

skin. These studies verify a conceptual rationale for employing naturally occurring dietary constituents as an approach to cancer chemoprevention.

5. Blot WJ, Chow WH, McLaughlin JK. Tea and cancer: a review of the epidemiological evidence. *Eur J Cancer Prev.* 1996 Dec;5(6):425-38.

Numerous recent reports of inhibition of carcinogenesis in experimental animals by tea or tea compounds raise the possibility that tea drinking may lower cancer risk in humans. Thus, studies around the world were reviewed to evaluate whether there is a consensus of epidemiologic evidence on the relation of tea drinking to cancer overall or to specific cancers. Ecological data suggest at most a modest benefit, since there is considerable international variation in tea consumption but generally small differences in cancer rates. More relevant case-control and cohort studies show mixed results. Detailed data from these studies on cancer risks according to amount and duration of tea intake are quite limited, and consistent dose-related patterns have yet to emerge. Nevertheless, several investigations point to the possibility of lowered risks of digestive tract cancers among tea drinkers, especially those consuming green tea. Further research, particularly in population with wide ranges of tea consumption, is needed before definitive conclusions on tea's impact upon cancer risk can be reached.

6. Bushman JL. Green tea and cancer in humans: a review of the literature. *Nutr Cancer.* 1998;31(3):151-159.

Researchers have investigated green tea as a potential protectant against cancer. This review focuses on studies of green tea in humans. Green tea contains polyphenols, chemicals that act as powerful antioxidants. Epidemiological and human studies have shown varying results. Thirty-one human studies and four reviews were examined. Among five studies reporting on colon cancer, three found an inverse association and one reported a positive association. For rectal cancer, only one of four studies reported an inverse association; increased risks were seen in two of the studies. An inverse association is suggested for urinary bladder cancer in two of two studies. Of 10 studies examining the association of green tea and stomach cancer, 6 suggest an inverse and 3 a positive association. The most comprehensive of these studies supports an inverse association of green tea and stomach cancer. Pancreatic cancer studies hint at an inverse association in two of three studies. A strong inverse effect was found with green tea and esophageal cancer. Lung cancer studies have shown an inverse effect with Okinawan tea, yet tentatively increased risk was shown in another study. Although human studies have their limitations, the research has warranted a further look into the effects of green tea and cancer.

7. *Consum Rep* Tonic in a teapot. 2003 Mar;68(3):40-3.

Here's the latest evidence tea is good for you and, because you have to drink it to reap the benefits, our taste tests of 19 green teas.

8. Craig WJ. Health-promoting properties of common herbs. *Am J Clin Nutr.* 1999 Sep;70(3 Suppl):491S-499S.

Herbs have been used as food and for medicinal purposes for centuries. Research interest has focused on various herbs that possess hypolipidemic, antiplatelet, antitumor, or immune-stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer. In different herbs, a wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides have been

identified. Several of these phytochemicals either inhibit nitrosation or the formation of DNA adducts or stimulate the activity of protective enzymes such as the Phase II enzyme glutathione transferase (EC 2.5.1.18). Research has centered around the biochemical activity of the *Allium* sp. and the Labiatae, Umbelliferae, and Zingiberaceae families, as well as flaxseed, licorice root, and green tea. Many of these herbs contain potent antioxidant compounds that provide significant protection against chronic diseases. These compounds may protect LDL cholesterol from oxidation, inhibit cyclooxygenase and lipoxygenase enzymes, inhibit lipid peroxidation, or have antiviral or antitumor activity. The volatile essential oils of commonly used culinary herbs, spices, and herbal teas inhibit mevalonate synthesis and thereby suppress cholesterol synthesis and tumor growth.

9. Demeule M, Michaud-Levesque J, Annabi B, Gingras D, Boivin D, Jodoin J, Lamy S, Bertrand Y, Beliveau R. Green tea catechins as novel antitumor and antiangiogenic compounds. *Curr Med Chem Anti-Canc Agents*. 2002 Jul;2(4):441-63.

The concept of cancer prevention by use of naturally occurring substances that could be included in the diet is under investigation as a practical approach towards reducing cancer incidence, and therefore the mortality and morbidity associated with this disease. Tea, which is the most popularly consumed beverage aside from water, has been particularly associated with decreased risk of various proliferative diseases such as cancer and atherosclerosis in humans. Various studies have provided evidence that polyphenols are the strongest biologically active agents in green tea. Green tea polyphenols (GTPs) mainly consist of catechins (3-flavanols), of which (-)-epigallocatechin gallate is the most abundant and the most extensively studied. Recent observations have raised the possibility that green tea catechins, in addition to their antioxidative properties, also affect the molecular mechanisms involved in angiogenesis, extracellular matrix degradation, regulation of cell death and multidrug resistance. This article will review the effects and the biological activities of green tea catechins in relation to these mechanisms, each of which plays a crucial role in the development of cancer in humans. The extraction of polyphenols from green tea, as well as their bioavailability, are also discussed since these two important parameters affect blood and tissue levels of the GTPs and consequently their biological activities. In addition, general perspectives on the application of dietary GTPs as novel antiangiogenic and antitumor compounds are also presented.

10. Fujiki H, Suganuma M, Okabe S, Sueoka N, Komori A, Sueoka E, Koza T, Tada Y, Suga K, Imai K, Nakachi K. Cancer inhibition by green tea. *Mutat Res*. 1998 Jun 18;402(1-2):307-10.

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Green tea is now an acknowledged cancer preventive in Japan. This paper discusses several important features of (-)-epigallocatechin gallate (EGCG), the main constituent of green tea and tea polyphenols. EGCG and other tea polyphenols inhibited growth of human lung cancer cell line, PC-9 cells with G2/M arrest. 3H-EGCG administered by p.o. intubation into mouse stomach revealed that small amounts of 3H-activity were found in various organs where EGCG and green tea extract had previously demonstrated their anticarcinogenic effects, such as skin, stomach, duodenum, colon, liver, lung and pancreas. Cancer onset of patients who had consumed over 10 cups of green tea per day was 8.7 years later among females and 3.0 years later among males, compared with patients who had consumed under three cups per day. The mechanisms of action of EGCG were briefly discussed with regard to inhibition of tumor necrosis factor-alpha (TNF-alpha) release.

11. Fujiki H. Two stages of cancer prevention with green tea. *J Cancer Res Clin Oncol.* 1999;125(11):589-97.

Cancer chemoprevention is a new and important medical science in its own right. On the occasion of my presentation entitled "Natural agents and cancer chemoprevention" at the 90th AACR Meeting in 1999, I summarized our recent results on cancer prevention with green tea. In this article, the present status of clinical trials supported by the Chemoprevention Branch of the National Cancer Institute in the United States is first described by way of introduction. Although various natural products are now under investigation in phase I clinical trials, green tea has, perhaps, the greatest potential for further development. In order to expand our understanding of the effects of tea polyphenols and green tea, I review their ability to inhibit growth and cause apoptosis of cancer cells, their distribution into target organs and their other cancer-preventing properties. In addition, the paper focuses on the significance of reducing tumor necrosis factor alpha (TNFalpha) gene expression in cells and TNFalpha release from cells as essential activities for cancer prevention. As for the amounts of green tea effective in cancer prevention, I present two results from our Research Institute: a prospective cohort study with over 8000 individuals in Saitama Prefecture revealed that the daily consumption of at least ten Japanese-size cups of green tea resulted in delayed cancer onset, and a follow-up study of breast cancer patients conducted at our Hospital found that stages I and II breast cancer patients consuming over five cups per day experienced a lower recurrence rate and longer disease-free period than those consuming fewer than four cups per day. Thus, I propose here, for the first time, the two-stage approach to analyzing cancer prevention with green tea: cancer prevention before cancer onset and cancer prevention following cancer treatment. As an additional example of cancer prevention with natural agents, kava, a daily beverage in Fiji, is mentioned. All the evidence reminds us of the significance of alternative medicine in practical cancer prevention.

12. Fujiki H, Suganuma M, Kurusu M, Okabe S, Imayoshi Y, Taniguchi S, Yoshida T. New TNF-alpha releasing inhibitors as cancer preventive agents from traditional herbal medicine and combination cancer prevention study with EGCG and sulindac or tamoxifen. *Mutat Res* 2003;523-524:119-125.

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Herbal medicines are now attracting attention as potential sources of cancer preventive agents. Based on accumulated results of green tea as a cancer preventive, the authors review two important results with EGCG: the synergistic effects of EGCG with sulindac or tamoxifen on cancer preventive activity in PC-9 cells, and cancer prevention of intestinal tumor development in multiple intestinal neoplasia (Min) mice by cotreatment using EGCG with sulindac. Overall, the encouraging and beneficial results indicate that both clinicians and medical researchers should consider green tea as a chemopreventive herbal medicine in the fight against cancer.

13. Fujiki H, Suganuma M, Okabe S, Komori A, Sueoka E, Sueoka N, Kozu T, Sakai Y. Japanese green tea as a cancer preventive in humans. *Nutr Rev* 1996 Nov;54(11 Pt 2):S67-70
Saitama Cancer Center Research Institute, Japan

In the opinions of the authors, green tea is now generally acknowledged as a dietary supplement to prevent cancer in Japan.

14. Fujiki H, Suganuma M, Okabe S, Kurusu M, Imai K, Nakachi K. Involvement of TNF-alpha changes in human cancer development, prevention and palliative care. *Mech Ageing Dev.* 2002 Nov;123(12):1655-63.

Cancer development and ageing are complex sciences. From the study on the process of rodent carcinogenesis, we identified tumor necrosis factor-alpha (TNF-alpha) as an important mediator of cancer development. This paper presents three clinical examples of TNF-alpha up-regulation: by cord factors of *Mycobacterium tuberculosis*, such as trehalose 6-monomycolate, as an activator of protein kinase C and by a cord factor like fraction of *Microsporium canis* obtained in the air inside houses in Thailand, both of which are risk factors in human lung cancer development, and by *Helicobacter pylori* gene product, H. pylori membrane protein 1 (HP-MP1) in relation to human stomach cancer. The second part of this paper deals with down-regulation of TNF-alpha by a wide variety of cancer preventive agents. Among the various agents, (-)-epigallocatechin gallate (EGCG) and green tea polyphenols inhibited TNF-alpha gene expression in the cells induced by tumor promoter, mediated through inhibition of NF-kappaB activation. Studying growth inhibition of human cancer cell lines by morphine, we found that morphine and the new morphine derivatives KT-90 and KT-87 have anticancer activity mediated through induction of apoptosis, in addition to analgesic action. We conclude that environmental and endogenous factors induce NF-kappaB activation mediated through expression of inflammatory cytokine genes, such as TNF-alpha, and that the expression pattern of the genes operates similarly in the aging process.

15. Fujiki H, Suganuma M, Okabe S, Sueoka E, Suga K, Imai K, Nakachi K. A new concept of tumor promotion by tumor necrosis factor-alpha, and cancer preventive agents (-)-epigallocatechin gallate and green tea--a review. *Cancer Detect Prev.* 2000;24(1):91-9.

The study of tumor promotion in rodent carcinogenesis using chemical tumor promoters has revealed various tumor promotion pathways, such as the 12-O-tetradecanoylphorbol-13-acetate (TPA) pathway mediated through activation of protein kinase C, and the okadaic acid pathway mediated through inhibition of protein phosphatases 1 and 2A (PP-1 and PP-2A). We previously demonstrated that application of TPA and okadaic acid induced tumor necrosis factor-alpha (TNF-alpha) gene expression in mouse skin, but that tautomycin, which is an inhibitor of PP-1 and PP-2A and not a tumor promoter on mouse skin, did not. Moreover, we found that TNF-alpha stimulated transformation of BALB/3T3 cells initiated with 3-methylcholanthrene 1,000 times stronger than did TPA (*Cancer Res.* 53, 1982-1985, 1993). This evidence demonstrates a link between the okadaic acid pathway and the endogenous tumor promotion pathway of TNF-alpha. Recently we presented the first evidence that tumor promotion in TNF-alpha(-/-) mice was significantly depressed compared with TNF-alpha(+/+) mice. Thus, in human carcinogenesis, we think that TNF-alpha and other inflammatory cytokines in preneoplastic lesion stimulate tumor promotion and progression of initiated cells as well as premalignant cells. The first part of this paper reports on this TNF-alpha tumor promotion pathway. In the second part, we report a promising screening method for cancer preventive agents, based on evidence that pretreatment with agents such as tamoxifen, sulindac, 1alpha, 25-(OH)₂ vitamin D₃, quercetin, caffeic acid phenethyl ester, and (-)-epigallocatechin gallate (EGCG) commonly inhibited TNF-alpha release from BALB/3T3 cells induced by okadaic acid. EGCG, the main constituent of Japanese green tea, and green tea itself are acknowledged cancer preventives in Japan, and this paper presents evidence of their effectiveness in both a high-risk group and the general population.

16. Higdon JV, Frei B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit Rev Food Sci Nutr.* 2003;43(1):89-143.

Increasing interest in the health benefits of tea has led to the inclusion of tea extracts in dietary supplements and functional foods. However, epidemiologic evidence regarding the effects of tea consumption on cancer and cardiovascular disease risk is conflicting. While tea contains a number of bioactive chemicals, it is particularly rich in catechins, of which epigallocatechin gallate (EGCG) is the most abundant. Catechins and their derivatives are thought to contribute to the beneficial effects ascribed to tea. Tea catechins and polyphenols are effective scavengers of reactive oxygen species *in vitro* and may also function indirectly as antioxidants through their effects on transcription factors and enzyme activities. The fact that catechins are rapidly and extensively metabolized emphasizes the importance of demonstrating their antioxidant activity *in vivo*. In humans, modest transient increases in plasma antioxidant capacity have been demonstrated following the consumption of tea and green tea catechins. The effects of tea and green tea catechins on biomarkers of oxidative stress, especially oxidative DNA damage, appear very promising in animal models, but data on biomarkers of *in vivo* oxidative stress in humans are limited. Larger human studies examining the effects of tea and tea catechin intake on biomarkers of oxidative damage to lipids, proteins, and DNA are needed.

17. Kazi A, Smith DM, Daniel K, Zhong S, Gupta P, Bosley ME, Dou QP. Potential molecular targets of tea polyphenols in human tumor cells: significance in cancer prevention. *In Vivo* 2002;16:397-403.

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Epidemiological studies have shown decreased cancer occurrence in those individuals who drink green tea regularly. A wealth of research suggests numerous mechanisms of action to explain these observations. The most abundant and popular compound studied in tea research is (-)-epigallocatechin-3-gallate (EGCG), which acts as a powerful antioxidant and can inhibit a number of tumor cell proliferation- and survival-related proteins. Tea polyphenols are known to inhibit the large multi-catalytic protease (the proteasome) and metalloproteinases, involved in tumor survival and metastasis, respectively. Additionally, tea polyphenols inhibit the activities of many tumor-associated protein kinases, including epidermal growth factor receptor, vascular endothelial growth factor receptor, platelet-derived growth factor receptor, mitogen-activated protein kinase, and I κ B kinase. Tea polyphenols have also been found to inhibit some cancer-related proteins that regulate DNA replication and transformation. At present, it is not known which of these activities of tea polyphenols are required for its cancer-preventive effects.

18. Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Boone CW, Malone WA, Hawk ET, Lieberman R, Lawrence JA, Kopelovich L, Ali I, Viner JL, Sigman CC. Progress in cancer chemoprevention. *Ann N Y Acad Sci.* 1999;889:1-13.

More than 40 promising agents and agent combinations are being evaluated clinically as chemopreventive drugs for major cancer targets. A few have been in vanguard, large-scale intervention trials--for example, the studies of tamoxifen and fenretinide in breast, 13-cis-retinoic acid in head and neck, vitamin E and selenium in prostate, and calcium in colon. These and other agents are currently in phase II chemoprevention trials to establish the scope of their chemopreventive efficacy and to develop intermediate biomarkers as surrogate end points for cancer incidence in future studies. In this group are

fenretinide, 2-difluoromethylornithine, and oltipraz. Nonsteroidal anti-inflammatories (NSAID) are also in this group because of their colon cancer chemopreventive effects in clinical intervention, epidemiological, and animal studies. New agents are continually considered for development as chemopreventive drugs. Preventive strategies with antiandrogens are evolving for prostate cancer. Anti-inflammatories that selectively inhibit inducible cyclooxygenase (COX)-2 are being investigated in colon as alternatives to the NSAID, which inhibit both COX-1 and COX-2 and derive their toxicity from COX-1 inhibition. Newer retinoids with reduced toxicity, increased efficacy, or both (e.g., 9-cis-retinoic acid) are being investigated. Promising chemopreventive drugs are also being developed from dietary substances (e.g., green and black tea polyphenols, soy isoflavones, curcumin, phenethyl isothiocyanate, sulforaphane, lycopene, indole-3-carbinol, perillyl alcohol). Basic and translational research necessary to progress in chemopreventive agent development includes, for example, (1) molecular and genomic biomarkers that can be used for risk assessment and as surrogate end points in clinical studies, (2) animal carcinogenesis models that mimic human disease (including transgenic and gene knockout mice), and (3) novel agent treatment regimens (e.g., local delivery to cancer targets, agent combinations, and pharmacodynamically guided dosing).

19. Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Malone WA, Boone CW, Kopelovich L, Hawk ET, Lieberman R, Lawrence JA, Ali I, Viner JL, Sigman CC. Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. *J Nutr.* 2000 Feb;130(2S Suppl):467S-471S.

Because of their safety and the fact that they are not perceived as "medicine," food-derived products are highly interesting for development as chemopreventive agents that may find widespread, long-term use in populations at normal risk. Numerous diet-derived agents are included among the >40 promising agents and agent combinations that are being evaluated clinically as chemopreventive agents for major cancer targets including breast, prostate, colon and lung. Examples include green and black tea polyphenols, soy isoflavones, Bowman-Birk soy protease inhibitor, curcumin, phenethyl isothiocyanate, sulforaphane, lycopene, indole-3-carbinol, perillyl alcohol, vitamin D, vitamin E, selenium and calcium. Many food-derived agents are extracts, containing multiple compounds or classes of compounds. For developing such agents, the National Cancer Institute (NCI) has advocated codevelopment of a single or a few putative active compounds that are contained in the food-derived agent. The active compounds provide mechanistic and pharmacologic data that may be used to characterize the chemopreventive potential of the extract, and these compounds may find use as chemopreventives in higher risk subjects (patients with precancers or previous cancers). Other critical aspects to developing the food-derived products are careful analysis and definition of the extract to ensure reproducibility (e.g., growth conditions, chromatographic characteristics or composition), and basic science studies to confirm epidemiologic findings associating the food product with cancer prevention.

