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NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN: TEA EXTRACTS GREEN TEA POLYPHENOLS EPIGALLOCATECHIN GALLATE

DRUG IDENTIFICATION

CAS Name (9CI): Tea Extracts
(*Thea sinensis L.*)
(*Camellia sinensis*)

Related Compounds:

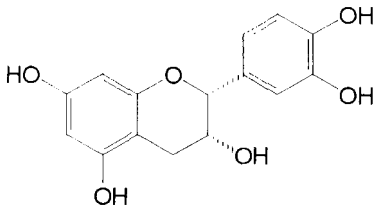
Green Tea Decaffeinated Extract (Decaffeinated GTE)
Green Tea Extract (GTE)
Green Tea Water Extract (WEGT)
Green Tea Polyphenols (GTP)
(–)-Epicatechin
(–)-Epicatechingallate
(–)-Epigallocatechin
Epigallocatechin Gallate (CAS No. 989-51-5)(2R-*cis*)-Benzoic Acid, 3,4,5-Trihydroxy-3,4-dihydro-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-2*H*-1-benzopyran-3-ester (9CI)

Black Tea Decaffeinated Extract (Decaffeinated BTE)
Black Tea Extract (BTE)
Black Tea Water Extract (WEBT)
Black Tea Unknown Polyphenols (BTP)
Black Tea Unknown Polyphenols Decaffeinated (Decaffeinated BTP)
Theaflavins
Thearubigins

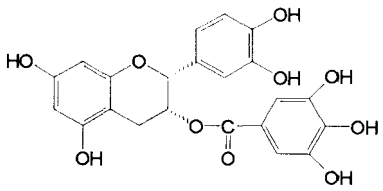
Tea, Oolong Extract

Molecular Wt.: 458.3 (Epigallocatechin Gallate)

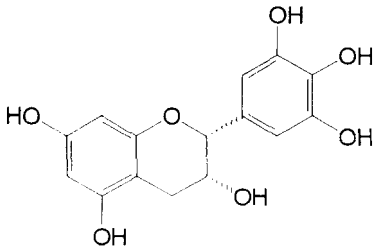
Figure 1. Fermentation Process and Structures of Tea Compounds



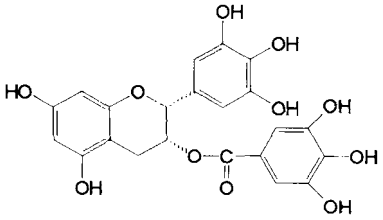
(–)-Epicatechin



(–)-Epicatechingallate



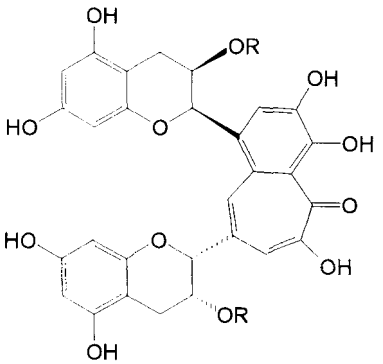
(–)-Epigallocatechin



(–)-Epigallocatechin Gallate

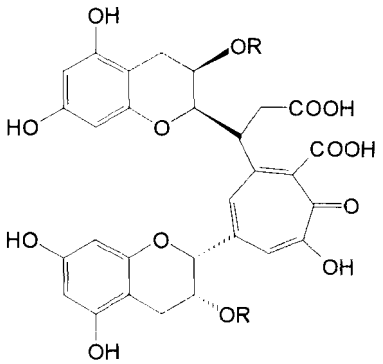
Major Components of Green Tea

Fermentation ↓ Tea Polyphenol Oxidase



Theaflavins

R = Galloyl or OH



Thearubigins (possible structure)

Major Components of Black Tea

EXECUTIVE SUMMARY

Tea is a beverage made from the leaves of *Camellia sinensis* species of the Theaceae family. This beverage is one of the most ancient and, next to water, the most widely consumed liquid in the world. Tea leaves are primarily manufactured as green, black, or oolong, with black tea representing approximately 80% of tea products consumed. Green tea is the non-oxidized/non-fermented product and contains several polyphenolic components such as epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG). EGCG is the major green tea polyphenol (GTP) (>40% dry weight). The major components of black tea (the fermented product) are theaflavins (1–2% dry weight) and thearubigins (10–20% dry weight). Theaflavins, which determine the quality and flavor of the tea, are formed by oxidation of quinones derived from the epicatechins. Thearubigins are highly colored flavonol oxidation products which are often bound to peptides or proteins. Oolong tea is the partially oxidized/fermented product which retains a considerable amount of the original polyphenolic material [1]. Figure 1 shows the polyphenol structures and the major components formed in the fermentation process.

Although not conclusive, epidemiological studies have suggested a protective effect of black or green tea consumption against human cancers of the breast [2], colon and rectum [3–5], gall bladder [6], liver [2], lung [2,4,7–9], nasopharynx [10], pancreas [11], stomach [4,10,12,13], and uterus [2,8]. In contrast, a number of other ecological, cohort, and case-control studies [*e.g.*, 4] have associated an increased risk for cancer of the breast, colorectum, esophagus, kidney, lung, pancreas, and stomach with tea intake. These inconsistencies may be attributed to consumption of salted or very hot tea (esophagus) or to geographical location, as observed with stomach cancer (*e.g.*, inhibition of endogenous formation of nitroso compounds which are a major cause of gastric cancer in some areas). Other confounding factors and variables may include the use of tobacco and alcohol and lack of information on the type of tea consumed (*e.g.*, black or green).

In published preclinical studies, tea, GTP, and EGCG have demonstrated antimicrobial, antimutagenic and anticarcinogenic activities [14,15]. In experimental animals, chemopreventive efficacy has been observed in a number of target organs including colon (GTP) [16–18] and large intestine (GTP, EGCG) [19], duodenum (EGCG) [20,21], esophagus (various black and green tea extracts) [22–25], forestomach (GTP, green tea water extract (WEGT)) [24,26–29], liver (EGCG, green tea in diet, decaffeinated WEGT and black tea water extract (WEBT) as main source of drinking water) [21,30,31], lung [GTP, EGCG, WEGT, and decaffeinated WEBT and WEGT] [24,26–28,30,32–37], mammary glands (GTP) [38, 39], and skin (EGCG, GTP, decaffeinated WEBT and WEGT, WEBT, and WEGT) [21,29, 40–54]. Additionally, modulation of intermediate biomarkers by tea compounds have been reported; epicatechin complex (a combination of four major catechins found in green tea), various green and black tea extracts, and GTP inhibited carcinogen-induced precancerous lesions in rat esophagus [55], GGT-positive foci in rat liver [56], and GST-p-positive foci in rat liver [57], respectively. Epidermal hyperplasia induced by such tumor promoters as TPA was also reduced by GTP administration [58]. Further, GTP inhibited aberrant hyperproliferation (an *in vitro* cellular biomarker for premalignant transformation) induced in mammary epithelial cell lines by carcinogens and oncogenes (*ras* or *myc*) [59]. Recently, topical application of EGCG to mouse skin and oral administration of WEGT in drinking water were shown to inhibit TPA-induced ODC, PKC, and c-*myc* expression and NNK-induced lung oncogene (c-*myc*, c-H-*ras*, and c-*raf*) expression, respectively [60]. Additional Chemoprevention Branch-funded studies in colon and bladder (black and green tea extract (BTE, GTE), EGCG, GTP, and theaflavin mixture), and esophagus models (BTP, EGCG, GTP, and theaflavin mixture), and the colon aberrant crypt focus assay (BTE, EGCG) are in progress. Preliminary results in the colon aberrant crypt assay show inhibition with low-dose BTP (360 ppm in drinking water) and high-dose EGCG (1200 ppm in drinking water). The Chemoprevention Branch has also carried out studies with tea compounds in several *in vitro* and mechanistic assays. Nearly all have shown inhibitory activity in the rat tracheal epithelial (RTE) cell transformation, mouse mammary organ culture, and human lung tumor A427 cell assays. Significant inhibitory activity was also observed in *in vitro* assays measuring formation of DNA adducts and free radicals, and enhancement of GSH levels and GST, ODC, and NAD(P)H:quinone reductase activities.

Published studies have also shown the antimutagenic activity of tea, GTP, and EGCG in standard

assays in *Salmonella typhimurium* (Ames assay), *Escherichia coli, Bacillus subtilis*, and V-79 cells [61–73]. Inhibitory effects on the formation [74,75] and mutagenicity [63] of food mutagens, as well as formation of endogenous nitrosation products, [76] and cell transformation [19,77,78] have also been reported. Recently, GTE was demonstrated to protect against DNA oxidative damage in rat colon measured by inhibition of DMH-induced 8-hydroxydeoxyguanosine (8-OHdG) formation [79]. In addition, several *in vivo* studies [*e.g.*, 4,80–87] have demonstrated the inhibitory effects of tea, GTP, and EGCG on tumor growth, as well as invasion and metastasis. Therefore, the available data are adequate to support the development of tea, GTP, and EGCG as chemopreventive drugs.

Several mechanisms [4,53,88–90] may be responsible for the antiinitiation and antipromotion properties of tea, GTP, and particularly EGCG. These include inhibition of radiation- and chemical-induced lipid peroxidation and free radical formation (antioxidant activity) [71,91–100], inhibition of radiation- and TPA-induced epidermal ODC [43,45,60], inhibition of lipoxygenase and cyclooxygenase activity [42,101], inhibition of PKC and cellular proliferation [60,83,102,103], inhibition of carcinogen-DNA binding and adduct formation [41,43], inhibition of inflammation including edema and IL-1α mRNA and protein expression [42,45,58,104], inhibition of type 1 5α-reductase activity [105], modulation of cytochrome P450 activity [35,69,106,107], enhancement of phase II (GST) [27], as well as GSH-Px, catalase [108], and NAD(P)H:quinone reductase [27] activities, and enhancement of gap junction intercellular communication [102,109,110]. Additionally, ECGC has a so-called "sealing effect" [111]; it inhibits interaction of tumor promoters, hormones, and various growth factors with their receptors.

Preclinical 28-day and 90-day toxicology studies in rats and dogs sponsored by the Chemoprevention Branch on decaffeinated BTP, EGCG, and GTP are in progress. No evidence of toxicity was found in the 28-day dog study; the highest dose levels selected for the 90-day dog study are in the range of 300 mg/kg-bw/day for EGCG and 600 mg/kg-bw/day for decaffeinated BTP and GTP. Only limited data have been published. Following review of the available epidemiological and animal testing data in 1989, the International Agency for Research on Cancer (IARC) Working Group [1] concluded that there is insufficient evidence for the carcinogenicity of tea consumption in humans and experimental animals.

