Chemoprevention of Head and Neck Cancer with Green Tea Polyphenols

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Abstract

Recently, squamous cell carcinoma of the head and neck (SCCHN) chemoprevention research has made major advances with novel clinical trial designs suited for the purpose, use of biomarkers to identify high-risk patients, and the emergence of numerous molecularly targeted agents and natural dietary compounds. Among many natural compounds, green tea polyphenols (GTPs), particularly (−)-epigallocatechin-3-gallate (EGCG), possess remarkable potential as chemopreventive agents. EGCG modulates several key molecular signaling pathways at multiple levels and has synergistic or additive effects when combined with many other natural or synthetic compounds. This review will provide an update of the potential of GTPs, particularly EGCG, for chemoprevention of SCCHN.

Keywords

Squamous cell carcinoma of the head and neck; Chemoprevention; EGCG; Natural compounds; Molecular targets

Introduction

Cancer is the leading cause of death for people under the age of 85 (1). About 47,000 cases of squamous cell carcinoma of the head and neck (SCCHN) were estimated to occur in the year 2009, with an expected 11,000 deaths from the disease (1,2). SCCHN causes significant morbidity and mortality with a five-year survival rate of less than 50% (2-5). A common cause of mortality in SCCHN survivors is second primary tumor, which occurs at an annual rate of 3-5% (6-8). SCCHN occurs as a consequence of accumulating genetic instabilities from exposure to various carcinogens, such as cigarettes, alcohol, marijuana and betel chewing (3, 9-11). Figure 1 illustrates the molecular progression of SCCHN.

Recently, SCCHN chemoprevention research has made major advances, including novel clinical trial designs suited for chemoprevention (12), use of biomarkers to identify high-risk patients (12), use of molecularly targeted agents and natural compounds in chemoprevention trials, and the development of an oral-specific carcinogenesis animal model (13). An ideal chemopreventive agent should be nontoxic, potent, inexpensive and easily available. Research over the last several decades has identified numerous natural compounds, many of which are present in the diet and have the potential to suppress the development of multiple cancers

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(14). Among many natural compounds, green tea polyphenols (GTPs) including (−)-epigallocatechin-3-gallate (EGCG) exhibit high promise for chemoprevention in epidemiological, preclinical and early clinical studies. EGCG also shows strong synergistic or additive antitumor activities with many natural or synthetic compounds. This review will provide an update of the potential of GTPs, particularly EGCG for chemoprevention of SCCHN.

Introduction to Green Tea Extract (GTE) and EGCG

Green tea is produced from the non-fermented leaves of the plant *Camellia sinensis*. Figure 2 showed the four major polyphenols present in green tea extract (GTE). EGCG is the most potent and abundant antioxidant present in green tea and has been extensively investigated for its chemopreventive and therapeutic potential (15). Several formulations of GTE have been used in clinical trials, in which the EGCG content varies from 13 to 70% (Table 1).

One of the first pieces of evidence of EGCG's chemopreventive effect was reported in 1987, when the inhibitory effects of EGCG on teleocidin-induced tumor promotion in mouse skin were demonstrated (16). Its antitumor effects were also demonstrated by regression of experimentally-induced skin papilloma in mice by orally administered green tea, intraperitoneally (i.p.)-administered GTP fraction or i.p. EGCG (17). Topical application of EGCG was shown to induce apoptosis in UVB-induced skin tumors in mice (18). Numerous preclinical studies have followed since showing the mechanisms of antitumor effects of GTP and EGCG in various cancer models.

