determined.

Pharmacodynamics Issues

Many of mechanisms shown for genistein *in vitro* require mg/ml or μg/ml plasma concentration levels, but even in Asian populations with traditional high-soy diets are only at ng/ml levels. The effects of genistein *in vivo* are complex and appear to vary with dose, the hormonal status of the individual, and possibly species. Inhibition of mammary carcinogenesis in prepubertal rats was associated with persistent estrogenic effects, such as early sexual maturity, longer estrus cycles, and accelerated differentiation of terminal end buds to lobules. Since the latter is eventually correlated to decreased proliferation, it is assumed to be the mechanism for chemoprevention in immature female rats. Thus, in the setting of low endogenous estrogen, the estrogenic effects of genistein are observed.

Chemopreventive efficacy has also been obtained in adult female rats with soybean protein containing genistein. Since epidemiological data show that Asian populations consuming soy products are at low breast cancer risk, the effect of genistein appears to be antagonistic in settings of higher estrogen. The mechanism may be an inhibitory effect on the hypothalamic-pituitary-gonadal axis demonstrated by prolongation of the menstrual cycle, specifically the follicular phase, and suppression of the LH and FSH mid-cycle surge. This is also seen in tamoxifen-treated breast cancer patients [*e.g.*, 104]. However, it is unknown if the same mechanism is responsible for mammary chemoprevention in adult female rats; a species difference may exist. An alternate mechanism is down-regulation of estrogen receptor mRNA. In support of this, pretreatment of MCF-7 cells with genistein for six days produced an attenuated stimulatory response to estradiol, concomitant with decreased steady-state estrogen receptor mRNA levels [29].

In postmenopausal women, dietary soy induced few hormonal effects [82]. Only estrogenic responses have been reported, including increased numbers of superficial cells in the vaginal epithelium and suppressed FSH levels [7,35]. Genistein may not be an effective breast cancer chemopreventive in this population.

At this point in time, the available chemopreventive efficacy, ADME, and toxicity data are insufficient for evaluating the pharmacodynamics of genistein. In the preclinical studies planned and in progress, considerable effort will be devoted to determining efficacious doses and tissue distribution of genistein, and in evaluating the safety margin in relation to doses causing unwanted estrogenic effects. These effects are expected to be the most frequent toxicities associated with the isoflavone.

Regulatory Issues

No specific regulatory issues have yet been identified for genistein.

Supply and Formulation Issues

Bulk purified soy isoflavone products containing approximately 43% and 90% genistein for the preclinical toxicity studies and the Phase I trial have been provided by Protein Technologies International (St. Louis, MO). The remaining drug substance consists of other isoflavones, fat and carbohydrates; no proteins, such as protease inhibitors, are present. The formulation for the clinical trial is under development. Stability studies on the final formulation will be completed before the Phase I trial begins.

Intermediate Biomarkers

Genistein has demonstrated modulation of histological intermediate biomarkers in the Chemoprevention Branch-funded rat AOM-induced ACF study and in published results on premalignant lesions in mouse prostate. Effects on a proliferation biomarker (PCNA) were also observed in the immature rat mammary gland. Since differentiation of tumor cells has been demonstrated *in vitro*, these types of biomarkers should also be evaluated in clinical trials. The biomarkers proposed for the breast cohort include histological (DCIS number and grade, nuclear morphometry), proliferation (MIB-1, ploidy, S-phase fraction, EGF), and genetic (c-*erb*B-2 expression) [106]. Estrogen receptor status evaluation should also be considered. Biomarker endpoints in prostate cancer patients include PIN and grade, ploidy, TGFα, EGFR, and MIB-1.

Clinical Studies Issues

As described above, previous epidemiological and experimental studies (primarily with soy products) suggest breast and prostate as logical targets for cancer chemoprevention by genistein. Pending favorable completion of preclinical toxicity studies and validation of analytical procedures to measure circulating genistein [105], the Phase I trial will be initiated. The Chemoprevention Branch is also considering short-term Phase II trials in presurgical breast and prostate cancer patients. The primary focus would be intermediate biomarker modulation as well as correlation of these changes with genistein dose and plasma levels. Based on the efficacy observed in the rat AOM-induced ACF study, colon cancer may also be a target for longer-term Phase II chemoprevention trials of genistein.