Limited data on the pharmacokinetics of tea in experimental animals are available [22, 112]. EGCG was detected in plasma at 0.08 μM after three weeks administration of 0.9% decaffeinated green tea to rats. Following a single ig dose of 50 mg EGCG to rats, C_{max} (200 ng/ml, or 0.44 μM) was reached after one hour [113].

No controlled clinical trials have been carried out with tea extracts, EGCG, BTP, or GTP. Limited human pharmacokinetics data are available in the literature; in four volunteers, the plasma EGCG level one hour after ingesting 1.2 g decaffeinated green tea (in 200 ml water) ranged from 46 to 268 ng/ml (0.13–0.75 μM) [112,114]. EGCG was not detected in the urine after 24 hours.

Green and black tea extracts, BTP, GTP, EGCG, and theaflavin mixture are being supplied to the Chemoprevention Branch by T.J. Lipton, Inc. (New Jersey). A significant effort in the development of the tea components is standardization and characterization of the type, source, and manufacturing and storage techniques, since these factors are expected to greatly impact the anticancer properties of such compounds. Part of the approach throughout the development work is parallel studies with a specific purified component of tea such as EGCG. Based on the results of the 90-day preclinical toxicology studies which are expected in late 1996, future clinical trials may evaluate the safety and efficacy of the tea compounds in such target tissues as colon, esophagus, lung (smokers), and skin (actinic keratoses).

PRECLINICAL EFFICACY STUDIES

The published *in vivo* (Table II) and *in vitro* preclinical efficacy studies, as well as a number of epidemiological, in particular, case-control (Table III) studies suggesting an inverse association of tea, GTP, and EGCG with cancer incidence, provide adequate support for further development of these agents as chemopreventive drugs.

Several Chemoprevention Branch-sponsored preclinical efficacy studies are in progress. These include studies in bladder and colon (several black and green tea extracts, EGCG, BTP, GTP) and esophagus (GTB, theaflavins, EGCG, and GTP) models of carcinogenesis. Preliminary data for the esophageal model indicates that theaflavins tested at 1200 ppm in drinking water significantly reduced tumor multi-

Table I. Constituents of the Tea Compounds Tested by the NCI, Chemoprevention Branch

Compound	Polyphenols Fraction (%)	EGCG (%)	Theaflavins (%)	Caffeine (%)	Unknown (%)
BTE, Caffeinated (freeze-dried hot water extract)	44.94	2.95	2.15	5	44.96
BTE, Decaffeinated (freeze-dried hot water extract)	41.9	2.67	3.39	<0.1	52.04
GTE, Caffeinated (freeze-dried hot water extract)	44.06	11.16	—	0.56	44.22
GTE, Decaffeinated (freeze-dried hot water extract)	44.72	10.33	—	<0.1	44.95
BTP	32 (unknown polyphenols), 2 (non-epicatechins), 5 (flavonol glycosides)	17	8	1.06	—
GTP	2 (non-epicatechins), 13 (other polyphenols), 1 (flavonol glycosides)	43	—	0.82	—
Theaflavin Mixture	—	—	7.65 30.59 (theaflavin gallate A) 19.45 (theaflavin gallate B) 32.17 (theaflavin digallate)	—	6.14 4 (water)

plicity. The constituents of the tea compounds tested in the Chemoprevention Branch-sponsored studies are listed in Table I.

In published studies, tea (WEGT, WEBT prepared by boiling in water and then extracting without use of any solvents), GTP, and EGCG have been reported to inhibit carcinogenesis induced by a wide variety of carcinogens using initiation, promotion, and progression protocols in rodent cancer models [2,14,21,24,37,53,54,88–90,115]. GTE (prepared by extraction with various solvents), EGCG and GTP have demonstrated cancer chemopreventive activity in colon, duodenum, esophagus, forestomach, large intestine, liver, lung, mammary glands, and skin. BTE (prepared by extraction with various solvents) has been tested and has shown chemopreventive activity in esophagus, lung, and skin cancer models. The published preclinical efficacy of tea compounds is summarized in Table II.

The major target organs for chemopreventive efficacy are forestomach, lung, and skin. The carcinogens used in these models require metabolic activation; therefore, effective tea compounds may act as antiinitiators. In the lung and forestomach models (NNK-, B(*a*)P-, DEN-induced), decaffeinated WEGT and WEBT, WEGT and GTP [26–28,30,32,33] demonstrated chemopreventive activity. Similar results were reported with decaffeinated WEGT and WEBT [28,34,35] and EGCG [36] in the NNK-induced lung model in mice. Efficacy has also been demonstrated by topical and oral administration of GTP against BPDE-2/TPA-[46,50], 3-MCA-[41,46, 51], and DMBA/TPA- or mezerein-[41,42,44,115] induced skin cancer. Similarly, topical application of EGCG was chemoprotective against DMBA-, TPA- or teleocidin-induced skin cancer [21,43]. Also, in several photocarcinogenesis (UV) studies [29], GTP [47], WEGT, and WEBT, as well as decaffeinated GTE and BTE [48,49] showed chemopreventive activity. The protective effect of topical application of GTP against malignant conversion of benign skin papillomas to carcinomas has also been reported [52].

In addition to forestomach, lung, and skin, the protective effects of tea extracts, GTP, and EGCG have been reported in other tissues. These include esophagus (several tea extracts) [22–24], duodenum (EGCG) [20], colon (GTP) [16–18], large intestine (GTP) [19], liver (green tea (NOS), decaffeinated WEGT and WEBT) [21,30,31], mammary glands (GTP) [38,39], and a multiorgan carcinogenesis model (GTP) [116].

In addition to published efficacy studies in animal cancer models, tea compounds have modulated intermediate biomarkers. For example, one Chemoprevention Branch-funded study to evaluate the effects of BTE and EGCG in the colon aberrant crypt focus assay is in progress. Preliminary results in this assay demonstrate an inhibitory activity with low-dose BTP (360 ppm in drinking water) and high-dose EGCG (1200 ppm in drinking water). Also, epicatechin complex, GTE and BTE were reported to inhibit DEN-induced precancerous lesions (*e.g.*, variant cell foci and nodes) [55] and aflatoxin B_1-induced GGT-positive foci [56] in rat liver, respectively. GTP reduced the number and area of GST-p-positive foci induced by Glu-P-1 in rat liver [57]. Additionally, epidermal hyperplasia measured in terms of thickness induced by tumor promoters such as TPA was also reduced significantly by GTP administration [58]. GTP also inhibited carcinogen- or oncogene-induced (*ras* or *myc*) aberrant hyperproliferation in mammary epithelial cell lines by >70% [59]. Further, Hu and co-workers [60] demonstrated that topical application of EGCG to mouse skin or oral administration of WEGT in drinking water inhibited TPA-induced ODC, PKC, and c-*myc* expression and NNK-induced lung oncogene (c-*myc*, c-H-*ras*, and c-*raf*) expression, respectively.

The Chemoprevention Branch has also carried out studies with the tea compounds in several *in vitro* and mechanistic assays. Positive agents in the rat tracheal epithelial (RTE) cell transformation assay include BTP, BTE (decaffeinated), GTE (including decaffeinated extract), GTP, and theaflavin. All the tea compounds except for GTE (including decaffeinated extract) were positive in the mouse mammary organ culture and the human lung tumor A427 cell assays. Significant inhibitory activity was also observed in *in vitro* assays measuring formation of DNA adducts (BTP, GTP, BTE, EGCG) and free radicals (GTP, BTE and decaffeinated BTE, GTE and decaffeinated GTE), and enhancement of GSH levels (BTP, GTE) and GST (EGCG, GTP, BTE, BTP), ODC (GTP, GTE), and NAD(P)H:quinone reductase (GTP, GTE, BTP) activities.

PRECLINICAL SAFETY STUDIES

Safety: Preclinical 28-day and 90-day toxicology studies sponsored by Chemoprevention Branch for

EGCG, and decaffeinated BTP and GTP are in progress. No evidence of toxicity was found in the 28-day dog study. Limited information has been published on the toxicity of tea compounds. Several epidemiological studies have associated tea consumption with increased risk for cancer; however, following a review of available data in 1989, the IARC Working Group concluded that there was insufficient evidence for the carcinogenicity of tea or tea component(s) in human or animals [1]. ECGC is presumed to have low toxicity; the reported mouse oral LD_{50} value is 2,170 mg/kg-bw [117]. In the rat esophageal efficacy study sponsored by the Chemoprevention Branch, a preliminary oral MTD of 4,500 ppm was established for BTP.

In the 28-day oral (capsules) dog study sponsored by the Chemoprevention Branch, no treatment-related toxicity was observed. In this study, EGCG at dose levels of 20, 75, and 150 mg/kg-bw/day and decaffeinated BTP and GTP at dose levels of 40, 150, and 300 mg/kg-bw/day were administered to one male and female dog/group for 28 or 29 days. Due to lack of toxicity following two weeks of treatment at the highest dose levels tested, animals in the lowest dose groups (20 and 40 mg/kg-bw/day) were treated with 300 mg/kg-bw/day EGCG and 600 mg/kg-bw/day decaffeinated BTP or GTP for the remaining two weeks. The only toxicity observed was sporadic vomiting in animals treated with 300 mg/kg-bw/day EGCG and a possible dose-related decrease in kidney weight in all the dose groups. However, no changes in kidney urea nitrogen and creatinine levels and histopathology (microscopic changes) were observed. On the basis of these results, the dose levels selected for the 90-day dog study are up to 300 mg/kg-bw/day for EGCG and 600 mg/kg-bw/day (equivalent to *ca.* 30 cups/day) for decaffeinated BTP and GTP.

In the multiorgan chemoprevention study cited above [116], an insignificant increase in incidence and number of lung adenomas and carcinomas was observed with 0.1% and 1% GTP administration. In the same study, the number and area of GST-p-positive foci in the liver were slightly, but significantly, increased in animals administered 0.1% and 1% GTP in diet both during and after carcinogen exposure; however, the incidence of hyperplastic nodules was not increased significantly compared with the carcinogen control.