20. Khokhar S, Magnusdottir SG. Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *J Agric Food Chem.* 2002 Jan 30;50(3):565-70.

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Levels of total phenol, catechins, and caffeine in teas commonly consumed in the United Kingdom have been determined using reversed phase high-performance liquid chromatography. Tea bags or tea leaves were purchased from local supermarkets and extracted in boiling water for 5 min. The resulting data showed considerable variability in both total phenols [80.5-134.9 mg/g of dry matter (DM) in black teas and 87-106.2 mg/g of DM in green teas] and catechins (5.6-47.5, 51.5-84.3, and 8.5-13.9 mg/g of DM in black, green, and fruit teas, respectively); this was most probably a result of differing agronomic conditions, leaf age, and storage during and after transport, as well as the degree of fermentation. Caffeine

contents of black teas (22-28 mg/g of DM) were significantly higher than in less fermented green teas (11-20 mg/g of DM). The relative concentration of the five major tea catechins ranked EGCG > ECG > EC > EGC > C. The estimated U.K. dietary intakes of total tea catechins, calculated on the basis of an average tea consumption of three cups of tea (200 mL cup, 1% tea leaves w/v), were 61.5, 92.7, and 405.5 mg/day from fruit teas, black teas, and green teas, respectively. The coefficients of variation were 19.4, 88.6, and 17.3%, respectively, indicating the wide variation in these intakes. The calculated caffeine intake ranged between 92 and 146 mg/day. In addition, many individuals will consume much larger quantities of tea, of various strengths (as determined by the brewing conditions employed). This broad spread of U.K. daily intakes further emphasizes the need for additional research to relate intake and effect in various population groups.

21. Kohlmeier L, Weterings KG, Steck S, Kok FJ. Tea and cancer prevention: an evaluation of the epidemiologic literature. *Nutr Cancer* 1997;27(1):1-13

Animal and in vitro studies provide evidence of an anticarcinogenic potential of active ingredients in teas. This review encompasses epidemiologic studies of stomach, colon, and lung cancer as well as the evidence of a relationship between tea drinking and cancer at large in humans. Cohort studies do not suggest a protective role for tea drinking in the total risk of cancer. Site-specific studies reveal a more complex picture. The epidemiologic studies on tea drinking and stomach cancer do not justify claims of a cancer-protective effect. A protective effect of green tea on the development of colon cancer is suggested. The evidence regarding black tea is less clear, with some indication of a risk of colon or rectal cancer associated with regular use of black tea. The studies on tea and lung cancer also suggest an increased risk with increased tea consumption. The range and crude categorization of tea consumption, choice of control groups, and inadequate control for confounding might have obscured possible relationships. From the limited studies that suggest a favorable effect from tea, it is likely that benefits are restricted to high intakes in high-risk populations.

22. La Vecchia C, Negri E, D'Avanzo B, Franceschi S. Food temperature and gastric cancer. *Int J Cancer*. 1990 Sep 15;46(3):432-4.

The relationship between preference for food temperature and the risk of stomach cancer was analysed using data from a case-control study conducted in Northern Italy on 563 histologically confirmed incident gastric cancers and 1,501 controls admitted to hospital for acute, non-neoplastic, non-digestive tract disorders. A specific question was related to food temperature, subjectively defined as "warm", "hot" or "very hot". Compared with subjects indicating preference for "warm" foods, the relative risk (RR) was 1.1 (95% confidence interval, CI, 0.9-1.4) for "hot" and 1.8 (95% CI = 1.3-2.4) for "very hot". The test for trend in risk was statistically significant, and the results were not appreciably modified by allowance for a number of identified potential distorting factors. The elevated risk, however, appeared to be restricted to the 17% of cases reporting a preference for "very hot" foods. This may be due to an absence of substantial misclassification between "warm" and "very hot", but also to the existence of a threshold temperature, below which no appreciable thermal irritation is evident. Thus, although the difficulties and uncertainties on measures of food temperature are substantial, these data suggest that thermal irritation may have a role in gastric carcinogenesis.

23. La Vecchia C, Negri E, Decarli A, D'Avanzo B, Gallotti L, Gentile A, Franceschi S. A case-control study of diet and colo-rectal cancer in northern Italy. *Int J Cancer*. 1988 Apr 15;41(4):492-8.

The relation between dietary factors and the risk of colorectal cancer was investigated in a case-control study conducted in Northern Italy on 339 cases of colon cancer, 236 cases of rectal cancer and 778 controls admitted to hospital for acute, non-neoplastic or digestive disorders. Consistent positive associations were observed with more frequent consumption of starchy foods (pasta or rice) (relative risk, RR = 3.0 for colon and 1.8 for rectum for highest vs. lowest tertile) and beef/veal meats (RR = 2.1 for colon, 2.3 for rectum), whereas reduced relative risks were observed in subjects reporting more frequent green vegetable consumption (RR = 0.5 for highest vs. lowest tertile), a few specific vegetable or fruit items, and coffee (RR = 0.6 for highest vs. lowest tertile). Various fats in seasonings were positively, but inconsistently, related to intestinal cancer risk, whereas no association was evident with measures of whole grain foods or alcohol intake. For both intestinal sites, a 4- to 5-fold difference in risk was evident between the extreme quintiles of a simple score obtained by algebraic sum of the 4 major groups of foods. These findings could not be explained in terms of confounding by socio-economic status or other major potential distorting factors, are in agreement with the results from previous studies of colo-rectal cancer in Southern Europe, and are consistent with various aspects of the descriptive epidemiology of intestinal cancer in Italy.

24. Le Marchand L. Cancer preventive effects of flavonoids--a review. *Biomed Pharmacother* 2002 ;56:296-301.

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A cancer protective effect from plant-derived foods has been found with uncommon consistency in epidemiologic studies. However, it has been difficult to identify specific components responsible for this effect. Many phytochemicals have been shown to be biologically active and they may interact to protect against cancer. In recent years, experimental studies have provided growing evidence for the beneficial action of flavonoids on multiple cancer-related biological pathways (carcinogen bioactivation, cell signaling, cell cycle regulation, angiogenesis, oxidative stress, inflammation). Although the epidemiologic data on flavonoids and cancer are still limited and conflicting, some protective associations have been suggested for flavonoid-rich foods (soy and premenopausal breast cancer; green tea and stomach cancer; onion and lung cancer). This review focuses on the biological effects of the main flavonoids, as well as the epidemiologic evidence that support their potential cancer protective properties.

25. McCarty MF. Selenium, calcium channel blockers, and cancer risk--the Yin and Yang of apoptosis? *Med Hypotheses*. 1998 May;50(5):423-33.

It is increasingly clear that apoptosis plays a crucial role in the promotional phase of cancer development. Initiated pre-neoplastic clones in rat liver experience a high rate of apoptosis, and this rate has an important impact on the survival and growth of these clones. Suppression of apoptosis appears to be a universal property of cancer promoters, suggesting conversely that agents which inhibit cancer induction during the promotional phase increase the rate of apoptosis in initiated cells. Modulation of apoptosis is a likely explanation for recent striking evidence that use of calcium channel blockers substantially increases, whereas supplemental selenium substantially decreases, human cancer incidence. Non-genotoxic measures which are likely to upregulate apoptosis in pre-neoplastic/neoplastic cells--and thus may be useful in prevention and/or therapy--include selenium, retinoids/carotenoids, green tea

polyphenols, caloric restriction, downregulation of IGF-I activity, high-dose tamoxifen and other protein kinase C antagonists, withdrawal or blockade of trophic hormones, isoflavones, limonene, vitamin D and cholecalciferol analogs, dietary fiber/sodium butyrate, hyperthermia, benzaldehyde derivatives, and creatine.

26. Meydani M. Nutrition interventions in aging and age-associated disease. *Ann N Y Acad Sci.* 2001 Apr;928:226-35.

The nutritional status and needs of elderly people are associated with age-related biological and often socioeconomic changes. Decreased food intake, a sedentary lifestyle, and reduced energy expenditure in older adults altogether become critical risk factors for malnutrition, especially protein and micronutrients. Surveys indicate that the elderly are particularly at risk for marginal deficiency of vitamins and trace elements. Changes in bodily functions, together with the malnutrition associated with advancing age, increase the risk of developing a number of age-related diseases. Chronic conditions pose difficulties for the elderly in carrying out the activities of daily living and may increase the requirements for certain nutrients due to changes in absorptive and metabolic capacity. Free radicals and oxidative stress have been recognized as important factors in the biology of aging and of many age-associated degenerative diseases. In this regard, modulation of oxidative stress by calorie restriction, as demonstrated in animal models, is suggested as one mechanism to slow the aging process and the decline of body functions. Therefore, dietary components with antioxidant activity have received particular attention because of their potential role in modulating oxidative stress associated with aging and chronic conditions. Several studies have indicated potential roles for dietary antioxidants in the reduction of degenerative disease such as vascular dementia, cardiovascular disease, and cancer. In support of epidemiological studies, our recent studies indicate that the antioxidant properties of vitamin E and polyphenols present in green tea may contribute to reducing the risk of cardiovascular disease, in part by reducing the susceptibility of low density lipoproteins to oxidation, decreasing the vascular endothelial cell expression of pro-inflammatory cytokines, and decreasing the expression of adhesion molecules and monocyte adhesion. Recently, we also demonstrated that these dietary antioxidants may have a preventive role in cancer, potentially through the suppression of angiogenesis by inhibiting interleukin-8 production and the cell junction molecule VE-cadherin. These findings concur with epidemiologic, clinical, and animal studies suggesting that the consumption of green tea and vitamin E is associated with a reduced risk of cardiovascular disease and cancer, the leading causes of morbidity and mortality among the elderly.

27. Mukhtar H, Ahmad N. Green tea in chemoprevention of cancer. *Toxicol Sci.* 1999 Dec;52(2 Suppl):111-7.

The concept of prevention of cancer using naturally occurring substances that could be included in the diet consumed by the human population is gaining increasing attention. Tea, next to water, is the most popularly consumed beverage in the world and it is grown in about 30 countries. Abundant data, amassed from several laboratories around the world in the last ten years, provided convincing evidence that polyphenolic antioxidants present in tea afford protection against cancer risk in many animal-tumor bioassay systems. The epidemiological studies, though inconclusive, have also suggested that the consumption of tea is associated with a lowered risk of cancer. Much of this work has been done on green tea; less is known about black tea. Green tea contains many polyphenolic antioxidants, and (-)-epigallocatechin-3-gallate (EGCG) is the key polyphenolic antioxidant believed to be responsible for most of the cancer chemopreventive properties of green tea. This review will discuss these effects and the molecular mechanisms associated with the biological response to green-tea polyphenols.

28. Mukhtar H, Ahmad N. Tea polyphenols: prevention of cancer and optimizing health. *Am J Clin Nutr* 2000;71(6 Suppl):1698S-702S; discussion 1703S-4S

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Epidemiologic observations and laboratory studies have indicated that polyphenolic compounds present in tea may reduce the risk of a variety of illnesses, including cancer and coronary heart disease. Most studies involved green tea, however; only a few evaluated black tea. Results from studies in rats, mice, and hamsters showed that tea consumption protects against lung, forestomach, esophagus, duodenum, pancreas, liver, breast, colon, and skin cancers induced by chemical carcinogens. Other studies showed the preventive effect of green tea consumption against atherosclerosis and coronary heart disease, high blood cholesterol concentrations, and high blood pressure. Because the epidemiologic studies and research findings in laboratory animals have shown the chemopreventive potential of tea polyphenols in cancer, the usefulness of tea polyphenols for humans should be evaluated in clinical trials. One such phase 1 clinical trial is currently under way at the MD Anderson Cancer Center in collaboration with Memorial Sloan-Kettering Cancer Center. This study will examine the safety and possible efficacy of consuming the equivalent of > or =10 cups (> or =2.4 L) of green tea per day. The usefulness of tea polyphenols may be extended by combining them with other consumer products such as food items and vitamin supplements. This "designer-item" approach may be useful for human populations, but it requires further study.

29. NCI, DCPC, Chemoprevention Branch and Agent Development Committee, Clinical development plan: tea extracts green tea polyphenols epigallocatechin gallate. *J Cell Biochemistry* 1996;26S:236-257.

Page 245 stated: ...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...

30. Park EJ, Pezzuto JM. Botanicals in cancer chemoprevention. *Cancer Metastasis Rev.* 2002;21(3-4):231-55.

Botanicals have been used for the treatment of various human diseases throughout history. In addition, botanicals play a role in disease prevention. For example, epidemiologic studies have suggested that a reduced risk of cancer is associated with high consumption of vegetables and fruits. Thus, the cancer chemopreventive potential of naturally occurring phytochemicals is of great interest. In this review, we discuss the cancer chemopreventive activity of cruciferous vegetables such as cabbage and broccoli, Allium vegetables such as garlic and onion, green tea, Citrus fruits, tomatoes, berries, ginger and ginseng, as well as some medicinal plants. In addition, methods for the discovery of active compounds from plant sources are described. Several lead compounds, such as brassinin (from cruciferous vegetables like Chinese cabbage), sulforaphane (from broccoli) and its analog sulforamate, withanolides (from tomatillos), and resveratrol (from grapes and peanuts among other foods), are in preclinical or clinical trials for cancer chemoprevention. Phytochemicals of these types have great potential in the fight against human cancer, and a variety of delivery methods are available as a result of their occurrence in nature.

31. Rosenberg L. Coffee and tea consumption in relation to the risk of large bowel cancer: a review of epidemiologic studies. *Cancer Lett.* 1990 Jul 31;52(3):163-71.

Most of the few epidemiologic investigations of the relation of methylxanthine ingestion to risk of large bowel cancer have concerned coffee consumption. A slightly increased risk in coffee drinkers was

suggested by one study, no association by another and an inverse association by four, but there was a statistically significant trend across levels of consumption in only one of the latter studies. Based on the data on hand, there is little reason for concern that coffee consumption increases the risk. Although some evidence suggests an inverse association, the data are not compelling and a biologic mechanism is not established. There is even less information on tea consumption and the relation of consumption of this beverage to risk of large bowel cancer is unknown.

32. Sano T, Sasako M. Green tea and gastric cancer. *N Engl J Med* 2001;344:675-6.

Several confounding factors may be considered in explaining the lack of chemopreventive effects of green tea consumption against the incidence of stomach cancer as reported by Tsubono Y, Nishino Y, Komatsu S, Hsieh CC, Kanemura S, Tsuji I, Nakatsuka H, Fukao A, Satoh H, Hisamichi S. Green tea and the risk of gastric cancer in Japan. *N Engl J Med* 2001;344(9):632-6

33. Shim JS, Kang MH, Kim YH, Roh JK, Roberts C, Lee IP. Chemopreventive effect of green tea (*Camellia sinensis*) among cigarette smokers. *Cancer Epidemiol Biomarkers Prev.* 1995 Jun;4(4):387-91.

Chemopreventive effects of green tea and coffee among cigarette smokers were examined in 52 clinically healthy male subjects between 20 and 52 years of age. Blood specimens were obtained from nonsmokers (group I), smokers (group II), smokers consuming green tea (group III), and smokers drinking coffee (group IV). The mean number of cigarette smoking years (> 10 cigarettes/day) in groups II-IV ranged from 13.4 to 14.7 years. Daily intake of green tea and coffee was 2-3 cups/day for 6 months (groups III and IV). The frequencies of sisterchromatid exchange (SCE) in mitogen-stimulated peripheral lymphocytes from each experimental group were determined and analyzed statistically. SCE rates were elevated significantly in smokers (9.46 +/- 0.46) versus nonsmokers (7.03 +/- 0.33); however, the frequency of SCE in smokers who consumed green tea (7.94 +/- 0.31) was comparable to that of nonsmokers, implying that green tea can block the cigarette-induced increase in SCE frequency. Coffee, in contrast, did not exhibit a significant inhibitory effect on smoking-induced SCE.

34. Stratton SP, Dorr RT, Alberts DS. The state-of-the-art in chemoprevention of skin cancer. *Eur J Cancer* 2000;36:1292-7.

Arizona Cancer Center, College of Medicine, University of Arizona, Tucson, AZ 85724, USA.

The incidence of skin cancer (both melanoma and non-melanoma) continues to grow at an alarming rate. The chemoprevention strategies proposed by the authors include the development of novel agents evaluated by (1) preclinical mechanistic studies in models of ultraviolet (UV) radiation-induced skin carcinogenesis; (2) clinical studies of immunohistochemical surrogate endpoint biomarkers in high-risk patients; and (3) randomised, placebo-controlled phase I, II and III clinical chemoprevention trials. Recent clinical results validate this development model. Molecular targets of chemopreventive strategies for melanoma and non-melanoma skin cancers include the ras and activator protein-1 (AP-1) signal transduction pathways. A transgenic murine melanoma model has been developed for evaluating potential agents in vivo. Agents at various stages of study include the green tea catechin epigallocatechin gallate (EGCG), the limonene derivative perillyl alcohol, the ornithine decarboxylase inhibitor alpha-difluoromethylornithine (DFMO), selenium, retinoids and salicylates. New chemopreventive agents that can be used to complement sunscreens may result in decreased incidence, morbidity and mortality of skin cancer.

35. Sueoka N, Suganuma M, Sueoka E, Okabe S, Matsuyama S, Imai K, Nakachi K, Fujiki H. A new function of green tea: prevention of lifestyle-related diseases. *Ann N Y Acad Sci.* 2001 Apr;928:274-80.