Mechanisms of Action / Preclinical Studies

EGCG exerts its chemopreventive actions by modulating multiple signaling pathways at various cellular levels, the ultimate outcomes of which are apoptosis, cell cycle arrest, growth inhibition, anti-angiogenesis and inhibition of metastasis. Figure 3 illustrates the molecular targets modulated by EGCG. At the cell membrane, EGCG inhibits activation of receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER)-2 and HER-3, insulin-like growth factor-1 receptor (IGF-1R) and vascular endothelial growth factor receptor (VEGFR), and their downstream effectors such as pAkt and pERK (19-29). Of these, EGFR appears to be the most active target of EGCG in both *in vitro and in vivo* SCCHN models (19,25,28). A correlation between pEGFR inhibition, Akt phosphorylation and tumor growth inhibition was observed in SCCHN xenografted tumor tissues (19). EGCG in combination with curcumin inhibited VEGFR-1 activation in a breast cancer xenograft model (30). EGCG also inhibited VEGFR-2 activation in a hepatocellular carcinoma xenograft model (31). Inhibition of VEGF by green tea preparations was also observed in animal models of breast (32,33) and prostate cancers (34). Downregulation of angiogenic stromal VEGF was observed in a clinical trial with GTE (35). GTP treatment also decreased serum VEGF levels in prostate cancer patients (36). Laminin receptor was identified as a potential receptor for EGCG to modulate several important intracellular signaling pathways (37-39).

The effects of EGCG on cytoplasmic signaling molecules in cell culture and animal models include inhibition of Akt, extracellular signal-related kinase (ERK)1/2 and MAP kinase or ERK kinase (MEK) phosphorylation (40-42), in addition to modulating phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) (41,43), and signal transducer and activator of transcription (STAT)-3 (25).

EGCG also modulates the function of certain transcription factors, namely nuclear factor-κB (NF-κB) and activator protein (AP)-1 in cell culture and animal studies (44-48). NF-κB facilitates the transcription of genes involved in inflammation, immunity, and carcinogenesis.

In a normal human epidermal keratinocyte model, pretreatment with EGCG caused significant inhibition of UVB-induced NF-κB/p65 activation and its nuclear translocation (47). As a consequence of AP-1 inhibition, expression of its target molecules, such as cyclin D1 and cyclooxygenase (COX)-2 are reduced, inducing apoptosis and reducing inflammatory response (21,25,49). Furthermore, EGCG induces apoptosis and G0/G1 arrest in several cell lines via activation of p53 and its downstream targets p21, p57 and Bax (50-53). Evidence also suggests that EGCG induces the expression of p73, which is important for apoptosis and expression of a subset of p53-target genes (54,55).

EGCG has shown a dose-dependent inhibition of invasion and migration of human oral cancer, which is thought to be related to decreased production of matrix metalloproteinase (MMP)-2/9 and urokinase plasminogen secretion (56). Topically administered GTP in UVB-induced tumors also inhibited the expression of MMP-2 and MMP-9 (57).

It seems that EGCG modulates multiple molecular targets, which largely depend on the experimental context. Moreover, the molecular targets affected by EGCG in cell cultures, animal models, and clinical samples are sometimes different. Furthermore, the doses of EGCG and administration routes used in these three situations are different. Although it is not currently clear which of these EGCG targets are most critical for its chemopreventive effects observed in clinical trials, this is an important issue that may become better elucidated once more clinical trial results are available.

Combination of EGCG with other compounds for SCCHN chemoprevention

EGCG and curcumin

Curcumin is another popular natural compound that has been studied extensively (58-62). EGCG showed synergistic effects with curcumin in SCCHN cells (63). The median effect analysis revealed that the combination of EGCG and curcumin exhibited synergistic growth inhibition of premalignant and malignant cells (63). Combination of topical curcumin and oral green tea also resulted in superior antitumor effects in 7,12-dimethylbenz[a]anthracene-induced carcinogenesis in Syrian hamsters (64). The combination significantly decreased the number and volume of visible oral tumors, purportedly mediated through suppression of cell proliferation, induction of apoptosis and inhibition of angiogenesis (64). This combination regimen has also demonstrated enhanced or synergistic effects in other cancer models such as a hormone receptor-positive breast cancer model (30). The combination induced greater tumor volume reduction and inhibition of VEGFR protein compared to the single agents in nude mice (30).

It also appears that the sequence of administration of these agents affects the synergistic effect. In an *in vitro* study in chronic lymphocytic leukemia-B cells, although each agent alone was active, simultaneous administration of the agents reduced apoptosis (65). However, treatment with EGCG followed by curcumin showed synergistic apoptotic effects (65).