REFERENCES

1. Naim, M., Gestetner, B., Kirson, I., Birk, Y. and Bondi, A. A new isoflavone from soya beans. *Phytochemistry* 12: 169–170, 1973.
2. Bradbury, R.B. and White, D.E. The chemistry of subterranean clover. Part I. Isolation of formononetin and genistein. *J. Chem. Soc.* 4: 3447–3449, 1951.
3. Bickoff, E.M., Livingston, A.L., Hendrickson, A.P. and Booth, A.N. Relative potencies of several estrogen-like compounds found in forages. *J. Agric. Food Chem.* 10: 410–412, 1962.
4. Setchell, K.D.R. Naturally occurring non-steroidal estrogens of dietary origin. In: McLachlan, J.A. (ed.) *Estrogens in the Environment.* New York: Elsevier Science Publishing Co., Inc., pp 69–85, 1985.
5. Rosenblum, E.R., Campbell, I.M., Van Thiel, D.H. and Gavaler, J.S. Isolation and identification of phytoestrogens from beer. *Alcohol. Clin. Exp. Res.* 16: 843–845, 1992.
6. Wang, H. and Murphy, P.A. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem.* 42: 1666–1673, 1994.
7. Cassidy, A. Physiological effects of phyto-oestrogens in relation to cancer and other human health risks. *Proc. Nutr. Soc.* 55: 399–417, 1996.
8. Severson, R.K., Nomura, A.M.Y., Grove, J.S. and Stemmermann, G.N. A prospective study of demographics, diet, and prostate cancer among men of Japanese ancestry in Hawaii. *Cancer Res.* 49: 1857– 1860, 1989.
9. Lee, H.P., Gourley, L., Duffy, S.W., Esteve, J., Lee, J. and Day, N.E. Dietary effects on breast cancer risk in Singapore. *Lancet* 337: 1197–1200, 1991.
10. Lee, H.P., Gourley, L., Duffy, S.W., Esteve, J., Lee, J. and Day, N.E. Risk factors for breast cancer by age and menopausal status: A case-control study in Singapore. *Cancer Causes Control* 3: 41–53, 1992.
11. Adlercreutz, H., Markkanen, H. and Watanabe, S. Plasma concentrations of phyto-oestrogens in Japanese men. *Lancet* 342: 1209–1210, 1993.
12. Adlercreutz, H., Fotsis, T., Bannwart, C., Wahala, K., Brunow, G. and Hase, T. Isotope dilution gas chromatographic-mass spectrometric method for the determination of lignans and isoflavonoids in human urine, including identification of genistein. *Clin. Chim. Acta* 199: 263–278, 1991.
13. Adlercreutz, H., Honjo, H., Higashi, A., Fotsis, T., Hamalainen, E., Hasegawa, T. and Okada, H. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am. J. Clin. Nutr.* 54: 1093–1100, 1991.
14. Baggott, J.E., Ha, T., Vaughn, W.H., Juliana, M.M., Hardin, J.M. and Grubbs, C.J. Effect of miso (Japanese soybean paste) and NaCl on DMBA-induced rat mammary tumors. *Nutr. Cancer* 14: 103–109, 1990.
15. Barnes, S., Grubbs, C., Setchell, K.D.R. and Carlson, J. Soybeans inhibit mammary tumors in models of breast cancer. *Prog. Clin. Biol. Res.* 347: 239–253, 1990.
16. Hawrylewicz, E.J., Huang, H.H. and Blair, W.H. Dietary soybean isolate and methionine supplementation affect

mammary tumor progression in rats. *J. Nutr.* 121: 1693–1698, 1991.