In the normal human life span, there occur lifestyle-related diseases that may be preventable with nontoxic agents. This paper deals with the preventive activity of green tea in some lifestyle-related diseases. Green tea is one of the most practical cancer preventives, as we have shown in various in vitro and in vivo experiments, along with epidemiological studies. Among various biological effects of green tea, we have focused on its inhibitory effect on TNF-alpha gene expression mediated through inhibition of NF-kappaB and AP-1 activation. Based on our recent results with TNF-alpha-deficient mice, TNF-alpha is an endogenous tumor promoter. TNF-alpha is also known to be a central mediator in chronic inflammatory diseases such as rheumatoid arthritis and multiple sclerosis. We therefore hypothesized that green tea might be a preventive agent for chronic inflammatory diseases. To test this hypothesis, TNF-alpha transgenic mice, which overexpress TNF-alpha only in the lungs, were examined. The TNF-alpha transgenic mouse is an animal model of human idiopathic pulmonary fibrosis which also frequently develops lung cancer. Expressions of TNF-alpha and IL-6 were inhibited in the lungs of these mice after treatment with green tea in drinking water for 4 months. In addition, judging from the results of a prospective cohort study in Saitama Prefecture, Japan, green tea helps to prevent cardiovascular disease. In this study, a decreased relative risk of death from cardiovascular disease was found for people consuming over 10 cups of green tea a day, and green tea also had life-prolonging effects on cumulative survival. These data suggest that green tea has preventive effects on both chronic inflammatory diseases and lifestyle-related diseases (including cardiovascular disease and cancer), resulting in prolongation of life span.

36. Suganuma M, Ohkura Y, Okabe S, Fujiki H. Combination cancer chemoprevention with green tea extract and sulindac shown in intestinal tumor formation in Min mice. *J Cancer Res Clin Oncol* 2001;127:69-72.

Saitama Cancer Center Research Institute, Ina, Kitaadachi-gun, Japan.

Green tea is the most effective beverage for cancer prevention in humans. Looking at the concept of combination cancer chemoprevention, we previously reported the synergistic effects of (-)-epigallocatechin gallate (EGCG) with sulindac, and the additive effects of EGCG with tamoxifen, on cancer-preventive activity in human lung cancer cell line PC-9. This paper reports confirmation of the synergistic effects of EGCG with sulindac on the inhibition of intestinal tumors in multiple intestinal neoplasia (Min) mice. Treatment with both green tea extract and sulindac significantly reduced the number of tumors from 72.3 +/- 28.3 to 32.0 +/- 18.7 tumors per mouse, a decrease of 44.3%, whereas treatment with green tea extract alone or with sulindac alone reduced it to 56.7 +/- 3.5 and 49.0 +/- 12.7, respectively. The results also indicated that green tea extract inhibited tumor growth in Min mice almost as potently as sulindac itself did. The three treated groups did not show any adenocarcinomas, whereas 10.8% of the control group did. Since cancer-preventive agents like sulindac and tamoxifen are associated with adverse effects, we discuss the possibility of non-toxic, combination cancer chemoprevention with green tea, looking at the goal of truly effective cancer prevention.

37. Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK, Lee SS. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. *Mutat Res* 2001;480-481:243-68.

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Cyclooxygenase-2 (COX-2) inducible and nitric oxide synthase (iNOS) are important enzymes that mediate inflammatory processes. Improper up-regulation of COX-2 and/or iNOS has been associated with pathophysiology of certain types of human cancers as well as inflammatory disorders. Since inflammation is closely linked to tumor promotion, substances with potent anti-inflammatory activities are anticipated to exert chemopreventive effects on carcinogenesis, particularly in the promotion stage. Examples are curcumin, a yellow pigment of turmeric (*Curcuma longa* L., Zingiberaceae), the green tea polyphenol epigallocatechin gallate (EGCG), and resveratrol from grapes (*Vitis vinifera*, Vitaceae) that strongly suppress tumor promotion. Recent studies have demonstrated that eukaryotic transcription factor nuclear factor-kappa B (NF-kappa B) is involved in regulation of COX-2 and iNOS expression. Several chemopreventive phytochemicals have been shown to inhibit COX-2 and iNOS expression by blocking improper NF-kappa B activation. Multiple lines of compelling evidence indicate that extracellular-regulated protein kinase and p38 mitogen-activated protein kinase are key elements of the intracellular signaling cascades responsible for NF-kappa B activation in response to a wide array of external stimuli. Curcumin, EGCG and resveratrol have been shown to suppress activation of NF-kappa B. One of the plausible mechanisms underlying inhibition of NF-kappa B activation by aforementioned phytochemicals involves repression of degradation of the inhibitory unit I kappa B alpha, which hampers subsequent nuclear translocation of the functionally active subunit of NF-kappa B.

38. Tosetti F, Ferrari N, De Flora S, Albini A. 'Angioprevention': angiogenesis is a common and key target for cancer chemopreventive agents. *FASEB J* 2002;16:2-14.

Molecular Biology Laboratory, National Cancer Research Institute (IST), Genova, Italy.

The potential to block tumor growth by inhibition of the neoangiogenic process represents an intriguing approach to the treatment of solid tumors. The high proliferation rate in the tumor deprived of proper vascularization would be balanced by cell death due to lack of diffusion of nutrients and oxygen. Matrix metalloproteinases (MMPs), angiogenic growth factors, and their receptors are the main targets of an increasing number of clinical trials approved to test the tolerance and therapeutic efficacy of antiangiogenic agents. The authors showed that epigallocatechin gallate (EGCG), a flavonoid from green tea that possesses chemopreventive activity in experimental and epidemiological studies, is a potent inhibitor of MMP-2 and MMP-9. Chemopreventive agents, like green tea, could exert antiangiogenic effects aimed at controlling tumor growth, and potentially useful in the clinic.

39. Tsubono Y, Takahashi T, Iwase Y, Itoi Y, Akabane M, Tsugane S. Dietary differences with green tea intake among middle-aged Japanese men and women. *Prev Med* 1997 Sep-Oct;26(5 Pt 1):704-10

BACKGROUND: Although several epidemiologic investigations have suggested a protective role of green tea against cardiovascular diseases and cancer, few studies examined how consumption of green tea was associated with intake of other dietary factors. **METHODS:** In the winters of 1989-1991, 880 men ages 40-49 years were randomly sampled from the general populations of five Public Health Center districts of Japan. Response rate was 72% (n = 634). A convenience sample of 373 spouses also consented to participate. They were interviewed on the frequency of consumption of green tea and 37 food items. A 3-day weighed food record was collected from a subgroup of the subjects (207 men and 164 women) to calculate daily intake of 22 nutrient variables. Consumption of the foods and nutrients was compared with three levels of green tea intake (< 1, 1-4, and > 4 cups/days) after adjustment for potential confounders. **RESULTS:** Among men, green tea was associated significantly with consumption of 10 foods (P < 0.05) and at borderline significance with 4 nutrients (P < 0.1). These foods and nutrients included fruits (apple, orange juice), vegetables (green, yellow, and pickled), total lipid, cholesterol, and

carotene. Among women, green tea was associated with 6 foods and total energy. **CONCLUSION:** The results indicate that consumption of green tea is associated with diets that could modify the risks of cardiovascular diseases and cancer, especially among men. When the health effects of green tea are examined by observational epidemiologic studies, potential confounding and effect modification by other dietary factors should be controlled thoroughly.

40. Webb T. Green tea experiments in lab, clinic yield mixed results. *J Natl Cancer Inst* 2000 ;92:1038-9.

In this report, various scientists began to pay serious attention to why green tea consumption has been associated in epidemiologic studies with decreased risk of many cancers, but other studies have suggested no benefits.

41. Weisburger JH, Chung FL. Mechanisms of chronic disease causation by nutritional factors and tobacco products and their prevention by tea polyphenols. *Food Chem Toxicol* 2002;40:1145-54.

The active components are polyphenols, mainly epigallocatechin gallate in green tea. The tea leaf polyphenol oxidase mediates oxidation to oolong and black tea, yielding other polyphenols, theaflavin and thearubigins. There is 40-50 mg caffeine in a 160-ml cup of tea. The chemopreventive effects of tea depend on: (1) its action as an antioxidant; (2) the specific induction of detoxifying enzymes; (3) its molecular regulatory functions on cellular growth, development and apoptosis; and (4) a selective improvement in the function of the intestinal bacterial flora. Many of cancers are caused by lifestyle elements. One is cigarette and tobacco use, leading to cancer in the oral cavity, esophagus and lung, inhibited by tea. Mice administered a tobacco nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), developed significantly fewer lung tumors than controls when given green tea or its major polyphenol, epigallocatechin gallate (EGCG). Tea suppressed the formation of 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative DNA damage, in the lung DNA of mice given NNK. Gastric cancer, caused by a combination of *Helicobacter pylori* and salted foods, is lower in tea drinkers. Western nutritionally-linked cancers of the breast, colon, prostate and pancreas can be inhibited by tea. The formation of genotoxic carcinogens for these target organs during the cooking of meats, heterocyclic amines, and their effects were decreased by tea. Tea inhibited the formation of reactive oxygen species and radicals and induced cytochromes P450 1A1, 1A2 and 2B1, and glucuronosyl transferase. The higher formation of glucuronides represents an important mechanism in detoxification. The developmental aspects and growth of cancers through promotion are decreased by tea. The regular use of a widely available, tasty, inexpensive beverage, tea, has displayed valuable preventive properties in chronic human diseases.

42. Weisburger JH. Approaches for chronic disease prevention based on current understanding of underlying mechanisms. *Am J Clin Nutr* 2000;71(6 Suppl):1710S-4S; discussion 1715S-9S.

In many parts of the world, particularly in the West, the major cancers associated with dietary habits involve the postmenopausal breast, distal colon, prostate, pancreas, ovary, and endometrium. Current evidence suggests that the genotoxic carcinogens for all but the last 2 of these diseases stem from the traditional intake of fried and broiled foods such as meats. The surface of these foods contains a class of powerful mutagens, heterocyclic amines, which are carcinogenic to the target organs in animal models. Fish-eating populations have lower incidences of heart disease and of many types of cancers than do other populations, which may be the result of the n-3 polyunsaturated oils found in fish. Among other dietary practices that may reduce the risk of cancer and cardiovascular disease are consuming 5-9 servings of fruits and vegetables daily, which provides antioxidants such as quercetin and isothiocyanates; having a high fiber intake, including bran cereal; and drinking 1.5-2.5 L of fluids daily. Tea polyphenols found in

black and green tea may have a protective effect against heart disease and some cancers. Concentrates of such desirable products have been made available in pill form to complement health-promoting personal lifestyles. Biomedical research funded by The National Institutes of Health and organizations such as the American Cancer Society has produced sound results that could lead to prevention of chronic disease. The public must heed this information to achieve long-term health.

43. Weisburger JH. Lifestyle, health and disease prevention: the underlying mechanisms. *Eur J Cancer Prev.* 2002 Aug;11 Suppl 2:S1-S7.

International studies in geographic pathology provide background information that a disease may have a quite different incidence and resulting mortality as a function of area of residence. Investigations in animals can model fairly precisely what is learned through such international research, and provide the basis for examining relevant hypotheses and, more importantly, possible mechanisms of action. These approaches can yield public health recommendations and health promotion activities. Regular intake of foods rich in saturated fats, such as meat and certain dairy products, raises the risk of coronary heart disease, especially in smokers. The total mixed fat intake is associated with a higher incidence of the nutritionally linked cancers (i.e. of the postmenopausal breast, distal colon, prostate, pancreas, ovary and endometrium). Monounsaturated oils, such as olive or canola oil, are low-risk fats, as shown in animal models, and through the finding that the incidence of coronary heart and neoplastic diseases is lower in the Mediterranean region, where such oils are customarily used. Fish and fish oils are protective. The associated genotoxic carcinogens for several of these cancers, and also in heart disease causation, are heterocyclic amines, produced during the broiling and frying of creatinine-containing foods such as meats. Excessive salt intake is associated with high blood pressure and with stomach cancer, especially with inadequate intake of potassium, from fruits and vegetables, and calcium from certain vegetables and low-fat dairy products. Bran cereal fiber intake, especially with adequate calcium, yields an increased stool bulk, eliminating factors involved in colon and breast cancer. Vegetables and fruits, as well as soy products, are rich in antioxidants that are essential to lower disease risk stemming from reactive oxygen species in the body. Green and black tea are excellent sources of such beneficial antioxidants of a polyphenol nature, as are cocoa and chocolates. Antioxidants also extend healthy aging and may protect against Alzheimer's and Parkinson's diseases. Nutritional lifestyles can be described for most populations in the world and offer the possibility of a healthy long life.

44. Weisburger JH. Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. *Food Chem Toxicol.* 1999 Sep-Oct;37(9-10):943-8.

Most chronic diseases, including coronary heart disease and many types of cancer depend on the *in vivo* conversion of cellular macromolecules or of carcinogens to specific reactive, oxidized forms. For that reason, health promoting nutrition involves the daily intake of five to 10 vegetables and fruits, fruit juices, red wine and tea that are rich sources of micronutrients with antioxidant properties, including the antioxidant vitamins C, E and beta-carotene. Tomatoes contain lycopene, a stable, active antioxidant. Many vegetables contain quercetin and related polyphenolic compounds. Tea is a source of epigallocatechin gallate, in green tea, and theaflavin and the associated thearubigins, in black tea. Red wine contains resveratrol. The diverse antioxidants in foods, red wine and tea provide the necessary antioxidant resources for the body to control oxidation reactions in the body with possible adverse consequences. For example, the oxidation of low density lipoprotein (LDL) cholesterol yields a product that damages the vascular system. Thus, a lower intake of saturated fats to decrease the levels of LDL cholesterol, together with an adequate intake of antioxidants, is the optimal approach to lower heart disease risk. Cancer of the stomach involves the consumption of salted, pickled foods yielding direct-acting carcinogens, and their formation is inhibited by vitamins C and E. Cancer in the colon, breast,

prostate and pancreas may be caused by a new class of carcinogens, the heterocyclic amines, formed during the broiling or frying of creatinine-containing foods, including fish and meats. Their formation and action can be inhibited by antioxidants such as those in soy, tea, vitamin C and also by the synthetic antioxidants BHA or BHT. The growth, cell proliferation and development of abnormal preneoplastic and neoplastic cells also involves oxidation reactions, including the formation of active oxygen or peroxy compounds. Such reactions can be inhibited by antioxidants, such as those in tea, tomatoes or vegetables. Even ageing and longevity in good health would be favoured by the availability of adequate amounts of varied antioxidants. Prevention of the formation and of action of reactive products by antioxidants as present in fruits, vegetables, tomatoes, red wine and tea is of great public health importance in decreasing the risk of major diseases. Prevention is the optimal approach to disease control, and also as an effective route to lower costs of medical care.

45. Weisburger JH. Tea and health: a historical perspective. *Cancer Lett.* 1997 Mar 19;114(1-2):315-7.

In many parts of the world, green tea and black tea are produced from the plant *Camellia sinensis*. Tea is one of the most widely consumed beverages, second only to water. It is one of the safest beverages since it is made with boiling, sterile water and has been popular for over 4000 years. Dogma has it that people knew it might have health promoting properties since it was frequently used as fluid supply for patients suffering from infectious diseases. However, detailed, focused research on the health benefits of tea is of recent vintage. Initially, such research was carried out in Japan and China and, because the local customs, this research involved green tea. Now, a number of other scientists in Europe and in the United States have conducted investigations on black tea, and in some laboratories exacting comparative studies were performed utilizing black and green tea. The major interest in tea and health stems from the high level of antioxidant tea polyphenols in green tea and black tea. The chemistry of the tea polyphenols has been worked out to some extent. Thus, their role in lowering the risk of heart disease and of a number of types of cancer begins to be understood. Most productive are multi-disciplinary approaches, considering data from epidemiology and field studies, and laboratory research in animal models for heart disease and cancers of various types, as well as through in vitro experiments.

46. Weisburger JH. Tea and health: the underlying mechanisms. *Proc Soc Exp Biol Med.* 1999 Apr;220(4):271-5.

Detailed multidisciplinary research on the effect of tea and the associated tea polyphenols has led to major advances on the underlying mechanisms. In most studies, green and black tea have similar effects, four of which are reviewed in this paper. 1) Tea polyphenols are powerful antioxidants that may play a role in lowering the oxidation of LDL-cholesterol, with a consequent decreased risk of heart disease, and also diminish the formation of oxidized metabolites of DNA, with an associated lower risk of specific types of cancer. 2) Tea and tea polyphenols selectively induce Phase I and Phase II metabolic enzymes that increase the formation and excretion of detoxified metabolites of carcinogens. 3) Tea lowers the rate of cell replication and thus the growth and development of neoplasms. 4) Tea modifies the intestinal microflora, reducing undesirable bacteria and increasing beneficial bacteria. The accumulated knowledge suggests that regular tea intake by humans might provide an approach to decrease the incidence of and mortality from major chronic diseases.

47. Weisburger JH. Worldwide prevention of cancer and other chronic diseases based on knowledge of mechanisms. *Mutat Res.* 1998 Jun 18;402(1-2):331-7.

International research, particularly as part of US/Japan programs, has led to major advances in knowledge of causes of heart disease, stroke, many types of cancer and diabetes, showing that individual lifestyle is associated with these diseases. In Japan, a major health problem is high blood pressure and stroke, and cancer of the stomach, from excessive use of salt and salted, pickled foods, and the relative low intake of protective fruits and vegetables. We identified a likely gastric carcinogen, 2-chloro-4-methylthiobutanoate, in salted, pickled fish. In the Western world, heart disease and cancer of the breast, colon, rectum, prostate, pancreas, ovary and endometrium relate to a nutritional tradition too high in total fat and fried or broiled meats, and too low in fiber, vegetables and fruits. The cooked meats contain genotoxic chemicals, heterocyclic amines, causative elements in heart disease and the nutritionally linked cancers. Decreasing total fat intake, from 40 to 20% of calories and a greater use of starches such as rice, pasta, potatoes and whole grain bread, as well as daily intake of five to nine vegetables and fruits would be beneficial. Adults should consume 2.5 l of fluids per day. Green or black tea and fruit juices have health promoting properties. Regular exercise contributes to good health, and to the avoidance of obesity, a major problem in the USA and of increasing importance in Japan. Avoidance of a risky lifestyle would likely prevent diseases important not only for the individual and his family, but with major impact in lowering medical care costs. Tobacco and cigarette use, particularly on a Western diet, involve a high risk of heart attacks, and cancers of the lung, pancreas, kidney, urinary bladder, and cervix, accounting for 35% of medical care expenditures.