In a lung chemoprevention study [36], A/J mice administered NNK and WEGT (2% in drinking water) had consistently lower body weight gains, although no significant differences in water and diet consumption were noted. This observation was attributed to caffeine, since similar results were obtained in animals treated with coffee; no such effect was observed in EGCG-treated animals (560 ppm, *ca.* 0.16 mmol/kg-bw/day).

In other studies [45, unpublished data], administration of WEGT (1.25% w/v) as the sole source of drinking water for 30 weeks was reported to be without hepatotoxic effects in SKH-1 mice.

Two investigators [118,119] reported an increase in SCEs with EGCG and tea extract treatment of cultured human lymphocytes; however, chromosome aberrations were not induced. Another study demonstrated a co-mutagenic effect (increased SCEs and chromosome aberrations) of high EGCG (20 μg/ml) in CHO cells treated with mitomycin C and UV-irradiation in the presence of microsomal activation (S-9) [120]. At lower concentrations, however, SCEs and chromosome aberrations induced by mitomycin C and UV-irradiation were inhibited. EGCG alone did not cause SCEs or chromosomal aberrations. The significance of these results is not known.

ADME: Recently published studies [22,112,113] have examined the pharmacokinetics of decaffeinated green tea and EGCG in rats. Decaffeinated tea was administered at a concentration of 0.9% in drinking water for three weeks. The EGCG, epigallocatechin, and epicatechin levels in plasma were 0.08, 0.18, and 0.07 μM, respectively. The levels of epigallocatechin and epicatechin in urine were 14.7 and 35.5 μM, respectively. Although investigated, no data on AUC and $t_{1/2}$ were reported [22,112]. In another study, following a single ig dose of 50 mg EGCG, C_{max} (200 ng/ml, or 0.44 μM) was reached after one hour in rats [113]. Within four hours after administration, EGCG levels in plasma could not be detected.

CLINICAL SAFETY: PHASE I STUDIES

No Phase I clinical trials with black or green tea extracts or their components have been funded by Chemoprevention Branch or reported in the literature.

Drug Effect Measurement: No drug effect measurements have been validated for black and green tea extracts and their components. In recently published animal and human studies [22,113,114], EGCG, epi-

gallocatechin and epicatechin were detected in plasma and urine after oral administration of decaffeinated green tea and EGCG. Further, green tea extract (NOS) [121], GTP (1.2–24 mg, top) [45,122], and EGCG (0.001, 0.005 mmol, top) [43,60] were reported to inhibit TPA-induced ODC activity in mouse skin. Recently, the dose-dependent inhibitory effects of GTP on testosterone-induced ODC activity in human prostate carcinoma LNCaP cells was also published [123]. Additionally, both cyclooxygenase (PGE_2, $PGF_{2\alpha}$, PGD2) and lipoxygenase (5-, 8-, 12-, 15-HETE) products induced by TPA in mouse epidermal microsomes were significantly inhibited by GTP (6 mg, top) *in vivo* [42].

In several published studies in experimental animals, administration of GTE and BTE, as well as GTP, enhanced phase II metabolic enzyme activities. GST, GSH-Px, catalase, and NAD(P)H:quinone reductase activities were enhanced in small bowel, liver, stomach, and lung by oral administration of GTP (0.2% w/v) and WEGT (2.5% w/v) in drinking water to A/J and SKH-1 hairless mice [27,108]. Similarly, administration of GTP (0.2% w/v) in drinking water enhanced the activity of liver GSH reductase in SKH-1 hairless mice [108]. Furthermore, F344 rats showed a significant increase in total serum GSH levels after administration of BTE (2% w/v); a nonsignificant increase was observed after GTE treatment at the same concentration [124]. Also, the activity of hepatic UDP-glucuronosyl transferase in rats was significantly enhanced by oral treatment with either black or green tea extracts (2%, 2.5% w/v) [124,125]. Therefore, if clinical development of black and green tea extracts, GTP, or EGCG continues, assessment of plasma and urine catechin levels, as well as serum GSH levels, prostaglandins, ODC activity, and enzymes such as GST and NAD(P)H:quinone reductase in skin or other accessible tissue may be useful drug effect measurements.

Safety: In a single case report, EGCG was found to be the major constituent of powdered green tea which provoked asthma and immediate skin reactions in three asthmatic patients who worked at a green tea factory [126]. Similar results were obtained with black and oolong teas. The concentration of EGCG found in green, oolong, and black teas were 8%, 4%, and 1%, respectively. Other studies [126] have shown inconclusive results, which may be attributed to method of testing, agent preparation, and condition of patients tested. These limited data suggest, but do not confirm, a low allergenic potential of tea components, although in experimental animals catechins such as EGCG and gallocatechin gallate have recently shown dose-dependent antiallergic activity [127].

ADME: In a single study [112,114], four human volunteers ingested 1.2 g decaffeinated green tea in 200 ml water. A sensitive coulochem electrode array system (CEAS) HPLC technique was developed to detect catechins in plasma and urine [114]. Plasma EGCG levels after one hour ranged from 46 to 268 ng/ml (0.13–0.75 μM); epigallocatechin and epicatechin plasma levels were 82–206 ng/ml (0.27– 0.67 μM) and 48–80 ng/ml (0.16– 0.27 μM), respectively. Epicatechin gallate was not detected in plasma. The maximum urinary excretion of epigallocatechin and epicatechin occurred at 3–6 hours. Most of the epigallocatechin and epicatechin was excreted after 9 hours, and the total after 24 hours was 2.8–3.2 mg and 1.6–2.3 mg, respectively. EGCG and epicatechin gallate were not detected in the urine; most of the catechins found in the plasma and urine were in their glucuronide and sulfate conjugated forms. The AUC and $t_{1/2}$ were also reportedly investigated, but no data were reported.

CLINICAL EFFICACY: PHASE II/III STUDIES

No Chemoprevention Branch-sponsored clinical studies have been planned for tea compounds. The epidemiology studies carried out with the tea compounds are summarized in Table III. Case-control studies suggesting an inverse relationship between tea consumption and cancers of colon and rectum [*e.g.*, 3–5], lung [2,4,7], pancreas [11], and stomach [4,12,13] have been published. Considering only case-control and cohort studies, five of 12 (42%) studies in colon and rectum [*e.g.*, 3,5,128,129] showed an inverse relationship. In other target organs such as kidney [*e.g.*, 9,10,130], lung [2,4,8, 9], and naso- and oropharynx [10,131], 9% (1/11), 25% (1/4), and 33% (2/6) of studies demonstrated such an association, respectively. In stomach [4, 9–13,132] and pancreas [11,133–135], 13% (2/15) and 18% (3/17) showed this relationship, respectively. One cohort study published recently [10] has shown inverse associations between non-herbal tea consumption and cancers of the stomach, oropharynx, esophagus, and kidney among 2,576 (non-skin cancer) postmenopausal women who were followed for seven years. Additionally, in a second recently pub-

lished study, consumption of green tea by cigarette smokers was demonstrated to result in reduced SCEs in mitogen-stimulated peripheral lymphocytes compared with the levels found in non-smokers [136]. Based on these and the animal efficacy studies described above, future clinical trials may evaluate the safety and efficacy of the tea compounds in such target tissues as colon, esophagus, lung (smokers), and skin (actinic keratosis patients).

PHARMACODYNAMICS

In experimental animals, tea extracts, GTP, and EGCG administered orally (in water and diet), ig, and topically were well-tolerated at efficacious doses. In most of the preclinical efficacy studies, tea extracts and GTP were administered either in drinking water or as the sole source of drinking water at concentrations ranging from 0.002 to 2.5%; 0.05% (w/v) is equivalent to four cups (approximately 800 ml) of tea consumed daily by an adult human. Additionally, data from studies measuring possible drug effect measurements (*e.g.*, ODC and arachidonic acid metabolites) suggest that oral doses of 2–2.5% tea extracts or GTP are effective without any toxicity.

Administration of EGCG at concentrations ranging from 0.016–0.32 mmol/kg-bw/day orally or 0.005–0.011 mmol/animal topically afforded protection in duodenum, liver, lung, and skin without major side effects. Intragastric administration of 2 mg EGCG/mouse (*ca.* 0.12 mmol/kg-bw/day) was also effective against DMH-induced large intestine tumors in Kunming mice [19]. In these studies, EGCG was well-tolerated. Assuming a typical cup (200 ml) of green tea contains 142 mg EGCG and 1 cup is consumed daily by an adult man (70 kg), a dose of 4.4 μmol/kg-bw/day is ingested. Consuming four cups (800 ml) of green tea daily results in ingestion of 17.7 μmol EGCG/kg-bw/day which is comparable to the lowest (0.016 mmol/kg-bw/day) effective dose in animal models.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

In published experimental animal studies, tea extracts, GTP, and EGCG have inhibited such enzymes as ODC, cyclooxygenase and lipoxygenase, as well as enhanced the activity of phase II metabolic enzymes (*e.g.*, GST, GSH-Px, catalase) and NAD(P)H:quinone reductase, and increased total serum GSH levels. On the basis of these results, if clinical development of black and green tea extracts, GTP, or EGCG continues, assessment of plasma and urine catechins, as well as serum GSH levels, prostaglandins, tissue ODC activity, and enzymes such as GST and NAD(P)H:quinone reductase in skin or other accessible tissue may be used as measurements. For example, in the case of DFMO, ODC activity in skin punch biopsies shows some potential as a drug effect measurement. In Phase I studies with oltipraz, the GSH level and activities of GST and NAD(P)H: quinone reductase are measured in lymphocytes and colorectal biopsies. It may be of interest to validate these measurements in animal models prior to initiation of Phase I clinical trials.

Safety Issues

There are no specific toxicity issues for tea extracts, GTP, and EGCG other than determining preclinical testing needed to fulfill FDA requirements for human testing (see Regulatory Issues).

Pharmacodynamics Issues

The primary target organs for clinical development of tea extracts, GTP, or EGCG are lung and skin; colon, esophagus, and mammary glands may also be considered on the basis of limited animal efficacy studies. Based on the efficacy data in a number of preclinical studies, attainment of an effective dose for EGCG in these tissues in humans should be possible without major side effects. Because of lack of preclinical toxicology, it is not possible to calculate a dose for Phase I/II clinical trials from a safety margin in animals; however, an oral dose of *ca.* 0.18 mmol/kg-bw/day was well-tolerated in the NNK-induced mouse lung model [36]. The lowest effective dose of 0.016 mmol EGCG/kg-bw/day in animal models is comparable to consumption of four cups of green tea or 17.7 μmol/kg-bw/day of EGCG by a 70 kg man; therefore, the well-tolerated dose of 0.18 mmol/kg-bw/day is approximately 10-fold the lowest effective dose. The results of the on-going subchronic toxicity tests in rats and dogs will provide adequate data for selection of doses for the Phase I/II clinical trials. These results are anticipated by December 1996, providing that sufficient quantities of EGCG and the tea extracts will be available for testing in the 90-day dog study.

