The effect of genistein aglycone on cancer and cancer risk: a review of in vitro, preclinical, and clinical studies

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In Asian epidemiological studies, health benefits, including reduced incidence of breast and prostate cancers, are attributed to soy food and isoflavone consumption. The recent increased intake of soy foods and supplements in the American diet has raised concerns about the possible estrogen-like effects of natural isoflavones and possible promotion or propagation of estrogen-sensitive cancers. These concerns are primarily based on in vitro and rodent data which suggest that genistein aglycone can stimulate tumor cell proliferation and growth in mice having deficient immune systems. In contrast, a recent nested case-control study and meta-analysis of numerous epidemiological studies show an inverse correlation between genistein intake and breast cancer risk. Furthermore, clinical studies in osteopenic and osteoporotic, postmenopausal women support the breast and uterine safety of purified naturally derived genistein administered for up to 3 years. In this review, we summarize the in vitro*, preclinical and clinical evidence for the safety of natural genistein.*

INTRODUCTION

There has been considerable interest in the putative cancer-preventing effects of genistein aglycone as well as other soy isoflavones based on epidemiological studies showing an inverse relationship between soy intake and risk of breast or prostate cancer.[1–3] Despite these suggestive epidemiological data, the results of the few dietary intervention studies conducted to date have been mixed. As a consequence, the National Cancer Institute is currently sponsoring several clinical trials investigating genistein as a therapeutic for treating prostate, bladder, and kidney cancer and for its chemopreventative effects in breast and endometrial cancer (see http://www.clinicaltrials.gov).

The predominant isoflavones in soy are genistin and daidzin, along with their minor aglycone forms genistein and daidzein.[4] Soy and soy products also contain a small amount of the isoflavone glycitin and its aglycone, glycitein.[5] The heightened concerns over the estrogenic-effect of soy isoflavone consumption is based on *in vitro* and rodent data suggesting that genistein may stimulate the proliferation of estrogen receptor (ER)-positive breast tumors.[6,7] Genistein has multiple molecular targets in the body, including various receptor, enzyme, and pathway interactions (Figure 1). Given the apparently complex relationship between isoflavone intake and cancer risk, this review is intended to give an overview of the literature investigating genistein's effects on cancer risk and established cancers at doses that have been shown to have an effect on bone mineral density and vasomotor symptoms in several clinical trials (35–54 mg/day).[8–12] Overall, the literature and data from clinical trials on purified genistein suggest that it is safe for use in peri- and postmenopausal women with no history of breast or reproductive organ cancers.

BACKGROUND

Hormone binding to estrogen receptors and consequences of ligand functional selectivity in relation to cancer

Estrogen receptors are members of the nuclear hormone family of intracellular receptors that are activated by the

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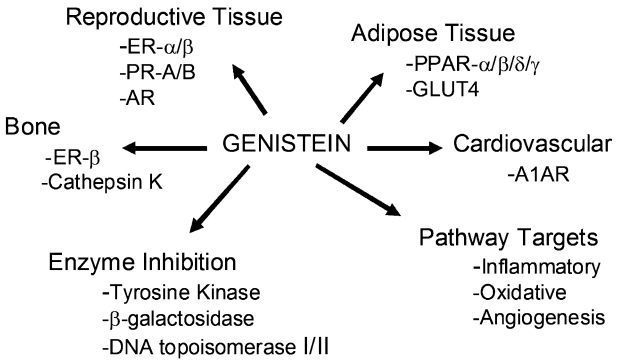

Figure 1 **Molecular, tissue and pathway targets with which genistein interacts.**
Abbreviations: ER, estrogen receptor; AR, androgen receptor; PR, progesterone receptor; PPAR peroxisome proliferator-activated receptors; GLUT, insulin-regulated glucose transporter; A1AR, A1 adenosine receptor.

hormone 17β-estradiol.[13] The main function of ERs is as DNA-binding transcription factors that regulate gene expression. Paradoxically, stimulation of ERs can be both beneficial and harmful; benefits include programming the breast and uterus for sexual reproduction, controlling cholesterol production to limit buildup of plaque in the coronary arteries, and preserving bone strength by helping to maintain the proper balance between bone formation and resorption; harmful effects are evident in that proliferation of breast and uterine tissue can occur.[14] The predominant type of receptor bound, the nature of the binding, and the affinity of a compound for the receptor seems to determine the observed outcome.

There are two different forms of the ER, referred to as α and β, each encoded by a separate gene (ESR1 and ESR2, respectively).[15–18] Hormone-activated ERs may form ERα (αα) and ERβ (ββ) homodimers or ERαβ (αβ) heterodimers, depending on their cellular expression.[19] Both ERs are widely expressed in different tissue types; however, there are some notable differences in their expression patterns. ERα is found in endometrium, breast, ovarian stroma, and in hypothalamic tissue. Expression of ERβ has been documented in kidney, brain, bone, heart, lungs, intestinal mucosa, prostate, and endothelial cells.[20–23] In the absence of hormone, ERs are located in the cytosol. Hormone binding to the ER induces a conformational change in the receptor that initiates a series of events starting with migration of the receptor from the cytosol into the nucleus, dimerization of the receptor, and subsequent binding of the receptor dimer to hormone response elements on DNA. The DNA/receptor complex then recruits transcriptional co-activator or co-repressor proteins, which, in turn, promote activation or repression of hormone-responsive genes.[24–26]

Estrogen receptors appear to have diverse biological functions and respond differently to various estrogenic compounds. The same ligand may be an agonist in some tissue (where co-activator proteins predominate) and an antagonist in other tissues (where co-repressor proteins dominate). For example, raloxifene is an antagonist in breast tissue and is therefore used to reduce the risk of certain breast cancers, but it is an agonist in bone (preventing osteoporosis) and in the endometrium (increasing uterine cancer risk).[27] Various ligands differ in their affinity for the ERα and ERβ isoforms: 17β-estradiol binds equally well to both receptors, raloxifene binds preferentially to Erα, while genistein binds preferentially to ERβ.[28,29] Genistein's affinity is from 7- to 48-fold more selective for binding to ERβ over ERα, depending on the assay system.[28–31] The relative estrogenic potency of genistein at ERβ is about 30-fold higher compared with the potency at ERα. When compared to 17β-estradiol affinity for ERα, the potency of genistein for this receptor is 130-fold lower. Despite the relatively low affinity and potency for ERα, genistein can act as an agonist at this receptor, and it exerts weak estrogen-like effects *in vitro* at high concentrations.[28,29,31] However, in clinical studies, estrogen-like effects on ERα are often not observed.[9,11,12,32–36] This discrepancy is not surprising since ER binding alone is a poor predictor of *in vivo* efficacy.[31,37]

Studies have shown that 17β-estradiol and genistein differ in their activation of genes due to different rates of ER binding to the estrogen response element (ERE), with genistein activating predominantly ERβ.[29,38] These data are important since ERβ has been shown by cluster analysis to upregulate the expression of genes that enhance cell cycle progression and that are important in the negative regulation of cellular proliferation. Activated ERβ has also been shown to affect the expression of genes associated with RNA processing and metabolism, G-protein coupled receptor signaling and integrin-mediated signaling, and to have a suppressive activity on ERα related to activities such as cellular proliferation.[39–41]

Structural studies have shown that binding events and different ER ligands induce distinct ER conformations that lead to the formation of ligand-dependent activation on functional surfaces and recruitment of co-activator or co-repressor proteins. Thus, ER transcriptional responses are unique to each ligand[37,42,43] and hence, tissue specific, based on ER and co-activator and co-repressor protein expression.[31,44–46] Genistein's selective transcriptional activation of genes under ERβ regulation, as compared to ERα, has been shown to be mediated by a greater capability to recruit co-regulator proteins, through a specific conformational change, and to form a transcriptionally competent activation function surface.[42,47] When genistein binds to ERβ, an antagonist-

induced conformation is adopted,[37] similar to, though unique from, the conformation adopted when raloxifene binds. These data suggest that genistein, at the molecular level, can act similarly to a selective estrogen receptor modulator (SERM) depending on tissue and the presence of ER co-regulator proteins that stabilize either an antagonist- or agonist-induced conformation.