EGCG and EGFR tyrosine kinase inhibitors

EGFR overexpression appears to play a role in the early part of SCCHN carcinogenesis, correlates with progression of dysplasia (66) and associates with poor clinical outcome in invasive SCCHN (67,68). Our laboratory has demonstrated synergistic growth inhibition of SCCHN by EGCG and erlotinib, mediated through greater inhibition of pEGFR and pAkt (19). The combination of erlotinib and EGCG was associated with greater tumor growth inhibition compared to single agent treatments (19). The mechanism of synergy was thought to be mediated through more sustained inhibition of Akt phosphorylation as compared with single agent treatment (19). A subsequent study suggested a critical role of activation of p53 and inhibition of NF-κB signaling pathways (69). The same combination also demonstrated

enhanced anti-proliferative effects in several erlotinb-sensitive and erlotinb-resistant non-small cell lung cancer (NSCLC) cell lines and in SCID mice bearing erlotinib-resistant NSCLC tumors (70).

Combination of EGCG with other compounds in other cancer models

The apoptosis-inducing effects of EGCG were drastically enhanced by sulindac and tamoxifen in lung cancer cell lines (71). Co-treatment with EGCG plus celecoxib induced synergistic apoptosis in lung cancer cell lines (72). Although neither EGCG nor celecoxib alone induced growth arrest or the expression of DNA damage-inducible-153 (GADD153) mRNA and protein, co-treatment with both compounds strongly induced the expression of both GADD153 mRNA and protein. Moreover, inhibition of ERK1/2 activation using chemical inhibitors inhibited the expression of GADD153 and apoptosis, suggesting that combination of EGCG and celecoxib induced synergistic apoptosis by upregulating GADD153 via ERK1/2 (72). Combined treatment with GTE and sulindac resulted in a significantly greater reduction in tumor number (from 72.3 +/− 28.3 to 32.0 +/− 18.7) than that achieved with either single agent alone (to 56.7 +/− 3.5 and 49.0 +/− 12.7 with GTE or sulindac alone, respectively) in multiple intestinal neoplasia mice that harbored a mutated *Apc* gene (73).

EGCG combined with the selective cyclooxygenase-2 inhibitor NS-398 resulted in synergistic cell growth inhibition, apoptosis induction, and inhibition of NF-κB in prostate cancer cell lines and enhanced tumor growth inhibition with reduced serum levels of prostate specific antigen (PSA) and insulin-like growth factor-1 in nude mice bearing androgen-sensitive prostate cancer cells (74).

Several studies have also demonstrated enhanced or synergistic anti-tumor effects in both *in vitro* and *in vivo* models of breast cancers when EGCG was combined with tamoxifen or soy phytochemical concentrate (75-77). Furthermore, in prostate cancer models, dihydrotesterone increased the sensitivity of prostate cancer cells to EGCG (78). Moreover, in a carcinogen-induced lung tumorigenesis mouse model, the combination of atorvastatin and EGCG at relatively low doses was able to induce synergistic tumor growth inhibition (79)

SCCHN Chemoprevention Clinical Trials with Green Tea Extracts

Despite mounting preclinical evidence to support the efficacy of GTPs, only a few clinical trials have been conducted (Table 1). The first clinical trial using green tea for oral premalignant lesions (OPL) was a double-blind, placebo-controlled randomized trial in patients with oral leukoplakia receiving either 760mg of mixed tea (40% GTPs) capsules q.i.d. plus 10% mixed tea ointment topically, or placebo plus topical glycerin (80). After 6 months of treatment, treatment groups achieved a response rate of 37.9%, compared to 10% in the control group (80). This finding correlated with histopathologic results showing a reduced number of EGFR-positive cells (80). Pisters *et al*. reported a phase I trial using a GTE in an attempt to find the maximum tolerated dose (81). The percentage of total catechins, EGCG, EGC, ECG, EC and caffeine contents of the GTE preparation were 26.9, 13.2, 8.3, 3.3, 2.2 and 6.8, respectively. Patients were given GTE either once or thrice a day for 4 weeks, up to a maximum of 6 months (81). The dose limiting toxicities were tremors, cough, constipation, and headache, which were thought to be related to caffeine components of the GTE (81). Although there was no clinical response observed, oral GTE at 1.0 g/m^2 thrice a day for at least 6 months was recommended (81).