48. Yang CS, Wang ZY. Tea and cancer. *J Natl Cancer Inst.* 1993 Jul 7;85(13):1038-49.

Tea is one of the most popular beverages consumed worldwide. The relationship between tea consumption and human cancer incidence is an important concern. This topic has been studied in different populations by many investigators, but no clear-cut conclusion can be drawn. Whereas some studies have shown a protective effect of tea consumption against certain types of cancers, other studies have indicated an opposite effect. Our purpose is to provide a critical review of this topic, covering basic chemistry and biochemical activity of tea, epidemiologic investigations, and laboratory studies, as well as possible directions for future research. Studies have demonstrated either a lack of association between tea consumption and cancer incidence at specific organ sites or inconsistent results. On the other hand, many laboratory studies have demonstrated inhibitory effects of tea preparations and tea polyphenols against tumor formation and growth. This inhibitory activity is believed to be mainly due to the antioxidative and possible antiproliferative effects of polyphenolic compounds in green and black tea. These polyphenolics may also inhibit carcinogenesis by blocking the endogenous formation of N-nitroso compounds, suppressing the activation of carcinogens, and trapping of genotoxic agents. The effect of tea consumption on cancer is likely to depend on the causative factors of the specific cancer. Therefore, a protective effect observed on a certain cancer with a specific population may not be observable with a cancer of a different etiology. On the basis of this concept, we suggest future laboratory and epidemiologic studies to elucidate the relationship between tea consumption and human cancer risk.

49. Zhang H, Spitz MR, Tomlinson GE, Schabath MB, Minna JD, Wu X. Modification of lung cancer susceptibility by green tea extract as measured by the comet assay. *Cancer Detect Prev.* 2002;26(6):411-8.

Green tea is widely consumed throughout the world and is known to possess various beneficial properties that may affect carcinogen metabolism, free radical scavenging, or formation of DNA adducts. Therefore, it is plausible that green tea extract may modify BPDE-induced DNA damage. In this report, we utilized the comet assay to (1) evaluate BPDE-induced DNA damage as a potential marker of cancer

susceptibility and (2) assess the ability of green tea to modify BPDE-induced DNA damage. DNA damage in individual comet cells was quantified by (1) visually measuring the proportion of cells exhibiting migration versus those without and (2) the length of damaged DNA migration (comet tail). We detected a dose-response between BPDE concentration and mean comet tail length in EBV-immortalized lymphoblastoid (lymphoid) cell lines. As the concentration of BPDE increased from 0.5 to 3 microM, the length of the mean comet tail length increased proportionally in the 3590P (derived from a healthy subject) and 3640P (derived from a patient with head and neck cancer) cell lines. In separate experiments using lymphoid cells from 21 lung cancer cases and 12 healthy subjects, the mean comet tail length was significantly higher in the lung cancer cases (80.19 +/- 15.55) versus the healthy subjects (59.94 +/- 14.23) ($P < 0.01$). Similar findings were observed when analyzing the mean percentage of comet induced cells (84.57 +/- 8.85 and 69.04 +/- 12.50, respectively) ($P < 0.01$). When green tea extract was added in conjunction with BPDE, there was a notable reduction of the mean comet tail length (13.29 +/- 0.97) as compared to BPDE treatment alone (80.19 +/- 15.55) ($P < 0.01$) in lung cancer cases. There were no statistical differences between the baseline (no treatments) (12.74 +/- 0.63) and the green tea extract treatment (13.06 +/- 0.97) ($P = 0.21$). These data suggest the modification of lung cancer susceptibility by the green tea extract. Similar results were observed for the percentage of induced comet cells and the statistical trends were similar for the 12 healthy subjects. This preliminary study demonstrated that the detection of BPDE-induced DNA damage via the comet assay may be a useful biologic marker of lung cancer susceptibility. The differential effects in BPDE-induced DNA damage between lung cancer cases and healthy subjects suggests predisposed cancer susceptibility to lung cancer risk. This reports also demonstrated the chemopreventive effects of green tea extract on BPDE-induced DNA damage. These observations provide further support for the application of the comet assay in molecular epidemiologic studies.

III. The scientific basis of anticancer effects of green tea with 36 references

The anticancer effects of green tea and some of its active components have been extensively studied. The exact anticancer mechanism at the molecular level and in the human body is still poorly understood. The potential mechanisms may include the following (1-12):

1. Green tea inhibits formation of N-Nitroso-compounds (carcinogens) in stomach.

Some food products, for example, salt-preserved fish and preserved meats containing nitrite, are nitrosated in the stomach, resulting in release of potent clastogenic and mutagenic compounds which are carcinogenic. Green tea inhibits this chemical reaction. Simultaneous intake of green tea with these food products has been shown to reduce the formation of mutagenic nitrosamine products in the stomach.

2. Green tea modulates gene expression of enzymes responsible for carcinogen metabolism.

Certain environmental substances require an endogenous enzyme or enzymatic system in the body to convert them into active carcinogen. For example, when sodium nitrite and methylbenzylamine are administered in rats, *in vivo* nitrosation will take place to form N-nitrosomethylbenzylamine, a carcinogen through enzymatic activities in the body. Green tea blocks the *in vivo* formation of the N-nitroso compounds and inhibits carcinogenesis.

In another example, green tea inhibits the gene expression of the hepatic cytochrome P450-dependent mixed-function oxidase system, which is closely associated with bioactivation of chemical carcinogens. It is well known that aryl hydrocarbon induces cancers in animals and its action can be blocked by green tea. The aryl hydrocarbon receptor (AhR) mediates the transcriptional activation of CYP1A1 and CYP1A2. Green tea inhibits the transcription of a human CYP1A promoter-driven reporter gene induced by the AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in a concentration-dependent manner and inhibits the induced accumulation of both CYP1A1 and CYP1A2 mRNAs. Green tea extract and (-)-epigallocatechin gallate (EGCG), the most abundant active catechin antioxidant in tea leaves, were able to inhibit TCDD-induced binding of the AhR to DNA and subsequent CYP1A transcription. The effects of green tea polyphenols against skin-tumor-initiating activity induced by polycyclic aromatic hydrocarbons (PAHs), the "pro-carcinogens" present in smoke from cigarettes, automobile emissions and grilled foods have been known since 1989.

On the other hand, green tea may activate numerous other detoxifying enzymes, such as quinone reductase, glutathione/glutathione-S-transferases, epoxide hydrolase, and UDP-glucuronosyltransferases, enhancing the activity of these so-called phase II enzymes, to metabolize the carcinogens.

3. Green tea inhibits tumor promoters (e.g. TPA, enhancer of the effects of many carcinogens).

Topical application of a green tea polyphenol fraction on the mouse skin can inhibit the effects of the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), on the initiation of tumor

induced by benzo[α]pyrene- and 7,12-dimethylbenz[α]-anthracene (DMBA). Topical application of the green tea polyphenol fraction also inhibits TPA-induced inflammation, ornithine decarboxylase activity, hyperplasia and hydrogen peroxide formation.

4. Green tea inhibits inducible NO-synthase (thus endogenous carcinogens).

Chronic inflammation is associated with excessive release of nitric oxide (NO) and superoxide anion which can react together to yield peroxynitrite. The latter compound can eventually cause damage to the DNA, inducing cancer formation. Green tea polyphenols are potent inhibitors of nitric oxide synthase gene expression, thus performing an important function of cancer prevention.

Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) are important enzymes that mediate inflammatory processes. Green tea suppresses improper up-regulation of COX-2 and/or iNOS, which is associated with pathophysiology of certain types of human cancers as well as inflammatory disorders. Green tea EGCG may inhibit COX-2 and iNOS expression by blocking improper activation of a transcription factor, nuclear factor-kappa B [NF-kappa B].

5. Green tea catechins are antioxidants to free radicals, thus reducing DNA damage by carcinogens, e.g. UV lights.

The most remarkable cancer chemopreventive effect of green tea (or its components) is due to its antioxidative activity. In the skin, oxidative stress induced by ultraviolet irradiation can be readily observed. The pathological changes may be in the form of erythema, edema, epithelial hyperplasia, dysplasia to skin cancer. Topical application of EGCG to human skin before UV irradiation decreases the UV-induced erythema, UV-induced production of hydrogen peroxide and nitric oxide in both epidermis and dermis. The degree of UV-induced infiltration of inflammatory leukocytes into the skin which are considered to be the major producers of reactive oxygen species is markedly reduced by EGCG pretreatment. The latter treatment also restores the UV-induced decrease in glutathione level and protects the antioxidant enzyme glutathione peroxidase. At high concentration, EGCG which is an antioxidant may have pro-oxidative activities causing generation of hydrogen peroxide, functioning as a mediator to apoptosis.

6. Green tea inhibits telomerase that co-determines the division capacity of a cell.

More than 85% of all cancers express telomerase activity whereas most somatic cells appear to lack detectable levels of telomerase. Germ line cells also express telomerase activity, but they have longer telomeres than cancer cells. A group of researchers discovered that EGCG is a strong inhibitor of telomerase, and suggested that telomerase inhibition may be one of the major mechanisms underlying the anticancer effects of tea.

7. Green tea inhibits DNA topoisomerases I and II which regulate DNA topology in cell division.

DNA topoisomerases I and II are essential for proper DNA rejoining and structural configuration during cell proliferation. DNA topoisomerases are the target of many anticancer drugs. Like

doxorubicin, EGCG is a DNA topoisomerase inhibitor against cancer cells, but without its toxicity to normal tissues. In addition, the special tea amino acid, theanine, is a biochemical modulator that has been shown to inhibit efflux of a DNA topoisomerase II inhibitor, doxorubicin, from the cancer cells, but not to reduce the outflow of the topoisomerase II inhibitor from the normal cells.

8. Green tea induces apoptosis via a mitochondrial pathway.

There are two distinct primary signaling pathways of apoptosis, one of which is the extrinsic or death receptor pathway controlled by caspase 8 and caspase 10 through a tumor necrosis factor receptor on surface of the cell membrane (TNF receptor).

The other is the intrinsic or mitochondrial pathway which occurs within the cell through release of cytochrome C from the mitochondria and activation of caspase 9. Normal Bcl-2 and Bcl-XL proteins in the mitochondrial membranes prevent pore formation and leakage of cytochrome C from the mitochondria to the cytoplasm. Cytochrome C activates caspase 9 which in turn activates other caspases, a series of proteases that digest the structural proteins in the cytoplasm, damage the DNA, and cause cell death. Bax protein is a Bcl-2 family member in the mitochondrial membranes, but is activated by this pathway to increase the permeability of the mitochondrial membrane, releasing cytochrome C to the cytoplasm.

EGCG decreases the Bcl-2 and Bcl-XL proteins, increases the Bax protein, and activates caspase 9 in the cancer cells. Therefore, its anticancer activity appears at least in part mediated by the mitochondrial pathway.

9. Green tea exerts its effects on signal transduction - to inhibit activation of transcription factors, e.g. nuclear factor-kappaB (NF- κ B), Cyclin D1, tumor-associated protein kinases, epidermal growth factor (EGF) receptors, and the release of tumor necrosis factor-alpha (TNF- α), an endogenous promoter for cancer genes.

Molecular signals, such as hormones or growth factors, are received by interaction between the signaling molecule (ligand) and a receptor specific for that signal on the surface of the cell. Through a series of steps, the message from that signal gets transmitted and amplified within the receiving cell, often leading to activation or deactivation of specific transcription factors in the nucleus, thus regulating the events in cell proliferation, differentiation and apoptosis, for example by controlling the gene expression of endogenous promoters like a tumor necrosis factor. This process is referred to as signal transduction pathways, involving the products of several genes (for example, Ras) that are mutant in cancer cells.

One of the key pathways is the mitogenic signal transduction through the cascade of mitogen-activated protein (MAP) kinase that also includes other transducing molecules such as MAP kinase (MEK) and Raf-1. The MAP kinase signaling, for instance, enhances cyclin D1 for cell proliferation, but also arrests cell growth by increasing expression of the cyclin kinase inhibitor p21 (Cip-1/MDA6/WAF1). The level and duration of MAP kinase expression appears to control this differential effect.

Green tea has been shown to inhibit activation of many transcription factors. For example, it inhibits the tumor necrosis factor- α (TNF- α) gene expression as well as the okadaic acid-induced AP-1 and NF-kappa B activation.

Green tea EGCG inhibits both the autocrine activation of epidermal growth factor receptor (EGFR) signaling and the activation of the signal transducer by exogenous transforming growth factor- α (TGF- α). As a consequence, EGCG also inhibits signaling to the extracellular regulated kinase (ERK) proteins and activation of transcription 3 (Stat 3) which lies downstream of the TGF- α /EGFR signaling pathway and apparently protects cancer cells from apoptosis.

10. Green tea regulates faulty apoptosis independent of the *p53* suppressor genes.

Green tea and its components significantly restore cancer cell apoptosis. They also affect *p53* gene mutations. However, the cancer chemopreventive efficacy of green tea may be independent of *p53* status of the cancer cells.

11. Green tea inhibits angiogenesis necessary for rapid tumor growth.

The components of green tea inhibit the process of forming new blood vessels which are needed to support the fast growing rate of a malignant tumor. The anticancer effect of EGCG is at least in part due to its inhibition of angiogenesis through blocking the induction of vascular endothelial growth factor (VEGF) in human colon cancer cells. EGCG, not other catechins, inhibits ErK-1 and ErK-2 activation in a dose dependent manner. Physiological concentrations (0.01-1 μ M) of EGCG induce a rapid and potent inhibition of VEGF-dependent tyrosine phosphorylation of VEGF receptor-2 (VEGFR-2). The inhibition of VEGFR-2 by EGCG is similar to that induced by Semaxanib (SU5416), a specific VEGFR-2 inhibitor.

12. Green tea inhibits proteolytic enzymes, urokinase and collagenase, needed to establish cancer metastasis.

Human cancers need proteolytic enzymes to invade other neighboring normal cells and form metastases. One of these enzymes is urokinase (uPA). Inhibition of uPA can decrease tumor size or even cause complete remission of cancers in mice. The known uPA inhibitors, for example, amiloride, are unlikely to be used in anticancer therapy because of their weak inhibitory activity or high toxicity. EGCG binds to uPA, blocking the amino acids His 57 and Ser 195 of the uPA catalytic triad and extending towards Arg 35 from a positively charged loop. Such localization of EGCG would interfere with the ability of uPA to recognize its substrates and inhibits its enzymatic activity. Based on laboratory studies, it has been recommended that drinking green tea containing 1,500 mg of EGCG per day may deliver more than adequate levels of EGCG to reduce the incidence of cancer in humans or the size of cancers already formed. A similar tea effect in suppressing cancer growth may be achieved by inhibition of type IV collagenase of the carcinoma cells by EGCG and some black tea components, such as theaflavin and theaflavin digallate.

An incomplete list of references in support of these above mechanisms is given below. These references, most of which were published in past five (5) years represent the result of a recent Internet search, including 31 publications with positive laboratory results demonstrating directly

or indirectly the anticancer effects of green tea or its components (References 1-31). In the same search printout, there were three references with laboratory results failing to demonstrate an anticancer effect (References 34-36).

References (1-36).

1. Agarwal R. Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. *Biochem Pharmacol* 2000;60:1051-9.
Center for Cancer Causation and Prevention, AMC Cancer Research Center, Denver, CO 80214, USA.

Treatment of cells with silymarin, genistein, and EGCG resulted in strong cell growth inhibition at lower doses, and complete inhibition at higher doses. In contrast to silymarin, higher doses of genistein also showed cell death. A more profound cytotoxic effect was observed in the case of EGCG, with strong cell death at lower doses and complete loss of viability at higher doses. Together, these results suggest that cell signaling and regulators of cell cycle are potential epigenetic molecular targets for prostate cancer prevention by dietary agents.

2. Bertolini F, Fusetti L, Rabascio C, Cinieri S, Martinelli G, Pruneri G. Inhibition of angiogenesis and induction of endothelial and tumor cell apoptosis by green tea in animal models of human high-grade non-Hodgkin's lymphoma. *Leukemia* 2000;14:1477-82.

Division of Hematology-Oncology, IRCCS European Institute of Oncology, Milan, Italy.

Recent reports suggest that green tea consumption may prevent or delay the growth of human cancer, possibly by impairing tumor invasion and/or by an anti-angiogenic effect. In NOD/SCID mice transplanted intraperitoneally with human non-Hodgkin's lymphoma (NHL) cell lines, Namalwa, RAP1-EIO and HS-Sultan, *green tea prevented 50% of Namalwa tumors (P = 0.0017 by log-rank) and significantly inhibited RAP1-EIO and HS-Sultan tumor growth. Notably, treatment with the chemotherapy drug cyclophosphamide at the maximum tolerable dose was unable to prevent Namalwa tumor occurrence. In the three models evaluated, the frequency of apoptotic endothelial and tumor cells was significantly increased in mice given green tea compared to controls. These results support further trials in NHL to evaluate whether green tea, alone or in combination with chemotherapy, may delay or prevent disease progression.*

3. Bode AM, Dong Z. Signal transduction pathways: targets for chemoprevention of skin cancer. *Lancet Oncol* 2000;1:181-8.

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Chemoprevention can be defined as the use of substances to interfere with the process of cancer development. Although substantial progress has been made in elucidating the basis of carcinogenesis, further advances are needed to identify molecular and cellular targets for effective use of chemopreventive agents. Hundreds of compounds have been identified as potential chemopreventive agents. However, the safety and efficacy of each substance must be thoroughly investigated. Carcinogenesis is a multistage process in which numerous genes are affected. Many of these genes regulate important cellular functions, so they are prime targets for chemopreventive agents. A major focus of our work has been the elucidation of mechanism(s) explaining the anticancer actions attributed to several chemopreventive compounds, especially 'natural compounds' that are considered safe because they are present in commonly consumed foods and beverages. Of particular interest are selected drugs (eg aspirin) and certain dietary factors (eg green and black tea, resveratrol) and their influence on cell-

signalling events coinciding with skin cancer promotion. This overview describes recent work from our laboratory and others focusing on molecular mechanisms of selected chemopreventive compounds in growth-related signal transduction pathways and skin cancer.