The subtle differences in ER conformation and co-regulator recruitment are a result of structural differences among ligands and how they bind in the ER ligand-binding domain.[24] Results of studies conducted by Manas et al.[43] showed that the ERβ selectivity of genistein is due to favorable steric interactions that result in subtle changes in the ligand-binding domain. In the ERα ligand-binding domain, however, unfavorable steric interactions occur, which lead to the decreased binding affinity and potency of genistein. The consequence of this selective receptor modulation is enhanced transcriptional activation or repression of promoters and genes under ERβ regulation, as compared to ERα. The binding interaction of certain molecules to specific receptors, however, can lead to adverse events.

The exact nature of ER stimulation to promote breast cancer is not well understood. Both direct stimulation of tissue growth by exposure to estradiol as well as the production of quinone derivatives from estradiol, genotoxic products which promote cancer, have been proposed.[48] In fact, there may be a combination of events that lead to cell proliferation, DNA mutations, and the development of neoplasms of the breast. Nearly 80% of all uterine carcinomas are associated with estradiol interactions with ERα initially inducing endometrial hyperplasia, while a minority seem to be non-estradiol related.[49] Similarly, ovarian cancer is driven predominantly by estradiol in about 90% of all cases arising from the epithelial layer, which contains a preponderance of ERα receptors.[50] These data support the role for ERα mediated binding events over ERβ in the development of reproductive neoplasms.

GENISTEIN EFFECTS ON CANCER AND CANCER RISK

Preclinical studies

There are multiple studies that have examined the effects of genistein on mutagenesis and carcinogenesis both *in vitro* and *in vivo*.

Mutagenesis

In an early study, genistein was shown to be non-mutagenic in the classic Ames test using *Salmonella typhimurium* histidine(-) variant strains, both with and without metabolic activation.[51] The Ames test results were replicated in a recent experiment demonstrating that genistein did not increase the number of revertant colonies across five *S. typhimurium* strains with or without metabolic activation.[52] In an *in vitro* mouse lymphoma cell assay system, genistein induced an increase in the number of resistant colonies to the mutagens 4-nitroquinoline 1-oxide (NQO) and methylmethansulfonate (MMS). These mutants were primarily small colonies, suggesting that genistein acted as a clastogen rather than a mutagen.[52] Another *in vitro* study found that genistein dose-dependently slowed and inhibited growth of Syrian hamster embryo cells, induced abnormal changes in cell shape, mutations, chromosome aberrations (including changes in chromosome number), and DNA adduct formation, suggesting carcinogenic potential.[53] However, the lowest concentration of genistein used in this experiment was 12.5 μM, a concentration well in excess of the peak serum concentrations of 1–3 μM found in humans following 50–60 mg/day dosing, as used in many recent human studies[9,36,54,55] and human pharmacokinetic experiments.[54,55] In another cell-based assay, genistein, at concentrations of 5–25 μM (again, in excess of conventional human doses) was found to induce micronuclei, a function that declined with higher concentrations of genistein, perhaps because of increasing cytotoxicity.[56] In contrast, there was no evidence of clastogenicity or mutagenicity following *in vivo* treatment of up to 20 mg/kg/day genistein in mice for 14 days and acute dosing of genistein (up to 2000 mg/kg) in rats.[52] In these *in vivo* experiments, exposure to genistein in mice and rats produced no increase in micronuclei.[51] Collectively, these results suggest that genistein can produce mutagenic or clastogenic effects under some *in vitro* experimental conditions of high genistein concentrations not achievable in humans through dietary administration of genistein.

Carcinogenesis and metastasis: effects on cancer induction

There are few preclinical research studies that have focused specifically on whether genistein inhibits or promotes the development of new cancers. The preponderance of existing general toxicological evidence, however, suggests that it does not promote the development of new cancers. In 1-year toxicology studies in both rats and dogs using up to 500 mg/kg/day of genistein, administered by oral gavage or consumed in the diet, there was no evidence of cancer in any organ, including reproductive tissues.[54,57] There was, however, some evidence of an estrogenic effect at doses from 50 to 500 mg/kg/day, including squamous metaplasia and hyperplasia of the uterus, changes in uterine and ovarian weights, and

ovarian atrophy. These changes, observed at doses of genistein that were 50- to 500-fold in excess of the 1 mg/kg/day dose commonly used in recent human studies, were not accompanied by any evidence of neoplasia.

The largest preclinical study investigating the toxicology, multigenerational reproductive effects, and influence of genistein on *de novo* carcinogenesis was sponsored by the National Toxicology Program (NTP).[57–61] The studies administered genistein to male and female Sprague-Dawley rats, mixed in the diet at concentrations of 0, 5, 100, and 500 parts per million (ppm), for 2 years. Genistein had no effect on survival at any dose. There was no evidence of increased cancer risk among males associated with any concentration of genistein in the feed corresponding to doses as high as 20 mg/kg/day. Among females, after 2 years of exposure to 100 ppm, the incidence of adenoma and adenocarcinoma was statistically indistinguishable from that among female rats on a control diet. At the 500 ppm dose of genistein, the incidence of mammary gland fibroadenoma was significantly decreased and the incidence of mammary adenoma or adenocarcinoma was significantly increased compared to female rats on the control diet. Therefore, at the exaggerated dose levels used in this study, there was a decrease in benign, possibly premalignant fibroadenomatous nodules but an increase in the incidence of histologically determined mammary adenomas and adenocarcinomas. The NTP reports concluded that there is "no evidence" for carcinogenic activity of genistein in male rats and "equivocal evidence" in females on the basis of an empirical investigation in Sprague-Dawley rats.

Although the NTP study provides valuable information concerning the potential health effect of life-long exposure to genistein, its findings likely do not accurately represent risk associated with purified genistein therapy in peri- and post-menopausal women. The concentration of genistein that was associated with increased risk of adenoma or adenocarcinoma in female rats is at least 29-fold greater than the amount taken by peri- and post-menopausal women in clinical studies investigating the effect of genistein on bone mineral density and vasomotor symptoms. Moreover, the timing of exposure was such that animals were exposed to high levels from conception until sacrifice. The extent to which extremely high doses during gestation and development may have influenced the induction of adenoma and adenocarcinoma is not known. Finally, it is interesting to note that the 500 ppm genistein group was associated with a decrease in benign fibroadenoma incidence. Therefore, though the NTP concluded that the evidence for carcinogenesis associated with lifetime genistein exposure is "equivocal" in female rats, dosing and timing issues associated with the study, as well as discrepant experimental results, do not provide evidence that genistein intake would produce an increase in breast cancer risk.

Effects on cancer cell lines (*in vitro*)

Numerous studies have shown an inhibitory effect of genistein on cancer cell and tumor growth *in vitro*. Treatment of NIH 3T3 cells, a mouse embryonic fibroblast cell line, with greater than 20 μM genistein induced apoptotic and necrotic cell death significantly affecting cell viability (Table 1).[62] Breast epithelial cells normally exist in homeostatic balance between cell proliferation and apoptosis.[63] A number of studies suggest that genistein may induce apoptosis in several breast cancer cell lines and produce synergistic inhibitory effects when combined with cancer therapies. For instance, genistein has been shown to induce apoptosis in the high-invasive (ER-negative) MDA-MB-231 and the low-invasive (ER-positive) MCF-7 breast cancer cell lines at relatively high concentrations of 10–100 μM (Table 1).[64–71] Synergistic pro-apoptotic effects have been shown when genistein has been combined with tamoxifen on tamoxifen-resistant BT-474 breast cancer cells that overexpress human epidermal growth factor receptor 2 (HER2)[72] (Table 1) and with adriamycin and docetaxel in MDA-MB-231 cells, a breast cancer line overexpressing ERβ (Table 1).[73] Furthermore, genistein showed synergistic effects in combination with cisplatin, docetaxel, or doxorubicin in PC-3 (prostate), MDA-MB-231 (breast), H460 (lung), and BxPC-3 (pancreas) cancer cell lines (Table 1).[74,75] Genistein increased the growth inhibition and apoptosis and reduced the adhesion and migration of these cancer cell lines by inhibiting nuclear factor-kappa B (NF-κB) and Akt transcription factors.[74,76,77] In the MDA-MB-231 and MCF-7 cell lines, genistein arrested invasiveness in the G2M (metastatic) phase through transcriptional downregulation of multiple matrix metalloproteinase genes (MMP) (Table 1).[78] Regenbrecht et al.[79] recently showed that when primary glioblastoma, rhabdomyosarcoma, hepatocellular carcinoma, or human embryonic carcinoma cells were exposed to a 50 μM genistein concentration, the cells underwent cell cycle arrest in M-phase. The inhibitory effects of genistein on cancer cell growth may also be due to non-estrogenic cellular effects (possibly including inhibition of protein tyrosine kinases,[80] DNA topoisomerase II,[1,81,82] and induction of p21),[83] or they could be due to increasing intracellular antioxidant potential.[84] A recent review concludes that many of the potential anti-cancer effects of genistein are due to gene expression modulation of Akt, NF-κB, MMPs, and Bax/Bcl-2 signaling pathways.[85]

