Selected Research
Polybotanical Formula for Breast Health*

Polybotanicals Shown to Be Beneficial for Breast Cellular Health

- Lowering inflammation
- Stimulating and supporting healthy immune function
- Supporting normal metabolism and blood sugar levels
- Promoting detoxification of xenobiotics
- Modifying CYP450 enzyme-mediated hormone metabolism toward favorable estrogen metabolites
- Modulating a multiplicity of cell signaling pathways in breast cancer to:
  - Reduce inflammation
  - Normalize cell behavior
  - Reduce aggressiveness and metastatic behavior of breast cancer cells
  - Induce apoptosis
  - Inhibit angiogenesis
  - Inhibit estrogen receptor stimulation

Formula Ingredients

Medicinal Herbs

- *Scutellaria barbata* extract
- Tumeric rhizome extract (*Curcuma longa*) (with enhanced bioavailable curcumin in the form of BCM-95®)
- *Astragalus membranaceus* root extract

Botanically Enhanced Medicinal Mushrooms

- The mushrooms are grown in controlled indoor environments on a medium which contains a blend of traditional herbs known for their immune enhancing & cancer fighting benefits along with organic brown rice.
- Coriolus (*Trametes versicolor*)
- Reishi (*Ganoderma lucidum*)
- *Phellinus linteus*

Purified Biologically Active Nutritional Compounds

- Diindolymethane (DIM)
- Quercetin (98% bioflavonoids)

Formula Research Summaries

Inhibition of Proliferation and Invasive Behavior of Highly Metastatic Human Breast Cancer Cells[1]

- Measures of cellular proliferation, cytotoxicity and expression of cell cycle regulatory genes were evaluated in a highly metastatic human breast cancer cell line treated with the polybotanical formula.
- Treatment resulted in significant inhibition of cell proliferation, cellular adhesion, migration and invasion.
- Mediated by the modulation of genetic expression: inhibition of proliferation was mediated by the upregulated expression of: CCNG1, CHEK1, CDKN1C, GADD45A E2F2 genes and downregulation of expression of CCNA1 (cyclin A1) and CDK6 genes.
- Inhibition of invasiveness was mediated by suppression of urokinase plasminogen activator (uPA) secretion and downregulation of CXCR4.

Suppresses Growth and Breast-to-Lung Cancer Metastasis in Human Breast Cancer Cells Implanted in Mice[2]

- Human breast cancer cells implanted into the mouse mammary pad (orthotopic model) most closely resembles conditions in humans.
- Demonstrated a significant decrease in tumor volume over time compared to the control group.
- Showed a marked decrease in the incidence of breast-to-lung cancer metastasis from 67% in the control to 20% with treatment.
- Result showed significantly less metastasis from 2.8 in the control group to 0 in the treated group.
- Demonstrated mechanisms of action including down regulation of PLAU, urokinase plasminogen activator (uPA) and CXCR4 genes in the breast tumors.

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- Oral administration of the formula via intragastric gavage showed no signs of organ or tissue toxicity.

Synergistic Effects of Modified Citrus Pectin (MCP) and a Polybotanical Compound for Breast Health[3]

- The combination of MCP with the polybotanical compound synergistically inhibited the metastatic phenotype of human breast cells.
- Dose dependent inhibition of cell migration by MCP was synergistically enhanced in combination with the formula.
- The combination of MCP with the formula suppressed the secretion of uPA from breast cells.
  - uPA is part of the plasminogen activator system, a complex system with multiple interactions and members participating in fibrinolysis, cell migration, angiogenesis, wound healing, tumor cell dissemination and metastasis in a variety of solid tumors.
  - Increased uPA in breast tumor tissue correlates with increased aggressiveness.
  - High tumor tissue antigen content of uPA or PAI-1 correlates with a lower probability of disease free and overall survival than in patients with low levels, serving as a prognostic marker.[4]

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3. Jiang J, Eliaz I, Silva D. Synergistic and additive effects of modified citrus pectin with two polybotanical compounds in suppression of invasive behavior of human breast and prostate cancer cells. *Integr Cancer Ther*. 2013;12(2):145-52.
4. Annecke K, Schmitt M, Euler U, *et al*. uPA and PAI-1 in breast cancer: review of their clinical utility and current validation in the prospective NNBC-3 trial. *Adv Clin Chem.* 2008;45:31-45.