Regulatory Issues

Preclinical 28-day and 90-day toxicity studies sponsored by the Chemoprevention Branch for decaffeinated BTP, GTP, and EGCG are in progress. It is likely that preclinical carcinogenicity studies will be required of any tea component advancing to clinical studies of more than one year duration. In addition, if further development of this agent is desired, Segment I, II and III reproductive toxicity studies may be required. However, no association between tea consumption and reproductive toxicity or teratogenicity has been found in pregnant women and their offspring [1].

Intermediate Biomarker Issues

In experimental animal studies published in the literature, epicatechin complex, GTE and BTE were reported to inhibit carcinogen-induced precancerous lesions [55] and GGT-positive foci in rat liver [56] and mouse epidermal cells [58]. GTP also inhibited aberrant hyperproliferation (an *in vitro* cellular marker for preneoplastic transformation) induced in mammary epithelial cell lines by carcinogens and oncogenes (*ras* or *myc*) [59]. Additionally, topical application of EGCG to mouse skin or oral administration of WEGT in drinking water was recently shown to inhibit TPA-induced ODC, PKC, and c-*myc* expression and NNK-induced lung oncogene (c-*myc*, c-H-*ras*, and c-*raf*) expression, respectively [60]. For clinical development of these agents, evaluation of their effects on precancerous lesions—particularly in targets that are likely to be affected such as lung, esophagus, and skin—may provide useful endpoints. It should be noted that preliminary results for NCI-funded colon aberrant crypt focus assay with BTP (low dose) and EGCG (high dose) indicate inhibitory activity.

Supply and Formulation Issues

T.J. Lipton, Inc. (New Jersey) has supplied green and black tea extracts, BTP, GTP, EGCG, and theaflavin mixture to the Chemoprevention Branch. The compositions of the green and black tea and GTP were established prior to testing (*e.g.*, amount of catechins present in green tea and GTP or theaflavins in black tea). There are a number of different types of green and black tea. They differ in genetic variety and production methods to yield, for example, those with high theaflavin content or strong aroma [63]. For instance, a typical cup of green tea (200 ml, gun powder, Hangzhou, China) contains 142 mg EGCG, 65 mg epigallocatechin, 28 mg epicatechin gallate, 17 mg epicatechin, and 76 mg caffeine [4]; a typical black tea beverage contains 3–10% catechins, 3–6% theaflavins, 12–18% thearubigins, and other components [88]. Another source of variability is the manufacturing conditions, which may affect antimutagenic and anticarcinogenic properties [*e.g.*, 25,70,71,101].

In most of the chemoprevention studies published in the literature, black and green tea extracts and GTP were administered either in drinking water or as the sole source of drinking water. The composition of these extracts, which in most cases were not quantitatively identified, differ according to source, preparation and brewing techniques. Therefore, a significant effort in the development of the tea components is to characterize and standardize the type, source, composition, method of manufacturing and preparation (if given in solution). Additionally, to minimize manufacturing variability, it would be desirable to produce and properly store green and black tea in large quantities.

Clinical Studies Issues

The efficacy of black and green tea extracts, GTP, and EGCG in several preclinical studies of lung and skin provide evidence that these are the most likely target organs for Phase II clinical chemoprevention trials. It may be of interest to examine the efficacy of the tea compounds in mammary gland carcinogenesis models to verify the chemopreventive activity in this target tissue prior to initiation of clinical trials. The on-going preclinical efficacy studies sponsored by the Chemoprevention Branch with green and black tea extracts, BTP, GTP, and EGCG in colon, bladder, and esophageal cancer models may also provide support for initiation of clinical trials in these target tissues. Future clinical trials may evaluate the safety and efficacy in such target tissues as colon, esophagus, lung (smokers), and skin (actinic keratosis patients).

REFERENCES

1. IARC Working Group. Tea. *IARC Monogr.* 51: 207–271, 1991.
2. Anonymous. Abstracts of papers presented at the first international symposium on the physiological and pharmacological effects of *Camellia sinensis* (tea). *Prev. Med.* 21: 331–333, 1992.
3. Fredrikson, M., Hardell, L., Bengtsson, N-L. and Axelson, O. Colon cancer and dietary habits—a case-control study. *Int. J. Oncol.* 7: 133–141, 1995.

4. Yang, C.S. and Wang, Z.-Y. Tea and cancer. *J. Natl. Cancer Inst.* 85: 1038–1049, 1993.
5. Kato, I., Tominaga, S., Matsuura, A., Yoshii, Y., Shirai, M. and Kobayashi, S. A comparative case-control study of colorectal cancer and adenoma. *Jpn. J. Cancer Res.* 81: 1101–1108, 1990.
6. Zatonski, W.A., La Vecchia, C., Prezewozniak, K., Maisonneuve, P., Lowenfels, A.B. and Boyle, P. Risk factors for gallbladder cancer: A Polish case-control study. *Int. J. Cancer* 51: 707–711, 1992.
7. Ohne, Y., Wakai, K., Genka, K., Ohmine, K., Kawamura, T., Tamakoshi, A., Aoki, R., Hayashi, Y., Nagao, K., Fukuma, S. and Aoki, K. Tea consumption and lung cancer risk: A case-control study in Okinawa, Japan. *Jpn. J. Cancer Res.* 86: 1027–1034, 1995.
8. Stocks, P. Cancer mortality in relation to national consumption of cigarettes, solid fuel, tea and coffee. *Br. J. Cancer* 24: 215–225, 1970.
9. Kinlen, L.J., Willows, A.N., Goldblatt, P. and Yudkin, J. Tea consumption and cancer. *Br. J. Cancer* 58: 397–401, 1988.
10. Zheng, W., Doyle, T.J., Hong, C.P., Kushi, L.H., Sellers, T.A. and Folsom, A.R. Tea consumption and cancer incidence in a prospective cohort study of postmenopausal women. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 278, abstract no. 1654, 1995.
11. Zatonski, W.A., Boyle, P., Prezewozniak, K., Maisonneuve, P., Drosik, K. and Walker, A.M. Cigarette smoking, alcohol, tea and coffee consumption and pancreas cancer risk: A case-control study from Opole, Poland. *Int. J. Cancer* 53: 601–607, 1993.
12. Kono, S., Ikeda, M., Tokudome, S. and Kuratsune M. A case-control study of gastric cancer and diet in northern Kyushu, Japan. *Jpn. J. Cancer Res.* 79: 1067–1074, 1988.
13. Hansson, L.-E., Nyren, O., Bergstrom, R., Wolk, A., Lindgren, A., Baron, J. and Adami, H.-O. Diet and risk of gastric cancer. A population-based case-control study in Sweden. *Int. J. Cancer* 55: 181–189, 1993.
14. Katiyar, S.K. and Mukhtar, H. Tea in chemoprevention of cancer: Epidemiologic and experimental studies (review). *Int. J. Oncol.* 8: 221–238, 1996.
15. Hamilton-Miller, J.M.T. Antimicrobial properties of tea (*Camellia sinensis* L.). *Antimicrobial Agents Chemotherapy* 39: 2375–2377, 1995.
16. Kim, M., Hagiwara, N., Smith, S.J., Yamamoto, T., Yamane, T. and Takahashi, T. Preventive effect of green tea polyphenols on colon carcinogenesis. *ACS Symp. Ser.* 47: 51–55, 1994.
17. Yamane, T., Hagiwara, N., Tateishi, M., Akachi, S., Kim, M., Okuzumi, J., Kitao, Y., Inagake, M., Kuwata, K. and Takahashi, T. Inhibition of azoxymethane-induced colon carcinogenesis in rat by green tea polyphenol fraction. *Jpn. J. Cancer Res.* 82: 1336–1339, 1991.
18. Narisawa, T. and Fukaura, Y. A very low dose of green tea polyphenols in drinking water prevents *N*-methyl-*N*-nitrosourea-induced colon carcinogenesis in F344 rats. *Jpn. J. Cancer Res.* 84: 1007–1009, 1993.
19. Komatsu, K., Kator, K., Mitsuda, Y., Mine, M. and Okumura, Y. Inhibitory effects of Rooibos tea, *Aspalathus linealis*, on x-ray-induced $C_3H10T_{1/2}$ cell trans formation. *Cancer Lett.* 77: 33–38, 1994.
20. Fujita, Y., Yamane, T., Tanaka, M., Kuwata, K., Okuzumi, J., Takahashi, T., Fujiki, H. and Okuda, T. Inhibitory effect of (–)-epigallocatechin gallate on carcinogenesis with *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine in mouse duodenum. *Jpn. J. Cancer Res.* 80: 503–505, 1989.
21. Fujiki, H., Yoshizawa, S., Horiuchi, T., Suganuma, M., Yatsunami, J., Nishiwaki, S., Okabe, S., Nishiwaki-Matsushima, R., Okuda, T. and Sugimura, T. Anticarcinogenic effects of (–)-epigallocatechin gallate. *Prev. Med.* 21: 503–509, 1992.
22. Wang, Z-Y., Wang, L-D., Lee, M-J., Ho, C-T., Huang, M-T., Conney, A.H. and Yang, C.S. Inhibition of *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats by green and black tea. *Carcinogenesis* 16: 2143–2148, 1995.
23. Yong, X. and Chi, H. The effect of Chinese tea on the occurrence of esophageal tumors induced by *N*-nitro somethylbenzylamine formed *in vivo*. *Biomed. Environ. Sci.* 3: 406–412, 1990.
24. Yang, C.S., Wang, Z.-Y. and Hong, J.-Y. Inhibition of tumorigenesis by chemicals from garlic and tea. *Adv. Exp. Med. Biol.* 354: 113–122, 1994.
25. Chen, J. The effects of Chinese tea on the occurrence of esophageal tumors induced by *N*-nitrosomethyl benzylamine in rats. *Prev. Med.* 21: 385–391, 1992.
26. Wang, Z.Y., Agarwal, W. R., Khan, W.A. and Mukhtar, H. Protection against benzo[*a*]pyrene- and *N*-nitroso diethylamine-induced lung and forestomach tumorigenesis in A/J mice by water extracts of green tea and licorice. *Carcinogenesis* 13: 1491–1494, 1992.
27. Katiyar, S.K., Agarwal, R., Zaim, M.T. and Mukhtar, H. Protection against *N*-nitrosodiethylamine- and benzo [*a*]pyrene-induced forestomach and lung tumorigenesis in A/J mice by green tea. *Carcinogenesis* 14: 849–855, 1993.
28. Wang, Z.Y., Hong, J.-Y., Huang, M.-T., Reuhl, K.R., Conney, A.H. and Yang, C.S. Inhibition of *N*-nitrosodiethylamine-and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone-induced tumorigenesis in A/J mice by green tea and black tea. *Cancer Res.* 52: 1943–1947, 1992.
29. Conney, A.H., Wang, Z.-Y., Huang, M.-T., Ho, C.-T. and Yang, C.S. Inhibitory effect of green tea on tumorigenesis by chemicals and ultraviolet light. *Prev. Med.* 21: 361–369, 1992.
30. Cao, J., Xu, Y., Chen, J. and Klaunig, J.E. Chemopreventive effects of green and black tea on pulmonary and hepatic carcinogenesis. *Fund. Appl. Toxicol.* 29: 244-250, 1996.
31. Li, Y. Comparative study on the inhibitory effect of green tea, coffee and levamisole on the hepatocarcinogenic action of diethylnitrosamine. *Chung Hua Chung Liu Tsa Chih* 13: 193–195, 1991.
32. Katiyar, S.K., Agarwal, R. and Mukhtar, H. Protective effects of green tea polyphenols administered by oral intubation against chemical carcinogen-induced forestomach and pulmonary neoplasia in A/J mice. *Cancer Lett.* 73: 167–172, 1993.
33. Katiyar, S.K., Agarwal, R. and Mukhtar, H. Protection against diethylnitrosamine (DEN)- and benzo(*a*)pyrene (BP)-induced forestomach and pulmonary neoplasia in A/J mice by limited oral intubation of green tea polyphenols. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 35: 626, abstract no. 3731, 1994.
34. Wang, Z.Y., Hong, J.-Y., Shi, S.T., Lee, M.J. Sun, Y., Conney, A.H. and Yang, C.S. Inhibitory effects of diallyl