4. Graham HN. Green tea composition, consumption, and polyphenol chemistry. *Prev Med* 1992;21:334-350.

Tea composition varies with climate, season, horticultural practices, variety, and the age of the leaf, i.e., the position of the leaf on the harvest shoot. The fresh green tea leaf contains up to 36% polyphenols and 7-13% (-)-epigallocatechin gallate (EGCG) in dry weight.

5. Gunning WT, Kramer PM, Lubet RA, Steele VE, Pereira MA. Chemoprevention of vinyl carbamate-induced lung tumors in strain A mice. *Exp Lung Res* 2000 ;26:757-72.

Department of Pathology, Block Health Science Building, Medical College of Ohio, 3035 Arlington Avenue, Toledo, OH 43614-5806, USA.

The ability of potential chemopreventive agents to prevent vinyl carbamate-induced lung tumors was determined in 2 different experiments. Female strain A mice administered intraperitoneally either a single injection of 60 mg/kg vinyl carbamate that induced 24.0 +/- 1.72 tumors/mouse at 24 weeks or 2 injections of 16 mg/kg vinyl carbamate each (32 mg/kg total dose) that induced 43.2 +/- 3.2 tumors/mouse at 20 weeks. Lung carcinomas were found as early as 16 weeks. Dexamethasone and piroxicam provided in the diet were found to significantly inhibit lung tumors induced by 60 mg/kg vinyl carbamate at 24 weeks whereas myo-inositol also provided in the diet, did not significantly inhibit tumor formation. In animals given 6 16-mg/kg doses of vinyl carbamate, tumor multiplicity was reduced roughly 25% by alpha-difluoromethylornithine and green tea and reduced 50% by dexamethasone and piroxicam. Combinations of these agents were also tested using a total dose of 32 mg/kg of vinyl carbamate. Although alpha-difluoromethylornithine and green tea did not result in a significant inhibition of lung tumor formation if used alone, the combination of alpha-difluoromethylornithine and green tea resulted in a significant reduction of tumor multiplicity. The combinations of alpha difluoromethylornithine or green tea with either dexamethasone or piroxicam or the combination of dexamethasone and piroxicam did not decrease tumor multiplicity greater than achieved by dexamethasone and piroxicam alone. In summary, selected chemopreventive agents previously shown to inhibit lung tumors by other chemical carcinogens also inhibited vinyl carbamate-induced lung tumors.

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Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205-2179, USA.

Hepatocellular carcinoma (HCC) is among the most prevalent and deadly cancers worldwide. Prominent risk factors for HCC include viral hepatitis infection; dietary exposure to hepatotoxic contaminants such as aflatoxins; alcoholism; smoking; and male gender. Agents exhibiting chemopreventive efficacy in preclinical HCC models include vitamins A, D, and E, herbal extracts, a 5alpha-reductase inhibitor, green tea, and D-limonene.

7. Hsu SD, Singh BB, Lewis JB, Borke JL, Dickinson DP, Drake L, Caughman GB, Schuster GS. Chemoprevention of oral cancer by green tea. *Gen Dent* 2002;50:140-6.

Department of Oral Biology and Maxillofacial Pathology, School of Dentistry, Medical College of Georgia, Augusta, USA.

Green tea has been a popular beverage for many centuries. Only recently, however, has the anti-cancer power of green tea constituents been unveiled. Green tea polyphenols are found to induce apoptosis (programmed cell death) in many types of tumor cells, including oral cancer cells. However, mechanisms that enable normal cells to evade the apoptotic effect still are not understood. In this study, cell growth and invasion assays combined with apoptosis assays were used to examine the effects of green tea extracts, green tea polyphenols, and the most potent green tea polyphenol, (-)-epigallocatechin-3-gallate (EGCG), on normal human keratinocytes and oral carcinoma cells. The results showed that green tea and its constituents selectively induce apoptosis only in oral carcinoma cells, while EGCG was able to inhibit the growth and invasion of oral carcinoma cells. These differential responses to green tea and its constituents between normal and malignant cells were correlated with the induction of p57, a cell cycle regulator. These data suggest that the chemopreventive effects of green tea polyphenols may involve a p57 mediated survival pathway in normal epithelial cells, while oral carcinoma cells undergo an apoptotic pathway. Therefore, regular consumption of green tea could be beneficial in the prevention of oral cancer.

8. Javed S, Shukla Y. Effects of black tea extract on transplantable and solid tumors in Swiss albino mice. *Biomed Environ Sci* 2000;13:213-8.

Environmental Carcinogenesis Division, Industrial Toxicology Research Centre, M. G. Marg, P.O. Box 80, Lucknow-226001, India.

The present set of investigations were initiated to study the anti-tumorigenic potential of aqueous black tea extract (ATE) in Swiss albino mice in in vivo animal bioassay, using 7, 12 dimethyl-benzanthracene (DMBA) as carcinogen. In the experimental group, 2% ATE was given orally as sole source of drinking water, while the control were allowed to drink normal water, ad lib. The results revealed that drinking of 2% ATE could effectively inhibit the onset of tumorigenesis, cumulative number of tumors and average number of tumors per mouse.

9. Jung YD, Ellis LM. Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea. *Int J Exp Pathol* 2001;82:309-16.

Chonnam University Research Institute of Medical Sciences, Chonnam National University Medical School, Kwangju, Korea.

Epidemiological studies have suggested that consumption of green tea may decrease cancer risk. In addition, abundant pre-clinical data from several laboratories have provided convincing evidence that polyphenols present in green tea afford protection against cancer in both in vivo and in vitro studies. Recently, epigallocatechin gallate (EGCG), a putative chemopreventive agent and a major component of green tea, was reported to inhibit tumour invasion and angiogenesis, processes that are essential for tumour growth and metastasis. Understanding the basic principles by which EGCG inhibits tumour invasion and angiogenesis may lead to the development of new therapeutic strategies, in addition to supporting the role of green tea as a cancer chemopreventive agent.

10. Katiyar SK, Ahmad N, Mukhtar H. Green tea and skin. *Arch Dermatol* 2000;136:989-94.

Department of Dermatology, Case Western Reserve University, Cleveland, OH 44106, USA.

The outcome of the several experimental studies suggests that green tea possess anti-inflammatory and anticarcinogenic potential, which can be exploited against a variety of skin disorders. Although more

clinical studies are needed, supplementation of skin care products with green tea may have a profound impact on various skin disorders in the years to come.

11. Katiyar SK, Bergamo BM, Vyalil PK, Elmetts CA. Green tea polyphenols: DNA photodamage and photoimmunology. *J Photochem Photobiol B* 2001;65:109-14.

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Green tea is a popular beverage consumed worldwide. The epicatechin derivatives, which are commonly called 'polyphenols', are the active ingredients in green tea and possess antioxidant, anti-inflammatory and anti-carcinogenic properties. Studies conducted by our group on human skin have demonstrated that green tea polyphenols (GTP) prevent ultraviolet (UV)-B-induced cyclobutane pyrimidine dimers (CPD), which are considered to be mediators of UVB-induced immune suppression and skin cancer induction. GTP treated human skin prevented penetration of UV radiation, which was demonstrated by the absence of immunostaining for CPD in the reticular dermis. The topical application of GTP or its most potent chemopreventive constituent (-)-epigallocatechin-3-gallate (EGCG) prior to exposure to UVB protects against UVB-induced local as well as systemic immune suppression in laboratory animals. Additionally, studies have shown that EGCG treatment of mouse skin inhibits UVB-induced infiltration of CD11b+ cells. CD11b is a cell surface marker for activated macrophages and neutrophils, which are associated with induction of UVB-induced suppression of contact hypersensitivity responses. EGCG treatment also results in reduction of the UVB-induced immunoregulatory cytokine interleukin (IL)-10 in skin as well as in draining lymph nodes, and an elevated amount of IL-12 in draining lymph nodes. These *in vivo* observations suggest that GTPs are photoprotective, and can be used as pharmacological agents for the prevention of solar UVB light-induced skin disorders associated with immune suppression and DNA damage.

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Topical treatment with EGCG on mouse skin also results in prevention of UVB-induced immunosuppression, and oxidative stress. The protective effects of green tea treatment on human skin either topically or consumed orally against UV light-induced inflammatory or carcinogenic responses are not well understood. Based on documented extensive beneficial effects of green tea on mouse skin models and very little in human skin, many pharmaceutical and cosmetic companies are supplementing their skin care products with green tea extracts. Therefore, the focus of this communication is to review and analyze the photoprotective effects of green tea polyphenols to skin.

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Random samples of green teas marketed in the United Kingdom were found to contain 2.0-4.2% (-)-epigallocatechin gallate (EGCG).

14. Lu YP, Lou YR, Lin Y, Shih WJ, Huang MT, Yang CS, Conney AH. Inhibitory effects of orally administered green tea, black tea, and caffeine on skin carcinogenesis in mice previously treated with ultraviolet B light (high-risk mice): relationship to decreased tissue fat. *Cancer Res* 2001;61:5002-9.

Laboratory for Cancer Research, Department of Chemical Biology, College of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854-8020, USA.

Treatment of SKH-1 hairless mice with ultraviolet B light (UVB; 30 mJ/cm²) twice a week for 22 weeks resulted in tumor-free animals with a high risk of developing malignant and nonmalignant skin tumors during the next several months in the absence of additional UVB treatment (high-risk mice). Oral administration of green tea or black tea (6 mg tea solids/ml) to UVB-pretreated high-risk SKH-1 mice for 23 weeks after stopping UVB treatment decreased the number of tumors/mouse, decreased the size of the parametrial fat pads, and decreased the thickness of the dermal fat layer away from tumors and directly under tumors. This is the first demonstration of a close association between inhibition of carcinogenesis and the lowering of tissue fat levels by a chemopreventive agent.

15. Masuda H, Suzui M, Weinstein IB. Effects of Epigallocatechin-3-gallate on Growth, Epidermal Growth Factor Receptor Signaling Pathways, Gene Expression, and Chemosensitivity in Human Head and Neck Squamous Cell Carcinoma Cell Lines. *Clin Cancer Res* 2001;7:4220-4229.

The antitumor effects of the green tea compound epigallocatechin-3-gallate (EGCG) have not been studied in detail previously in head and neck squamous cell carcinoma (HNSCC) cells. Overexpression of the epidermal growth factor receptor (EGFR) occurs frequently in HNSCC, which is an adverse prognostic factor. Therefore, we examined in detail the molecular effects of EGCG on two human HNSCC cell lines, YCU-N861 and YCU-H891, focusing on the EGFR signaling pathway. The 70% lethal dose (IC₇₀) of EGCG for both cell lines was 10 microg/ml. Treatment with EGCG increased the proportion of cells in the G(1) phase of the cell cycle and induced apoptosis. In cells treated with EGCG, there was a decrease in the cyclin D1 protein, an increase in the p21(Cip1) and p27(Kip1) proteins, and a reduction in the hyperphosphorylated form of pRB, changes that may account for the arrest in G(1). EGCG also caused a decrease in the Bcl-2 and Bcl-X(L) proteins, an increase in the Bax protein, and activation of caspase 9, suggesting that EGCG induces apoptosis via a mitochondrial pathway. Treatment with EGCG inhibited phosphorylation of the EGFR, signal transducer and activator of transcription3 (Stat3), and extracellular regulated kinase (ERK) proteins and also inhibited basal and transforming growth factor-alpha-stimulated c-fos and cyclin D1 promoter activity. EGCG at 0.1 microg/ml (a concentration found in serum after oral administration) markedly enhanced the growth-inhibitory effects of 5-fluorouracil. Taken together, these findings provide insights into molecular mechanisms of growth inhibition by EGCG and suggest that this naturally occurring compound may be useful, when used alone or in combination with other agents, in the chemoprevention and/or treatment of HNSCC.

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Laboratoire du Controle Metabolique et Nutritionnel en Oncologie Digestive de l'Universite Louis Pasteur, Institut de Recherche contre les Cancers de l'Appareil Digestif, 67091 Strasbourg, France.

We determined the effects of a crude green tea extract given as drinking fluid on the promotion/progression phase of colon carcinogenesis in rats after induction of the neoplastic process by azoxymethane. Our data demonstrate that green tea extract reduces cyclooxygenase (COX)-2 activity and suppresses the formation of colonic preneoplastic lesions. They provide new insights into the mechanism of chemopreventive and anti-inflammatory properties of green tea.

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Other tea components were found to be more effective than catechins in suppressing *umu* gene expression of the SOS response in *Salmonella typhimurium* TA1535/pSK1002 induced by four nitroarenes.

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(-)-Epigallocatechin gallate (EGCG), a major constituent of green tea, has been shown to exhibit anti-cancer activity. Sulindac is also well known as a cancer-preventive agent against colon cancer, but its usage is restricted because of its adverse effects, as exemplified by gastrointestinal bleeding. In the present study, the authors demonstrated a synergistic effects of EGCG and sulindac for cancer preventive activity for rat colon carcinogenesis induced by azoxymethane (AOM). They concluded EGCG is a suitable candidate for use in combination with cancer-preventive agents, such as sulindac, to reduce their adverse effects.

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Department of Biochemistry, Boston University School of Medicine, 715 Albany Street, Boston, MA 02118-2394, USA.

Overexpression of the epidermal growth factor receptor family member Her-2/neu in breast cancer is associated with poor prognosis. With evidence accumulating for a chemopreventive role of green tea polyphenols, the effects of epigallocatechin-3 gallate (EGCG) on Her-2/neu-overexpressing breast cancer cells were examined. EGCG inhibited mouse mammary tumor virus (MMTV)-Her-2/neu NF639 cell growth in culture and soft agar. EGCG reduced signaling via the phosphatidylinositol 3-kinase, Akt kinase to NF-kappaB pathway because of inhibition of basal Her-2/neu receptor tyrosine phosphorylation. EGCG similarly inhibited basal receptor phosphorylation in SMF and Ba/F3 2 + 4 cells, which suggests the potential beneficial use of EGCG in adjuvant therapy of tumors with Her-2/neu overexpression.

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In the latter study, it was concluded that a dose of green tea extract (GTE) at 1.0 g/m⁽²⁾ tid (equivalent to 7 to 8 Japanese cups [120 mL] of green tea three times daily) was recommended for future studies. The side effects of this preparation of GTE were caffeine related. Oral GTE at the doses studied can be taken safely for at least 6 months in adults.

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School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka, Japan.

Components of green tea had previously been shown to be useful modulators in combination with doxorubicin (DOX). The authors confirmed that theanine, a glutamate analogue in tea, enhances the antitumor activity of DOX due to inhibition of DOX efflux from tumor cells. Like dihydrokainate (DHK), theanine is an inhibitor of glutamate transporter across the cell membrane. Both theanine and DHK may be useful modulators for inducing enhancement of antitumor activity of DOX.

22. Sartippour MR, Shao ZM, Heber D, Beatty P, Zhang L, Liu C, Ellis L, Liu W, Go VL, Brooks MN. Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. *J Nutr* 2002;132:2307-11.

Department of Surgery, Division of Oncology and. Center for Human Nutrition, University of California, Los Angeles 90095, USA.

Investigators have shown that green tea and its main catechin epigallocatechin-3 gallate (EGCG) may decrease the risk of cancer. Previous study of the authors showed that green tea extract (GTE) as well as its individual catechin components inhibited MDA-MB231 breast cancer cell and human umbilical vein endothelial cell (HUVEC) proliferation. Further evidence is provided in this article that inhibition of VEGF transcription appeared to be one of the molecular mechanism(s) involved in the antiangiogenic effects of green tea, which may contribute to its potential use for breast cancer treatment and/or prevention.

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Department of Pathology, Medical College of Ohio, Toledo 43614-5806, USA.

The heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is formed during the cooking of proteinaceous animal foods (meat, chicken, and fish). PhIP is a carcinogen in the Fischer 344 (F-344) rat; it induces mammary tumors in female rats and lymphomas and colon and prostate tumors in male rats. In F-344 rats, PhIP forms DNA adducts in various organs, including the target organs. Inhibition of PhIP-DNA adduct formation is likely to lead to inhibition of PhIP tumorigenicity. The authors found that green tea and black tea are potential chemopreventive agents in PhIP-induced tumorigenesis in the F-344 rat.

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Drug Discovery Program, H. Lee Moffitt Cancer Center and Research Institute, Interdisciplinary Oncology Program and Department of Biochemistry and Molecular Biology, College of Medicine, University of South Florida, Tampa, FL 33612, USA.

Here we report that the tea polyphenol (-)-epigallocatechin (EGC) inhibits DNA replication in three leukemia cancer cell lines, Jurkat T, HL-60 and K562. Among all the tested tea polyphenols, EGC was found to be the most potent in accumulation of S phase cells and inhibition of the S-G2 progression. In addition, EGC-mediated inhibition of S phase progression results in induction of apoptosis, as determined

by sub-G1 cell population, breakage of endonuclear DNA, cleavage of poly(ADP-ribose) polymerase and loss of cell viability. When used in cells containing low S and high G1 and G2/M populations, EGC did not induce apoptosis. Furthermore, EGC did not inhibit M-G1 transition. Our finding that EGC inhibits S phase progression that results in leukemia cell death provides a novel and plausible molecular mechanism for how green tea may inhibit the growth of rapidly proliferating neoplastic cells.

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The human biliary tract carcinoma cells (TGBC-2, SK-ChA-1, and NOZC-1) were treated with different doses of EGCG (0, 25, 50, 100, and 200 mM) for 48 hours in cell medium. Cell proliferation was analyzed by WST-1 colorimetric assay. For the cell-invasion analysis, the cells were incubated with 100 mM of EGCG for 2 hours. The cells were then added into a Matrigel-coated Cell Insert. After incubation at 37 degrees C for 24 hours, the cells visible through the Matrigel were counted under the microscope. All human biliary tract cancer cells studied showed a significant suppression of cell growth by EGCG treatment in a dose-dependent manner. Epigallocatechin-3-gallate treatment also produced a significant suppression of invasive ability of the carcinoma cells. These data indicated that EGCG might be a potent biological inhibitor of human biliary tract cancers, reducing their proliferative and invasive activities.

26. Takada M, Nakamura Y, Koizumi T, Toyama H, Kamigaki T, Suzuki Y, Takeyama Y, Kuroda Y. Suppression of human pancreatic carcinoma cell growth and invasion by epigallocatechin-3-gallate. *Pancreas* 2002;25:45-8.