Recently, it was reported that genistein induces the expression of BRCA-1 and BRCA-2 breast tumor suppressor genes in breast and prostate cancer cells[67,86] and

Table 1 **Effects of genistein on markers of cancer risk observed from cell experiment studies performed *in vitro*.**

Reference	Cell type	Description	Measure of interest	Results
Rucinska (2008)[62]	NIH 3T3 cells	Mouse embryonic fibroblasts	Morphological, antiproliferative and apoptotic effects	GEN reduced cell viability, caused cell morphological changes, and induced apoptotic and necrotic cell death Oxidative modification of protein increased in a dose- and time-dependent manner
Fioravanti (1998), Le Bail (1998), Hsieh (1998), and Jeune (2005)[64–67]	MDA-MB-231	ER-negative (estrogen-independent) human BRCA cells	Antiproliferative and apoptotic effects; cell motility; protease secretion; NF-κB, AP-1, Bcl-2, MMP, BRCA-1, BRCA-2, ERα, ERβ, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	GEN caused accumulation of cells in the S and G2/M cell cycle phases and induced apoptotic cell death in a dose- and time-dependent manner
Caetano (2006)[87]	MDA-MB-231	ER-negative (estrogen-independent) human BRCA cells	Antiproliferative and apoptotic effects; cell motility; protease secretion; NF-κB, AP-1, Bcl-2, MMP, BRCA-1, BRCA-2, ERα, ERβ, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	Counteracted the growth-stimulatory effects exerted by estradiol
Liu (2005)[68]	MDA-MB-231	ER-negative (estrogen-independent) human BRCA cells	Antiproliferative and apoptotic effects; cell motility; protease secretion; NF-κB, AP-1, Bcl-2, MMP, BRCA-1, BRCA-2, ERα, ERβ, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	Increased expression of Bax protein and the decreased expression of erbB-2 protein
Valachovicova (2004)[71]	MDA-MB-231	ER-negative (estrogen-independent) human BRCA cells	Antiproliferative and apoptotic effects; cell motility; protease secretion; NF-κB, AP-1, Bcl-2, MMP, BRCA-1, BRCA-2, ERα, ERβ, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	GEN suppressed cell adhesion, migration and motility by inhibiting the constitutively active transcription factors NF-κB and AP-1, suppression of secretion of urokinase-type plasminogen activator (uPA)
Satoh (2003)[73]	MDA-MB-231	ER-negative (estrogen-independent) human BRCA cells	Antiproliferative and apoptotic effects; cell motility; protease secretion; NF-κB, AP-1, Bcl-2, MMP, BRCA-1, BRCA-2, ERα, ERβ, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	Synergistic proapoptotic effects when combined with adriamycin and docetaxel
Li (2005) and Mohammad (2006)[74,75]	MDA-MB-231	ER-negative (estrogen-independent) human BRCA cells	Antiproliferative and apoptotic effects; cell motility; protease secretion; NF-κB, AP-1, Bcl-2, MMP, BRCA-1, BRCA-2, ERα, ERβ, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	Synergistic proapoptotic effects when combined with cisplatin, docetaxel, or doxorubicin
Satoh (2003)[78]	MDA-MB-231	ER-negative (estrogen-independent) human BRCA cells	Antiproliferative and apoptotic effects; cell motility; protease secretion; NF-κB, AP-1, Bcl-2, MMP, BRCA-1, BRCA-2, ERα, ERβ, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	GEN arrested invasiveness in the G2M cell cycle phase through transcriptional downregulation of multiple matrix metalloproteinase genes (MMP)
Caetano (2006)[87]	MDA-MB-231	ER-negative (estrogen-independent) human BRCA cells	Antiproliferative and apoptotic effects; cell motility; protease secretion; NF-κB, AP-1, Bcl-2, MMP, BRCA-1, BRCA-2, ERα, ERβ, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	Increased BRCA-1, BRCA-2, p53, p21, GADD45, BAX, Jun, Rb1, p300, BARD1, BAP1, RAD51, TNF and Ki-67 expression; decreased pS2 expression
Mai (2007)[70]	MCF-7	ER-positive (estrogen-dependent) human BRCA cells	Antiproliferative and apoptotic effects	GEN inhibited cell growth and caused accumulation of cells in the G1 cell cycle phase
Fan (2006)[86]	MCF-7	ER-positive (estrogen-dependent) human BRCA cells	BRCA-1, BRCA-2, ERα, ERβ, EGFR, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	GEN/tamoxifen combination produced synergistic inhibitory effects GEN time- and dose-dependently induced expression of both BRCA-1 and BRCA-2
Caetano (2006)[87]	MCF-7	ER-positive (estrogen-dependent) human BRCA cells	BRCA-1, BRCA-2, ERα, ERβ, EGFR, pS2, p53, p21, GADD45, BAX, jun, Rb1, p300, BARD1, BAP1, RAD51, TNF, and Ki67 expression	Decreased ERα and EGFR expression; decreased ERα expression and increased pS2, p21, GADD45, and BAX expression
Seo (2006)[6]	MCF-7	ER-positive (estrogen-dependent) human BRCA cells	Stimulation of cell proliferation	GEN stimulated cellular proliferation after 6 days in growth culture with MCF-7 cells and blocked the anti-proliferative effect of Tamoxifen
Hsieh (1998)[66]	MCF-7	ER-positive (estrogen-dependent) human BRCA cells	Stimulation of cell proliferation	Decreased ERα mRNA and protein levels GEN stimulated cellular proliferation
Farina (2006)[69]	F3II cells	Mouse BRCA cell line	Tumor cell motility	GEN reduced tumor cell migration/motility and decreased urokinase-type plasminogen activator and increased tissue-type plasminogen activator secretion
Farina (2006)[69]	B16 cells	Mouse melanoma cell line	Tumor cell motility protease secretion	GEN reduced tumor cell migration/motility and decreased urokinase-type plasminogen activator and increased tissue-type plasminogen activator secretion
Mai (2007)[72]	BT-474	Human BRCA cells overexpressing human epidermal growth factor receptor-2 (HER2)	Antiproliferative and apoptotic effects; survivin, EGFR, HER2, and ERα expression	GEN/Tamoxifen synergistically inhibited cell growth, caused accumulation of cells in the G1 cell cycle phase and induced apoptosis; expression of survivin, EGFR, HER2, and ERα were downregulated
Li (2005)[74]	PC-3	Human prostate	Antiproliferative and apoptotic effects	Synergistic proapoptotic effects when combined with cisplatin, docetaxel, or doxorubicin
Mohammad (2006)[75]	BxPC-3, H460	Pancreatic and lung cancer cell lines	Antiproliferative and apoptotic effects	Synergistic proapoptotic effects when combined with cisplatin, docetaxel, or doxorubicin
Schwartz (1998)[91]	HeLa	Human cervical cancer cells transiently transfected with human ERα	Stimulation of cell proliferation	GEN stimulated cellular proliferation

Abbreviations: AP-1, activator protein-1; BARD1, BRCA-1 associated RING domain-1; BAP1, BRCA-1 associated protein-1; BAX, bax protein; BRCA, breast cancer; BRCA-1, breast cancer susceptibility gene-1; BRCA-2, breast cancer susceptibility gene-2; Bcl-2, bcl-2 protein; EGFR, epidermal growth factor receptor; ERα, estrogen receptor-α; ERβ, estrogen receptor-β; GADD45, p53-regulated stress protein; GEN, genistein; HER2, epidermal growth factor receptor-2; jun, c-jun protein; Ki67, MKI67 protein; NF-κB, nuclear factor-kappa beta; MMP, matrix metalloproteinase; Ki67 protein; pS2, breast cancer-associated pS2 protein; p53, p53 tumor suppressor protein; p21, ras-oncogene-encoded p21 protein; p300, p300 protein; RAD51, rad51 recombination protein; Rb1, retinoblastoma tumor suppressor protein; TNF, tumor necrosis factor.