sulfone ($DASO_2$) and green tea on lung tumorigenesis induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in A/J mice. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 35: 625, abstract no. 3728, 1994.

35. Tao, S., Wang, Z.-Y., Smith, T.J., Hong, J.-Y., Chen, W.-F., Ho, C.-T. and Yang, C.S. Effects of green tea and black tea on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone bioactivation, DNA methylation, and lung tumorigenesis in A/J mice. *Cancer Res.* 54: 4641–4647, 1994.
36. Xu, Y., Ho, C.-T., Amin, S.G., Han, C. and Chung, F.-L. Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. *Cancer Res.* 52: 3875–3879, 1992.
37. Chung, F.L., Morse, M.A., Eklind, K.I., Xu, Y., Rylander, R. and Krinsky, N. Inhibition of the tobacco-specific nitrosamine-induced lung tumorigenesis by compounds derived from cruciferous vegetables and green tea. *Ann. N.Y. Acad. Sci.* 686: 186–202, 1993.
38 Hirose, M., Hoshiya, T., Akagi, K., Futakuchi, M. and Ito, N. Inhibition of mammary gland carcinogenesis by green tea catechins and other naturally occurring antioxidants in female Sprague-Dawley rats pretreated with 7,12-dimethylbenz[*a*]anthracene. *Cancer Lett.* 83: 149–156, 1994.
39. Hirose, M., Akagi, K., Hasegawa, R., Yaono, M., Satoh, T., Hara, Y., Wakabayashi, K. and Ito, N. Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine(PhIP) -induced mammary gland carcinogenesis by antioxidants in F344 female rats. *Carcinogenesis* 16: 217–221, 1995.
40. Suganuma, M., Yoshizawa, S., Yatsunami, J., Nishiwaki, S., Furuya, H., Okabe, S., Nishiwaki-Matsushima, R., Frenkel, K., Troll, W., Verma, A.K. and Fujiki, H. Mechanisms of action of new antitumor promoters. *Basic Life Sci.* 61: 317–323, 1993.
41. Wang, Z.Y., Khan, W.A., Bickers, D.R. and Mukhtar, H. Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols. *Carcinogenesis* 10: 411–415, 1989.
42 Katiyar, S.K., Agarwal, R., Wood, G.S. and Mukhtar, H. Inhibition of 12-*O*-tetra-decanoylphorbol-13-acetate-caused tumor promotion in 7,12-dimethylbenz[*a*]anthracene-initiated SENCAR mouse skin by a poly phenolic fraction isolated from green tea. *Cancer Res.* 52: 6890–6897, 1992.
43. Katiyar, S.K., Agarwal, R., Wang, Z.Y., Bhatia, A.K. and Mukhtar, H. (–)-Epigallocatechin-3-gallate in *Camellia sinensis* leaves from Himalayan region of Sikkim: Inhibitory effects against biochemical events and tumor initiation in SENCAR mouse skin. *Nutr. Cancer* 18: 73–83, 1992.
44. Katiyar, S.K., Agarwal, R. and Mukhtar, H. Inhibition of both stage I and stage II skin tumor promotion in SENCAR mice by a polyphenolic fraction isolated from green tea: Inhibition depends on the duration of polyphenol treatment. *Carcinogenesis* 14: 2641–2643, 1993.
45. Huang, M.-T., Ho, C.-T., Wang, Z.Y., Ferraro, T., Finnegan-Olive, T., Lou, Y.-R., Mitchell, J., Laski, J.D., Newmark, H., Yang, C.S. and Conney, A.H. Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation in mouse skin. *Carcinogenesis* 13: 947–954, 1992.
46. Wang, Z.Y., Khan, W.A., Bickers, D.R. and Mukhtar, H. Anticarcinogenic activity of green tea polyphenols in murine skin. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 29: 132, abstract no. 527, 1988.
47. Wang, Z.Y., Agarwal, R., Bickers, D.R. and Mukhtar, H. Protection against ultraviolet B radiation-induced photocarcinogenesis in hairless mice by green tea polyphenols. *Carcinogenesis* 12: 1527–1530, 1991.
48. Wang, Z.Y., Huang, M.-T., Lou, Y.-R., Xie, J.-G., Reuhl, K.R., Newmark, H.L., Ho, C.-T., Yang, C.S., and Conney, A.H. Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz[*a*]anthracene-initiated SKH-1 mice. *Cancer Res.* 54: 3428–3435, 1994.
49. Wang, Z.-Y., Huang, M.-T., Ferraro, T., Wong, C.-Q., Lou, Y.-R., Reuhl, K., Iatropoulos, M., Yang, C.S. and Conney, A.H. Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-*O*-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. *Cancer Res.* 52: 1162–1170, 1992.
50. Khan, W.A., Wang, Z.Y., Athar, M., Bickers, D.R. and Mukhtar, H. Inhibition of the skin tumorigenicity of (+/–)-7β,8α-dihydroxy-9α,10I28α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene by tannic acid, green tea poly phenols and quercetin in SENCAR mice. *Cancer Lett.* 42: 7–12, 1988.
51. Wang, Z.Y., Bickers, D.R. and Mukhtar, H. Inhibition of 3-methylcholanthrene-induced skin tumorigenesis by green tea polyphenols. In: *Eleventh International Symposium on Polynuclear Aromatic Hydrocarbons.* Gaithersburg, MD.: NIH/National Bureau of Standards, p. 8, 1987.
52. Katiyar, S.K., Agarwal, R. and Mukhtar, H. Protection against malignant conversion of chemically induced benign skin papillomas to squamous cell carcinomas in SENCAR mice by a polyphenolic fraction isolated from green tea. *Cancer Res.* 53: 5409–5412, 1993.
53. Mukhtar, H., Katiyar, S.K. and Agarwal, R. Cancer chemoprevention by green tea components. *Adv. Exp. Med. Biol.* 354: 123–134, 1994.
54. Conney, A.H., Wang, Z.Y., Huang, M.-T., Lou, Y.-R. and Yang, C.S. Inhibitory effects of green and black tea on carcinogenesis. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 704, 1995.
55. Xu, Y. and Han, C. Prevention of *in vivo* formation of NMBzA and the formation of oesophageal tumour by tea in rats. *Chung Hua Yu Fang I Hsueh Tsa Chih* 25: 201–204, 1991.
56. Chen, Z.Y., Yan, R.Q., Qin, G.Z. and Qin, L.L. Effect of six edible plants on the development of AFB_1-induced -glutamyltranspeptidase-positive hepatocyte foci in rats. *Chung Hua Chung Liu Tsa Chih* 9: 109–111, 1987.
57. Hirose, M., Hasegawa, R., Kimura, J., Akagi, K., Yoshida, Y., Tanaka, H., Miki, T., Satoh, T., Wakabayashi, K., Ito, N. and Shirai, T. Inhibitory effects of 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ), green tea catechins and other antioxidants on 2-amino-6-methylpyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-1)-induced rat hepatocarcinogenesis and dose-dependent inhibition by HTHQ of lesion induction by Glu-P-1 or 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx). *Carcinogenesis* 16: 3049–3055, 1995.
58. Katiyar, S.K., Rupp, C.O., Korman, N.J., Agarwal, R. and Mukhtar, H. Inhibition of 12-*O*-tetradecanoyl phorbol-13-acetate and other skin tumor-promoter-caused induction of epidermal interleukin-1α mRNA and protein expression in SENCAR mice by green tea polyphenols. *J. Invest. Dermatol.* 105: 394–398, 1995.
59. Katdare, M.S., Singhal, H., Newmark, H., Osborne, M.P.

and Telang, N.T. Aberrant hyperproliferation in mouse mammary epithelial cells: A biomarker for chemoprevention of preneoplastic transformation. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 124, abstract no. 738, 1995.

