[Frontiers in Bioscience 12, 2309-2315, January 1, 2007]

Green tea polyphenols in the prevention of colon cancer

Nagi Kumar[1], David Shibata[2], James Helm[2], Domenico Coppola [3] , Mokenge Malafa[2]

[1]Division of Health Outcomes and Behavior,H. Lee Moffitt Cancer Center and Research Institute, University of South Florida College of Medicine, Tampa, Florida, [2]Gastrointestinal Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, University of South Florida College of Medicine, Tampa, Florida, [3]Anatomical Pathology, Dept. of Interdisciplinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, University of South Florida College of Medicine, Tampa, Florida

TABLE OF CONTENTS

1. ABSTRACT

Several plant-based nutrients and non-nutrients that can inhibit mutagenesis and proliferation have been identified. Some of the most promising nutrients identified as chemopreventive agents in colon cancer prevention include isoflavones, curcumin, calcium, Vitamin D and more recently Green tea polyphenols (GTP). In addition to inhibiting mutagenesis and proliferation, these compounds are relatively non-toxic, are of low cost and can be taken orally or as a part of the daily diet. Epidemiological and laboratory studies have identified epigallocatechin gallate (EGCG) in green tea polyphenols (GTP), as the most potent chemopreventive agent that can induce apoptosis, suppress the formation and growth of human cancers including colorectal cancers (CRC). It is only logical then, that future clinical studies should focus on examining the efficacy of phytochemicals such as EGCG in cancer chemoprevention as an alternative to pharmacological agents, especially in populations where administration of COX-2 inhibitors, Aspirin and NSAIDS is contraindicated. The goal of this review is to provide the rationale, and discuss the use of EGCG in GTP as a chemopreventive agent for prevention of colon carcinogenesis and present evidence for the efficacy and safety of these agents based on epidemiological, animal, *in vitro* studies and Phase I clinical trials.

2. INTRODUCTION

It is estimated that in 2006, 148,610 new cases of colorectal cancer (CRC) will be diagnosed in the US and 55,170 deaths are expected with this disease making colorectal cancer the third leading cause of cancer death in the US (1). Several chemoprevention trials were initiated utilizing promising agents such as NSAIDS and COX 2 inhibitors to reverse, suppress or delay the process of colorectal carcinogenesis, all of which were stopped early for demonstrating a 2.5-fold increased risk of major fatal and non-fatal cardiovascular events for participants taking the drug compared to those on a placebo (2). Long-term use of Aspirin and NSAIDS, although observed to be effective in colorectal cancer prevention, carries with it a substantial risk of toxicity including gastro-intestinal (GI) symptoms such as dyspepsia, gastro intestinal hemorrhage and peptic ulcers (3). Furthermore, large multicenter trials suggest that some of the NSAIDs produce a small, but significant increase in serious cardiovascular events (4).

Colon carcinogenesis is a multi-step process with aberrant crypt foci (ACF) as an intermediate step in the development of cancer (6). ACF are the earliest visually detectable lesions in this pathway to colorectal cancer. An ACF is defined as a collection of crypts with abnormal lumina and thickened epithelium. Conceptually, dysplastic

ACF can be considered to be "microadenomas": they are prevalent in subjects with familial adenamatous polyps (FAP), share common gene mutations with more advanced CRC, and can potentially be modulated by chemopreventive agents. Furthermore, the median number of rectal ACF has been observed to be higher in subjects with sporadic adenomas and among CRC subjects compared to controls (5,7). Although definitive proof is still lacking, such observations strongly implicate ACF as an intermediate endpoint biomarker of CRC. Moreover, the accuracy with which this endpoint can be measured is now optimized with the introduction of the new generation of magnification chromoendoscopes with high specificity (97%) and sensitivity (100%) (8). These features of CRC, namely, high prevalence, long latency, and significant mortality and morbidity, the lack of safe chemopreventive agents and the availability of ACF as an intermediate stage of CRC progression, provide the most opportunistic and promising approach for evaluating agents for chemoprevention.

3. GREEN TEA POLYPHENOLS: PROMISING NUTRIENTS FOR COLON CANCER PREVENTION

The health effects of brewed green tea are attributed to numerous polyphenolic compounds, which represent 30% of dried leaf extract (69) . These compounds include flavonols, flavandiols, flavonoids, and phenolic acids; however, most of the polyphenols found in green tea are monomeric flavan-3-ols, better known as catechins, including: (+)-catechin (C), (–)- epicatechin (EC), (+) gallocatechin (GC), (–)-epigallocatechin (EGC), (–)- epicatechin gallate (ECG), and (–)- epigallocatechin gallate (EGCG). Among the catechins in green tea, one has predominated in scientific scrutiny and is the target of promising anticancer research. It is EGCG. The others are found to be both less abundant and less pharmacologically active (70-71).