17. Barnes, S., Peterson, G., Grubbs, C. and Setchell, K. Potential role of dietary isoflavones in the prevention of cancer. *Adv. Exp. Med. Biol.* 354: 135–147, 1994.
18. Troll, W., Wiesner, R., Shellabarger, C.J., Holtzman, S. and Stone, J.P. Soybean diet lowers breast tumor incidence in irradiated rats. *Carcinogenesis* 1: 469–472, 1980.
19. Messina, M.J., Persky, V., Setchell, K.D.R. and Barnes, S. Soy intake and cancer risk: A review of the *in vitro* and *in vivo* data. *Nutr. Cancer* 21: 113–131, 1994.
20. Henderson, B.E., Ross, R. and Bernstein, L. Estrogens as a cause of human cancer: The Richard and Hinda Rosenthal Foundation Award lecture. *Cancer Res.* 48: 246–253, 1988.
21. Murrill, W.B., Brown, N.M., Zhang, J.-X., Manzolillo, P.A., Barnes, S. and Lamartiniere, C.A. Prepubertal genistein exposure suppresses mammary cancer and enhances gland differentiation in rats. *Carcinogenesis* 17: 1451–1457, 1996.
22. Brown, N.M. and Lamartiniere, C.A. Xenoestrogens alter mammary gland differentiation and cell proliferation in the rat. *Environ. Health Perspect.* 103: 708–713, 1995.
23. Shutt, D.A. and Cox, R.I. Steroid and phyto-oestrogen binding to sheep uterine receptors *in vitro*. *J. Endocrinol.* 52: 299–310, 1972.
24. Verdeal, K., Brown, R.R., Richardson, T. and Ryan, D.S. Affinity of phytoestrogens for estradiol-binding proteins and effect of coumestrol on growth of 7,12-dimethylbenz[*a*]anthracene-induced rat mammary tumors. *J. Natl. Cancer Inst.* 64: 285–290, 1980.
25. Kvetnansky, R., Sun, C.L., Lake, C.R., Thoa, N., Torda, T. and Kopin, I.J. Effect of handling and forced immobilization on rat plasma levels of epinephrine, norepinephrine, and dopamine-β-hydroxylase. *Endocrinology* 103: 1868–1874, 1978.
26. Cheng, E., Story, C.D., Yoder, L., Hale, W.H. and Burroughs, W. Estrogenic activity of isoflavone derivatives extracted and prepared from soybean oil meal. *Science* 118: 164–165, 1953.
27. Folman, Y. and Pope, G.S. The interaction in the immature mouse of potent oestrogens with coumestrol, genistein and other utero-vaginotrophic compounds of low potency. *J. Endocrinol.* 34: 215–225, 1966.
28. Markiewicz, L., Garey, J., Adlercreutz, H. and Gurdipe, E. *In vitro* bioassays of non-steroidal phytoestrogens. *J. Steroid Biochem. Mol. Biol.* 45: 399–405, 1993.
29. Wang, T.T.Y., Sathyamoorthy, N. and Phang, J.M. Molecular effects of genistein on estrogen receptor mediated pathways. *Carcinogenesis* 17: 271–275, 1996.
30. Mousavi, Y. and Adlercreutz, H. Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture. *Steroids* 58: 301–304, 1993.
31. Campbell, D.R. and Kurzer, M.S. Flavonoid inhibition of aromatase enzyme activity in human preadipocytes. *J. Steroid Biochem. Mol. Biol.* 46: 381–388, 1993.
32. Makela, S., Davis, V.L., Tally, W.C., Korkman, J., Salo, L., Vihko, R., Santti, R. and Korach, K.S. Dietary estrogens act through estrogen receptor-mediated processes and show no antiestrogenicity in cultured breast cancer cells. *Environ. Health Perspect.* 102: 572–578, 1994.