the overexpression of many genes involved in the BRCA-1 and BRCA-2 pathways such as GADD45A, BRCA associated ring finger domain1 protein (BARD1), ERα and ERβ, p53, and tumor necrosis factor-α (TNFα) in MCF-7 and MDA-MB-231 breast cancer cells (Table 1).[87] Also, a study using BRCA-1 antisense blocked (AS4) and unblocked (NEO) BG-1 ER-positive ovarian cancer cells showed that genistein caused concentration-dependent growth inhibition through two different pathways, depending on the presence or absence of BRCA-1, producing comparable cytotoxic effects via both pathways.[88] Genistein inhibited ERα expression and activated BARD1 in BRCA-1 blocked AS4 cells but activated ERβ and FAS expression and induced caspase-8-dependent apoptotic pathway when BRCA-1 was present in NEO cells. Therefore, the use of genistein in both BRCA-1 mutated and wild-type cancers seems rational.

Despite numerous studies supporting a chemopreventive profile for genistein, there is also some *in vitro* evidence that genistein can stimulate ER-positive breast tumors. Several studies suggest that genistein can stimulate proliferation and estrogen-sensitive gene expression of the ER-positive MCF-7 breast cancer cell line at concentrations of 1–10 μM (Table 1).[6,65,66,89–91] At similar concentrations, genistein was particularly mitogenic to tamoxifen-sensitive cells[89,92,93] and antagonized the antitumor effect of tamoxifen.[92–94] In contrast, genistein has been shown to have either no effect or an inhibitory effect on the growth of ER-negative and tamoxifen-resistant breast cancer cells.[6,89,95]

It should be emphasized that *in vitro* studies represent an artificial milieu that typically do not mimic the dynamics of cancer cell growth in an intact organism. In particular, cells are exposed to fixed and notably higher concentrations of genistein in culture over long periods of time. While in an intact organism, the majority of circulating genistein following oral administration is conjugated to glucoronide and sulfate moieties following biotransformation in the intestine and liver.[55] These metabolites are approximately 100-fold less potent at stimulating proliferation of MCF-7 cells than genistein,[91] suggesting that *total* (i.e., aglycone and conjugated) circulating genistein would produce smaller effects on cancer cell proliferation than the pure non-conjugated genistein used in *in vitro* experiments.

Methodological differences among these studies may account, in part, for contradictory findings with respect to whether genistein stimulates or inhibits cancer cell growth *in vitro*. Furthermore, there are documented differences between different MCF-7 isolates in their sensitivities to estrogens and anti-estrogens, differential expression of ERs, ER mRNA, and progesterone receptor as well as differences in tumorigenicity and proliferation rates.[96] Without knowledge of the historical lineage of the particular MCF-7 isolate or whether it is, in fact, a clonal isolate at all, makes it difficult to interpret and compare these experimental data. Therefore, these results must be interpreted with caution. The consistent finding from the literature, however, is that concentrations of genistein below 10 μM appear to stimulate cancer cell growth while concentrations greater than 10 μM inhibit cancer cell growth.

Effects on models of induced cancer (*in vivo*)

The carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) has been used to induce carcinomas in standard animal models of cancer and these animal models have been used for the identification of chemopreventative agents. In DMBA-treated mice, genistein reduced the incidence of skin tumors by about 20% by inhibition of DNA adduct formation, inhibition of oxidative damage,[97,98] and by inhibition of the initiation phase of DMBA-induced carcinogenesis (Table 2).[99] Female rats exposed to genistein in the early neonatal period and challenged with DMBA at day 50 post-partum had a reduced incidence of mammary tumors (Table 2).[98] Histologic examination showed more terminal end buds and mature ducts and more proliferative activity in all terminal mammary structures, suggesting that the protective effect of genistein was due to early maturation of terminal duct structures and possible alteration of the endocrine environment to reduce cellular proliferation. These data suggest that genistein exposure early in life may reduce the risk of mammary/breast cancer.[98] Another study found no difference in incidence or multiplicity of tumors between rats fed a standard diet or a diet enriched with genistein at approximately 200 mg/kg/day and later challenged with DMBA (Table 2).[100] In contrast, when the isoflavone daidzein was substituted for genistein in the same DMBA-induced animal model, no protective effect was observed.[98,99,101] In this animal model of cancer development, circulating estradiol and progesterone levels, as well as oocyte and follicle counts were not different from controls, suggesting that genistein suppressed the development of chemical-induced mammary cancer without toxicity to the endocrine or reproductive systems. Genistein and daidzein also inhibited hepatic sulfonotransferase, thus altering the balance of estrogens to inactive estrogen sulfonates.[102] These results imply that, although the structures of genistein and daidzein are similar, differences in their chemical structure results in distinctly different effects *in vivo*.[103]

In nude mice orthotopically implanted with MDA-MB-435 cells, there was a 10-fold lower metastatic burden when their diets were supplemented with genistein as compared to control feed (Table 2).[104] Although it was long believed that MDA-MB-435 cells are a breast cancer

Table 2 **Effects of genistein on markers of cancer risk observed from animal studies performed *in vivo*.**