4. EGCG IN COLON CANCER: EVIDENCE FROM EPIDEMIOLOGICAL STUDIES

Recent epidemiological studies have also demonstrated the cancer preventive properties of green tea polyphenols (18) in CRC. In a large population-based case-control study conducted in Shanghai, China, newly diagnosed cancer cases (931 colon, 884 rectum and 451 pancreas) during 1990-1993 among residents 30-74 years of age were compared to controls (n = 1,552) selected among Shanghai residents and frequency-matched to cases by gender and age. Multivariate odds ratios (ORs) and 95% confidence intervals (Cls) of each cancer associated with green tea consumption were derived after adjustment for age, income, education and cigarette smoking. Additional adjustment for dietary items and body size was found to have minimal impact. An inverse association with each cancer was observed with increasing amount of green tea consumption, with ORs among those in the highest tea consumption category (≥300 g/month) were 0.82 for colon cancer, 0.72 for rectal cancer and 0.63 for pancreatic cancer, with p values for trend being 0.38, 0.04 and 0.04, respectively. For women, the respective ORs for the highest consumption category (≥200 g/month) were 0.67, 0.57 and 0.53, with the respective p values for trend being 0.07, 0.001 and 0.008. These findings provide evidence that green tea drinking may lower the risk of colorectal cancers (18). Of all the tea produced worldwide, about 20% of green tea is consumed in Asian countries such as China, Japan, Korea and India, where populations regularly drinking more cups of tea, have a lower risk of colon, prostate and breast cancers (19-20) In contrast, a number of other ecological, cohort, and case-control studies have associated an increased risk for cancer of the breast, colorectum, esophagus, kidney, lung, pancreas, and stomach with tea intake (21-24). The conflicting epidemiological results have been attributed to geographic variations in active ingredients in the teas consumed in the in various parts of the world and confounding factors related to consumption of coffee and other nutrients that may have an impact on the CR epithelia. Other confounding factors include consumption of salted or very hot tea, use of tobacco and alcohol, and lack of standardization of quantities and compositions of the tea products consumed, varying data collection instruments used to measure tea intake and observation vs. reported measures of exposure (25). Thus the potential preventive properties of GTP in CRC, as demonstrated by evidence from epidemiological studies although limited, appears promising.

5. EGCG IN COLON CANCER: EVIDENCE FROM ANIMAL STUDIES

Several published nonclinical studies using green tea, green tea leaves, green tea extracts, green tea polyphenol mixtures, green tea catechin mixtures, and the individual catechins have demonstrated chemopreventive efficacy in several cancers, including colorectal cancers. Suppression of azoxymethane-induced preneoplastic lesions and inhibition of cyclooxygenase-2 activity in the colonic mucosa of rats drinking a crude green tea extract demonstrated that GTE reduces cox-2 and suppresses the formation of colonic preneoplastic lesion, providing insight into the mechanism of chemopreventive activity and anti-inflammatory properties of GTP (26). Using a similar model Ju *et al* (27)observed that GT inhibits ACF formation even in mice fed a high corn diet, suggesting the potential to inhibit colon carcinogenesis in population such as those in the US that consume a high fat diet. In another study to investigate the chemopreventive effect of green tea and tea pigments on DMH-induced rat colorectal carcinogenesis, a significant reduction in ACF formation was observed at the end of 16 weeks ($P<0.01$) (28), in the group on green tea and green tea pigments compared to controls, and that these effects may be related to the suppression of cell proliferation in the intestinal crypts (13). Other data also support the synergistic chemopreventive effect of GTP administered with sulindac against intermediate and late stages of colon cancer, via effects on the betacatenin/Tcf signaling pathway in the Apc (min) mouse model (29) and in rats treated with AOM by enhancing apoptosis (30), using ACF as the IEB. Similar synergistic effects have been observed in reduction in number of ACF with green tea and phytic acid (31). Studies on the efficacy of GTP in rodent models appear promising

and have elucidated the effectiveness and several potential mechanisms of action in addition to validating ACF as a IEBs of CRC progression.