Ingredient Selected Research Summaries

Scutellaria barbata Extract

- Anticancer effects including:
  - Modulation of multiple signaling pathways
  - Induction of oxidative stress
  - Inhibition of glycolysis
  - Promotion of mitochondria-mediated apoptosis
- An aqueous extract of *Scutellaria barbata* in ER positive and ER negative cell, lines inhibited cell proliferation, induced cell death and G2 cycle arrest, induced oxidative stress, hyper activated PARP and inhibited glycolysis, causing depletion of ATP and NAD(H), inhibiting metabolic pathways that are preferentially activated in tumor cells.[5]
- A Phase 1B, multicenter, dose escalation study conducted on heavily pretreated patients with Stage IV breast cancer demonstrated that *Scutellaria barbata* extract was safe and well tolerated with promising clinical evidence of anticancer activity, with some participants showing stable disease as well as tumor regression.[6]
- An extract of *Scutellaria barbata* induced cell death selectively in breast cancer cells via oxidative stress, DNA damage and activation of death-promoting genes. Oxidative damage led to hyperactivation of PARP, depletion of ATP and NAD, and inhibition of glycolysis. Because tumor cells frequently rely on glycolysis for energy production, the observed inhibition is likely a key factor in the energetic collapse and necrotic cell death that occurred selectively in breast cancer cells.[7]
- *Scutellaria* species have been found to contain compounds that selectively modulate the immune system, supporting centuries-old traditional use in Traditional Chinese Medicine.[4]

- Preclinical studies showed that an aqueous extract of *Scutellaria barbata* induced strong growth inhibition and apoptosis in breast cancer cell lines.[8]
- A Phase I trial of heavily pretreated Stage IV breast cancer patients administered *Scutellaria barbata* extract resulted in 25% of patients having stable disease (SD) for >90 days with 19% SD for >180 days. Five patients had objective tumor regression, demonstrating encouraging clinical activity with a favorable toxicity profile.[9]
- *Scutellaria barbata* is widely used as an antitumor agent in China. Ethanol extracts were found to have dose dependent cytotoxicity on six cancer cell lines.[10]

5. Klawitter J, Klawitter J, Gurshtein J, *et al*. Bezielle (BZL101)-induced oxidative stress damage followed by redistribution of metabolic fluxes in breast cancer cells: a combined proteomic and metabolomic study. *Int J Cancer*. 2011 Dec 15;129(12):2945-57.
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7. Fong S, Shoemaker M, Cadaoas J. Molecular mechanisms underlying selective cytotoxic activity of BZL101, an extract of Scutellaria barbata, towards breast cancer cells. *Cancer Biol Ther*. 2008 Apr;7(4):577-86.
8. Tan BK, Vanitha J. Immunomodulatory and antimicrobial effects of some traditional chinese medicinal herbs: a review. *Curr Med Chem*. 2004 Jun;11(11):1423-30.
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10. Yu J, Liu H, Lei J *et al*. Antitumor activity of chloroform fraction of Scutellaria barbata and its active constituents. *Phytother Res*. 2007 Sep;21(9):817-22.

Tumeric Rhizome Extract

- The bioactive component of turmeric (*Curcuma longa*) has a wide spectrum of activities:
  - Antioxidant
  - Anti-inflammatory
  - Anti-cancer through its unique multifocal signal modulatory effects
  - Chemoprevention
  - Chemosensitizer
  - Radiosensitizer
- Extensive *in vivo* and *in vitro* research has shown that curcumin can sensitize tumors to different chemotherapeutic agents including doxorubicin, 5-FU, paclitaxel, vincristine, melphalan, butyrate, cisplatin, celecoxib, vinorelbine, gemcitabine, oxaliplatin, etoposide, sulfinosine, thalidomide and bortezomib.[11]
- Chemosensitization has been observed in cancers of the breast, colon, pancreas, gastric, liver, lung, prostate, bladder, cervix, ovary, head and neck, brain and multiple myeloma, leukemia and lymphoma.[11]
- Curcumin has been shown to sensitize a variety of tumors to gamma radiation including glioma, neuroblastoma, cervical carcinoma, epidermal carcinoma, prostate cancer and colon cancer.[13]
- Curcumin down regulates various growth regulatory pathways and specific genetic targets including NF-*k*β, STAT3, COX2, Akt, antiapoptotic proteins, growth factor receptors and multidrug-resistance proteins.[11]
- Curcumin has also been show to protect normal organs from chemotherapy and radiotherapy induced toxicity mediated through its ability to induce activation of NRF2 and induce expression of antioxidant enzymes including glutathione, and directly quench free-radicals.[11]
- Curcumin has been shown to inhibit a multitude of cell signaling pathways including: NF-*k*β, AP-1, STAT3, Akt, Bcl-2, Bcl-X (L), caspases, PARP, IKK, EGFR, HER2, JNK, MAPK, COX2 and 5-LOX.[12]
- Curcumin represents a multi-targeted therapy with its ability to activate apoptosis, down regulate cell survival gene products and up

regulate p53, p21 and p27, as well as inhibit COX-2, HER2, tumor necrosis factor, EGFR, Bcr-abl, proteasome, and VEGF. Multiple studies have shown that even low levels of physiological concentration may be sufficient for its chemopreventive and chemotherapeutic activity.[13]