33. Faber, K.A. and Hughes, C.L., Jr. The effect of neonatal exposure to diethylstilbestrol, genistein, and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. *Biol. Reprod.* 45: 649–653, 1991.
34. Kaldas, R.S. and Hughes, C.L., Jr. Reproductive and general metabolic effects of phytoestrogens in mammals. *Reprod. Toxicol.* 3: 81–89, 1989.
35. Baird, D.D., Umbach, D.M., Lansdell, L., Hughes, C.L., Setchell, K.D.R., Weinberg, C.R., Haney, A.F., Wilcox, A.J. and McLachlan, J.A. Dietary intervention study to assess estrogenicity of dietary soy among postmenopausal women. *J. Clin. Endocrinol. Metab.* 80: 1685–1690, 1995.
36. Evans, B.A.J., Griffiths, K. and Morton, M.S. Inhibition of 5α-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. *J. Endocrinol.* 147: 295–302, 1995.
37. Ross, R.K., Bernstein, L., Lobo, R.A., Shimizu, H., Stanczyk, F.Z., Pike, M. C. and Henderson, B.E. 5α-Reductase activity and risk of prostate cancer among Japanese and US white and black males. *Lancet* 339: 887–889, 1992.
38. Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S.-I., Itoh, N., Shibuya, M. and Fukami, Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.* 262: 5592–5595, 1987.
39. Sibley, D.R., Benovic, J.L., Caron, M.G. and Lefkowitz, R.J. Regulation of transmembrane signaling by receptor phosphorylation. *Cell* 48: 913–922, 1987.
40. Palangat, M. and Roy, D. Nuclear protein tyrosine kinases: Isolation, characterization and their probable role as markers in carcinogenesis/toxicity. *Toxicologist* 14: 361 abst. no. 1418, 1994.
41. Peterson, G. and Barnes, S. Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. *Prostate* 22: 335–345, 1993.
42. Kiguchi, K., Constantinou, A.I. and Huberman, E. Genistein-induced cell differentiation and protein-linked DNA strand breakage in human melanoma cells. *Cancer Commun.* 2: 271–278, 1990.
43. Chang, C.-J. and Geahlen, R.L. Protein-tyrosine kinase inhibition: Mechanism-based discovery of antitumor agents. *J. Nat. Prod.* 55: 1529–1560, 1992.
44. Dean, N.M., Kanemitsu, M. and Boynton, A.L. Effects of the tyrosine-kinase inhibitor genistein on DNA synthesis and phospholipid-derived second messenger generation in mouse 10T1/2 fibroblasts and rat liver T51B cells. *Biochem. Biophys. Res. Commun.* 165: 795–801, 1989.
45. Zwiller, J., Sassone-Corsi, P., Kakazu, K. and Boynton, A.L. Inhibition of PDGF-induced c-*jun* and c-*fos* expression by a tyrosine protein kinase inhibitor. *Oncogene* 6: 219–221, 1991.
46. Nakafuku, M., Satoh, T. and Kaziro, Y. Differentiation factors, including nerve growth factor, fibroblast growth factor, and interleukin-6, induce an accumulation of an active *ras*-GTP complex in rat pheochromocytoma PC12 cells. *J. Biol. Chem.* 267: 19448–19454, 1992.
47. Honma, Y., Okabe-Kado, J., Kasukabe, T., Hozumi, M. and Umezawa, K. Inhibition of *abl* oncogene tyrosine kinase induces erythroid differentiation of human myelogenous leukemia K562 cells. *Jpn. J. Cancer Res.* 81: 1132–1136, 1990.