Reference	Species	Treatment	Study length	Measure of interest	Results
Mai (2007)[70]	Severe combined immune deficient mice	Implanted MCF-7 cells, diet supplemented with 0.07% GEN	85 days	Tumor growth progression; ERα, pS2, and EGFR expression	GEN inhibited tumor growth GEN/tamoxifen combination produced additive to synergistic growth-inhibitory effects, induced MCF-7 cell apoptosis, and decreased ERα and EGFR expression
Ju (2002)[110]	Ovariectomized athymic BALB/c (nude) mice	Injected MCF-7 cells into two sites on the flank of the mouse, 0.25 mg E2 pellets implanted, diet supplemented with 1000 ppm GEN	32 weeks	Effect of GEN on E2-dependent breast tumor growth in animals treated with tamoxifen; pS2, PR, and cyclinD1 mRNA expression	GEN negated the reduction in tumor surface area, pS2, PR and cyclinD1 mRNA expression achieved by tamoxifen treatment
Ju (2006)[111]	Ovariectomized athymic BALB/c (nude) mice	MCF-7 cells injected into mice, silastic implants inserted s.c. containing 1:31, E2:cholesterol, diet supplemented with 500 ppm GEN	17 weeks	Effect of GEN on E2-dependent breast tumor growth	At 17 weeks GEN or E2 alone and GEN combined with E2 significantly increased tumor surface area and uterine weight
Vantyghem (2005)[104]	Female athymic nude mice	MDA-MB-435/HAL human breast carcinoma cells injected into mammary fat pad of 8-week-old mice, diet supplemented with 750 μg/g GEN	70 days	Efficacy of adjuvant treatment with GEN to inhibit the outgrowth of metastases unresected and post-resection	In mice with unresected tumors, GEN-supplemented diet significantly reduced tumor volume from day 10 to day 30 At day 35, the mean tumor volume was not significantly different from DMBA-treated control By day 70, mice on the GEN-supplemented diet had significantly lower metastatic burden in the lungs, significantly decreased percentages of proliferating tumor cells within lung metastases, and increased percentages of tumor cells undergoing apoptosis
Vantyghem (2005)[104]	Female athymic nude mice	MDA-MB-435/HAL human breast carcinoma cells injected into mammary fat pad of 8-week-old mice, diet supplemented with 750 μg/g GEN	70 days	Efficacy of adjuvant treatment with GEN to inhibit the outgrowth of metastases unresected and post-resection	Post-resection adjuvant treatment with GEN significantly limited the metastatic burden of the total lung volume, reducing metastatic burden in the lungs 10-fold, and completely inhibited lymph node metastases
Upadhyaya (1998)[97]	Female CD rats	DMBA-induced carcinogenesis, diet supplemented with 111 ppm GEN	50 days	DMBA-DNA binding in liver and mammary tissues; DNA-adduct formation	GEN inhibited DMBA-DNA binding in mammary tissue Total binding was inhibited because of reduced formation of three major adducts: anti-diol epoxide deoxyguanosine, syn-diol epoxide deoxyadenosine, and anti-diolepoxide deoxyadenosine
Lamartiniere (1995) and Lamartiniere (2002)[98,99]	Female Sprague-Dawley CD rats	Injected s.c. with 5 mg GEN on days 2, 4, and 6 postpartum. At day 50, they were exposed to 80 μg DMBA/g body weight	50 days	Mammary cell proliferation; mammary histomorphometry; reproductive maturation; serum progesterone and 17-beta estradiol	Neonatal GEN exposure increased latency and reduced incidence and multiplicity of DMBA-induced mammary adenocarcinomas, fewer mammary terminal end buds, lower percentages and total numbers of cells in S-phase in terminal end buds, terminal ducts, lobules I and lobules II Vaginal openings occurred earlier, uterine-ovarian weights were smaller In 21-day-old GEN-treated rats, mammary glands were larger and there were more terminal end buds and terminal ducts, and more proliferative activity in all terminal ductals structures In 50-day-old GEN-treated rats there were atretic antral follicles, fewer corpora lutea, and lower circulating progesterone but not 17-beta estradiol concentrations
Constantinou (2001)[100]	Female Sprague-Dawley CD rats	GEN (200 mg/kg diet), for 1-week prior to mammary carcinomas induced with DMBA	120 days	DMBA-induced tumor incidence, multiplicity, latency and survival	GEN-treated group showed no significant difference in tumor incidence or survival, nor was there a significant reduction in tumor multiplicity
Wood (2004)[106]	Ovariectomized adult female cynomolgus monkeys	The SPI treatment group received SPI containing isoflavones equivalent to 129 mg/d (~91 mg GEN, 31 mg daidzein, and 7 mg glycitein).	36 months	Breast and uterine proliferation markers; apoptosis; Ki67, MK167 and PR expression; serum estrogens; uterine size; vaginal maturation in either the follicular or luteal phase; *ERα* and *ERα*-driven genes: *TFF1*, *CXCL12*, and *PR*	Three years of SPI + isoflavones did not significantly alter epithelial area, Ki67 expression, or sex steroid receptor expression in the breast or uterus E_1 and E_2 serum concentrations were significantly decreased.
Wood (2006)[107]	Ovariectomized adult female cynomolgus monkeys	The SPI treatment group received SPI containing isoflavones equivalent to 129 mg/d (~91 mg GEN, 31 mg daidzein, and 7 mg glycitein	12 months	Breast and uterine proliferation markers; apoptosis; Ki67, MK167, and PR expression; serum estrogens; uterine size; vaginal maturation in either the follicular or luteal phase; *ERα* and *ERα*-driven genes: *TFF1*, *CXCL12*, and *PR*	No significant effect on: mean cycle length; serum E_2 or progesterone; breast epithelial area, proliferation, apoptosis; PR expression; or uterine size or vaginal maturation
Wood (2006)[108]	Ovariectomized adult female cynomolgus monkeys	The treatment group received isoflavones equivalent to 0, 60, 120, or 240 mg/d (0, 31, 74, or 148 mg/d GEN) with oral E_2 at doses equivalent to either 0.09 mg/d (low E_2) or 0.5 mg/d (high E_2)	4 months	Breast and uterine proliferation markers; apoptosis; Ki67, MK167, and PR expression; serum estrogens; uterine size; vaginal maturation in either the follicular or luteal phase; *ERα* and *ERα*-driven genes: *TFF1*, *CXCL12*, and *PR*	No significant estrogen-like isoflavone effects were identified Isoflavones antagonized E_2-induced proliferation in the breast and uterus in a dose-dependent manner and decreased serum E_2 concentrations In the low E_2 environment, isoflavones significantly lowered vaginal maturation values, the 240 mg isoflavone dose decreased pS2 expression In the high E_2 environment isoflavones had no effect on vaginal maturation values, decrease in pS2 expression was dose-dependent The 240 mg isoflavone dose resulted in significantly higher PR expression in lobular breast epithelium
Wood (2006)[109]	Ovariectomized adult female cynomolgus monkeys	The treatment group received 509 mg/d of the isoflavones GEN (333 mg), daidzein (149 mg), and glycitein (27 mg)	1 month	Breast and uterine proliferation markers; apoptosis; Ki67, MK167 and PR expression; serum estrogens; uterine size; vaginal maturation in either the follicular or luteal phase; *ERα* and *ERα*-driven genes: *TFF1*, *CXCL12*, and *PR*	Endometrial progesterone receptor gene expression was significantly increased No significant effect on MKI67, PR, or ERα expression in breast epithelium In uterine tissue, CXCL12, ESR1, and PR gene expression was significantly higher In breast tissue, expression of TFF1 and PR was unchanged

Abbreviations: CXCL12, **s**tromal cell-derived factor-1 gene; DMBA, 7,12-dimethylbenz[a]anthracene; E_2, estrogen; EGFR, epidermal growth factor receptor; ERα, estrogen receptor-α; *ESR1*, estrogen receptor-α gene; GEN, genistein; Ki67, MKI67 protein; MK167, Ki67 protein; PR, progesterone receptor; pS2, breast cancer associated pS2 protein; SPI, soy protein isolate.

cell line, a recent study convincingly showed that MDA-MB-435 cells are of melanoma origin.[105] These data provide preliminary evidence that genistein at high concentrations may inhibit metastasis of some established cancers, though additional work is needed to fully evaluate this possibility.

With regard to the potentially estrogenic effects of genistein in estrogen-responsive tissues in humans, several studies in non-human female primates have failed to demonstrate estrogenic effects of soy protein isolate or soy isoflavone mixtures that included approximately 10 mg genistein/kg/day and resulted in serum concentrations of genistein equivalent to those achieved from high-dose soy ingestion in women (Table 2).[106–109]

In contrast to the chemopreventative effects observed with genistein in some studies, other studies in rodents suggest that dietary genistein intake may stimulate proliferation of implanted human breast cancer cells. A study in athymic nude mice, lacking proper cellular surveillance immunity, showed that 1000 ppm genistein supplemented in mouse chow produced increases in implanted MCF-7 tumor size compared to control animals with implanted tumors fed a diet lacking genistein. Also, 1000 ppm dietary genistein negated the inhibitory effect of tamoxifen on stimulation of implanted MCF-7 cells (Table 2).[110] However, all animals had initial tumor growth stimulated by a subcutaneous (s.c.) estradiol pellet and subsequent tumor growth attributed to genistein was 6-fold lower following pellet removal compared to non-removal of the estradiol pellet.[111] The mean plasma level of total (i.e., free and aglycone) genistein among the genistein-treated animals was approximately 2.1 μM, a level that falls in the physiological range and could potentially be attained through dietary intake.

In an ovariectomized athymic mouse model with low circulating estradiol levels purported to mimic the hormonal state of postmenopausal women, genistein produced dose-dependent stimulation of MCF-7 tumor size at concentrations achievable with diet, beginning at 150 ppm (Table 2).[7,95,111] This finding was replicated in a chemically induced 1-methyl-1-nitrosourea (MNU) tumor model in ovariectomized Sprague-Dawley rats.[94] However, an analogous experiment showed that dietary genistein did not stimulate the growth of ER-negative MDA-MB-231 cells implanted into mice.[93] These data suggest that physiologically attainable concentrations of genistein may stimulate the growth of induced or established mammary tumors in animals, but at a lower rate compared to estradiol and suggests that any *in vivo* stimulatory effect on cancer cell growth may be limited to ER-positive cells.

On the surface, the agonist activity of genistein at ERα seems to reinforce the tumor-promoting effects observed in these animal models. However, one can presume that lower binding affinity and potency of genistein at ERα may lead to a lower level of ERα transcriptional activation and result in a safety buffer in postmenopausal women. A lower level of transcriptional activation would be less mutagenic in postmenopausal women than hormone replacement therapy (HRT). Furthermore, cancer is multifactorial and normal postmenopausal women have intact cellular surveillance immunity unlike several of the animal models that used immunocompromised athymic mice that may have led to an exaggerated effect of genistein in these studies.