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INTRODUCTION: The consumption of green tea is associated with a lower risk of several types of human carcinomas. A number of studies have focused on the possible mechanisms of cancer prevention by tea extracts, especially polyphenols such as epigallocatechin-3-gallate (EGCG). **AIMS AND METHODOLOGY:** Green tea-derived EGCG was tested in human pancreatic carcinoma cells. The cells (PANC-1, MIA PaCa-2, and BxPC-3) were treated with different doses of EGCG (0, 25, 50, 100, and 200 micromol/L) for 48 hours in culture medium. Proliferation of pancreatic carcinoma cells was measured by means of the WST-1 colorimetric assay. For the study of cell invasion, the cells were incubated with 100 micromol/L EGCG for 2 hours. Then, the cells were added into the cell insert, coated with Matrigel basement membrane matrix. After incubation at 37 degrees C for 24 hours, the cells that had invaded through the Matrigel were counted visually under the microscope. **RESULTS:** The growth of all three pancreatic carcinoma cells was significantly suppressed by EGCG treatment in a dose-dependent manner. EGCG treatment caused significant suppression of the invasive ability of pancreatic carcinoma cells PANC-1, MIA PaCa-2, and BxPC-3 but did not affect the cell cycle protein cyclin D1. **CONCLUSION:** EGCG may be a potent biologic inhibitor of human pancreatic carcinomas, reducing their proliferative and invasive activities.

27. Trosko JE, Chang CC. Mechanism of up-regulated gap junctional intercellular communication during chemoprevention and chemotherapy of cancer. *Mutat Res* 2001;480-481:219-29

To develop a strategy for efficacious intervention in order to prevent or treat various cancers, one must understand the basic mechanism(s) by which various anticancer dietary factors prevent or reverse the tumor promotion or progression phases. Carcinogenesis is a multistage, multimechanism process, involving the irreversible alteration of a stem cell (the "initiation" phase), followed by the clonal proliferation of the initiated stem cell (the "promotion" phase), from which the acquisition of the invasive and metastatic phenotypes are generated (the "progression" phase). While intervention to prevent or treat

cancer could occur at each step, the objective of this presentation will focus on the rate limiting step, the promotion phase. Gap junctional intercellular communication (GJIC) has been hypothesized to regulate growth control, differentiation and apoptosis. Most normal, contact-inhibited cells have functional GJIC, while most, if not all, tumor cells have dysfunctional homologous or heterologous GJIC. Cancer cells are characterized by the lack of growth control, by the inability to terminally differentiate and by resistance to apoptosis. Chemical tumor promoters (phorbol esters, DDT, phenobarbital, unsaturated fatty acids, saccharin, etc.) inhibit GJIC in a reversible fashion and at doses above particular chemical thresholds. Various oncogenes (e.g. ras, raf, neu, src, mos) down-regulate GJIC while several tumor suppressor genes can up-regulate GJIC. Antitumor promoters (retinoids, carotenoids, green tea components) and antioncogene drugs (i.e. lovastatin) can up-regulate GJIC. Transfection of gap junction genes ("connexins") into GJIC-deficient tumor cells can restore GJIC, growth control and reduce tumorigenicity. On the other hand, antisense gap junction genes can convert the phenotype of a non-tumorigenic cell to that of a tumorigenic one. Recently, a specific connexin knockout mouse was shown to have a higher frequency of spontaneous and induced liver cancers. Evidence from these studies clearly suggests that dietary factors can modulate GJIC by inducing various signal transducing systems.

28. Wang YC, Bachrach. The specific anti-cancer activity of green tea (-)-epigallocatechin-3-gallate (EGCG). *Amino Acids* 2002;22: 131-143.

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Green tea which is widely consumed in China, Japan and India, contains polyphenolic compounds, which account for 30% of the dry weight of the leaves. Most of the polyphenols are flavanols, of which (-)-epigallocatechin-3-gallate (EGCG) is most abundant. Epidemiological studies revealed that the incidences of stomach and prostate cancers are the lowest in the world among a population that consumes green tea on a regular basis. It has also been reported that the quantity of green tea consumed, plays an important role in reducing cancer risk and in delaying cancer outbreak and recurrence. Various systems were used to confirm anti-cancer activities of green tea and/or EGCG. These included experimental animals in which cancer was induced chemically. Cultured cells transformed chemically or by oncogenes were also used. These studies clearly demonstrated that green tea or EGCG have anticancer and cancer preventive properties. The mechanisms of these activities have also been studied in details. It has been shown that green tea and its active components interfere with signal transduction pathways. Thus the activities of various protein kinases are inhibited, the expression of nuclear proto-oncogenes declines and the activity of ornithine decarboxylase (ODC) is reduced. ODC, which catalyzes the rate-limiting step in the biosynthesis of polyamines is closely linked with cellular proliferation and carcinogenesis. Inhibitors of ODC, like alpha-difluoromethylornithine (DFMO) have long been used for cancer prevention and therapy. It has been suggested that polyamine depletion by green tea could offer one explanation for its anti-cancer activities.

The authors in the second report stated that the level of ornithine decarboxylase, which is a signal for cellular proliferation, was reduced by EGCG in the transformed but not in the normal cells. EGCG also showed strong inhibition of tyrosine kinase and mitogen-activated protein kinase activities in the transformed cells without affecting the kinases in the normal cells. EGCG inhibited the proliferation of leukemic cells. The research findings suggest that EGCG has therapeutic potential in the combat against cancer.

29. Wargovich MJ, Woods C, Hollis DM, Zander ME. Herbs, cancer prevention and health. *J Nutr* 2001 131(11 Suppl):3034S-6S

In the authors' opinion, herbal products may act in a pathway similar to pharmaceuticals yet without side effects. Natural anti-inflammatory compounds abound in the herbal world and are found in green tea, the

spice turmeric and rosemary, feverfew and others. Because the use of nonsteroidal anti-inflammatory drugs (NSAID) is associated with a reduced risk for several cancers, it is at least plausible that natural NSAID should be explored for possible use as cancer preventives.

30. Williams SN, Shih H, Guenette DK et al. Comparative studies on the effects of green tea extracts and individual tea catechins on human CYP1A gene expression. *Chem Biol Interact* 2000;128:211-229.

Modulation of human CYP1A expression by green tea extracts cannot be attributed to the action of a single tea catechin, but rather is due to the effects of a complex mixture.

31. Yanaga H, Fujii T, Koga T, Araki R, Shirouzu K. Prevention of carcinogenesis of mouse mammary epithelial cells RIII/MG by epigallocatechin gallate. *Int J Mol Med* 2002;10:311-5.

Department of Surgery, School of Medicine, Kurume University, Asahimachi Kurume, Fukuoka 830-0011, Japan.

The authors investigated the chemopreventive effect of EGCG in vitro and in vivo using mouse mammary epithelial cells RIII/MG. In the in vitro experiment, crude catechin (catechin) containing 50% or more EGCG significantly inhibited the growth of RIII/MG cells, which were precancerous cultured cells. Many cells died, and a DNA ladder was observed. In the in vivo experiment, RIII/MG cells formed a tumor after 13 weeks in a group without catechin treatment, and the tumor formation rate in the 20th week was 40%. In a group treated with 0.1% catechin, a tumor began to grow in the 13th week, and the tumor formation rate in the 20th week was 20%. In a group treated with 1% catechin, no tumor was detected even in the 20th week. There was no significant difference in the change in body weight between the catechin treatment groups and the non-treatment group during the observation period. Tissue samples were stained by the nick-end-labeling method and apoptosis was observed in many cells. Based on the above findings, EGCG inhibited growth in the mouse viral mammary epithelial carcinogenesis model RIII/MG, and induced apoptosis, suggesting a clinical usefulness of EGCG as a chemopreventive substance.

32. Yang CS, Maliakal P, Meng X. Inhibition of carcinogenesis by tea. *Annu Rev Pharmacol Toxicol* 2002;42:25-54.

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Tea has received a great deal of attention because tea polyphenols are strong antioxidants, and tea preparations have inhibitory activity against tumorigenesis. The inhibition of tumorigenesis by green or black tea preparations has been demonstrated in animal models on different organ sites such as skin, lung, oral cavity, esophagus, forestomach, stomach, small intestine, colon, pancreas, and mammary gland. Epidemiological studies, however, have not yielded clear conclusions concerning the protective effects of tea consumption against cancer formation in humans. The discrepancy between the results from humans and animal models could be due to 1) the much higher doses of tea used in animals in comparison to human consumption, 2) the differences in causative factors between the cancers in humans and animals, and 3) confounding factors limiting the power of epidemiological studies to detect an effect. It is possible that tea may be only effective against specific types of cancer caused by certain etiological factors. Many mechanisms have been proposed for the inhibition of carcinogenesis by tea, including the modulation of signal transduction pathways that leads to the inhibition of cell proliferation and transformation, induction of apoptosis of preneoplastic and neoplastic cells, as well as inhibition of tumor invasion and angiogenesis. These mechanisms need to be evaluated and verified in animal models or humans in order to gain more understanding on the effect of tea consumption on human cancer.

33. Zhang G, Miura Y, Yagasaki K. Effects of dietary powdered green tea and theanine on tumor growth and endogenous hyperlipidemia in hepatoma-bearing rats. *Biosci Biotechnol Biochem* 2002 ;66:711-6

Department of Applied Biological Science, Tokyo Noko University, Fuchu, Japan.

The effects of dietary powdered green tea (PGT) and theanine on in vivo hepatoma growth and cancerous hyperlipidemia were investigated in rats that had been implanted with a rat ascites hepatoma cell line of AH109A cells. The hepatoma-bearing rats were fed with a 20% casein diet (20C), 20C containing 2% PGT, or 20C containing 0.1% theanine for 14 days. Dietary PGT significantly and time-dependently reduced the solid tumor volume and weight as did dietary theanine. The hepatoma-induced endogenous hyperlipidemia, which was characterized by rises in the serum cholesterol (hypercholesterolemia) and triglyceride (hypertriglyceridemia) levels, was significantly suppressed by PGT and theanine supplementation. Bile acid excretion into the feces was significantly higher in the PGT- and theanine-fed rats than in the control rats. This inhibition of hypercholesterolemia may have resulted from tumor growth suppression as well as increased excretion of steroids from the body. These results suggest that PGT had both anti-proliferative activity toward hepatoma cells and hypolipidemic activity in the hepatoma bearing rats. They also suggest that theanine was, at least in part, responsible for the PGT actions.

34. Caderni G, De Filippo C, Luceri C, Salvadori M, Giannini A, Biggeri A, Remy S, Cheynier V, Dolara P. Effects of black tea, green tea and wine extracts on intestinal carcinogenesis induced by azoxymethane in F344 rats. *Carcinogenesis* 2000;21:1965-9.

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We investigated whether polyphenolic extracts from black tea, green tea or red wine affect azoxymethane (AOM)-induced intestinal carcinogenesis in rats. The data indicate that black tea and wine extracts, but not green tea extracts, can protect against AOM-induced colon carcinogenesis by a mechanism probably involving increased apoptosis in tumours.

35. Hirose M, Yamaguchi T, Mizoguchi Y, Akagi K, Futakuchi M, Shirai T. Lack of inhibitory effects of green tea catechins in 1,2-dimethylhydrazine-induced rat intestinal carcinogenesis model: comparison of the different formulations, administration routes and doses. *Cancer Lett* 2002;188:163-70.

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Differences in the modifying effects of green tea catechins (GTC) on intestinal carcinogenesis by different formulations, doses and administration routes were investigated in male rats pretreated with 1,2-dimethylhydrazine (DMH). One hundred and eighty nine F344 male rats received subcutaneous injections of DMH at 40 mg/kg body weight twice a week for 3 weeks. Three days after completion of the carcinogen treatment, they were divided into nine groups. Each was administered a different source of 0.1% or 0.01% of GTC (Mitsui Norin Co. (M) or Taiyo Kagaku Co. (T)) either in the diet (D) or the drinking water (W), or basal diet and tap water alone without GTC for 33 weeks and then killed for autopsy. The survival rate tended to be lower with 0.01% MGTC (W) group than in the other groups. In the large intestine, although the multiplicity and/or incidences of adenomas showed tendencies for dose-dependent decrease in all GTC groups, and the average volumes of tumors tended to be decrease dose-dependently in the MGTC (W) and TGTC (W) groups, the multiplicity of carcinomas did not show such a trend, rather being significantly increased in the 0.01% MGTC (D) and 0.1% TGTC (W) groups. In the small intestine, the incidence and the multiplicity of tumors in all GTC treated groups had a tendency to

decrease. On the other hand, the volume of tumors was increased with statistical significance in the 0.01% MGTC (W) and 0.1% TGTC (W) groups. Thus it can be concluded that GTC does not exert chemopreventive effects on intestinal carcinogenesis irrespective of its formulation, dose or route of administration.

36. Witschi H. Successful and not so successful chemoprevention of tobacco smoke-induced lung tumors. *Exp Lung Res* 2000;26:743-55.

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Strain A/J mice underwent whole body exposure for 6 hours a day, 5 days a week, for 5 months to a mixture of cigarette sidestream and mainstream smoke (89%-11%; total suspended particulates 80-150 mg/m³), then were kept for another 4 months in air before being killed for scoring of lung tumors. In 7 independent experiments, lung tumor multiplicity was significantly increased in all 7 trials and lung tumor incidence in 5. When animals were kept for 9 months in smoke, lung tumor multiplicity was not significantly higher than in controls, although lung tumor incidence was. The following chemopreventive agents were evaluated: green tea, phenethyl isothiocyanate (PEITC), acetylsalicylic acid (ASA), N acetylcysteine (NAC), p-XSC (1,4-phenylenebis[methylene]selenocyanate), d-limonene (DL), and a mixture of PEITC and BITC (benzyl isothiocyanate). In animals exposed to tobacco smoke, none of these agents reduced lung tumor multiplicity or incidence. As a control, the effects of the same agents were examined in A/J mice initiated with 4-(methylnitrosamino)-1-(3pyridyl)-1-butanone (NNK) or urethane. In mice injected with NNK, green tea and ASA did not reduce lung tumor multiplicities and NAC had no effect on urethane-induced lung tumors, whereas PEITC, p-XSC and DL reduced NNK-induced tumor multiplicities to 20% to 50% of control values. On the other hand, dietary mixture of myoinositol and dexamethasone was not only highly protective against NNK, but reduced lung tumor multiplicities and incidence in smoke-exposed animals to control values. This effect was also seen when the animals were fed the myo-inositol-dexamethasone mixture once they were removed from smoke. It is concluded that in animal studies it might be preferable to evaluate the effectiveness of putative chemopreventive agents against full tobacco smoke rather than against selected model compounds. The observations made with myo-inositol-dexamethasone suggest that people who have recently quit smoking might benefit the most from active chemoprevention.

IV. The scientific evidence in support of the proposed health claim outweighs the scientific evidence against such claim

Practically all of the scientific publications in the public domain have affirmed that green tea and its active ingredients inhibit cancer cell growth or cancer cell spreading at the molecular and cell biology level in the laboratory and in experimental animal model studies provided the tea and the tea ingredients used for the study are properly prepared. In human epidemiological studies, a benefit of cancer prevention is usually observed in a population known to consume a large volume, i.e. a minimum of 1200 ml equivalent to three large mugs, 400 ml each, or four medium-sized mugs, 300 ml each, of high quality green tea regularly, as in Saitama, Aichi and Shizuoka, the well-known tea-producing prefectures in Japan, but not obvious among the residents surveyed in Miyagi, a northern rural region of Japan and in large poorly controlled cohort studies in areas where the quality of the green tea is in question. Similar cancer preventive benefits have been observed in populations living in cities and provinces of other countries where quality green teas are readily available to the study participants. The fact that the quality of the tea and the dose of the tea consumed may determine the outcomes of the cancer chemoprevention in a tea-drinking population only became known in recent years.

Of the 135 reports involving epidemiological or human subject studies, ninety-five (95) stated that black tea was used solely or was included in the study, or that green tea was not the target of the study with significant conclusions. For these 95 studies, the type of tea listed in the questionnaire surveys was identified as black tea, or a combination of black tea and green tea, or simply "tea". Therefore, they are treated as a heterogeneous group and referred to as the "study series including black tea" since the majority of the study participants in these reports were derived from populations in which most tea drinkers consume black tea, for example, in America and Europe. Forty (40) of the 135 reports available in the public domain clearly stated that the study participants were green tea drinkers. This latter group is referred to as the "green tea series" for the analyses in this petition.

It is of great interest to note that in 79 of the 95 of the reports (79/95) of the study series including black tea, the authors did not find evidence to support the claim that drinking tea is associated with a reduced cancer risk. However, in 32 of the 40 reports (32/40) of the green tea series, the authors found a significant reduction in cancer incidence among regular tea drinkers, especially among the heavy green tea drinkers living in areas near a tea plantation and especially for those tea drinkers who have a habit of renewing the tea batch in the pot more frequently. This difference in the outcome of chemoprevention between study series including black tea and green tea series is further confirmed by organ-specific analyses which show that drinking green tea is more often associated with reduction of the cancer rates in 10 organs, including cancers of the esophagus (4/6 reports), stomach (13/18 reports), pancreas (2/2 reports), colorectum (6/7 reports), urinary bladder (3/4 reports), lung (2/2 reports), breast (4/4 reports), liver (3/3 reports), uterus (1/1 report) and ovary (1/1 report). No green tea epidemiological data were available for cancer of the prostate and kidney.

Of the 135 publications reviewed, the authors in 40 articles, i.e. No. 20, 21, 23, 24, 27, 28, 32, 39, 44, 45, 46, 47, 52, 53, 54, 55, 60, 61, 62, 63, 87, 89, 90, 91, 92, 95, 96, 97, 104, 106, 117,

119, 120, 121, 123, 125, 126, 127, 133 and 135, specifically stated that their research or observation was based on human consumption of green tea as a potential chemopreventive agent against cancer risk. In eight (8) of these publications whose reference numbers are underlined, the authors did not find a significant inverse relationship between drinking green tea and cancer risks. The reasons for the lack of an apparent inverse relationship may be presented as follows.

Article # 89. Nagano J, Kono S, Preston DL, Mabuchi K. A prospective study of green tea consumption and cancer incidence, Hiroshima and Nagasaki (Japan). *Cancer Causes Control* 2001;12:501-8.