48. Fotsis, T., Pepper, M., Adlercreutz, H., Fleischmann, G., Hase, T., Montesano, R. and Schweigerer, L. Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis. *Proc. Natl. Acad. Sci. USA* 90: 2690–2694, 1993.

49. Coyne, D.W. and Morrison, A.R. Effect of the tyrosine kinase inhibitor, genistein, on interleukin-1 stimulated PGE2 production in mesangial cells. *Biochem. Biophys. Res. Commun.* 173: 718–724, 1990.

50. Glaser, K.B., Sung, A., Bauer, J. and Weichman, B.M. Regulation of eicosanoid biosynthesis in the macrophage. Involvement of protein tyrosine phosphorylation and modulation by selective protein tyrosine kinase inhibitors. *Biochem. Pharmacol.* 45: 711–721, 1993.

51. Takano, T., Takada, K., Tada, H., Nishiyama, S. and Amino, N. Genistein, a tyrosine kinase inhibitor, blocks the cell cycle progression but not Ca^{2+} influx induced by BAY K8644 in FRTL-5 cells. *Biochem. Biophys. Res. Commun.* 190: 801–807, 1993.

52. Majumdar, A.P.N. Role of tyrosine kinases in gastrin induction of ornithine decarboxylase in colonic mucosa. *Am. J. Physiol.* 259: G626–G630, 1990.

53. Atluru, S. and Atluru, D. Evidence that genistein, a protein tyrosine kinase inhibitor, inhibits CD28 monoclonal-antibody-stimulated human T cell proliferation. *Transplantation* 51: 448–450, 1991.

54. Katagiri, K., Katagiri, T., Kajiyama, K., Uehara, Y., Yamamoto, T. and Yoshida, T. Modulation of monocytic differentiation of HL-60 cells by inhibitors of protein tyrosine kinases. *Cell. Immunol.* 140: 282–294, 1992.

55. Makishima, M., Honma, Y., Hozumi, M., Sampi, K., Hattori, M., Umezawa, K. and Motoyoshi, K. Effects of inhibitors of protein tyrosine kinase activity and/or phosphatidylinositol turnover on differentiation of some human myelomonocytic leukemia cells. *Leuk. Res.* 15: 701–708, 1991.

56. Wei, H., Wei, L., Frenkel, K., Bowen, R. and Barnes, S. Inhibition of tumor promoter-induced hydrogen peroxide formation *in vitro* and *in vivo* by genistein. *Nutr. Cancer* 20: 1–12, 1993.

57. Wei, H. Isoflavones scavenge reactive oxygen species and protect DNA bases from oxidative DNA damage. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 35: 619, abstract no. 3690, 1994.

58. Wei, H., Cai, Q. and Rahn, R.O. Inhibition of UV light- and Fenton reaction-induced oxidative DNA damage by the soybean isoflavone genistein. *Carcinogenesis* 17: 73–77, 1996.

59. Okura, A., Arakawa, H., Oka, H., Yoshinari, T. and Monden, Y. Effect of genistein on topoisomerase activity and on the growth of [VAL 12]Ha-*ras*-trans formed NIH 3T3 cells. *Biochem. Biophys. Res. Commun.* 157: 183–189, 1988.

60. Chae, Y.-H., Marcus, C.B., Ho, D.K., Cassady, J.M. and Baird, W.M. Effects of synthetic and naturally occurring flavonoids on benzo[*a*]pyrene metabolism by hepatic microsomes prepared from rats treated with cytochrome *P*-450 inducers. *Cancer Lett.* 60: 15–24, 1991.

61. Lee, H., Wang, H.-W., Su, H.-Y. and Hao, N.J. The structure-activity relationships of flavonoids as inhibitors of cytochrome P-450 enzymes in rat liver microsomes and the mutagenicity of 2-amino-3-methyl-imidazo[4,5-*f*]quinoline. *Mutagenesis* 9: 101–106, 1994.

62. Giri, A.K. and Lu, L.-J.W. Inhibition of 7,12-dimethyl benz(*a*)anthracene (DMBA)-induced sister chromatid exchanges (SCE) by soya isoflavone genistein but not daidzein in female ICR mice. *Environ. Mol. Mutagen.* 23: 21, 1994.

63. Finlay, G.J., Holdaway, K.M. and Baguley, B.C. Comparison of the effects of genistein and amsacrine on leukemia cell proliferation. *Oncol. Res.* 6: 33–37, 1994.