6. EGCG IN COLON CANCER: EVIDENCE FROM *IN VITRO* STUDIES

Tea and tea compounds reduce growth and/or induce apoptosis in several human cancer cell lines *in vitro*, including stomach, lung, prostate, colon, leukemia, oral tumor, liver, breast, and cervix, as well as HPV-immortalized cervical epithelial cells. EGCG has been shown to affect several signal transduction pathways *in vitro* in the colon and/or colon cancer cell lines. The ubiquitin/proteasome system plays an important role in the degradation of cellular proteins. This proteolytic system includes two distinct steps: ubiquitination and degradation (9-10). By using various proteasome inhibitors, several recent studies have suggested that the ubiquitin/proteasome pathway plays an essential role in the regulation of apoptosis. Activation of the cellular apoptotic program is a current strategy for treatment of human cancers. Our Drug Discovery group found that CEP1612, a dipeptidyle proteasome inhibitor, was able to rapidly induce apoptosis in all the human cancer cell lines tested, including breast, prostate, colon, leukemia, lung, bone, brain, and head and neck, but not in human normal fibroblasts and normal breast cells (9-10, 32-33). The proteasome has been proven to be an excellent target for developing anticancer drugs (34-35) . For example, in animal studies, proteasome inhibitors suppress tumor growth and angiogenesis as either a single drug or in combination with other cytotoxic agents (36-37). More recent studies of proteasome inhibitors such as PS-341 (bortezomib, Velcade) in animal models and in patients with hormone refractory prostate cancer, resulted in both PSA and tumor volume decrease demonstrating clinical application in prostate cancer therapy. However, there are several side effects including nausea, fatigue, diarrhea, peripheral neuropathy, rapidly reversible reduction in platelets, and reversible thrombocytopenia observed with Velcade and Bortezomib.(38-41). Therefore, it is necessary to identify less toxic proteasome inhibitors with similar potency to PS-341 or different novel proteasome inhibitors. As observed by our group at the Moffitt Cancer Center , EGCG, a nutrient-derived agent, potently and selectively inhibits the proteasome activity in intact human colon cancer cells and consequently accumulates IkB-α and p27 proteins, leading to growth arrest (9-10) proving its potential as a promising proteasome inhibitor without the toxicities of Velcade and Bortezomib, thus warranting further evaluation in clinical trials.

In addition, EGCG has been shown to inhibit activation of the RTKs EGFR, HER2 and HER3, which are associated with inhibition of multiple downstream signaling pathways. The IGF/IGF-1R system, which is also an RTK, and includes IGF, IGF-1R and IGFBPs proteins has been shown to play an important role in the development of CRC. EGCG is an inhibitor of critical RTKs involved in cell proliferation (42), EGCG has also been shown to inhibit angiogenesis through blocking the induction of VEGF (Jung YD *et al*) and to inhibit topoisomerase I activity in human colon carcinoma cells (44) thus demonstrating anti-tumor properties. Research has demonstrated that GTP can affect the cyclooxygenase (COX)-and lipoxygenase (LOX)-dependant arachidonic acid metabolism in human colon mucosa and colon tumors, which may alter the risk of CRC (45). Induction of apoptosis in colon cancer cells has also been observed via the TGF-beta superfamily protein , NAG-1 (Non-Steroidal anti-inflammatory drug Activated Gene(46). Other effects of EGCG in downregulating genes involved in the inflammatory pathways include impairment of chemokine production in colon epithelial cells (47). In summary, the *in vitro* studies indicate that EGCG is a promising chemopreventive agent, with several cellular effects, which are both genomic and non-genomic, which are critical elements required to effect chemoprevention.

7. EGCG IN COLON CANCER: EVIDENCE FROM CLINICAL TRIALS

Several Phase I studies, sponsored by the National Cancer Institute NCI, DCP, comparing the pharmacokinetics and safety of oral green tea, Polyphenon E and EGCG, have been completed and published (48-50,64-65). By conducting a Phase I trial of oral green tea extract (caffeine-free) in adult patients with solid tumors, Pisters *et al.* (59) reported that a safe dose of green tea extract was equivalent to 7-8 Japanese cups (120 ml) of green tea three times daily for six months. They concluded that the side effects (neurological and gastrointestinal effects) of the green tea extract preparation were caffeine-related, and not from EGCG. The average cup of green tea contains 10-50 mg of caffeine therefore, over-consumption may have a stimulatory effect in some individuals. Thus, although green tea is practical, non-toxic and has the potential to be developed for chemoprevention, one has to consume 8-10 cups to obtain these benefits and the side effects due to caffeine are a concern.