In this study, the subjects were 38,540 people (14,873 men, mean age 52.8 years; 23,667 women, mean age 56.8 years) who responded to a mail survey carried out between 1979 and 1981. A self-administered questionnaire ascertained consumption frequency of green tea using precoded answers (never, once per day, twice to four times per day, and five or more times per day). Nagano et al. in the concluding remarks stated: **“Our failure to find a clear, protective association between green tea and cancer may be due to some crudeness in the assessment of green tea intake; green tea consumption was determined only in terms of self-reported daily frequency of drinking, and the highest category was five or more cups per day. Bioactivity of a cup of green tea obviously differs by the amount of green tea leaves used to brew it and the frequency of renewing a tea batch in the pot.”** **“In Shizuoka prefecture, which has the highest production of green tea leaves in Japan, residents of towns with low mortality from stomach cancer were found not only to drink green tea more frequently, but also to renew tea leaves more frequently than those of a town with high mortality from stomach cancer”**.

Petitioner’s Note: A decreased risk of cancer incidence in association with drinking green tea apparently only reaches statistical significance among the people with high consumption, e.g., 10 or more cups per day, of green tea (44, 61) who live near the green tea-producing regions where high quality green teas are readily available to consumers (20, 90).

Article # 23. Galanis DJ, Lee J, Kolonel LN. **Comments** on: The influence of cigarette smoking, alcohol, and green tea consumption on the risk of carcinoma of the cardia and distal stomach in Shanghai, China. *Cancer*. 1997 May 1;79(9):1840-1.

In this Correspondence to the Editor, the authors stated “Preliminary findings from our research among a cohort of residents of Hawaii indicates an increased risk of gastric cancer among men who consumed green tea. In the fulltext report published by the authors (listed in #22), the questionnaire for survey only asked the question if the participant consumed one cup, or 2 or more cups of green tea a day. In addition, approximately half of the participants of the study were current or former cigarette smokers. Smoking has been shown to be a significant risk factor for stomach cancer by many investigators. Therefore, the quantity and quality of green tea consumed by the participants in this study might not have reached the level of efficacy for cancer risk reduction. And cigarette smoking might have played a role of being a confounding factor because of the high percentage of smokers among the participants.

Article #32. Hara N, Sakata K, Nagai M, Fujita Y, Hashimoto T, Yanagawa H. Statistical analyses on the pattern of food consumption and digestive-tract cancers in Japan. *Nutr Cancer*. 1985;6(4):220-8.

In this analysis, the cancer mortality data were acquired on a prefectural basis from the standardized cancer mortality ratios (SMR) calculated by the Research Committee of the Distribution of Adult Diseases from raw data of Japanese vital statistics. The data on food consumption were obtained from the Fourth National Survey of Family Income and Expenditures, in which data for each prefecture were available. Twenty-six (26) food items were chosen for comparative studies with the SMR calculated from six digestive-tract cancers. Esophageal cancer was found to be positively associated with pork, oil, popular-grade sake, and green tea; cancer of the biliary passages positively associated with pork, popular-grade sake, and green tea. This is a very crude statistical study because the amount of green tea consumption per person per month in each prefecture was expressed in *yen (expense)* (Table 2), not in quantity or quality of the tea consumed. Factors other than green tea, such as cigarette smoking and sake consumption might also play a role in those patients who died of cancers of the esophagus and the biliary passages.

Article #39. Hoshiyama Y, Kawaguchi T, Miura Y, Mizoue T, Tokui N, Yatsuya H, Sakata K, Kondo T, Kikuchi S, Toyoshima H, Hayakawa N, Tamakoshi A, Ohno Y, Yoshimura T; Japan Collaborative Cohort Study Group. A prospective study of stomach cancer death in relation to green tea consumption in Japan. *Br J Cancer* 2002;87:309-13.

To evaluate whether green tea consumption provides protection against stomach cancer death, relative risks were calculated using Cox proportional hazards regression analysis in the Japan Collaborative Study for Evaluation of Cancer Risk, sponsored by the Ministry of Health and Welfare (JACC Study). The study was based on 30 370 men and 42 481 women aged 40-79. The authors found no inverse association between green tea consumption and the risk of stomach cancer death.

This was a nation-wide multicentre prospective study to evaluate risks on cancer incidence and mortality, involving forty-five (45) municipalities in Japan. However, the number of cups of green tea consumed per person per day in various regions in the country might not constitute a valid uniform dosing category to group tea drinkers for cancer risk comparison because the quality of the green tea leaves and the method of drinking tea varied from region to region. As the authors pointed out in the discussion, *Helicobacter pylori* infection is a strong risk factor for stomach cancer and was not taken into consideration in this study. These factors might have contributed to the conclusion of "no inverse association between green tea consumption and the risk of stomach cancer death".

Article #46. Inoue M, Tajima K, Hirose K, Kuroishi T, Gao CM, Kitoh T. Life-style and subsite of gastric cancer--joint effect of smoking and drinking habits. *Int J Cancer*. 1994 Feb 15;56(4):494-9.

This study compared 668 histologically confirmed gastric cancer cases [123 cardia, 218 middle (body), 256 antrum, and 71 unclassified] with 668 controls using a common questionnaire about life-styles as related to smoking, drinking, dietary habits and frequency of food intake. In

addition to numerous food items surveyed, green tea was listed as one of five (5) “beverage preference and habitual smoking” items in the questionnaire for the participants to respond to. The OR for total stomach cancer was found to be 1.09 (95% CI 0.83-1.43) as listed in Table IV. The frequency and quantity of green tea consumption were not taken into account for the analysis. The same leading researchers of this article subsequently published another report (Article #45 in 1998) stating that the odds ratio (OR) of stomach cancer decreased to 0.69 (95% CI=0.48-1.00) with high intake of green tea (seven cups or more per day).

Article #60. Koizumi Y, Tsubono Y, Nakaya N, Nishino Y, Shibuya D, Matsuoka H, Tsuji I. No association between green tea and the risk of gastric cancer: pooled analysis of two prospective studies in Japan. *Cancer Epidemiol Biomarkers Prev.* 2003 May;12(5):472-3.

This report consisted of a pooled analysis of two population-based prospective cohort studies in rural northern Japan, including 31,345 and 47,605 subjects respectively. Self-administered questionnaires asked about frequency categories of green tea consumption ranging from “never” to “5 cups or more” per day. No inverse relationship was found between consumption of green tea and risk of stomach cancer. It has been suggested that the epidemiological data can be easily influenced by the quality of the green tea and the quantity of green tea catechins consumed in addition to the number of cups per day (see Articles #20 and #90). The lack of inverse relationship reported by Koizumi et al. can be explained by the fact that the subjects living in rural northern Japan may not have ready access to quality green teas as the Japanese living in the South in the tea-producing prefectures.

Article #117. Tsubono Y, Nishino Y, Komatsu S, Hsieh CC, Kanemura S, Tsuji I, Nakatsuka H, Fukao A, Satoh H, Hisamichi S. Green tea and the risk of gastric cancer in Japan. *N Engl J Med* 2001;344(9):632-6.

In January 1984, a total of 26,311 residents in three municipalities of Miyagi Prefecture in northern Japan completed a self-administered questionnaire that included questions about the frequency of consumption of green tea. During 199,748 person-years of follow-up, through December 1992, no association between green-tea consumption and the risk of gastric cancer was found. Miyagi Prefecture is a northern rural region of Japan with no tea plantations in the area. The quality of the green teas consumed by the local population in the North is probably not comparable to that consumed by the Japanese who live in the South near the tea plantations and thus have ready access to fresh quality green teas. Since quality green teas in Japan are rather expensive, the northern rural residents, even if they have access to quality green teas, may not have renewed the batch of green tea leaves in the tea pot as frequently as the southerners, which may in turn result in consumption of less green tea catechins per person per day in northern Japan.

Article #121. Watanabe Y, Tada M, Kawamoto K, Uozumi G, Kajiwara Y, Hayashi K, Yamaguchi K, Murakami K, Misaki F, Akasaka Y, et al. [A case-control study of cancer of the rectum and colon] *Nippon Shokakibyō Gakkai Zasshi.* 1984 Feb;81(2):185-93. [Article in Japanese with English translation attached]

The goal of this study was to elucidate risk factors of cancer of the rectum and colon. Drinking green tea might be associated with an increase in colorectal cancers, as suggested by

the data included in Table 9 and Table 10. The difference between only a few matched cases and controls was not significant. The authors of the article did not even attempt to make a comment on this finding in the discussion.

In conclusion, the lack of association between drinking green tea and a reduction of cancer risk in the 8 of the 40 reports cited above was probably the results of an insufficient intake of the green tea bioactive ingredients by the participants, inappropriate research designs and the influence of confounding factors in these studies.

Two of the 135 epidemiological references need special comments. These are references # 56 and # 70.

56. Kinjo Y, Cui Y, Akiba S, Watanabe S, Yamaguchi N, Sobue T, Mizuno S, Beral V. Mortality risks of oesophageal cancer associated with hot tea, alcohol, tobacco and diet in Japan. J Epidemiol. 1998 Oct;8(4):235-43.

This report is concerned with drinking tea at high temperature as a potential cause of esophageal cancer. The study used green tea as the liquid medium for the temperature effects, but did not target the physiological/pharmacological green tea effects on cancer risk per se. This article has been included in this petition in the section of Organ-specific analysis of the published data, Esophagus, Green tea series and is classified as Green Tea, Not supportive for completeness, but is not included in the 40 green tea publications for statistical analysis.

70. Lee HH, Wu HY, Chuang YC, Chang AS, Chao HH, Chen KY, Chen HK, Lai GM, Huang HH, Chen CJ. Epidemiologic characteristics and multiple risk factors of stomach cancer in Taiwan. Anticancer Res 1990 Jul-Aug;10(4):875-81.

This is a hospital-based matched case-control study carried out in Taipei metropolitan areas showing a positive association of stomach cancer with blood type A, chronic gastric diseases, cigarette smoking, alcohol drinking, green tea drinking (only 10 patients with a questionable significant p value between 0.05 and 0.10), as well as consumption of salted meat, cured meat, smoked food, fried food and fermented beans. It included 210 stomach cancer patients in the study, in which green tea was used in less than 2% of hospital controls. Because Chinese patients with various illnesses often avoid tea drinking when certain medications are taken, particularly herbal medicines, and may be encouraged to consume more nutritious drinks (e.g., chicken soup and milk), the findings from this study are difficult to interpret (see comments in Author Reply of Ref. # 23 by Bu-Tian Ji et al.). Furthermore, in Taiwan and southern China oolong teas are customarily referred to as green tea by the general public and tea merchants. Traditionally, southern Chinese and Taiwanese tend to avoid drinking green tea because of its notorious "harmful" physiological effects in depleting body fat during famine years, a remarkably accurate observation made by the Chinese peasants over the past three centuries. This fact is reflected in Table VI of this article that the ratios of half-processed tea drinkers: green tea drinkers were 70:10 in the Case Number group and 285:14 in the Control Number group, respectively. In view of these confounding factors, this report is not classified as an epidemiological study primarily based on green tea.

Based on the published epidemiological and laboratory data presented above, the preponderance of the scientific evidence available in the public domain indicates that daily consumption of green tea* brewed with quality natural tea leaves of *Thea sinensis* in a sufficient dose, for example about 40 ounces per day, may reduce the risk of certain forms of cancer, in particular cancer of the esophagus, stomach, pancreas, colorectum, urinary bladder, lung, breast, liver, uterus and ovary, the proposed health claim, although the evidence is not conclusive.

Note * A typical cup of green tea contains 710 µg/ml natural (-)-epigallocatechin gallate (EGCG) [National Cancer Institute: *J Cell Biochemistry* 1996;26S:236-257].

When the **Interim Evidence-based Ranking System for Scientific Data Guidance for Industry and FDA** issued in July 2003 by CFSAN was applied to rate and rank the above quoted 40 epidemiological studies on green tea, the results were obtained as follows.

Nine (9) of the 40 studies were classified as “Not a primary report” (Ref. No. 20, 21, 23, 28, 54, 60, 63, 95, and 106) and therefore excluded from the ranking.

In twelve (12)* of the 40 studies, either the number of the study subjects observed or the substance/disease relationship targeted for the study, or both, did not reach the level of a moderate comfortable strength of evidence. Nevertheless, ten (Ref. 27, 46, 55, 61, 62, 96, 119, 120, 125, 127) of these 12 studies provided results in support of the proposed claim; and two (Ref. 32, 121) did not provide results in support of the claim.

In twelve (12)** studies in which a study design type **Three** or **Two** was used with a sufficient number of study subjects and with at least a moderate study quality, eleven (Ref. 24, 45, 52, 53, 87, 97, 104, 126, 123, 133, 135) of them provided results in support of the proposed claim; and one (Ref. 39) did not provide results in support of the claim.

In seven (7)*** studies in which a study design type **Two** or **One** was used with a sufficient number of study subjects and with a high study quality, five (Ref. 44, 47, 90, 91, 92) of them provided results in support of the proposed claim; and two (Ref. 89, 117) did not provide results in support of the claim.

Therefore, the scientific evidence in support of the health claim as proposed in this petition outweighs the scientific evidence against such claim among studies of every level of evidence-based rank. Studies of similar or different design would probably result in similar findings and benefits in support of the proposed health claim provided the intake of the dietary supplement, green tea, is in sufficient dose and the green tea is of a “typical” quality as defined in this petition.

A table for Evidence-based Rating and Ranking of 40 Publications analyzed on the relationship between Green Tea and Cancer Risk is presented on the next page.

Evidence-based Rating and Ranking of 40 Publications on Green Tea and Cancer Risk

Ref. No. and Author(s)	Design type	Quality	Strength of Evidence
20. Fujiki H, Suganuma M, et al.		<i>Not a primary report</i>	
21. Fujiki H, Suganuma M, et al.		<i>Not a primary report</i>	
23. Galanis DJ, Lee J, Kolonel LN.		<i>Not a primary report</i>	
24. Gao YT, McLaughlin JK, et al.	Three	+	**
27. Goto R, Masuoka H, et al.	Three	Ø	*
28. Gupta S, Ahmad N, Mukhtar H.		<i>Not a primary report</i>	
32. Hara N, Sakata K, Nagai M, et al.	Four	-	*
39. Hoshiyama Y, Kawaguchi T, et al.	Two	Ø	**
44. Imai K, Suga K, Nakachi K.* 2	Two	+	***
45. Inoue M, Tajima K, Hirose K, et al.	Three	+	**
46. Inoue M, Tajima K, Hirose K, et al.	Three	-	*
47. Inoue M, Tajima K, Mizutani M, et al.	One	+	***
52. Ji BT, Chow WH, Hsing AW, et al.	Three	+	**
53. Ji BT, Chow WH, Yang G, et al.	Three	+	**
54. Kamat AM, Lamm DL.		<i>Not a primary report</i>	
55. Kato I, Tominaga S, et al.	Three	Ø	*
60. Koizumi Y, Tsubono Y, et al.		<i>Not a primary report</i>	
61. Kono S, Ikeda M, Tokudome S, et al.	Three	Ø	*
62. Kono S, Shinchi K, Ikeda N, et al.	Four	-	*
63. Kono S.		<i>Not a primary report</i>	
87. Mu LN, Zhou XF, Ding BG, et al.	Three	Ø	**
89. Nagano J, Kono S, Preston DL, et al.	Two	+	***
90. Nakachi K, Eguchi H, Imai K, et al.	Two	+	***
91. Nakachi K, Matsuyama S, et al.	Two	+	***
92. Nakachi K, Suemasu K, Suga K, et al.	One	+	***
95. Oguni I, Cheng SJ, Lin PZ, Hara Y.		<i>Not a primary report</i>	
96. Ohno Y, Aoki K, Obata K, et al.	Three	-	*
97. Ohno Y, Wakai K, Genka K, et al.	Three	+	**
104. Setiawan VW, Zhang ZF, et al.	Three	+	**
106. Shibata K, Moriyama M, et al.		<i>Not a primary report</i>	
117. Tsubono Y, Nishino Y, et al.	Two	+	***
119. Wakai K, Ohno Y, Obata K, Aoki K.	Three	-	*
120. Wang M, Guo C, Li M.	Three	Ø	*
121. Watanabe Y, Tada M, et al.	Three	-	*
123. Wu AH, Yu MC, Tseng CC, et al.	Three	+	**
125. Ye WM, Yi YN, Luo RX, et al.	Three	Ø	*
126. Yu GP, Hsieh CC, Wang LY, et al.	Three	+	**
127. Yu GP, Hsieh C.	Three	Ø	*
133. Zhang M, Binns CW, Lee AH.	Three	+	**
135. Zhong L, Goldberg MS, et al.	Three	+	**

Note for Table contents:

Ref. No. refers to the No. of References with abstract attached to Section **B. I.** (pages 38-96).

Study Design Type **One:** Randomized, controlled intervention trials.

Two: Prospective observational cohort studies.

Three: Case-control studies; nonrandomized intervention trials with concurrent or historical controls.

Four: Cross-sectional studies; analyses of secondary disease endpoints in intervention trials; case series.