64. Matsukawa, Y., Marui, N., Sakai, T., Satomi, Y., Yoshida, M., Matsumoto, K., Nishino, H. and Aoike, A. Genistein arrests cell cycle progression at G2-M. *Cancer Res.* 53: 1328–1331, 1993.

65. McCabe, M.J., Jr. and Orrenius, S. Genistein induces apoptosis in immature human thymocytes by inhibiting topoisomerase-II. *Biochem. Biophys. Res. Commun.* 194: 944–950, 1993.

66. Yanagihara, K., Ito, A., Toge, T. and Numoto, M. Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. *Cancer Res.* 53: 5815–5821, 1993.

67. Spinozzi, F., Pagliacci, M.C., Migliorati, G., Moraca, R., Grignani, F., Riccardi, C. and Nicoletti, I. The natural tyrosine kinase inhibitor genistein produces cell cycle arrest and apoptosis in Jurkat T-leukemia cells. *Leuk. Res.* 18: 431–439, 1994.

68. Adlercreutz, H., Fotsist, T., Schweigerert, L., Pepper, M., Attalla, H., Zhang, Y., Wahala, K., Montesano, R., Nawroth, P.P. and Hase, T. Isoflavonoids and 2-methoxyestradiol: Inhibitors of tumor cell growth and angiogenesis. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 35: 693–694, 1994.

69. Peterson, G. and Barnes, S. Genistein inhibition of the growth of human breast cancer cells: Independence from estrogen receptors and the multi-drug resistance gene. *Biochem. Biophys. Res. Commun.* 179: 661–667, 1991.

70. Reddy, B.S., Wang, C.-X., Aliaga, C., Rao, C.V., Lubet, R.A., Steele, V.E. and Kelloff, G.J. Potential chemopreventive activity of perillyl alcohol and enhancement of experimental colon carcinogenesis by folic acid and genistein. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 37: 271, abstract no. 1849, 1996.

71. Lamartiniere, C.A., Moore, J.B., Brown, N.M., Thompson, R., Hardin, M.J. and Barnes, S. Genistein suppresses mammary cancer in rats. *Carcinogenesis* 16: 2833–2840, 1995.

72. Upadhyaya, P. and El-Bayoumy, K. Effects of dietary soy protein isolate and genistein on the levels of 7,12-dimethylbenz(*a*)anthracene (DMBA)-DNA binding in mammary glands of female CD rats. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 597, abstract no. 3553, 1995.

73. Holland, M.B. and Lamartiniere, C.A. Neonatal diethylstilbestrol chemoprevents mammary adenocarcinomas in rats. Proc. Annu. *Meet. Am. Assoc. Cancer Res.* 33: 167, abstract no. 1000, 1992.

74. Holland, M.B. and Lamartiniere, C.A. Neonatal estrogen treatment prevents dimethyl-benz(*a*)anthracene (DMBA) and *N*-nitroso-*N*-methylurea (NMU) induced mammary adenocarcinomas in Sprague Dawley (SD) female rats. *Toxicologist* 14: 35, abstract no. 45, 1994.

75. Constantinou, A., Thomas, C., Mehta, R., Runyan, C. and Moon, R. The effect of genistein and daidzein on MNU-induced mammary tumors in rats. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 115, abstract no. 684, 1995.

76. Wei, H.C., Bowen, R., Cai, Q., Barnes, S. and Wang, Y.

Antioxidant and antipromotional effects of the soybean isoflavone genistein. *Proc. Soc. Exp. Biol. Med.* 208: 124–130, 1995.