In a single-dose study, each subject received 200, 400, 600 or 800 mg EGCG provided by each of two formulations separated by a two-week wash-out period (49). Plasma EGCG levels increased with dose, and formulation had no effect on EGCG pharmacokinetics. Although little EGCG circulated in conjugated form, EGC and EC were highly conjugated. In a completed multidose study, healthy subjects with sun-sensitive skin received 800 mg EGCG alone or as Polyphenon E in one or divided daily doses for four weeks [50]. Adverse effects were predominantly mild gastrointestinal complaints. Plasma levels of EGCG were significantly higher in the 800 mg qd versus 400 mg bid dose group for both formulations. Plasma antioxidant levels and the UV light-induced minimum erythema dose (MED) were unaffected by any treatment. In the third completed Phase I study, the effect of fasting on pharmacokinetics was examined in healthy adults taking 400, 800 or 1200 mg EGCG as Polyphenon E. Plasma levels of free EGCG were dramatically higher when taking Polyphenon E in the fasting state compared to the fed state for all three dose levels; the average Cmax increased more than 3.6-fold, and the average AUC

increased more than 2.3-fold when taking Polyphenon E on an empty stomach. In a study to test if oral bioavailability of green tea catechins can be enhanced when consumed in the absence of food, Chow *et al* observed that greater bioavailability of free catechins can be achieved by taking the Polyphenon E capsules on an empty stomach after an overnight fast thus optimizing the biological effects of tea catechins. (48). With its initial safety information and the significantly reduced caffeine content (~ <1%), Polyphenon E has potential to be developed into an effective cancer-preventative and therapeutic drug with little or no toxicity. However, to date, human clinical trials evaluating the effectiveness of green tea polyphenols in a colon cancer model are not available. Evidence from these phase I studies on safety warrants further evaluating the safety and efficacy of this agent in phase II clinical trials as the logical next step.

8. INTERMEDIATE ENDPOINT BIOMARKERS FOR COLON CANCER

The malignant potential of aberrant crypt foci (ACF), especially dysplastic ACF, is widely accepted as precursors of CRC morphologically, histologically, biologically and genetically. ACF was first described by Bird *et al* as lesions consisting of large, thick crypts in methylene blue-stained specimens of colon from mice treated with azoymethane. Since this report, several reports have been published demonstrating progression of ACF with cancer promoters in rodent models, growing larger with more marked nuclear atypia or dysplasia and suppression with chemopreventive agents such as aspirin (51) and other nutrient-derived chemoprevention agents such as green tea, trans-resveratrol (52) indole-3 carbinol (53), miso (54)and naringin in grapefruit (55). Similar to those observed in the rodent model, ACF have also been reported in colonic mucosa in humans and reportedly more frequently observed in patients with colon cancer than those with non-cancerous lesions [51] and displays variable histologic features, ranging from hyperplasia to dysplasia. ACF in human colon are more frequently located in the distal relative to the proximal parts, which is in accordance with those with CRC. The immunohistochemical expressions of carcnoembryonic antigen (CEA), B-catenin, placental cadherin (P-cadherin), epithelial cadherin (E-cadherin), inducible nitric oxide synthetase(iNOS),cyclooxygenase (COX-2), and P16 ^{INK4a} are found to be altered. Genetic mutations of K-ras, APC and p53, and epigenetic alterations of CpG island methylation of ACF have also been demonstrated. Genomic instabilities due to the defect of mismatch repair (MMR) system are detectable in ACF. Based on the evidence from the literature, two distinct hypotheses have been proposed- the dysplasia ACF-adenoma-carcinoma sequence and the herteroplastic ACF -adenoma-carcinoma sequence. In the classic report of Yokota *et al* (1997) (57), the application of magnification chromocolonoscopy made it possible to detect "*in vivo*" ACF, which has now become a standard clinical screening tool to identify high-risk populations for CRC. ACF is thus identified as a valid intermediate endpoint biomarker of CRC in chemoprevention trials, especially with the introduction of the new generation of magnification chromoendoscopes with high specificity (97%) and sensitivity (100%)(8).

9. FUTURE DIRECTIONS

Although there is evidence from epidemiological and preclinical studies that specific components of GTP such as EGCG are linked to CRC and prevention of other cancers, the specific mechanisms and sites of action of these nutrient-derived substances continue to remain elusive. Currently, although several clinical trials using green tea or (-)-EGCG have been, or are currently being conducted in prostate and other cancers, to our knowledge, none are examining this agent in CRC using ACF and the primary IEB nor the proteasome, as a molecular target for CRC in a phase II clinical trial. There is an urgent need to continue to address how non-toxic bioactive food components such as EGCG regulate cancer progression, working in collaboration with a multidisciplinary team of scientists in an effort to ultimately optimize effective dietary intervention strategies for cancer prevention.