Recently, Tsao *et al*. reported a randomized phase II trial using the same GTE preparation as Pisters *et al*. (81) for patients with high risk OPLs (35). Patients were randomized into one of the four arms – 500, 750 or 1,000 mg/m^2 GTE, or placebo, t.i.d. for 12 weeks, and clinical response was measured by the Response Evaluation Criteria in Solid Tumors (35). At 12 weeks,

there was 50% OPL clinical response rate in the treatment arms, *vs*. 18.2% in the control arm. A higher response rate was observed in the two higher dose GTE arms, of 58.8% *vs* 36.4% in the lower dose arm, suggesting dose-response effects of GTE (35). GTE was very well tolerated with only three grade 3 toxicities, including insomnia, diarrhea and oral/neck pain, and no grade 4 toxicity. Analysis of patients' demographics among clinical responders showed a higher response rate in never-drinkers (p = 0.001). Other demographic characteristics did not affect the clinical response. At a median follow-up of 27.5 months, there was no difference in oral cancer-free survival between the GTE and placebo arms. Baseline biomarker characteristics, such as higher stromal VEGF expression, were associated with clinical but not histologic response. Interestingly, stromal VEGF and cyclin D1 expression were downregulated in clinically responsive GTE patients and upregulated in nonresponsive patients (35). These biomarker characteristics would be important predictive factors in profiling the patient population that would benefit most from GTE treatment.

Patients with high grade-prostate intraepithelial neoplasia (HG-PIN) received either 200mg of green tea catechin (GTC) containing approximately 103.6mg of EGCG, orally, t.i.d., or placebo. The primary endpoint was prevalence of prostate cancer. After 1 year follow-up, 3.3% patients were diagnosed with prostate cancer in the treatment group compared with 30% in the placebo group (82). Multivariate analysis including age, PSA, prostate volume, HG-PIN, and monofocal or plurifocal HG-PIN lesions showed no significant differences between the two arms. In a 2-year follow-up, only one prostate cancer was diagnosed among 13 GTC-treated patients and 2 among 9 placebo-treated patients (83). A daily dose of 311 mg of EGCG in GTC formulation was able to induce a clinical response in these patients. Although the results of this clinical study for prostate cancer prevention are dramatic and highly promising, the data need to be validated in larger randomized trials.

Oral administration of GTE, three 500 mg tablets, each containing 52.5 mg EGCG, 12.3 mg EC, 34.6 mg EGC, 11.1 mg ECG, and 15.7 mg caffeine, daily for 12 months, in addition to a tea drinking lifestyle, demonstrated its efficacy in preventing incidence of metachronous adenoma in patients 1 year post polypectomy (84). Twelve-month follow-up colonoscopy showed 31% incidence of colonic adenoma in the control arm *vs*. 15% in the GTE arm (84). It was not reported whether the GTE treatment had any impact on the incidence of colon cancer. Furthermore, patients with HPV-infected cervical premalignant lesions were treated with various formulations of GTE: polyphenon E 200mg orally t.i.d., EGCG 200mg orally t.i.d., polyphenon E 200mg orally t.i.d. plus topical polyphenon E twice a week, or polyphenon E topical treatment only twice a week for 8 to 12 weeks (85). Compared with 4 out of 39 untreated patients, 35 out of 51 treated patients achieved a clinical response in reduction in HPV DNA titer or improvement in cytology or tissue biopsy after treatment (85).

Although these clinical trials used different GTE formulations with different percentages of EGCG content, none have encountered serious adverse effects. It appears that a GTE formulation containing EGCG levels as low as 158mg a day for 12 months was able to induce a clinical response (84). GTE is also well tolerated at doses as high as $4200mg/m^2$, containing $554.4mg/m^2$ of EGCG, a day for at least 6 months (81).