60. Hu, G., Han, C. and Chen, J. Inhibition of oncogene expression by green tea and (–)-epigallocatechin gallate in mice. *Nutr. Cancer* 24: 203–209, 1995.
61. Yen, G.-C. and Chen H.-Y. Relationship between anti mutagenic activity and major components of various teas. *Mutagenesis* 11: 37–41, 1996.
62. Yamada, J. and Tomita, Y. Antimutagenic activity of water extracts of black tea and oolong tea. *Biosci. Biotech. Biochem.* 58: 2197–2200, 1994.
63. Apostolides, Z. and Weisburger, J.H. Screening of tea clones for inhibition of PhIP mutagenicity. *Mutat. Res.* 326: 219–225, 1995.
64. Stich, H.F., Chan, P.K.L. and Rosin, M.P. Inhibitory effects of phenolics, teas, and saliva on the formation of mutagenic nitrosation products of salted fish. *Int. J. Cancer* 30: 719–724, 1982.
65. Kada, T., Kaneko, K., Matsuzaki, S., Matsuzaki, T. and Hara, Y. Detection and chemical identification of natural bio-antimutagens. A case of the green tea factor. *Mutat. Res.* 150: 127–132, 1985.
66. Okuda, T., Mori, K. and Hayatsu, H. Inhibitory effect of tannins on direct-acting mutagens. *Chem. Pharm. Bull.* 32: 3755–3758, 1984.
67. Wang, Z.Y., Cheng, S.J., Zhou, Z.C., Athar, M., Khan, W.A., Bickers, D.R. and Mukhtar, H. Antimutagenic activity of green tea polyphenols. *Mutat. Res.* 223: 273–285, 1989.
68. Jain, A.K., Shimoi, K., Nakamura, Y., Kada, T., Hara, Y. and Tomita, I. Crude tea extracts decrease the mutagenic activity of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine *in vitro* and in intragastric tract of rats. *Mutat. Res.* 210: 1–8, 1989.
69. Bu-Abbas, A., Clifford, M.N., Walker, R. and Ioannides, C. Marked antimutagenic potential of aqueous green tea extracts: Mechanism of action. *Mutagenesis* 9: 325–331, 1994.
70. Yen, G.-C. and Chen, H.-Y. Comparison of antimutagenic effect of various black tea extracts (green, oolong, pouchong, and black tea). *J. Food Protect.* 57: 54–58, 1994.
71. Yen, G.-C. and Chen, H.-Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* 43: 27–32, 1995.
72. Hayatsu, H., Inada, N., Kakutani, T., Arimoto, S., Negishi, T., Mori, K., Okuda, T. and Sakata, I. Suppression of genotoxicity of carcinogens by (–)-epigallocatechin gallate. *Prev. Med.* 21: 370–376, 1992.
73. Hayatsu, H., Negishi, T. and Arimoto, S. Dietary inhibitors against mutagenesis and carcinogenesis. *Basic Life Sci.* 61: 387–418, 1993.
74. Weisburger, J.H., Nagao, M., Wakabayashi, K. and Oguri, A. Prevention of heterocyclic amine formation by tea and tea polyphenols. *Cancer Lett.* 83: 143–147, 1994.
75. Weisburger, J.H. Practical approaches to chemoprevention of cancer. *Drug Metab. Rev.* 26: 253–260, 1994.
76. Stich, H.F., Dunn, B.P., Pignatelli, B., Ohshima, H. and Bartsch, H. Dietary phenolics and betel nut extracts as modifiers of *N*-nitrosation in rat and man. *IARC Sci. Publ.* 57: 213–222, 1983.
77. Nakamura, Y., Harada, S., Hara, Y. and Tomita, I. Inhibitory effect of crude extracts of green tea leaves on the promotion of neoplastic transformation in JB6 cells by TPA. *Mutat. Res.* 182: 369–370, 1987.
78. Telang, N.T., Inoue, S., Bradlow, H.L., Fujiki, H. and Osborne, M.P. Antipromotional effect of a green tea polyphenol on c-*myc*-transfected mammary epithelial cells. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 35: 619, abstract no. 3692, 1994.
79. Inagake, M., Yamane, T., Kitao, Y., Oya, K., Matsumoto, H., Kikuoka, N., Nakatani, H., Takahashi, T., Nishimura, H. and Iwashima, A. Inhibition of 1,2-dimethylhydrazine-induced oxidative DNA damage by green tea extract in rat. *Jpn. J. Cancer Res.* 86: 1106–1111, 1995.
80. Sazuka, M., Murakami, S., Isemura, M., Satoh, K. and Nukiwa, T. Inhibitory effects of green tea infusion on *in vitro* invasion and *in vivo* metastasis of mouse lung carcinoma cells. *Cancer Lett.* 98: 27–31, 1995.
81. Sakamoto, K., Deepika, R., Hara, Y. and Milner, J.A. Impact of green or black tea polyphenols on canine mammary tumor cells in culture. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 595, abstract no. 3542, 1995.
82. Nishida, H., Omori, M., Fukutomi, Y., Ninomiya, M., Nishiwaki, S., Suganuma, M., Moriwaki, H. and Muto, Y. Inhibitory effects of (–)-epigallocatechin gallate on spontaneous hepatoma in C3H/HeNCrj mice and human hepatoma-derived PLC/PRF/5 cells. *Jpn. J. Cancer Res.* 85: 221–225, 1994.
83. Komori, A., Yatsunami, J., Okabe, S., Abe, S., Hara, K., Suganuma, M., Kim, S.-J. and Fujiki, H. Anticarcinogenic activity of green tea polyphenols. *Jpn. J. Clin. Oncol.* 23: 186–190, 1993.
84. Taniguchi, S., Fujiki, H., Kobayashi, H., Go, H., Miyado, K., Sadano, H. and Shimokawa, R. Effect of (–)-epigallocatechin gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines. *Cancer Lett.* 65: 51–54, 1992.
85. Wang, Z.Y., Huang, M.-T., Ho, C.-T., Chang, R., Ma, W., Ferraro, T., Reuhl, K.R., Yang, C.S. and Conney, A.H. Inhibitory effect of green tea on the growth of established skin papillomas in mice. *Cancer Res.* 52: 6657–6665, 1992.
86. Oguni, I., Nasu, K., Yamamoto, S. and Nomura, T. On the antitumor activity of fresh green tea leaf. *Agric. Biol. Chem.* 52: 1879–1880, 1988.
87. Miyamoto, K., Kishi, N., Koshiura, R., Yoshida, T., Hatano, T. and Okuda, T. Relationship between the structures and the antitumor activities of tannins. *Chem. Pharm. Bull.* 35: 814–822, 1987.
88. Stoner, G.D. and Mukhtar, H. Polyphenols as cancer chemopreventive agents. *J. Cell. Biochem.* 22: 169–180, 1994.
89. Mukhtar, H., Wang, Z.Y., Katiyar, S.K. and Agarwal, R. Tea components: Antimutagenic and anticarcinogenic effects. *Prev. Med.* 21: 351–360, 1992.
90. Han, J. Highlights of the cancer chemoprevention studies in China. *Prev. Med.* 22: 712–722, 1993.
91. Chan, M.-Y. M., Ho, C.-T. And Huang, H.-I. Effects of three dietary phytochemicals from tea, rosemary, and turmeric on inflammation-induced nitrite production. *Cancer Lett.* 96: 23–29, 1995.
92. Lee, S.F., Liang, Y. C. and Lin, J.K. Inhibition of 1,2,4-benzenetriol-generated active oxygen species and induction of

phase II enzymes by green tea polyphenols. *Chem. Biol. Interact.* 98: 283–301, 1995.