10. REFERENCES

1.Cancer Facts and Figures. American Cancer Society, Atlanta, Georgia (2006)

2. NIH Halts Use of COX-2 Inhibitor in Large Cancer Prevention Trial**.** National Cancer Institute. http://www.nih.gov/news/pr/dec2004/od-17Q&A.htm December 17, 2004.

3. C. A. Silagy, J. J. McNeil, G. A. Donnan, A. M. Tonkin, B. Worsam, K. Campion: Adverse effects of low-dose aspirin in a healthy elderly population. *Clin Pharmacol Ther* 54(1), 84-89 (1993)

4. D. Henry, L. L. Lim, L. A. Garcia Rodriguez, S. Perez Gutthann, J. L. Carson, M. Griffin, R. Savage, R. Logan, Y. Moride, C. Hawkey, S. Hill, J. T. Fries: Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. *BMJ* 312(7046), 1563-1566 (1996)

5. L. Cheng: Aberrant crypt foci as microscopic precursors of colorectal cancer. *World J Gastroenterol* 9(12), 2642-2649 (2003)

6. R. Nascimbeni, V. Villanacci, P. P. Mariani, E. Di Betta, M. Ghirardi, F. Donato, B. Salerni: Aberrant crypt foci in the human colon: frequency and histologic patterns in patients with colorectal cancer or diverticular disease. *Am J Surg Pathol* 23(10), 1256-1263 (1999)

7. T. Takayama, S. Katsuki, Y. Takahashi, M. Ohi, S. Nojiri, S. Sakamaki, J. Kato, K. Kogawa, H. Miyake, Y. Niitsu: Aberrant crypt foci of the colon as precursors of adenoma and cancer. *N Engl J Med* 339(18),1277-1284 (1998)

8. Kiesslich R, Neurath MF. Chromo- and magnifying endoscopy for colorectal lesions.Eur J Gastroenterol Hepatol. 2005 Aug;17(8):793-801. Review.

9. S. Nam, D. M. Smith, Q. P. Dou: Ester Bond-containing Tea Polyphenols Potently Inhibit proteasome Activity *in vitro* and *in vivo*. *The Journal of Biological Chemistry* 276(16),13322-13330 (2001)

10. A. Kazi, D. M. Smith, K. G. Daniel, Q. Zhong, P. Gupta, M. E. Bosley and Q. P. Dou: Potential Molecular Targets of Tea Polyphenols in Human Cancer: Significance in Cancer Prevention (invited review). *IN VIVO* 16, 1-7 (2002)

11. W. S. Jeong, I. W. Kim, R. Hu, A. N. Kong: Modulatory properties of various natural chemopreventive agents on the activation of NF-kappaB signaling pathway. *Pharm Res* 21(4), 661-670 (2004)

12. N. Ahmad, S. Gupta, H. Mukhtar: Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappaB in cancer cells versus normal cells. *Arch Biochem Biophys* 376(2), 338-346 (2000)

13. X. Jia, C. Han: Effects of green tea on colonic aberrant crypt foci and proliferative indexes in rats. *Nutr Cancer* 39(2), 239-243 (2001)

14. N. B. Kumar, A. Cantor, K. Allen, D. Riccardi, K. Besterman-Dahan, J. Seigne, M. Helal, J. Salup & J. Powsang: The Specific Role of Isoflavones in Reducing Prostate Cancer Risk. *Prostate* 59, 2 (2004)

15. N. B. Kumar, K. A. Allen, A. Cantor, D. Riccardi & C. E. Cox: The Specific Role of Isoflavones on Estrogen Metabolism in Pre-menopausal Women. *Cancer* 94(4), 1166-1174 (2002)

16. A. Kazi, K. G. Danie, D. M. Smith, N. B. Kumar, Q. P. Dou: Inhibition of the proteasome activity, a novel mechanism associated with the tumor cell apoptosis-inducing ability of genistein. *Biochem Pharmacol* 66(6), 965-976 (2003)

17. A. Kazi, D. A. Urbizu, D. J. Kuhn, A. L. Acebo, E. R. Jackson, G. P. Greenfelder, N. B. Kumar, Q. P. Dou: A natural musaceas plant extract inhibits proteasome activity and induces apoptosis selectively in human tumor and transformed, but not normal and non-transformed, cells. *Int J Mol Med* 12(6), 879-887 (2003)
87.