Collectively, the *in vitro* and *in vivo* data generally support a protective effect or no effect for genistein against induced breast cancer. Circulating concentrations in excess of the 1–2 μM achievable through dietary genistein intake may be necessary to produce these effects, though it is difficult to directly compare concentrations used *in vitro* with those attainable *in vivo*.

Clinical studies

There is direct evidence in support of genistein's safety for breast and endometrial health following cohorts of postmenopausal osteopenic and osteoporotic women given purified genistein for up to 3 years. In a clinical study investigating the effects of purified genistein on menopausal vasomotor symptoms over 1 year, women taking genistein ($n = 122$) showed no change in vaginal cytology (measured by change in Maturation Value score) compared to women taking placebo ($n = 125$) or baseline. These data are important since they were the first to directly assess the effects of purified genistein on predictors of cancer in sex hormone responsive tissues. A daily dose of 54 mg of purified genistein does not appear to stimulate mammary tissue and may, in fact, exert a modest antiestrogenic effect in endometrial tissue (Table 3).[11]

Recently published clinical trials included secondary endpoints to determine whether purified genistein alters breast density, endometrial thickness, and/or vaginal cytology (Table 3).[9,11,36] These safety measures were used to assess possible estrogenic effects of 54 mg/day of purified genistein as indices of breast and uterine cancer risk. Subjects ($n = 389$) took either purified genistein ($n = 198$) or placebo ($n = 191$) for 2 years. After randomization, mammograms and uterine ultrasounds for endometrial thickness were obtained at baseline and after 24 months. There were no reported cases of breast cancer or changes in uterine thickness in either group at the end of the 24 months. In addition, none of the participants have reported having breast cancer since the trial concluded in Sept 2005, though dosing stopped at that time. In a third year extension of this trial, the study participants consumed purified genistein ($n = 71$) or

Table 3 **Effects of genistein on markers of cancer risk observed from human clinical studies.**

Reference	Subjects (*n*)	Number/intervention (mg/day)	Study length (months)	Primary/secondary endpoints	Results
D'Anna (2007)[11]	90 post-menopausal women	HRT ($n = 30$; 1 mg 17 beta-estradiol combined with 0.5 mg norethisterone acetate), GEN ($n = 30$; 54 mg), or placebo ($n = 30$)	12	BMD in the femoral neck and lumbar spine / Urinary excretion of PYR and DPYR; serum levels of B-ALP and BGP; and adverse events	At 6 and 12 months, GEN significantly increased BMD in the femoral neck and lumbar spine; significantly reduced the excretion of PYR and DPYR cross-links; and significantly increased serum B-ALP and BGP No significant effects on endometrial thickness, vaginal bleeding, or breast tenderness
Marini (2007)[9]	389 post-menopausal women	Placebo ($n = 191$; 1000 mg calcium and 800 mg vitamin D) or GEN ($n = 198$; 54 mg GEN, 1000 mg calcium, and 800 mg vitamin D)	24	BMD in the femoral neck and lumbar spine / Urinary excretion of PYR and DPYR; serum levels of B-ALP and IGF-1; endometrial thickness and adverse events	At 12 and 24 months GEN significantly increased BMD at the lumbar spine and the femoral neck; significantly decreased urinary excretion of PYR and DPYR; significantly increased levels of B-ALP and IGF-1 Endometrial thickness was unchanged compared with placebo. Mild-to-moderate GI side effects were reported
Marini (2007), Marini (2008), and Atteritano (2008)[9,36,114]	138 post-menopausal women	Placebo ($n = 67$; 1000 mg calcium and 800 mg vitamin D) or GEN ($n = 71$; 54 mg GEN, 1000 mg calcium, and 800 mg vitamin D)	36	BMD in the femoral neck and lumbar spine / Urinary excretion of PYR, DPYR and CTX, sRANKL; serum levels of B-ALP, IGF-1 and OPG; breast density, endometrial thickness, BRCA-1 and BRCA-2 mRNA expression, sister chromatid exchanges and adverse events	At 24 and 36 months GEN significantly increased BMD at the lumbar spine and the femoral neck; significantly decreased urinary excretion of PYR, CTX, and sRANKL; significantly increased levels of B-ALP, IGF-1, and OPG. Breast density and endometrial thickness were unchanged compared with placebo BRCA-1 and -2 mRNA expression was preserved and sister chromatid exchanges were decreased Adverse events were similar to placebo group
Maskarinec (2004)[115]	220 post-menopausal women	Placebo ($n = 111$) or isoflavone intervention ($n = 109$; ~50 mg isoflavones)	24	Breast density	At 24 months the isoflavone group had increased breast area and decreased breast density; neither was significant when compared to placebo
Maskarinec (2003) and Maskarinec (2002)[116,117]	34 pre-menopausal women	Placebo ($n = 17$) or soy intervention ($n = 17$; 100 mg isoflavone supplement, ~76 mg aglycones)	12	Breast density / Serum levels of estrone, estradiol, estrone sulfate, progesterone, sex hormone-binding globulin, follicle-stimulating hormone, and luteinizing hormone	At 12 months there was a non-significant decrease in breast area and no change in dense areas or percent breast density No significant changes in any hormone levels
Hargreaves (1999) and McMichael-Phillips (1998)[122,127]	84 pre-menopausal women	Placebo ($n = 23$) or soy intervention ($n = 28$; 60 mg, ~45 mg isoflavones)	14 days	Ki67, ER, and PR expression; ApoD and pS2 measurement in nipple aspirates	Nipple aspirate levels of apolipoprotein D were significantly lowered and pS2 levels increased in response to soy supplementation No effect of soy supplementation on breast epithelial cell proliferation, estrogen, and progesterone receptor status, apoptosis, mitosis, or Bcl-2 expression was detected.

Abbreviations: ApoD, apolipoprotein D; B-ALP, bone-specific alkaline phosphatase; BGP, osteocalcin (bone Gla protein [BGP]); BMD, bone mineral density; BRCA, breast cancer; BRCA-1, breast cancer susceptibility gene-1; BRCA-2, breast cancer susceptibility gene-2; Bcl-2, bcl-2 protein; CTX, carboxy terminal telopeptide; DPYR, deoxypyridinoline; ER, estrogen receptor; GEN, genistein; IGF-1, insulin-like growth factor 1; Ki67, MKI67 protein; OPG, osteoprotegerin; PR, progesterone receptor; pS2, breast cancer-associated pS2 protein; PYR, pyridinoline; sRANKL, soluble receptor activator of nuclear factor kappa B ligand; TNF, tumor necrosis factor.

placebo ($n = 67$) and, again, did not demonstrate any evidence of breast or uterine carcinogenicity (Table 3).[36] There are no reports of reproductive cancer from the entire study population.

The data from these studies suggest that purified genistein does not exert adverse estrogenic effects on breast tissue when consumed at a dose of 54 mg/day. There was no treatment-related difference in endometrial thickness between the genistein and placebo groups following 3 years of daily intake in the larger cohort or the extension group. In fact, whereas endometrial thickness remained consistent in the placebo group, the genistein group showed a time-dependent reduction that achieved statistical significance at the 36-month follow-up (approximately 12% reduction, $P < 0.01$).[36] In addition, gene expression levels of BRCA-1 and -2, breast tumor suppressor genes,[112,113] were maintained over the 3-year period in the group administered genistein, whereas the placebo group showed decreased levels of both BRCA-1 and -2 gene products (Table 3).[36,114] Genistein also significantly decreased sister chromatid exchanges, in peripheral blood lymphocytes isolated from the study participants, suggesting that genistein may inhibit genotoxicity and consequent mutagenesis (Table 3).[114] Whether this effect has clinical relevance for cancer prevention remains to be determined.