93. Uchida, S., Ohta, H., Edamatsu, R., Hiramatsu, M., Mori, A., Nonaka, G.-I., Nishioka, I., Niwa, M., Akashi, T. and Ozaki, M. Active oxygen free radicals are scavenged by condensed tannins. *Prog. Clin. Biol. Res.* 280: 135–138, 1988.
94. Zhao, B., Li, X., He, R., Cheng, S. and Xin, W. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys.* 14: 175–185, 1989.
95. Uchida, S., Edamatsu, R., Hiramatsu, M., Mori, A., Nonaka, G.-I., Nishioka, I., Niwa, M. and Ozaki, M. Condensed tannins scavenge active oxygen free radicals. *Med. Sci. Res.* 15: 831–832, 1987.
96. Uchida, S., Ozaki, M., Suzuki, K. and Shikita, M. Radioprotective effects of (−)-epigallocatechin 3-*O*-gallate (green-tea tannin) in mice. *Life Sci.* 50: 147–152, 1992.
97. Nanjo, F., Honda, M., Okushio, K., Matsumoto, N., Ishigaki, F., Ishigami, T. and Hara, Y. Effects of dietary tea catechins on α–tocopherol levels, lipid peroxidation, and erythrocyte deformability in rats fed on high palm oil and perilla oil diets. *Biol. Pharm. Bull.* 16: 1156–1159, 1993
98. Shiraki, M., Hara, Y., Osawa, T., Kumon, H., Nakayama, T. and Kawakishi, S. Antioxidative and antimutagenic effects of theaflavins from black tea. *Mutat. Res.* 323: 29–34, 1994.
99. Yoshino, K., Hara, Y., Sano, M. and Tomita, I. Antioxidative effects of black tea theaflavins and thearubigin on lipid peroxidation of rat liver homogenates induced by *tert*-butyl hydroperoxide. *Biol. Pharm. Bull.* 17: 146–149, 1994.
100. Katiyar, S.K., Agarwal, R. and Mukhtar, H. Inhibition of spontaneous and photo-enhanced lipid peroxidation in mouse epidermal microsomes by epicatechin derivatives from green tea. *Cancer Lett.* 79: 61–66, 1994.
101. Ho, C.-T., Chen, Q., Shi, H., Zhang, K.-Q. and Rosen, R.T. Antioxidative effect of polyphenol extract prepared from various Chinese teas. *Prev. Med.* 21: 520–525, 1992.
102 Klaunig, J.E. Chemopreventive effects of green tea components on hepatic carcinogenesis. *Prev. Med.* 21: 510–519, 1992.
103 Lea, M.A., Xiao, Q., Sadhukhan, A.K., Cottle, S., Wang, Z.Y. and Yang, C.S. Inhibitory effects of tea extracts and (−)-epigallocatechin gallate on DNA synthesis and proliferation of hepatoma and erythroleukemia cells. *Cancer Lett.* 68: 231–236, 1993.
104. Katiyar, S.K., Agarwal, R., Ekker, S., Wood, G.S. and Mukhtar, H. Protection against 12-*O*-tetradecanoyl phorbol-13-acetate-caused inflammation in SENCAR mouse ear skin by polyphenolic fraction isolated from green tea. *Carcinogenesis* 14: 361–365, 1993.
105. Liao, S. and Hiipakka, R.A. Selective inhibition of steroid 5α-reductase isoenzymes by tea epicatechin-3-gallate and epigallocatechin-3-gallate. *Biochem. Biophys. Res. Comm.* 214: 833–838, 1995.
106. Wang, Z.Y., Das, M., Bickers, D.R. and Mukhtar, H. Interaction of epicatechins derived from green tea with rat hepatic cytochrome P-450. *Drug Metab. Dispos.* 16: 98–103, 1988.
107. Bu-Abbas, A., Clifford, M.N., Walker, R. and Ioannides, C. Selective induction of rat hepatic CYP1 and CYP4 proteins and of peroxisomal proliferation by green tea. *Carcinogenesis* 15: 2575–2579, 1994.
108. Khan, S.G., Katiyar, S.K., Agarwal, R. and Mukhtar, H. Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: Possible role in cancer chemoprevention. *Cancer Res.* 52: 4050–4052, 1992.
109. Ruch, R.J., Cheng, S.-J. and Klaunig, J.E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 10: 1003–1008, 1989.
110. Sigler, K. and Ruch, R.J. Enhancement of gap junctional intercellular communication in tumor promoter-treated cells by components of green tea. *Cancer Lett.* 69: 15–19, 1993.
111. Fujiki, H., Suganuma, M., Komori, A., Yatsunami, J., Okabe, S., Ohta, T. and Sueoka, E. A new tumor promotion pathway and its inhibitors. *Cancer Detect. Prev.* 18: 1–7, 1994.
112. Yang, C.S., Lee, M.-J., Chen, L.-S., Li, H., Gobbo, S., Balentine, D. and Wang, Z.Y. Analysis of plasma and urinary tea polyphenols in animals and humans. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36: 117, abstract no. 696, 1995.
113. Unno, T. and Takeo, T. Absorption of (−)-epigallocatechin gallate into the circulation system of rats. *Biosci. Biotech. Biochem.* 59: 1558–1559, 1995.
114. Lee, M-J., Wang, Z-Y., Li, H., Chen, L., Sun, Y., Gobbo, S., Balentine, D.A. and Yang, C.S. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol. Biomarkers Prev.* 4: 393–399, 1995.
115. Mukhtar, H., Katiyar, S.K. and Agarwal, R. Green tea and skin—Anticarcinogenic effects. *J. Invest. Dermatol.* 102: 3–7, 1994.
116. Hirose, M., Hoshiya, T., Akagi, K., Takahashi, S., Hara, Y. and Ito, N. Effects of green tea catechins in a rat multi-organ carcinogenesis model. *Carcinogenesis* 14: 1549–1553, 1993.
117. RTECS. *Registry of Toxic Effects of Chemical Substances.* Available online via National Library of Medicine (NLM). Latest update, 4/95.
118. Jain, A.K. and Sethi, N. Chromosomal aberrations and sister chromatid exchanges in cultured human lymphocytes II. Induced by epigallocatechin gallate. *Cytologia* 56: 539–542, 1991.
119. Jain, A.K. and Sethi, N. Chromosomal aberrations and sister chromatid exchanges in cultured human lymphocytes IV. Concluding remarks. *Cytologia* 56: 549–554, 1991.
120. Imanishi, H., Sasaki, Y.F., Ohta, T., Watanabe, M., Kato, T. and Shirasu, Y. Tea tannin components modify the induction of sister-chromatid exchanges and chromosome aberrations in mutagen-treated cultured mammalian cells and mice. *Mutat. Res.* 259: 79–87, 1991.
121. Cheng, S.J. Inhibitory effect of green tea extract on promotion and related action of TPA. *Chung Kuo I Hsueh Ko Hsueh Yuan Hsueh Pao* 11: 259–264, 1989.
122. Agarwal, R., Katiyar, S.K., Zaidi, S.I.A. and Mukhtar, H. Inhibition of skin tumor promoter-caused induction of epidermal ornithine decarboxylase in SENCAR mice by polyphenolic fraction isolated from green tea and its individual epicatechin derivatives. *Cancer Res.* 52: 3582–3588, 1992.
123. Mohan, R.R., Khan, S.G., Agarwal, R. and Mukhtar, H. Testosterone induces ornithine decarboxylase (ODC) activity and mRNA expression in human prostate carcinoma cell

line LNCaP: Inhibition by green tea. *Proc. Am. Assoc. Cancer Res.* 36: 274, abstract no. 1633, 1995.

124. Sohn, O.S., Surace, A., Fiala, E.S., Richie, J.P., Jr., Colosimo, S., Zang, E. and Weisburger, J.H. Effects of green and black tea on hepatic xenobiotic metabolizing systems in the male F344 rat. *Xenobiotica* 24: 119–127, 1994.
125. Bu-Abbas, A., Clifford, M.N., Ioannides, C. and Walker, R. Stimulation of rat hepatic UDP-glucuronosyl transferase activity following treatment with green tea. *Food Chem. Toxicol.* 33: 27–30, 1995.
126. Shirai, T., Sato, A. and Hara, Y. Epigallocatechin gallate. The major causative agent of green tea-induced asthma. *Chest* 106: 1801–1805, 1994.
127. Ohmari, Y., Ito, M., Kisji, M., Misutani, H., Katada, T. and Konishi, H. Antiallergic constituents from oolong tea stem. *Bio. Pharm. Bull.* 18: 683–686, 1995.
128. Heilbrun, L.K., Nomura, A. and Stemmerman, G.N. Black tea consumption and cancer. *Br. J. Cancer* 58: 677–683, 1986.
129. La Vecchia, C., Negri, E., Franceschi, S., D'Avanzo, B. and Boyle, P. Tea consumption and cancer risk. *Nutr. Cancer* 17: 27–31, 1992.
130. McLaughlin J.K., Mandel, J.S., Blot, W.J., Schuman, L.M., Mehl, E.S. and Fraumeni, J.F. Jr. A population-based case-control study of renal cell carcinoma. *J. Natl. Cancer Inst.* 72: 275–284, 1984.
131. Franceschi, S., Barra, S., La Vecchia, C., Bidoli, E., Negri, E. and Talamini, R. Risk factors for cancer of the tongue and the mouth. A case-control study from North Italy. *Cancer* 70: 2227–2233, 1992.
132. Lee, H.H., Wu, H.-Y., Chuang, Y.-C., Chuang, A.-S., Chao, H.-H., Chen, K.-Y., Chen, H.-K., Lai, G.-M., Huang, H.-H., and Chen, C.-J. Epidemiologic characteristics and multiple risk factors of stomach cancer in Taiwan. *Anticancer Res.* 10: 875–881, 1990.
133. Kinlen, L.J. and McPherson, K. Pancreas cancer and coffee and tea consumption: A case-control study. *Br. J. Cancer* 49: 93–96, 1984.
134. Mizuno, S., Watanabe, S., Nakamura, K., Omata, M., Oguchi, H., Ohashi, K., Ohyanagi, H., Fujiki, T. and Motojima, K. A multi-institute case-control study on the risk factors of developing pancreatic cancer. *Jpn. J. Clin. Oncol.* 22: 286–291, 1992.
135. Bueno De Mesquita, H.B., Maisonneuve, P., Moerman, C.J., Ruini, S. and Boyle, P. Lifetime consumption of alcoholic beverages, tea and coffee and exocrine carcinoma of the pancreas: A population-based case-controlled study in the Netherlands. *Int. J. Cancer* 50: 514–522, 1992.
136. Shim, J.S., Kang, M.H., Kim, Y.H., Roh, J.K., Roberts, C. and Lee, I.P. Chemopreventive effect of green tea (*Camellia sinensis*) among cigarette smokers. *Cancer Epidemiol. Biomarkers Prev.* 4: 387–391, 1995.
137. Ewertz, M. Breast cancer in Denmark. Incidence, risk factors, and characteristics of survival. *Acta Oncol.* 32: 595–615, 1993.
138. Baron, J.A., de Verdier, M.G. and Ekbom, A. Coffee, tea, tobacco, and cancer of the large bowel. *Cancer Epidemiol. Biomarkers Prev.* 3: 565–570, 1994.
139. Ghadirian, P., Ekoe, J.-M. and Thouez, J.-P. Food habits and esophageal cancer: An overview. *Cancer Detect. Prev.* 16: 163–168, 1992.
140. Siddiqi, M., Kumar, R., Fazili, Z., Spiegelhalder, B. and Preussmann, R. Increased exposure to dietary amines and nitrate in a population at high risk of oesophageal and gastric cancer in Kashmir (India). *Carcinogenesis* 13: 1331–1335, 1992.
141. Dhar, G.M., Shah, G.N., Naheed, B. and Hafiza. Epidemiological trend in the distribution of cancer in Kashmir Valley. *J. Epidemiol. Comm. Health* 47: 290–292, 1993.
142. Gao, Y.T., McLaughlin, J.K., Blot, W.J., Ji, B.T., Dai, Q. and Fraumeni, J.F., Jr. Reduced risk of esophageal cancer associated with green tea consumption. *J. Natl. Cancer Inst.* 86: 855–858, 1994.
143. Hu, J., Nyren, O., Wolk, A., Bergstrom, R., Yuen, J., Adami, H.-O., Guo, L., Li, H., Huang, G., Xu, X., Zhao, F., Chen, Y., Wang, C., and Qin, H. Risk factors for oesophageal cancer in Northeast China. *Int. J. Cancer* 57: 38–46, 1994.
144. Benhamou, S., Lenfant, M.-H., Ory-Paoletti, C. and Flamant, R. Risk factors for renal-cell carcinoma in a French case-control study. *Int. J. Cancer* 55: 32–36, 1993.
145. Mellemgaard, A., Engholm, G., McLaughlin, J.K. and Olsen, J.H. Risk factors for renal cell carcinoma in Denmark. I. Role of socioeconomic status, tobacco use, beverages, and family history. *Cancer Causes Control* 5: 105–113, 1994.
146. Olshan, A.F., Breslow, N.E., Falletta, J.M., Grufferman, S., Pendergrass, T., Robison, L.L., Waskerwitz, M., Woods, W.G., Vietti, T.J. and Hammond, G.D. Risk factors for Wilms tumor. Report from the National Wilms Tumor Study. *Cancer* 72: 938–944, 1993.
147. Huang, C., Zhang, X., Qiao, Z., Guan, L., Peng, S., Liu, J., Xie, R. and Zheng, L. A case-control study of dietary factors in patients with lung cancer. *Biomed. Environ. Sci.* 5: 257–265, 1992.
148. Mashberg, A., Boffetta, P., Winkelman, R. and Garfinkel, L. Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. Veterans. *Cancer* 72: 1369–1375, 1993.
149. Shibata, A., Mack, T.M., Paganini-Hill, A., Ross, R.K. and Henderson, B.E. A prospective study of pancreatic cancer in the elderly. *Int. J. Cancer* 58: 46–49, 1994.
150. Slattery, M.L. and West, D.W. Smoking, alcohol, coffee, tea, caffeine, and theobromine: Risk of prostate cancer in Utah (United States). *Cancer Causes Control* 4: 559–563, 1993.
151. Memik, F., Nak, S.G., Gulten, M. and Ozturk, M. Gastric carcinoma in Northwestern Turkey: Epidemiologic characteristics. *J. Environ. Pathol. Toxicol. Oncol.* 11: 335–338, 1992.
152. Ito, N. and Imaida, K. Strategy of research for cancer-chemoprevention. *Teratogenesis Carcinog. Mutagen.* 12: 79–95, 1992.