Future Directions and Conclusions

Since the first clinical chemoprevention study in 1986 (86), the field of SCCHN chemoprevention has made remarkable advances and entered into mainstream cancer research. Although none of the agents has yet been translated into clinical practice, there are several agents under clinical investigation that hold strong promise. Amongst the most promising compounds are GTPs. The recent phase II clinical trial by Tsao *et al,* showing a dose-response relationship of GTE against OPLs that correlates with biomarker response, has generated a

tangible momentum in SCCHN chemoprevention (35). Several approaches should be pursued in order to maximize yields from this promising agent in future investigations. First, GTE should be used in combination to generate synergy and to reduce toxicity since this agent has shown synergistic/additive antitumor effects in many cancer models (19,63,65,72,74,76,79).

Second, investigations to enhance the bioavailability and potency of GTE should be encouraged and validated more vigorously in preclinical studies. One approach is the use of a pro-drug formulation of EGCG, many of which have shown increased activity and bioavailability *in vitro* and *in vivo* (87-90). Another promising approach is to formulate nanoparticles for effective delivery. Siddiqui *et al*, have demonstrated about 10-fold higher potency of nanoparticle-encapsulated EGCG (91). Both of these approaches are novel and hold high promise for chemoprevention, thus warranting further validation in animal studies and clinical settings. However, efforts must be made not to compromise the safety or cost of this agent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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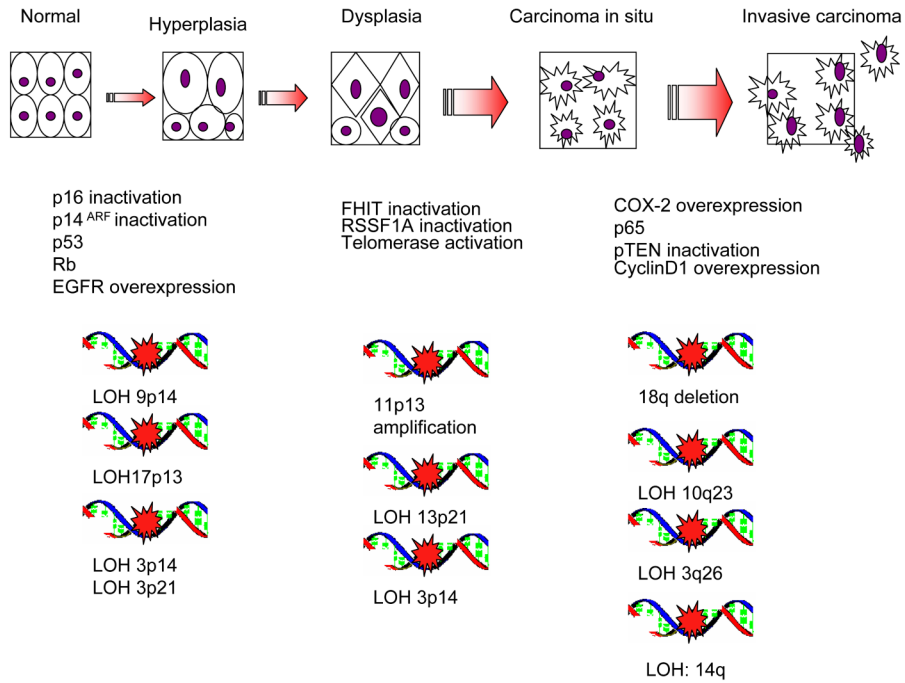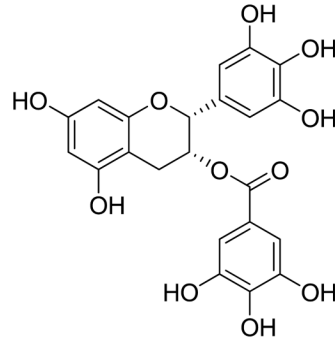
Figure 1. Model of genetic instability and progression in head and neck cancer
Genetic instability increases and accumulates as a lesion acquires its malignant potential. With increasing heterogeneity of genetic instability, inactivation of tumor suppressor genes and activation of oncogenes account for the phenotypic changes of SCCHN carcinogenesis through normal histology, hyperplasia, dysplasia, carcinoma *in situ* and invasive carcinoma.
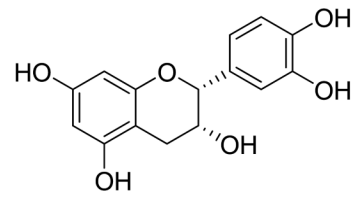
Epicatechin
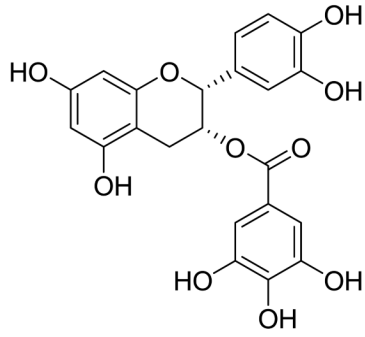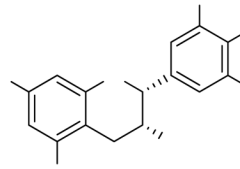
Epicatechin-3-gallate   Epigallocatechin-3-gallate