A few dietary intervention studies have tested the effects of soy on breast density and other markers for breast cancer risk. An obvious limitation of these trials is that it is impossible to know to what extent genistein contributed to any observed effects on experimental endpoints. Moreover, the majority of trials were conducted in pre-menopausal women and isoflavones may have different effects in post-menopausal women due to changes in ambient hormone levels. One of the largest published dietary intervention trials was a 2-year study in which pre-menopausal subjects ($n = 220$) were randomized to an intervention arm or a control arm. The intervention involved recommendations to increase soy food intake and was reinforced by frequent dietary counseling with study personnel, and verified by increased urinary soy isoflavone excretion.[115] The intervention group consumed approximately 58 mg of isoflavones/day, whereas the control group consumed only 5 mg of isoflavones/day. There was no significant difference in mammographic density between the intervention and control groups (Table 3). Interestingly, the study assessed soy consumption throughout adulthood by way of a retrospective questionnaire and found that the strongest predictor of decreased breast density was low soy intake early in life and high consumption of soy throughout adulthood. This study corroborates a previous exploratory study that found no difference in breast density among women who consumed a 100 mg/day isoflavone supplement over a 1 year period (Table 3).[116] Furthermore, in these same women, there were no significant changes in serum levels of estrone, estradiol, estrone sulfate, progesterone, sex hormone-binding globulin, follicle-stimulating hormone, and luteinizing hormone (Table 3).[117] The lack of change in mammographic density agrees with the negative findings on hormone levels. In the 2-year clinical study by Marini et al.,[9] with a third year extension,[36] there were decreases in breast density both in the placebo control group as well as the genistein treated group. There were no statistically significant differences between the two groups.[9,36] Taken as a whole, these data support the safety profile obtained with purified genistein, as chronic intake of high amounts of soy isoflavones did not significantly alter breast density in these studies.

Ambient hormone levels are predictors of breast cancer risk. For instance, high estradiol levels are associated with increased breast cancer risk,[118] as are the levels of 4- and 16α-hydroxylated estradiol metabolites.[119] In contrast, levels of the estradiol metabolite 2-hydroxyestrone are negatively associated with risk of breast cancer.[119] There is evidence that soy isoflavones containing high levels of genistein may favorably influence the ovarian hormone profile, at least in pre-menopausal women. In a crossover design, it was found that intake of a soy-rich diet containing 85 mg/day of genistein and its glucoside genistin for 1 month reduced plasma estradiol and progesterone levels in a small cohort ($n = 10$) of pre-menopausal women.[120] The reduction in estradiol following genistein treatment was statistically associated with plasma genistein concentration, suggesting that genistein in the soy diet was responsible for the observed decrease in plasma estradiol and progesterone. A subsequent study also reported reduced estradiol levels among women consuming an isoflavone-free soy diet suggesting that non-genistein components of soy also appear to contribute to its estradiol-lowering effects.[121] In another study, an isoflavone-rich soy diet produced increases in the 2-hydroxyestrone:16α-hydroxyestrone ratio, suggesting that soy isoflavones may produce protective effects by modifying expression or activity of estradiol-metabolizing enzymes.[119] Limitations of these trials are that they were conducted in a small number of subjects ($n = 6$–10) although the effects are statistically reliable across trials. Moreover, the reduction in ovarian hormone levels must be evaluated in the context of other markers for cancer risk and in postmenopausal women to gain a comprehensive picture of the effects of genistein on cancer risk.

In contrast to the data presented thus far, a few investigations have found pro-estrogenic effects of dietary soy supplementation on breast tissue. Hargreaves et al.[122] found that the estrogen-regulated proteins pS2 and apo-

lipoprotein D (ApoD) were significantly increased and decreased, respectively, in the nipple aspirates of pre-menopausal women who received 45 mg/day of soy isoflavones for 14 days (Table 3). It has been well documented that pS2 and ApoD are under transcriptional control, regulated by estrogen,[123–126] and the changes in pS2 and ApoD levels suggest that soy isoflavone supplementation has an estrogenic effect on breast tissue. However, there were no significant differences between treatment and control groups with respect to *ex vivo* proliferation of epithelial breast tissue, ER binding, or Bcl2 expression. In contrast, another study from the same group using a similar protocol found that soy protein supplementation increased proliferation of breast tissue epithelium and expression of progesterone receptors (Table 3).[127] These data also suggest an estrogenic effect of soy isoflavones on reproductive tissues although the study consisted of pre-menopausal patients undergoing surgery for breast cancer and benign breast conditions. The relevance of these data for healthy postmenopausal women without breast cancer or with benign breast conditions is therefore unknown.

Whether genistein and other soy isoflavones are safe for breast cancer patients or breast cancer survivors is not well established. Although genistein appears safe and may even have a protective role against primary cancers, there is concern regarding its use in women with established ER-positive breast cancers due to its agonist activity at ERα.[128] The data from clinical studies suggesting isoflavones have an estrogenic effect on gene expression and cell proliferation support the possible stimulatory effects of genistein on established cancers.[122,127]

A few studies have administered isoflavones to breast cancer patients. Sartippour et al.[129] found that women given 200 mg of isoflavones/day (genistein content not specified) for 14 days showed a trend towards increased apoptotic to mitotic ratio of breast cancer cells compared to historical untreated case-controls. Results from this study suggest a modest beneficial effect of isoflavones on the course of breast tumors, including among 88% of subjects who had ER-positive breast cancer. These data support the *in vitro* and *in vivo* experimental models showing that genistein induces apoptosis of cancer cells.[64–70] No safety concerns were reported in several studies with use of 114 mg/day of isoflavones among breast cancer patients over periods of up to 3 months.[130–133] Although direct measures of breast safety were not taken, there was no reported incidence of breast-related adverse events. These data must be treated with caution, however, due to the small sample sizes, short study durations, and lack of a true control group in the Sartippour et al. study.[129]

Overall, the published data in humans supports the safety of genistein and suggests that there may be some chemopreventative effects. Furthermore, it is highly unlikely that institutional review boards would have granted approval to the numerous ongoing clinical trials involving genistein if there was convincing evidence of carcinogenicity. This is highlighted by the fact that there are several federally funded trials being conducted to try to solve some of the unanswered questions regarding the therapeutic potential for genistein in treating or preventing prostate, bladder, kidney, breast, and endometrial cancers (see http://www.clinicaltrials.gov). For example, 1) Gemcitabine Hydrochloride and Genistein in Treating Women with Stage IV Breast Cancer (NCT00244933); 2) Genistein in Preventing Breast Cancer in Women at High Risk for Breast Cancer (NCT00290758); 3) Genistein in Preventing Breast or Endometrial Cancer in Healthy Postmenopausal Women (NCT00099008); 4) Genistein in Patients Who Are Undergoing Surgery for Bladder Cancer (NCT00118040); 5) Genistein in Treating Patients with Stage II, Stage III, or Stage IV Prostate Cancer (NCT00005827).

Epidemiological studies

Asian women have a markedly lower incidence of breast cancer than Western women, possibly due to a diet high in soy and fiber and low in fat.[134] Although genetic differences could account for some of this protective effect, it has been noted that within a few generations following immigration to Western countries, Asian women have an incidence of breast cancer comparable to their Western counterparts. This is primarily attributable to dietary changes.[135] These data strongly implicate dietary and lifestyle factors as a significant determinant underlying the lower incidence of breast cancer among Asian women. A similar geographic disparity has been found with respect to the incidence of prostate cancer among Japanese and American men.[136] Japanese men living in Hawaii consume less soy and have a higher incidence of prostate cancer than Japanese men living in Japan suggesting that soy isoflavones may be chemopreventive against prostate cancer as well.[137] It has also been hypothesized that consumption of soy products while young may be necessary to confer a protective effect since breast cancer risk increases with subsequent generations of women following immigration to the United States.[135] These epidemiological data have generated considerable interest in the putative chemopreventive properties of soy, especially its isoflavone constituents.

Due to the large variations in isoflavone consumption among Asian individuals, the Asian population represents an ideal setting for determining whether an association exists between relatively high-dose isoflavone intake and breast cancer risk. In a recent nested case-control study (the Japan Public Health Center-based prospective study), Japanese women ($n = 24,226$) were

studied over a 10-year period to determine the association of high- and low-isoflavone dietary intake with breast cancer risk. The study used a health questionnaire to assess dietary intake of the isoflavones, and health check-up and blood collection to assess plasma levels of the aglycones genistein and daidzein at baseline, 5 years, and 10 years. There were 144 new cases of breast cancer identified among the cohort. When these cases were stratified by plasma genistein and daidzein levels, a statistically significant inverse association was found between genistein and the risk of breast cancer (*P* for trend, 0.02), and no statistically significant association for daidzein (*P* for trend, 0.54). Furthermore, the results did not change after adjustment for other dietary isoflavone intake, age at menarche, menopausal status at baseline, age at menopause, height, body mass index, and alcohol consumption.[138] A 65% reduction in breast cancer risk was observed in the highest plasma genistein quartile group compared with the lowest quartile (353.9 ng/mL; 28.5 mg/day; odds ratio = 0.34, 95% confidence interval 0.16–0.74), but no decrease was noted in the lower plasma genistein quartile groups, indicating that only the highest group benefited from risk reduction (Table 4).[138] The statistically significant inverse association between plasma genistein and the risk of breast cancer, but no association for plasma daidzein suggests that genistein and not daidzein may play an important role in the etiology of breast cancer. Furthermore, these data suggest that even at the relatively low concentrations achievable from dietary intake alone, genistein poses a risk-reducing rather than a risk-enhancing effect on breast cancer.