18. B. T. Ji, W. H. Chow, A. W. Hsing, J. K. McLaughlin, Q. Dai, Y. T. Gao, W. J. Blot, J. F. Fraumeni Jr.: Green tea consumption and the risk of pancreatic and colorectal cancers. *Int J Cancer* 70(3), 255-258 (1997)

21. P. Terry, A. Wolk: Tea consumption and the risk of colorectal cancer in Sweden. *Nutr Cancer* 39(2), 176-9 (2001)

22. T. J. Hartman, J. A. Tangrea, P. Pietinen, N. Malila, M. Virtanen, P. R. Taylor: Albanes D. Tea and coffee consumption and risk of colon and rectal cancer in middle-aged Finnish men. *Nutr Cancer* 31(1), 41-48 (1998)

23. Y. Suzuki, Y. Tsubono, N. Nakaya, Y. Koizumi, Y. Suzuki, D. Shibuya, I. Tsuji: Green tea and the risk of colorectal cancer: pooled analysis of two prospective studies in Japan. *J Epidemiol* 15(4), 118-124 (2005)

24. J. R. Cerhan, S. D. Putnam, G. D. Bianchi, A. S. Parker, C. F. Lynch, K. P. Cantor: Tea consumption and risk of cancer of the colon and rectum. *Nutr Cancer* 41(1-2), 33-40 (2001)

25. L. Arab, D. Il'yasova: The epidemiology of tea consumption and colorectal cancer incidence. *J Nutr* 133(10), 3310S-3318S. Review. (2003)

26. N. Metz, A. Lobstein, Y. Schneider, F. Gosse, R. Schleiffer, R. Anton, F. Raul: Suppression of azoxymethane-induced preneoplastic lesions and inhibition of cyclooxygenase-2 activity in the colonic mucosa of rats drinking a crude green tea extract. *Nutr Cancer* 38(1), 60-64 (2000)

27. J. Ju, Y. Liu, J. Hong, M. T. Huang, A. H. Conney, C. S.Yang: Effects of green tea and high-fat diet on arachidonic acid metabolism andaberrant crypt foci formation in an azoxymethane-induced colon carcinogenesis mouse model. *Nutr Cancer* 46(2), 172-8 (2003)

28. X. Jia, W. Wang, W. Cui, C. Han: [Effects of tea on aberrant crypt foci and colorectal tumors in rats] *Wei Sheng Yan Jiu* 29(1), 54-56 (2000)

29. G. A. Orner, W. M. Dashwood, C. A. Blum, G. D. Diaz, Q. Li, M. Al-Fageeh, N. Tebbutt, J. K. Heath, M. Ernst, R. H. Dashwood: Response of Apc(min) and A33 (delta N beta-cat) mutant mice to treatment with tea, sulindac, and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). *Mutat Res* 506-507, 121-127 (2002)

30. T. Ohishi, Y. Kishimoto, N. Miura, G. Shiota, T. Kohri, Y. Hara, J. Hasegawa, M. Isemura: Synergistic effects of (-)-epigallocatechin gallate with sulindac against colon carcinogenesis of rats treated with zoxymethane. *Cancer Lett* 177(1), 49-56 (2002)

31. A. Challa, D. R. Rao, B. S.: Interactive suppression of aberrant crypt foci induced by azoxymethane in ratcolon by phytic acid and green tea. *Carcinogenesis* 18(10), 2023-2026 (1997)

32. Q. P. Dou, T. F. McGuire, Y. Peng, B. An: Proteasome inhibition leads to significant reduction of Bcr-Abl expression and subsequent induction of apoptosis in K562 human chronic myelogenous leukemia cells. *J Pharm. Exp Ther* 289, 781-790 (1999)

33. B. An, R. H. Goldfarb, R. Siman, Q. P. Dou: Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. *Cell Death Differ* 5, 1062-75 (1998)

34. C. Tan, T. A. Waldmann: Proteasome inhibitor PS-341, a potential therapeutic agent for adult T-cell leukemia. *Cancer Res* 62(4), 1083-1086 (2002)

35. J. Adams: Development of the proteasome inhibitor PS-341. *Oncologist* 7, 9-16 (2002)

36. Q. P. Dou, & R. H. Goldfarb: Evaluation of the proteasome inhibitor MLN-341 (PS-341). *Curr Drugs Ltd* In Press (2002)

37. J. Adams. Potent and selective inhibitors of the proteasome: dipeptidyl boronic acids. *Bioorg Med Chem Lett* 8, 333-338. (1998)