Table II. Summary of Preclinical *In Vivo* Efficacy Studies

Target Organ	Species	Carcinogen	Tea Compound	Dose[1]	Result	Reference
Colon	Rat, F344	AOM	GTP	0.01, 0.1%	+	[17]
Colon	Rat, F344	AOM	GTP	0.01, 0.1%	+	[16]
Colon	Rat, F344	MNU	GTP	0.002, 0.01, 0.05%	+	[18]
Duodenum	Mouse, C57BL/6	ENNG	EGCG	0.005% (*ca.* 0.016 mmol/ kg-bw/day)	+	[20,21]
Esophagus	Rat, Wistar	Sodium Nitrite + Methyl-benzyl-amine	Various Black, Green Tea Water Extracts	0.5–1 g dried tea	+	[23,24,25]
Esophagus	Rat, Wistar	MBN	Various Black, Green Tea Water Extracts	0.5–1 g dried tea	NE	[23,24,25]
Esophagus	Rat	MBN	Various Black, Green Tea Infusions	2%	+	[24]
Esophagus	Rat, Sprague-Dawley	MBN	Decaffeinated Black Tea or Decaffeinated or Regular Green Tea Water Extracts	0.6%, 0.9%	+	[22,24]
Forestomach	Mouse, A/J	B(*a*)P, DEN	WEGT	1.2%	+	[26]
Forestomach	Mouse, A/J	B(*a*)P, DEN	GTP, WEGT	GTP: 0.2% WEGT: 2.5%	+	[27]
Forestomach	Mouse, A/J	DEN	WEGT	0.63%, 1.25%	+	[24,28,29]

Table II. Summary of Preclinical *In Vivo* Efficacy Studies *(continued)*

Target Organ	Species	Carcinogen	Tea Compound	Dose[1]	Result	Reference
Intestine, Large	Mouse, Kunming	DMH	GTP, EGCG	1–3 mg/mouse ig (EGCG: 2 mg/mouse, *ca.* 0.12 mmol/ kg-bw/day)	+	[19]
Liver	Rat, Wistar	DEN	Green Tea (NOS)	2.5% in diet	+	[31]
Liver	Mouse, C3H	DEN	Decaffeinated WEGT, WEBT	WEGT: 0.63%, 1.25%, WEBT: 1.25%	+	[30]
Liver	Mouse, C3H/HeN	Spontaneous	EGCG	0.05%, 0.1% (*ca.* 0.16–0.32 mmol/kg-bw/ day)	+	[21]
Lung	Mouse, A/J	B(*a*)P, DEN	WEGT	1.2%	+	[26]
Lung	Mouse, A/J	B(*a*)P, DEN	GTP	5 mg/0.2 ml H_2O, ig	+	[32,33]
Lung	Mouse, A/J	B(*a*)P, DEN	GTP, WEGT	GTP: 0.2% WEGT: 2.5%	+	[27]
Lung	Mouse, A/J	DEN	WEGT	0.63%, 1.25%	+	[28]
Lung	Mouse, C3H	DEN	Decaffeinated WEGT, WEBT	WEGT: 0.63%, 1.25%, WEBT: 1.25%	+	[30]
Lung	Mouse, A/J	NNK	Decaffeinated WEBT, WEGT	0.3%, 0.6%	+	[24,28,29, 34]
Lung	Mouse, A/J	NNK	Decaffeinated WEGT	0.6%	+	[35]

Table II. Summary of Preclinical *In Vivo* Efficacy Studies *(continued)*

Target Organ	Species	Carcinogen	Tea Compound	Dose[1]	Result	Reference
Lung	Mouse, A/J	NNK	WEGT, EGCG	2%, EGCG: 560 ppm (*ca.* 0.18 mmol/ kg-bw/day)	+	[24,36,37]
Mammary Glands	Rat, Sprague-Dawley	DMBA	GTP	1% in diet	+	[38]
Mammary Glands	Rat, F344	PhIP	GTP	1% in diet	+ (Tumor Size only)	[39]
Multiorgan Carcinogene-sis Model (Colon, Lung Small Intestine)	Rat, F344	DEN+ MNU+ DMH+ BBN+DHPN	GTP	0.1%, 1% in diet	+ (Intestine) NE (Colon Lung)	[116,152]
Skin	Mouse, SENCAR	DMBA/TPA	GTP	10 mg/ 0.2 ml acetone, top or 0.5 g/l in H_2O	+	[41]
Skin	Mouse, SENCAR	DMBA/TPA	GTP	1–24 mg/ 0.2 ml acetone/ animal, top	+	[42]
Skin	Mouse, SENCAR	DMBA/TPA	EGCG	5 μmol/ 0.2 ml acetone/ animal, top	+	[43]
Skin	Mouse, SENCAR	DMBA/TPA, DMBA/TPA/ Mezerein	GTP	6 mg/animal, top	+	[44]
Skin	Mouse, CD-1	DMBA/TPA, B(*a*)P/TPA	GTP	1.2, 3.6 mg/200 μl acetone, top	+	[29,45]
Skin	Mouse, SENCAR	DMBA/TPA	GTP	top, oral	+	[46]

Table II. Summary of Preclinical *In Vivo* Efficacy Studies *(continued)*

Target Organ	Species	Carcinogen	Tea Compound	Dose[1]	Result	Reference
Skin	Mouse, CD-1	DMBA/ Teleocidin	EGCG	5 mg/mouse, top (*ca.* 0.011 mmol)	+	[53]
Skin	Mouse, SKH-1 hairless	UV	GTP	10 mg, top and 0.1% in H_2O	+	[47]
Skin	Mouse, SKH-1 hairless	DMBA/UV	WEGT, WEBT, Decaffeinated WEGT, Decaffeinated WEBT	0.63%, 1.25%	+	[48,54]
Skin	Mouse, SKH-1 hairless	DMBA/UV, DMBA/TPA, UV/TPA	WEGT	1.25%	+	[29,49]
Skin	Mouse, SENCAR	BPDE-2/TPA	GTP	24 mg/mouse, top	+	[46,50]
Skin	Mouse, BALB/c	3-MCA	GTP	1.2 mg/ 0.2 ml acetone, top	+	[41,46]
Skin	Mouse, BALB/c	3-MCA	GTP	5 mg/animal	+	[51]
Skin	Mouse, SENCAR	Malignant Conversion Protocol: DMBA/TPA, Benzoyl Peroxide, NQO, Spontaneous	GTP	6 mg/ 0.2 ml acetone, top	+ (NE, Spontaneous)	[52]

[1] Agent administered in or as the sole source of water, unless otherwise noted.

Table III. Summary of Epidemiology Studies

Target Organ	Association with Drinking Tea[1]	Type of Study (Number of Studies) 1 = Ecological 2 = Cohort 3 = Case-control	Reference
Bladder	No Relationship	1 (1) 2 (2) 3 (16)	[4,8,9,128]
Breast	Positive No Relationship Negative	1 (1) 3 (5) 1 (1)	[2,4,8,137]
Colon, Intestine & Rectum	Positive No Relationship Negative	1 (1) 2 (1) 2 (1) 3 (5) 3 (5)	[3–5,8,9,128,129,138]
Esophagus	Positive Positive (High Temperature, Salted Tea) No Relationship (Normal or Hot Temperature Tea) Negative	1 (3) 1 (4) 3 (7) 1 (1) 6 (3) 2 (1)	[4,8,10,129,139–143]
Gall Bladder	Negative	3 (1)	[6]
Kidney	Positive No Relationship No Relationship (Wilms Tumors, Maternal Consumption) Negative	2 (1) 3 (1) 3 (7) 2 (1) 2 (1)	[4,9,10,130,144–146]
Leukemia	No Relationship	1 (1)	[8]
Liver	No Relationship Negative	1 (1) 2 (1) 3 (1) 1 (1)	[2,8,128,129]

Table III. Summary of Epidemiology Studies *(continued)*

Target Organ	Association with Drinking Tea[1]	Type of Study (Number of Studies) 1 = Ecological 2 = Cohort 3 = Case-control	Reference
Lung	Positive No Relationship Negative	1 (1) 2 (1) 3 (2) 1 (1) 3 (1)	[2,4,7–9,147]
Nasopharynx Oropharynx (Oral Cavity, Mouth, Tongue)	Positive No Relationship Negative	3 (1) 3 (3) 1 (1) 3 (1) 2 (1)	[4,8,10,131,148]
Ovary	No Relationship	1 (1)	[8]
Pancreas	Positive Positive (Green Tea) No Relationship No Relationship (Black Tea) Negative	3 (1) 3 (1) 2 (3) 1 (1) 3 (7) 2 (2) 3 (1) 3 (2)	[4,8,11,133–135,149]
Prostate	No Relationship	3 (1) 2 (1)	[8,9,150]
Stomach	Positive No Relationship Negative	2 (1) 3 (1) 1 (1) 3 (8) 1 (2) 3 (4) 2 (1)	[4,8–10,12,13,132,151]
Uterus	Negative	1 (2)	[2,8]

[1] Association with drinking tea: Positive: increased cancer incidence with tea intake; Negative: decreased cancer incidence with tea intake; No Relationship: tea intake had no effect on cancer incidence.

TEA EXTRACTS AND POLYPHENOLS DEVELOPMENT STATUS