77. Naik, H.R., Lehr, J.E. and Pienta, K.J. An *in vitro* and *in vivo* study of antitumor effects of genistein on hormone refractory prostate cancer. *Anticancer Res.* 14: 2617–2620, 1994.
78. Carlo-Stella, C., Regazzi, E., Garau, D., Mangoni, L., Rizzo, M.T., Bonati, A., Dotti, G., Almici, C. and Rizzoli, V. Effect of the protein tyrosine kinase inhibitor genistein on normal and leukaemic haemopoietic progenitor cells. *Br. J. Haematol.* 93: 551–557, 1996.
79. Brown, A. and Wei, H. Effect of genistein on cell proliferation, differentiation and apoptosis in neuroblastoma cells. *Toxicologist* 15: 217, abstract no. 1165, 1995.
80. Pereira, M.A., Barnes, L.H., Rassman, V.L., Kelloff, G.V. 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.
81. Steele, V.E., Pereira, M.A., Sigman, C.C. and Kelloff, G.J. Cancer chemoprevention agent development strategies for genistein. *J. Nutr.* 125: 713S–716S, 1995.
82. Messina, M. and Barnes, S. The role of soy products in reducing risk of cancer. *J. Natl. Cancer Inst.* 83: 541– 546, 1991.
83. Carter, M.W., Matrone, G. and Smart, W.W.G., Jr. The effect of genistin and its aglycone on weight gain in the mouse. *Br. J. Nutr.* 14: 301–304, 1960.
84. Constantinou, A., Kiguchi, K. and Huberman, E. Induction of differentiation and DNA strand breakage in human HL-60 and K-562 leukemia cells by genistein. *Cancer Res.* 50: 2618–2624, 1990.
85. Bartholomew, R.M. and Ryan, D.S. Lack of mutagenicity of some phytoestrogens in the *Salmonella*/mammalian microsome assay. *Mutat. Res.* 78: 317– 321, 1980.
86. Nagao, M., Morita, N., Yahagi, T., Shimizu, M., Kuroyanagi, M., Fukuoka, M., Yoshihira, K., Natori, S., Fujino, T. and Sugimura, T. Mutagenicities of 61 flavonoids and 11 related compounds. *Environ. Mutagen.* 3: 401–419, 1981.
87. East, J. The effect of genistein on the fertility of mice. *J. Endocrinol.* 13: 94–100, 1955.
88. Whitten, P.L. and Naftolin, F. Dietary estrogens: A bio logically active background for estrogen action. *Serono Symp. Publ. Raven Press* 74: 155–167, 1991.
89. Leavitt, W.W. and Meismer, D.M. Sexual development altered by non-steroidal oestrogens. *Nature* 218: 181– 182, 1968.
90. Farmakalidis, E. and Murphy, P.A. Oestrogenic response of the CD-1 mouse to the soya-bean isoflavones genistein, genistin and daidzin. *Food Chem. Toxicol.* 22: 237–239, 1984.
91. Manzolillo, P.A., Murrill, W.B., Zhang, J., Brown, N.M. and Lamartiniere, C.A. Genistein toxicity in the female rat reproductive system. *Fundam. Appl. Toxicol.* 63: 144, abstract no. 736, 1996.
92. Levy, J.R., Faber, K.A., Ayyash, L. and Hughes, C.L., Jr. The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. *Proc. Soc. Exp. Biol. Med.* 208: 60–66, 1995.
93. Hackett, A.M. The metabolism of flavonoid compounds in mammals. *Prog. Clin. Biol. Res.* 213: 177–194, 1986.
94. King, R.A., Broadbent, J.L. and Head, R.J. Absorption and excretion of the soy isoflavone genistein in rats. *J. Nutr.* 126: 176–182, 1996.
95. Supko, J.G. and Malspeis, L. Plasma pharmacokinetics of genistein in the mouse and beagle dog. *Proc. Am. Assoc. Cancer Res.* 36: 382 abstract no. 2279, 1995.
96. Yasuda, T., Mizunuma, S., Kano, Y., Saito, K. and Ohsawa, K. Urinary and biliary metabolites of genistein in rats. *Biol. Pharm. Bull.* 19: 413–417, 1996.
97. Lu, L.-J.W., Grady, J.J., Marshall, M.V., Ramanujam, V.M.S. and Anderson, K.E. Altered time course of urinary daidzein and genistein excretion during chronic soya diet in healthy male subjects. *Nutr. Cancer* 24: 311–323, 1995.
98. Lu, L.-J.W., Anderson, K.E., Grady, J.J. and Nagamani, M. Effects of soya consumption for one month on steroid hormones in premenopausal women: Implications for breast cancer risk reduction. *Cancer Epidemiol. Biomarkers Prev.* 5: 63–70, 1996.
99. Adlercreutz, H., Fotsis, T., Lampe, J., Wahala, K., Makela, T., Brunow, G. and Hase, T. Quantitative determination of lignans and isoflavonoids in plasma of omnivorous and vegetarian women by isotope dilution gas chromatography-mass spectrometry. *Scand. J. Clin. Lab. Invest.* 53: 5–18, 1993.
100. Xu, X., Wang, H.-J., Murphy, P.A., Cook, L. and Hendrich, S. Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. *J. Nutr.* 124: 825–832, 1994.
101. Lu, L.-J.W., Hokanson, J.A., Anderson, K.E., Marshall, M.V., Hu, D.M.-C. Kinsky, M.P. and Ramanujum, S.V.M. Urinary excretion of isoflavones in healthy subjects after soymilk consumption. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 34: 556, abstract no. 3314, 1993.
102. Kelly, G.E., Nelson, C., Waring, M.A., Joannou, G.E. and Reeder, A.Y. Metabolites of dietary (soya) isoflavones in human urine. *Clin. Chim. Acta* 223: 9–22, 1993.
103. Adlercreutz, H., van der Wildt, J., Kinzel, J., Attalla, H., Wahala, K., Makela, T., Hase, T. and Fotsis, T. Lignan and isoflavonoid conjugates in human urine. *J. Steroid Biochem. Mol. Biol.* 52: 97–103, 1995.
104. Cassidy, A., Bingham, S. and Setchell, K.D.R. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am. J. Clin. Nutr.* 60: 333–340, 1994.
105. Barnes, S., Peterson, T.G. and Coward, L. Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer. *J. Cell. Biochem.* 22: 181–187, 1995.
106. Lagios, M.D. Evaluation of surrogate endpoint biomarkers for ductal carcinoma *in situ*. *J. Cell. Biochem.* 19: 186–188, 1994.