38. A. Tahmatzopoulos, C. Gudegast, M. Stockle, B. Wullich, G. Unteregger, U. Zwergel, T. Zwergel: Proteasome inhibitors: induction of apoptosis as new therapeutic option in prostate cancer. *Aktuelle Urol* 35(6), 491-496 (2004)

39. C. N. Papandreou, C. J. Logothetis: Bortezomib as a potential treatment for prostate cancer. *Cancer Res* 64(15), 5036-5043 (2004)

40. J. Adams, M. Kauffman: Development of the proteasome inhibitor Velcade (Bortezomib). *Cancer Invest* 22(2), 304-311 (2004)

41. C. N. Papandreou, D. D. Daliani, D. Nix, H. Yang, T. Madden, X. Wang, C. S. Pien, R. E. Millikan, S. M. Tu, L. Pagliaro, J. Kim, J. Adams, P. Elliott, D. Esseltine, A. Petrusich, P. Dieringer, C. Perez, C. J. Logothetis: Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. *J Clin Oncol* 22(11), 2108-2121 (2004)

42.M. Shimizu, A. Deguch, A. K. Joe, J. F. Mckoy, H. Moriwaki, I. B. Weinstein: EGCG inhibits activation of HER3 and expression of cyclooxygenase-2 in human colon cancer cells. *J Exp Ther Oncol* 5(1), 69-78 (2005)

43. Y. D. Jung, M. S. Kim, B. A. Shin, K. O. Chay, B. W. Ahn, W. Liu, C. D. Bucana, G. E. Gallick, L. M. Ellis: EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. *Br J Cancer* 84(6), 844-850 (2001)

44. S. J. Berger, S. Gupta, C. A. Belfi, D. M. Gosky, H. Mukhtar: Green tea constituent (--)-epigallocatechin-3-gallate inhibits topoisomerase I activity in human colon carcinoma cells. *Biochem Biophys Res Commun* 288(1), 101-105 (2001)

45. J. Hong, T. J. Smith, C. T. Ho, D. A. August, C. S. Yang: Effects of purified green and black tea polyphenols on cyclooxygenase- and lipoxygenase-dependent metabolism of arachidonic acid in human colon mucosa and colon tumor tissues. *Biochem Pharmacol* 62(9),1175-1183 (2001)

46. S. J. Baek, J. S. Kim, F. R. Jackson, T. E. Eling, M. F. McEntee, S. H. Lee: Epicatechin gallate-induced expression of NAG-1 is associated with growth inhibition and apoptosis in colon cancer cells. *Carcinogenesis* 2004 25(12),2425-2432 Epub (2004)

47. D. Porath, D. Riegger, J. Drewe, J. Schwager: Epigallocatechin-3-gallate impairs chemokine production in human colon epithelial cell lines. *J Pharmacol Exp Ther* 315(3),1172-1180 Epub (2005)

48. H. H. Chow, I. A. Hakim, D. R. Vining, J. A. Crowell, J. Ranger-Moore, W. M. Chew, C. A. Celaya, S. R. Rodney, Y. Hara, D. S. Alberts: Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. *Clin Cancer Res* 2005 11(12), 4627-4633 (2005)

49. H. H. Chow, Y. Cai, D. S. Alberts, I. Hakim, R. Dorr, F. Shahi, J. A. Crowell, C. S. Yang, Y. Hara: Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiol Biomarkers Prev* 10(1), 53-58 (2001)

50. H. H. Chow, Y. Cai, I. A. Hakim, J. A. Crowell, F. Shahi, C. A. Brooks, R. T. Dorr, Y. Hara, D. S. Alberts: Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin Cancer Res* 9(9), 3312-3319 (2003)

52. A. Kazi, Z. Wang, N. Kumar, S. C. FalsettiC, T. H. Chan, Q. P. Dou: Structure-activity relationships of synthetic analogs of (-)-epigallocatechin-3-gallate as proteasome inhibitors. *Anticancer Res* 24(2B), 943-954.(2004)

53. S. B. Moyers, N. B. Kumar: Green Tea Polyphenols and Cancer Chemoprevention: Multiple Mechanisms and Endpoints for Phase II Trials. *Nutrition Reviews* 62(5), 204-211 (2004)

54. N. B. Kumar, A. Cantor, K. Allen, & C. E. Cox: Android Obesity at diagnosis and breast carcinoma survival. *Cancer* 88(12),2751-2757 (2000)