A recent meta-analysis of 18 published epidemiological studies supports the hypothesis that soy isoflavones may reduce breast cancer risk.[3] Across all investigations, high soy intake was associated with an odds ratio of 0.86 (95% confidence interval 0.75–0.99%) for the development of breast cancer (Table 4). It is interesting to note that a slightly stronger protective effect was observed among Western than Asian women (odds ratios 0.84 and 0.89, respectively) given that soy intake is much higher in Asian populations. This may suggest that intervention with soy protein or isoflavones can have a positive effect over a short period of time rather than requiring prior consumption over a period of years. There was considerable variability in the amount of soy consumed that conferred a protective effect and the way that intake was quantified, so that a threshold for soy and/or isoflavone intake could not be determined from this study. Although the contribution of many potential confounding factors cannot be excluded in this analysis, it is notable that despite differences in methodology, soy intake level, and subject demographics, an overall significant inverse association between soy intake and breast cancer remained.

Case-control data support the hypothesis that high isoflavone, and in particular genistein intake, may be associated with reduced breast cancer risk. Interestingly, a recent prospective study ($n = 766$) found that plasma genistein levels were inversely correlated with subsequent incidence of breast cancer in both pre- and postmenopausal Dutch women (Table 4).[139] In this investigation, the relative risk of developing breast cancer was 32% lower (odds ratio = 0.68, 95% confidence interval 0.47–0.98) among women in the highest tertile of plasma genistein levels as compared to women in the lowest tertile. This association remained significant when demographic and lifestyle factors as well as plasma levels of other isoflavones were controlled. There was a similar, but non-significant trend for the daidzein metabolite equol, but no statistical relationship was shown between any other isoflavone or lignan and reduction in breast cancer risk. Importantly, the association between high genistein levels and reduced breast cancer incidence is consistent with other studies showing an inverse relationship between soy intake and breast density,[140–142] a major breast cancer risk factor.[143] Though daidzein did not lead to any statistically significant increases in cancer, equol, the gastrointestinal metabolic product of daidzein conversion produced in 45–50% of people,[144] appears to slightly elevate cancer risk.

Equol is more potent at ERα in comparison to daidzein or genistein.[38] Equol also produces greater increases in bone mineral density (BMD) in postmenopausal women[145] who convert daidzein compared to those who do not. In a recent study, statistically higher levels of equol were found in the urine of women with ER-positive tumors,[146] which may relate to its greater affinity for ERα (Table 4). The overall risk of breast cancer was also increased when the urinary levels of all isoflavones was elevated. Genistein levels in the same study, however, were not specifically associated with any increase in breast cancer.

CONCLUSION

The preponderance of clinical and epidemiological data related to soy consumption by both Asian and US populations demonstrate the safety of soy proteins and isoflavones. Summarized below are the data that favor soy and naturally derived genistein, specifically, for its safety:1) Genistein has been shown to cause cancer cell apoptosis *in vitro* and protect against the development of carcinomas in animal models. 2) Rodent and non-human primate studies indicate that genistein is safe in normal populations of animals. 3) Human and animal studies suggest that, unlike HRT, genistein does not cause the development of new, estrogen-dependent breast or reproductive tissue cancers. 4) Clinical studies have

Table 4 **Effects of genistein on markers of cancer risk observed from epidemiological studies.**

Reference	Subjects (*n*)	Intervention	Study length (years)	Primary/secondary endpoints	Results
Iwasaki (2008)[138]	*n* = 24,226, Japanese women	Nested case-control study (the Japan Public Health Center-based prospective study)	10	The association of high- and low-isoflavone dietary intake with breast cancer risk	144 new cases of breast cancer identified among the cohort Stratification by plasma GEN and daidzein levels yielded a statistically significant inverse association between GEN and the risk of breast cancer (*P* for trend, 0.02), and no statistically significant association for daidzein (*P* for trend, 0.54) The results did not change after adjustment for other dietary isoflavone intake, age at menarche, menopausal status at baseline, age at menopause, height, body mass index, and alcohol consumption. A 65% reduction in breast cancer risk was observed in the highest plasma GEN quartile group compared with the lowest quartile (353.9 ng/mL; 28.5 mg/day; odds ratio = 0.34, 95% confidence interval 0.16–0.74).
Trock (2006)[3]		Meta-analysis of 18 published epidemiological studies		Association of isoflavone dietary intake with breast cancer risk	High soy intake was associated with an odds ratio of 0.86 (95% confidence interval 0.75–0.99%) for the development of breast cancer. A stronger protective effect was observed among Western compared to Asian women (odds ratios 0.84 and 0.89, respectively).
Verheus (2007)[139]	*n* = 766, pre-, peri-, and post-menopausal women	Nested case-control study within the Prospect cohort, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition		Association of isoflavone dietary intake with breast cancer risk	Plasma GEN levels were inversely correlated with incidence of breast cancer in both pre- and post-menopausal Dutch women. The relative risk of developing breast cancer was 32% lower (odds ratio = 0.68, 95% confidence interval 0.47–0.98) among women in the highest tertile of plasma GEN levels compared to women in the lowest tertile
Ward (2008)[146]	*n* = 237 breast cancer cases and 952 control individuals (aged 45 to 75 years, both male and female)	Nested case-control study within European Prospective into Cancer-Norfolk cohort		The association of serum and urine isoflavone and particularly equol levels with ER-positive tumors	The risk of breast cancer was slightly increased among individuals with elevated urinary isoflavones (odds ratio = 1.08 (95% confidence interval = 1.00–1.16), *P* = 0.055) For those with ER-positive tumors, the risk of breast cancer was increased elevated urinary equol (odds ratio = 1.07 (95% confidence interval = 1.01 to 1.12), *P* = 0.013)

shown that genistein therapy produced no negative effects on endometrial thickness or vaginal cytology, changes in breast tissue density or breast cancer in studies up to 3 years in duration in postmenopausal women. 5) Elevated intake of soy decreases incidence of breast cancer in Asian populations. 6) Extensive epidemiological data have shown that intake of soy isoflavones, and genistein in particular, may protect against the development of breast cancer.

There are, however, conflicting data from *in vitro* and animal studies concerning the effects of genistein on cancer cell lines and estrogen-sensitive induced mammary tumors *in vivo*: 1) Genistein stimulates the growth of estrogen-sensitive cancer cell lines *in vitro* under specific, artificial conditions, namely using immunocompromised animals lacking proper cellular surveillance immunity that had chemically induced or implanted cancer. 2) These findings give pause only in that they raise a question about the stimulatory effects of genistein on cancer cells under artificial experimental conditions, not necessarily under conditions present in peri- or post-menopausal women.

Although extensive research has been performed to provide a detailed description of the possible mechanisms of action of genistein and other naturally derived isoflavones for their efficacy and safety, the literature suggests that genistein does not act by a single mechanism to achieve its effects. More studies are required to fully elucidate the mechanism of action of isoflavones, particularly genistein, including effects on estrogen-metabolizing enzymes, cell cycle, cell differentiation, proliferation, apoptosis, the inflammatory response and various other cell signaling pathways in order to fully evaluate the biological relevance of experimental findings.

Results from the numerous ongoing clinical trials are eagerly awaited, not only to expand the growing body of evidence supporting the safety of genistein but also to establish whether the chemotherapeutic/chemopreventative effects of genistein observed in laboratory and epidemiological data can be translated into definite clinical benefits.

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Declaration of interest. RML and BPB are employees of Primus Pharmaceuticals, Inc. which manufactures and sells a prescription medical food, containing genistein aglycone, for the metabolic management of osteopenia and osteoporosis.

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