+ high scientific quality; Ø moderate quality; - quality uncertain;

*** High strength of evidence; ** moderate strength of evidence; * low strength of evidence

V. Answers to specific questions and summary remarks

1. Is there an optimum level of the particular substance to be consumed beyond which no benefit would be expected?

Dry green tea leaves are natural plant products. There are more than 60 volatile chemicals in the fresh tea leaves and this number increases to more than 300 in the dry tea leaves after heat processing. Scientists have focused on the study of the tea polyphenols, namely the antioxidant catechins, especially the most active and most abundant catechin, (-)-epigallocatechin gallate (EGCG) and an amino acid, theanine which may be associated with health benefits of tea drinking. Although EGCG is the most extensively studied anticancer antioxidant in green tea, other ingredients may play equally important roles in anti-carcinogenesis (Ohe T, Marutani K, Nakase S. Catechins are not major components responsible for anti-genotoxic effects of tea extracts against nitroarenes. *Mutat Res* 2001;496:75-81). The whole green tea infusion prepared according to the traditional tea-preparing methods is more effective in suppressing cancer cell growth in the laboratory than any of its individual antioxidant catechins because the anticancer activity of green tea is the effect of a complex mixture (Williams SN, Shih H, Guenette DK et al. Comparative studies on the effects of green tea extracts and individual tea catechins on human CYP1A gene expression. *Chem Biol Interact* 2000;128:211-229). Drinking at least 1200 ml, or three mugs of tea, 400 ml each, of high quality green tea per day is needed for an effective chemoprevention of cancer based on the Japanese experience. Most people use a 1:100 w/v ratio of dry tea leaves to water to make tea infusion for beverage preparation. A volume of 1200 ml green tea would represent a hot water infusion of about 12 gm dry green tea leaves per day. A high quality (potency) or "typical" green tea leaf contains at least 7% of EGCG in dry weight if a 1:100 w/v ratio of dry tea leaves to water is used to make tea infusions. The latter percentage is derived from a reference statement by the US NCI, DCPC Chemoprevention Branch and Agent Development Committee and published in the Journal of Cellular Biochemistry 1996;26S:236-257. Therefore, daily consumption of a minimum volume of green tea infusion containing about 800 mg EGCG is needed for an effective chemoprevention against cancer. In some populations more than 2400 ml of green tea a day is consumed regularly (Graham HN. Green tea composition, consumption, and polyphenol chemistry. *Prev Med* 1992;21:334-350). The optimum level and the upper beneficial limit of green tea consumption have not been determined in clinical trials.

2. Is there any level at which an adverse effect from the substance or from foods containing the substance occurs for any segment of the population?

According to the preclinical 28-day and 90-day toxicology studies in rats and dogs sponsored by the Chemoprevention Branch of NCI, no evidence of toxicity was found; the highest dose levels selected for the 90-day dog study were in the range of 300 mg/kg-bw/day for EGCG (NCI, DCPC Chemoprevention Branch and Agent Development Committee *J Cell Biochem* 1996;26S:236-257). Several phase 1 and phase 2 clinical trials using various green tea products for the treatment of solid tumors with the FDA permission have been organized in a few

medical centers in the USA [Mukhtar H, Ahmad N. Tea polyphenols: prevention of cancer and optimizing health. *Am J Clin Nutr* 2000;71 (6 Suppl):1698S-702S; discussion 1703S-4S-reference under B.II. and Pisters KM, Newman RA, Coldman B, Shin DM, Khuri FR, Hong WK, Glisson BS, Lee JS. Phase I trial of oral green tea extract in adult patients with solid tumors. *J Clin Oncol*. 2001;19:1830-8-reference under B.III.] In the latter study, it was concluded that a dose of green tea extract (GTE) at 1.0 g/m² tid (equivalent to 7 to 8 Japanese cups [120 mL] of green tea three times daily) was recommended for future studies. The side effects of this preparation of GTE were caffeine related. Oral GTE at the doses studied can be taken safely for at least 6 months in adults.

A PubMed literature search at the website www.ncbi.nih.gov using a combination of the key search words, "Adverse reactions to green tea" on October 14, 2003 yielded no scientific reports on the subject except one title of publication (attached as page 151a) : Weisburger JH. Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. *Food Chem Toxicol*. 1999 Sep-Oct;37(9-10):943-8. Copy of the fulltext of this article is enclosed as Reference 44 in Section B. II. of this petition. In this article, no adverse reactions due to green tea were listed.

Some medical practitioners are concerned that the vitamin K in green teas may interact with warfarin, an anticoagulant drug. However, green tea catechins and (-)-epigallocatechin gallate also have antithrombotic activities without changing the coagulation parameters such as activated partial thromboplastin time, prothrombin time, and thrombin time, suggesting that the modes of antithrombotic action of green tea may be due to its antiplatelet function, but not to its anticoagulation activities. The patients on anticoagulative drugs should inform their health care professionals if they drink green tea as a dietary supplement so that the optimum status of blood coagulation can be maintained. (No clinical adverse reactions have been reported- see page 151 a)

Green tea may interfere with absorption of inorganic ferrous iron and lipids. Therefore, regular consumption of large doses of green tea is not recommended for children and for pregnant women without medical consultation.

3. Are there certain populations that must receive special consideration?
Tea has certain pharmacologic effects and may interact with some drugs, such as warfarin. Tea contains a low level of caffeine. Tea may cause allergic reactions and reduce inorganic iron absorption. Green tea is not recommended for people under starvation, pregnant women and children because it inhibits lipid absorption. Regular tea consumers should inform their physicians that they are drinking tea as a dietary supplement.
4. What other nutritional or health factors (both positive and negative) are important to consider when consuming the substance?

Based on the chemical composition of the fresh green tea leaf, the positive and negative nutritional or health factors in drinking green tea as a dietary supplement are considered to be negligible other than the polyphenol effects and caffeine-related effects.

According to the 1993-1997 composite statistics, last figures available, published by the American Cancer Society, the cancer incidence rate and the mortality rate per 100,000 in the US population was 347.8 and 140.0 for women, and 475.5 and 209.7 for men, respectively. Since environmental elements are thought to account for 70% of the risk factors in the development of human malignant tumors and 35% of these factors are diet-related, reduction of cancer rates may be accomplished by adopting a healthy life style and a healthy dietary habit. However, much half-true information incorrectly extrapolated from the anticancer effects of green tea observed in laboratory and experimental animal research has been circulated in the lay news media, which, for example, stated: **"Green tea is healthy, yes. You are crazy if you don't drink green tea. Or eat it. Or pop it"** (The New York Times Magazine/January 19, 2003, page 53, by Jonathan Reynolds). Green tea leaf powder had been reported to be effective in reducing the risk of human cancers if it was swallowed or sprinkled on foods or ice cream. Most American consumers who read these lay articles might be led to believe that any green tea beverage prepared with a dust-grade green tea in an infusion tea bag brewed in hot water at 170 to 175°F for less than two minutes is good enough as a chemopreventive daily dietary supplement in the fight against cancer. This belief is far from the truth. High quality green teas have never reached the populace in North America because they degrade at uncontrollable rates when exposed to atmospheric oxygen, especially in the dust form when the total cut surface of the tea leaf fragments increases exponentially from intact leaves to dusts during manufacturing of the infusion tea bags. There is an urgent need to disseminate correct information on drinking green tea for health promotion among the public consumers who want to manage their own health by adopting a healthy lifestyle and a healthy diet. To serve this need of disseminating health information is the purpose of this petition.

Thanks to several groups of serious researchers in Asia where most high quality green teas are available in the regions near the tea plantations, it has been observed that regular consumption of high quality green tea at least 1200 ml a day is associated with reduced cancer risks, compared to non-tea-drinkers. However, the technology to duplicate this practice is not readily available in the USA because there are no tea plantations in this country. The petitioner intends to make this time-proven technology of drinking high quality green tea to promote the health in mind and body available to the American public by implementing appropriate quality control measures based on the results of scientific research published in the past five years. In particular, the petitioner is introducing a high quality green tea which is packaged under vacuum with nitrogen flushing at the tea plantation immediately after harvesting and processing in order to preserve the high levels of antioxidants in the dry tea leaves before being transported out of the plantation site. With these quality control measures, the general consumers in the US will be able to gain the same access to quality green teas as the Asian residents living in the tea-producing prefectures or provinces.

In addition to the minimum contents of 7% EGCG in the dry weight, the tea leaves used regularly for chemoprevention of cancer should contain no pesticide or heavy-metal contaminants; or the residues of these contaminants should be below the US EPA allowable limits. These incidental environmental contaminant residues in the green tea specified in this petition are non-detectable or substantially less than all these allowable limits.

C. ANALYTICAL DATA

Dry green tea leaves are natural plant products. To date, there is no standard chemical specification for green teas. However, customarily, green tea is brewed at a 1:100 w/v dry tea leaf-to-water ratio for most green tea drinkers. According to an NCI, DCPC, Chemoprevention Branch and Agent Development Committee document (NCI, DCPC, Chemoprevention Branch and Agent Development Committee, Clinical development plan: tea extracts green tea polyphenols epigallocatechin gallate. *J Cell Biochemistry* 1996;26S:236-257-reference grouped under **B. II.**), a typical cup of green tea contains 710 $\mu\text{g/ml}$ (-)-epigallocatechin gallate (EGCG) when a quality Chinese green tea was used for the brewing. Therefore, it is reasonable to assume that this is the typical green tea which is consumed by the green-tea-drinking residents living in the tea-producing prefectures and provinces in Japan and China where most of epidemiological studies referred to in this petition were conducted. A green tea containing 708 $\mu\text{g/ml}$ EGCG has been used successfully to suppress the *in vivo* growth of a human malignant lymphoma transplanted to mice (Bertolini F, Fusetti L, Rabascio C, Cinieri S, Martinelli G, Pruneri G. Inhibition of angiogenesis and induction of endothelial and tumor cell apoptosis by green tea in animal models of human high-grade non-Hodgkin's lymphoma. *Leukemia* 2000;14:1477-82). In order to provide the consumers a green tea containing about 700 $\mu\text{g/ml}$ EGCG when the tea is brewed at a 1:100 w/v dry tea leaf-to-water ratio, the petitioner establishes that the green tea leaves must contain at least 7% EGCG in dry weight. The green tea leaves that the petitioner intends to market and that will bear the proposed model health claim will meet this specification in the contents of EGCG.

Representative samples of the quality green teas which will bear the proposed model health claim have been analyzed by a qualified independent laboratory, Center for Advanced Food Technology, Rutgers University. The analytical results showed that the contents of EGCG in six random green tea samples provided by Fleminger, Inc. were as follows:

Sample a. #1 tea	%EGCG = 8.15+/-0.46
Sample b. #2 tea	%EGCG = 7.61+/-0.26
Sample c. #3 tea	%EGCG = 8.21+/-0.47
Sample d. A tea	%EGCG = 9.25+/-0.59
Sample e. B tea	%EGCG = 8.95+/-0.70
Sample f. C tea	%EGCG = 8.41+/-0.61

Since there are no available methods from the Association of Official Analytical Chemists (AOAC), an assay method for determination of EGCG in green tea and data establishing the validity of the method for assaying EGCG contents in the green tea leaf samples are also submitted in this filing.

See separate report issued by Center for Advanced Food Technology, Rutgers University, for green tea EGCG analyses and validation of the analytical method dated January 5, 2004 and addressed to Dr. Paulina Chan, Dr. Lee's Tea For Health, Fleminger, Inc.

D. MODEL HEALTH CLAIM

The petitioner seeks granting of one FDA Category B Qualified Health Claim as follows, including all words in bold face letters:

Daily consumption of 40 ounces of typical green tea* may reduce the risk of certain forms of cancer. Although there is scientific evidence supporting the claim, the evidence is not conclusive.

Note * A typical cup of green tea contains 710 µg/ml natural (-)-epigallocatechin gallate (EGCG) [National Cancer Institute: *J Cell Biochemistry* 1996;26S:236-257].

1. A brief capsulized statement of the relevant conclusions of the summary:

A survey of the world scientific literature showed that in the 40 epidemiological studies, drinking green tea has been found to be inversely associated with risk of human cancers in 32 of the 40 studies, indicating a protective effect of green tea against cancer development. Eight (8) of the 40 studies did not provide evidence for an association between drinking green tea and a reduction of cancer incidence. As analyzed under **B. Summary of Scientific Data** pages 136-139, the lack of association between drinking green tea and a reduction of cancer risk reported in these 8 of the 40 articles cited above was probably the results of an insufficient intake of the green tea bioactive ingredients by the participants- a reflection of dose-dependent relationship, inappropriate research designs or the influence of confounding factors in these studies. After careful review of the world literature, the scientific evidence in support of the proposed model health claim outweighs the scientific evidence against such claim, i.e. regular consumption of green tea brewed with quality natural tea leaves of *Thea sinensis* in sufficient quantity may reduce the risk of certain forms of cancer, in particular cancer of the esophagus, stomach, pancreas, colorectum, urinary bladder, lung, breast, liver, uterus and ovary. No adverse reaction has been observed in association with green tea consumption as a dietary supplement.

2. A statement of how this substance helps the consumers to attain a total dietary pattern or goal associated with the health benefit that is provided.

The substance claimed is dry natural green tea leaf, commonly known as green tea, processed by lightly heating fresh tea leaves at the plantation site after harvest to inactivate the intrinsic polyphenol oxidase activity to preserve the tea antioxidants. EGCG is the most abundant and active antioxidant in green tea. EGCG and other tea catechins have degraded to various degrees during manufacturing of black tea and oolong tea by oxidation. Although the mechanism of the anticancer effect of green tea is still poorly understood and may involve multiple focuses at the molecular and cellular level by blocking the initiation, promotion and progression of cancer development, the most studied anticancer ingredient of green tea is EGCG. However, other components in green

tea, such as theanine and other forms of polyphenols and catechins, even tea caffeine, may play a complex role in concert in combat of cancers.

Green tea inhibits the formation of carcinogen from pro-carcinogens in the foodstuff ingested in the stomach, inhibits the hepatic enzymes which are responsible for converting pro-carcinogens into active carcinogens in the body, activates the phase II enzymes to detoxify the carcinogens, inhibits the effects of exogenous tumor promoters, inhibits the formation of endogenous carcinogens, reduces the DNA damage caused by carcinogens, e.g. ultraviolet irradiation, through neutralization of free radicals, inhibits the telomerase activity, inhibits DNA topoisomerases, induces cancer cell apoptosis via the mitochondrial pathway, inhibits activation of transcription factors in the signal transduction pathway, inhibits angiogenesis needed for rapid tumor growth, and inhibits the proteolytic enzymes needed to establish cancer metastases. The anticancer activities of green tea are widely ranged starting at the formation of exogenous carcinogens in the stomach to the stage of tumor metastasis, the most dangerous phase that often ends the life of the patient.

The anticancer effects of green tea or its components, especially EGCG, can be readily demonstrated in the research laboratories and experimental animal models under ideal controlled conditions, as evidenced by the increasing number of scientific reports published offering such proof in the past five years. However, epidemiological studies have not yielded unanimous results in the chemopreventive benefits of drinking green tea against human cancers. For example, a benefit of cancer prevention is usually observed in a population known to consume a large volume, i.e. a minimum of 1200 ml of high quality green tea regularly, as in Saitama, Aichi and Shizuoka, the well-known tea-producing prefectures in Japan, but not obvious among the residents surveyed in Miyagi, a northern rural region of Japan where there are no tea plantations. The fact that the quality of the tea and the dose of the tea consumed may determine the outcomes of the cancer chemoprevention in a tea-drinking population only became known in the past few years.

Based on the typical concentration adopted by the National Cancer Institute, DCPC Chemoprevention Branch and Agent Development Committee for green tea epigallocatechin gallate in a report published in 1996, the subsequent animal studies and phase I clinical trial protocols both using this typical green tea concentration, the petitioner has introduced the 7% EGCG contents as the minimum level in dry weight for the specification of quality green tea leaves. The experience of the petitioner with several responsible tea plantations has shown that when properly processed, the green tea leaves should contain 7-14% EGCG in dry weight at the site of harvest, depending on the growing and environmental conditions of the plantation, as demonstrated in the reports of analysis attached in part 21 C.F.R.101.70(f)(C) of this petition.

E. ATTACHMENTS

1. A PubMed literature search at the website www.ncbi.nih.gov using two combinations of key search words, "Green tea and cancer risk" and "Green tea and cancer epidemiology" to search the Internet on June 12, 2003 yielded a total of 136 and 55 titles of publications, respectively. Copies of these two printouts are attached herewith.

2. Copies of the articles cited in the literature searches and other information as follows:

- a) Copies of 135 epidemiological publications which were relied upon for the support of the health claim relating to organ-specific and overall cancer rate analyses.

Copies of 49 publications including review articles and comments made by experts on using green tea as a chemopreventive beverage in cancer risk reduction.

Copies of 36 scientific publications relating to laboratory and experimental animal research on the anticancerous mechanisms of green tea and its components.

- b) All information concerning adverse consequences: A PubMed literature search at the website www.ncbi.nih.gov using a combination of the key search words, "Adverse reactions to green tea" on October 14, 2003 yielded one title of publication:

Weisburger JH. Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. Food Chem Toxicol. 1999 Sep-Oct;37(9-10):943-8. (attached as following page 151 a).

Copy of the fulltext of this article is enclosed as Reference 44 in Section **B. II.** of this petition. In this article, no adverse reactions due to green tea were listed.

- c) Table of statistics of the 1993-1997 Cancer Incidence and Mortality Rates in Connecticut and in the United States of America is attached (as following page 151 b). It shows that the combined incidence rate of cancers of the breast, colorectum, liver, lung, pancreas, stomach and urinary bladder, the malignant tumors against which green tea has been shown to be protective in this petition, is 395.3 per 100,000, about a half of the total human cancer cases in the US. Since green tea is a non-toxic chemopreventive beverage, when used properly it may benefit a large segment of the population by lowering their cancer risk without any adverse effects.

F. DECLARATION OF CATEGORICAL EXCLUSION from an environmental assessment (EA) or an environmental impact statement (EIS) [(21 C.F.R. 101. 70 (f)(F)] :

The Petitioner declares that the proposed health claims are categorically excluded from an EA or EIS under 21 C.F.R. 25.32 (p).

21 C.F.R.101.70(c) and 21 C.F.R.101.70(d) declaration

To the best of the Petitioner's knowledge, all non-clinical studies relied upon were conducted in compliance with the good laboratory practice regulations as set forth in 21 C.F.R. Part 58, and all clinical or other human investigations relied upon were either conducted in accordance with the requirements for institutional review set forth at 21 C.F.R. Part 56 or were not subject to such requirements in accordance with 21 C.F.R. 56.104 or 56.105, and were conducted in conformance with the requirements for informed consent set forth in 21 C.F.R. Part 50.

TO THE BEST OF THE KNOWLEDGE, INFORMATION AND BELIEF OF THE UNDERSIGNED, THIS PETITION CONSTITUTES A REPRESENTATIVE AND BALANCED SUBMISSION THAT INCLUDES UNFAVORABLE AS WELL AS FAVORABLE INFORMATION KNOWN TO THE UNDERSIGNED TO BE PRETINENT TO THE EVALUATION OF THE PROPOSED HEALTH CLAIM.

Yours very truly,

Petitioner: Fleminger, Inc.

By 
Sin Hang Lee, MD, President