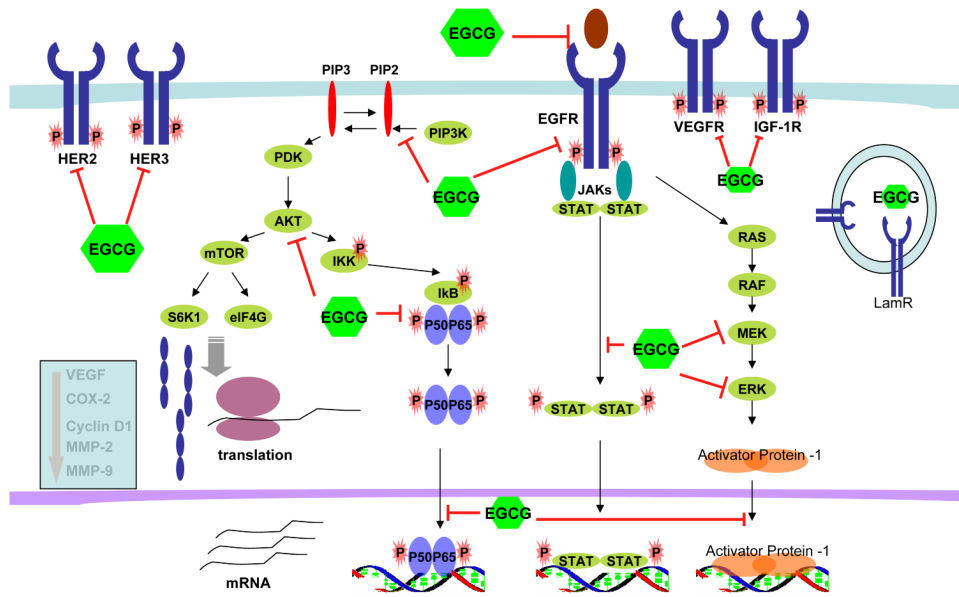**Figure 2. Chemical structures of green tea polyphenols**

Figure 3. Molecular targets of EGCG
Several signaling pathways are affected by EGCG at multiple levels. EGCG inhibits the ligand binding of EGFR and inhibits phosphorylation/activation of the receptor tyrosine kinases. It also inhibits several intracellular signaling pathways, including PI3K/Akt/mTOR, JAK/STAT, RAF/MEK/ERK/AP-1, and Akt/NF-κB. At the nuclear level, EGCG also inhibits the DNA binding of effector transcription factors, such as NF-κB, AP-1 and STAT. As a consequence, expression of molecules that are involved in cell proliferation, angiogenesis, invasion and inflammation are reduced.