- Curcumin has been found to inhibit proliferation of various tumor cell lines in culture, prevent carcinogen-induced cancers in rodents and inhibits growth of human tumors in animal models. Phase I and II trials indicate that curcumin is quite safe.[14]

11. Goel A, Aggarwal BB. Curcumin, the golden spice from Indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. *Nutr Cancer*. 2010;62(7):919-30.
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13. Goal A, Jhurani S, Aggarwal BB. Multi-targeted therapy by curcumin: how spicy is it? *Mol Nutr Food Res*. 2008 Sep;52(9):1010-30.
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Research on BCM-95®, an Enhanced Bioavailable Form of Curcumin

- BCM-95 was tested on human volunteers in a cross over study with two week washout period. Normal curcumin (95% extract) was used as a control. BCM-95 was also compared with a combination of curcumin-lecithin-peperine, which was earlier shown to provide enhanced bioavailability. The bioavailability of BCM-95 was shown to be approximately 6.93 fold compared to normal curcumin (95% extract) and about 6.3 fold compared to the curcumin-lecithin-piperine formula.[15]
- Curcumin extract in the highly bioavailable form of BCM-95 decreased the concentration of plasma AGP (alpha 1-acid glycoprotein), and APP (plasma acute phase protein) (which is mainly expressed by the liver), improving obesity-related inflammatory state *in vivo*.[16]
- In a clinical study of 45 patients with active rheumatoid arthritis, curcumin in the form of BCM-95 was found to have superior clinical effects compared to a standard of care anti-inflammatory diclofenac sodium (NSAID COX-1/COX-2 inhibitor), demonstrating curcumin's clinically significant anti-inflammatory effect as shown in significant improvement in standard scores assessing joint pain, swelling, disability. Curcumin significantly lowered CRP.[17]

15. Antony B, Merina B, Iyer VS, *et al*. A Pilot Cross-Over Study to Evaluate Human Oral Bioavailability of BCM-95CG (Biocurcumax), A Novel Bioenhanced Preparation of Curcumin. *Indian J Pharm Sci*. 2008 Jul-Aug;70(4):445-9.
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Astragalus membranaceus Root Extract

- Enhances and modulates immune function
- Adaptogenic
- Antinflammatory
- Supports hematopoiesis
- Neuroprotective
- Hepatoprotective
- Antiviral
- Supports healthy glucose regulation
- Anticancer activity
  - Direct anti-tumor activity
  - Modulation of multiple signaling pathways
  - Modulation of genetic expression

- Astragalus polysaccharide inhibited the proliferation of a basal-like breast cancer cell line, down regulating expression of Akt phosphorylation, and up regulating expression of p53 and PTEN.[18]
- Astragalus root polysaccharides, used historically as immunomodulation agent for over 2000 years in Traditional Chinese Medicine. Modern photochemistry has elucidated multiple beneficial properties including immunomodulation, antioxidant, antitumor, anti-diabetes, antiviral hepatoprotective, anti-inflammatory, anti-atherosclerosis, hematopoiesis and neuroprotective.[19]
- Astragalosides, the active compounds from *Astragalus membranaceus* have immune modulatory activity enhancing T cell activation in immunosuppressed mice without affecting B cell proliferation.[20]
- Astragalus was shown to promote the secretion of IL-2, IL-12 and TNF-α, and decrease IL-10 levels in tumor bearing mice, exerting its antitumor activity in part via improving immune responses of the host organism.[21]

18. Ye MN, Chen HF, Zhou RJ, *et al*. Effects of astragalus polysaccharide on proliferation and Akt phosphorylation of the basal-like breast cancer cell line. *Zhong Xi Yi Jie He Xue Bao*. 2011 Dec;9(12):1339-46.
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Medicinal Mushrooms

- Immune modulation
- Anticancer effects including:
  - Act via multiple signaling pathways
  - Effective in both estrogen dependent and estrogen independent breast cancer cells
  - Benefit in inflammatory breast cancer
  - Improve survival in advanced breast cancer patients
  - Inhibit angiogenesis and metastatic behavior
- Enhanced xenobiotic clearance
- A number of compounds present in medicinal mushrooms have been shown to potentiate the host's innate (non-specific) and acquired (specific) immune responses, and activate many types of immune cells that are important for the maintenance of homeostasis, including:[22]
  - Host cells such as cytotoxic macrophages, monocytes, neutrophils, natural killer cells, and dendritic cells
  - Lymphocytes governing antibody production and cell-mediated cytotoxic T-cells
  - Inducing cytokines such as interleukins, interferon, colony stimulating factors
  - Inducing gene expression of various immunomodulatory cytokines and cytokine receptors
- A review of ganoderic acid triterpines derived from *Ganoderma lucidum* showed enhanced detoxification of carcinogens.[22]
- An *in vitro* study tested a blend of mushroom mycelia from the species *Agaricus blazei*, *Cordyceps sinensis*, *Coriolus versicolor*, *Ganoderma lucidum*, *Grifola frondosa* and *Polyporus umbellatus*, and 1,3 beta-D-glucan isolated from the yeast, *Saccharomyces cerevisiae*. The study found cytostatic effects via inhibition of cell proliferation and cell cycle arrest in highly invasive human breast cancer cells. A number of cell cycle regulatory genes were found to be down regulated. Metastatic behavior was suppressed by the inhibition of cell adhesion, cell migration and cell invasion.[23]
- An *in vitro* study using an alcohol extract of *Ganoderma lucidum* showed inhibition of cell