Table I. Clinical Trials of Genistein 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-65117 Phase I Single and Multidose Safety, Pharmacokinetic, and Efficacy Clinical Studies of Genistein in Prostate Neoplasia (Dr. Steven Zeisel, University of North Carolina) 9/96–11/97 New IND	---	Single dose: Healthy volunteers 24–30 subjects (3/dose/product) Multidose: Healthy volunteers and Stage C or D prostate cancer patients 24–30 subjects (6/subject type/product)	Two purified soy isoflavone products containing 90% and 43% genistein Single dose: 3–4 dose escalations Multidose: Dose chosen from single dose study for 3 months	Single and multidose Safety, pharmacokinetics of genistein and genistin (plasma and urine)	Protocol being finalized
Phase II (Dose titration, efficacy, intermediate biomarkers)					
Planned Study Genistein in Prostate Neoplasia: Administration During the Period Between Diagnostic Core Biopsy and Definitive Surgery	Prostate	Men scheduled for diagnostic biopsy for prostate cancer diagnosis 100 patients (50/arm)	Dose to be determined for the 3–8 weeks between diagnostic biopsy and surgery	Efficacy: Intermediate biomarkers (*e.g.*, PIN grade, ploidy, PSA, PCNA, MIB-1, S-phase fraction, nuclear morphometry)	Study not yet designed.
Planned Study Genistein in Breast Neoplasia: Administration During the Period Between Diagnostic Biopsy and Definitive Surgery	Breast	Women with mammogram suspicious for DCIS or early stage breast cancer 100 patients (50/arm)	Dose to be determined for the 1–4 weeks between diagnostic biopsy and surgery	Efficacy: Intermediate biomarkers (*e.g.*, DCIS grade, ploidy, PCNA, MIB-1, S-phase fraction, nuclear morphometry)	Study not yet designed.

GENISTEIN DEVELOPMENT STATUS