55. N. B. Kumar, G. H. Lyman, K. Allen, C. E. Cox & D. V. Schapira: Timing of Weight Gain and Breast Cancer Risk. *Cancer* 2:76, (1995)

56. N. B. Kumar, K. A. Allen, D. Riccardi, B. Bercu, A. Cantor, S. Minton, L. Balducci & P. Jacobsen: Fatigue, Weight Gain, Lethargy and Amenorrhea in Breast Cancer

Patients on Chemotherapy: is Subclinical Hypothyroidism the Culprit? *Breast Cancer Research & Treatment* 83,149-159 (2004)

57.C. Papaconstantinou, J. P. Krischer: An automated patient registration and treatment randomization system. *J Med Syst* 19(6), 445-456 (1995)

58. Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. Cancer Res. 15;66(2):1234-40 (2006)

59. K. M. W. Pister, R. A. Newman, B. Coldman., D. M. Shin, R. F. Khuri, W. K. Hong, B. S. Blisson, J. S. Lee: Phase I Trial of Oral Green Tea Extract in Adult Patients with Solid Tumors. *Journal of Clinical Oncology* 19,1830-1838 (2001)

60. J. Sun, S. Nam, C. S. Lee, B. Li, D. Coppola, A. D. Hamilton, Q. P. Dou, S. M. Sebti: CEP1612, a dipeptidyl proteasome inhibitor, induces p21WAF1 and p27KIP1 expression and apoptosis and inhibits the growth of the human lung adenocarcinoma A-549 in nude mice. *Cancer Res* 61(4),1280-1284 (2001)

61. N. B. Kumar, A. Cantor, K. Allen & C. E. Cox: Android Obesity at diagnosis and breast carcinoma survival. *Cancer* 88(12), 2751-2757 (2000)

62. N. B. Kumar, G. H. Lyman, K. Allen, C. E. Cox & D. V. Schapira: Timing of Weight Gain and Breast Cancer Risk. *Cancer*. 2, 76 (1995)

63. N. B. Kumar, K. Allen, A. Cantor, C. E. Cox, H. Greenberg, S. Shah & G. H. Lyman: Weight gain associated with adjuvant tamoxifen therapy in stage I and II breast cancer: Fact or Artifact. *Breast Cancer Research & Treatment* 44,135-143 (1997)

64. U. Ullmann, J. Haller, J. D. Decour, J. Girault, V. Spitzer, P. Weber: Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. *Int J Vitam Nutr Res* 74(4), 269-78 (2004)

65. U. Ullmann, J. Haller, J. P. Decourt, N. Girault, J. Girault, A. S. Richard-Caudron, B. Pineau, P. Weber: A single ascending dose study of epigallocatechin gallate in healthy volunteers. *J Int Med Res* 31(2), 88-101 (2003)

66. J. J. Dalluge, B. C. Nelson, J. B. Thomas, L. C. Sander: Selection of column and gradient elution system for the separation of catechins in green tea using high-performance liquid chromatography. *J Chromatogr A* 793(2), 265-274 (1998)

67. W. E. Bronner, G. R. Beecher: Method for determining the content of catechins in tea infusions by high-performance liquid chromatography. *J Chromatogr A* 805(1-2), 137-142 (1998)

68. Umegaki K, Sugisawa A, Yamada K, Higuchi M. Analytical method of measuring tea catechins in human plasma by solid-phase extraction and HPLC with electrochemical detection. J Nutr Sci Vitaminol (Tokyo). 2001 Dec;47(6):402-8.

69. J. K. Lin, Y. C. Liang, S. Y. Lin-Shiau: Cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade. *Biochem Pharmacol* 58, 911–915 (1999)

70. National Cancer Institute, Division of Cancer Prevention and Control, Chemoprevention Branch, and Agent Development Committee: Clinical development plan: tea extracts green tea polyphenols epigallocatechin gallate. *J Cell Biochem* 26S, 236–257 (1006)

71. D. A. Balentine, S. A. Wiseman, L. C. M. Bouwens: The chemistry of tea flavonoids. *Crit Rev Food Sci Nut*r 37, 693–704 (1997)

Key Words: Green Tea Polyphenols, Chemoprevention, Colon Cancer, Epigallocatechin Gallate, Aberrant Crypt Foci, Intermediate Endpoint Biomarker

Send correspondence to: Dr Nagi Kumar, Cancer Control, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa Florida 33612, USA, Tel: 813-903-6885, Fax: 813-903-7183, E-mail: kumar@moffitt.usf.edu

http://www.bioscience.org/current/vol12.htm