Table 1

Clinical Trials with Green Tea Extracts

Organ Site	Type of Clinical Trial	Subjects	GTE Formulation	Treatments	Results	Comments
Oral Leukoplakia Li 1999 (80)	Randomized, double-blind, placebo-controlled.	59 patients with oral mucosa leukoplakia	Mixed tea capsule prepared by the Institute of Tea Science and Research, Chinese Academy of Agricultural Science. Each capsule contained 40% green tea polyphenols EGCG content not reported.	3 grams of mixed tea a day (760 mg mixed tea capsule, QD) plus topical treatment with mixed tea in glycerin at 10%, TID.	37.9 % response rate in treatment arm *vs* 10% in control arm	n/a
High Risk OPLs Tsao 2009 (35)	Phase II trial	41 patients with high risk OPLs	THEA-FLAN 30 ARG supplied by Ito En Ltd. Each capsule contains 350mg of GTE, 13.2% EGCG	Placebo TID, vs GTE capsule 500mg/m² TID vs 750mg/m² TID vs 1000mg/m² TID (132 mg/m² TID, EGCG)	50% response rate in treatment arm vs 18.2% in placebo arm	Higher 12-week histologic response rate in never drinkers (p=0.01) Neither clinical or histologic response to GTE intervention was associated with oral cancer development
Refractory Solid Tumors Pisters 2001 (81)	Phase I trial	49 patients with solid tumors refractory to standard treatment	GTE capsule Supplied by Ito En Ltd. Each capsule contained 13.2% EGCG	GTE capsule, 0.5 to 5.05g/m² a day	No major clinical response. Maximally tolerated dose was 4.2 g/ m² of GTE a day for up to 6 months. Dose limiting toxicities were caffeine-related.	Optimal dose suggested was 1 g/m² TID.
Advanced Lung Cancer Laurie 2005 (92)	Phase I trial	17 patients with advanced lung cancer	GTE capsule Supplied by Ito En Ltd. Each capsule contained 13.2% EGCG	0.5 g/m² GTE with dose escalation-scheme upto 8 g/m² GTE.	No major clinical response MTD was 3 g/m² of oral GTE, once	n/a

Organ Site	Type of Clinical Trial	Subjects	GTE Formulation	Treatments	Results	Comments
					daily.	
High risk Cervical Lesions Ahn 2003 (85)	Pilot study	51 patients with cervical lesions	Content of Poly E ointment or capsule was not reported.	Poly E ointment twice a week vs. Poly E 200mg oral daily + Poly E ointment twice a week vs., Poly E 200mg orally daily, vs., EGCG 200mg orally daily vs Untreated	Overall 69% (35/51) in treatment arms vs 10% (4/39) patients in untreated control (p<0.05)	n/a
Colorectal Adenomas Shimizu 2008 (84)	Randomized trial	136 patients status 1 year post polypectomy	GTE tablet provided by the Green Tea Union of Saitama. One 500mg tablet contains 52.5mg EGCG.	GTE tablet 1500mg (157.5 mg EGCG) a day for 12 months	Incidence of metachronous adenoma was 31% in the control group and 15% in the GTE group. (p<0.05)	n/a
Prostate Cancer McLarty 2009 (36)	Single arm phase II trial	26 men with positive prostate biopsy awaiting prostectomy	Polyphenon E provided by Mitsui-Norin Co Ltd / Polyphenon E International,Inc. Each Polyphenon E 325mg capsule contains 200mg EGCG	Polyphenon E 1300mg (800mg EGCG) a day during the interval between the prostate biopsy and radical prostectomy. Median period was 34.5 days	Serum levels of HGF, VEGF, IGF-BP3, IGF-I and PSA were decreased No liver toxicities were observed.	n/a
High-Grade Prostate Intraepitheli al Neoplasia Bettuzzi 2006 (82)	Double-blind placebo-controlled trial	60 patients with HG-PIN	Green tea catechin capsules. Each capsule contains 51.8% EGCG.	Green tea catechins capsule 600mgs a day (311 mg, EGCG) vs. placebo for 1 year	Incidence of prostate cancer was 30.0% in the control group vs 3.3% in the GTE group (p<0.01)	GTE treatment did not significantly affect PSA values