proliferation, and direct induction of apoptosis, anti-tumor effects via multiple underlying mechanisms.[24]

- *In vitro Ganoderma lucidium* inhibited the proliferation of estrogen independent breast cancer cells by downregulating akt/NF-*k*β signaling, resulting in cell cycle arrest.[25]
- *Ganoderma lucidum* inhibited the proliferation of estrogen receptor positive human breast cancer cells via modulation of estrogen receptor alpha (ER) and NF-*k*β signaling without affecting ERβ. Suppression of proliferation of estrogen dependent as well as estrogen independent cancer cells was accomplished via its modulatory effect on multiple signaling pathways.[26]
- Ganoderic acids (GA-A, and GA-H), two structurally related lanostane-type hydroxylated triterpenes identified in *Ganoderma lucidum*, were found to suppress cell proliferation, colony formation, adhesion, migration and invasion of highly invasive human breast cancer cells via downregulation of signaling pathways AP-1 and NF-*k*β which then down regulate Cdk 4 and uPA.[27]
- *Ganoderma lucidum* demonstrated activity against inflammatory breast cancer, selectively inhibiting cancer cell viability without affecting non-cancerous epithelial cells. *Ganoderma* Induced apoptosis, inhibited cell invasion and disrupted the cellular architecture unique to IBC invasive pathology as well as decreasing expression of genes involved in cell survival and proliferation including BCL-2, PDGFβ, and decreasing invasion and metastasis via suppression of MMP-9.[28]
- Ganoderic acids extracted from *Ganoderma lucidum* suppressed growth, angiogenesis and invasiveness of highly invasive and metastatic breast cancer cells *in vivo* and *in vitro* via modulation of the signaling pathway of NF-*k*β. Ganoderic acids suppressed NF-*k*β activity, as well as inhibiting the expression profile of its downstream genes, including those involved in cell proliferation (cyclin D1 and c-Myc), anti-apoptosis (Bcl-2), invasion (MMP-9), and angiogenesis (VEGF, IL-6 and IL-8).[29]
- *Ganoderma Lucidum* down-regulated the expression of NF-*k*β-regulated urokinase plasminogen activator (uPA and uPA receptor (uPAR), which resulted in the suppression of cell migration of highly invasive human breast cancer cells, also inhibiting cell growth via multiple mechanisms including cell cycle arrest.[30]
- *Coriolus versicolor* extract demonstrated suppression of proliferation in three of four breast cancer cell lines tested, comparable to the chemotherapeutic drug mitomycin C. Multiple cell signaling pathways were involved including upregulation of p53 expression and downregulation of Bcl-2 expression in different cell lines.[31]
- A meta-analysis of thirteen clinical trials provided strong evidence that *Trametes versicolor* had survival benefit in cancer patients, particularly in carcinoma of breast, gastric and colorectal. There was a 9% absolute reduction in 5 year mortality with one additional patient alive for every 11 patients treated with chemotherapy.[31]
- Extracts of *Trametes versicolor* mushroom have shown significant immunological and oncologic activity in breast cancer with clinical benefit, suggesting they have a role in the primary and secondary prevention of breast cancer.[33]
- Hispolon, one of the most important functional compounds that forms *Phellinus linteus* was found to inhibit breast cancer cell growth regardless of p 53 status.[34]
- *Phellinus linteus* (PL) exhibits anticancer activity through stimulation of the immune system as well as induction of apoptosis. PL inhibited proliferation and colony formation of highly invasive human breast cancer cells via cell cycle arrest and up-regulation of p27 expression. PL also inhibited cell adhesion, migration and invasion via suppression of uPA secretion, as well as mark edly inhibiting early angiogenesis through down-regulation of multiple signaling pathways, including VEGF, and inhibition of AKT signaling.[35]
- An *in vivo* study investigated the modulatory effect of *Ganoderma lucidum* on expression of xenobiotic enzymes, oxidant-antioxidant and hormonal status in toxin induced mammary carcinoma in rats. Oral administration significantly diminished levels of lipid

peroxidation, enhanced antioxidants and positively regulated the estrogen receptor hormone levels to near normal when compared with controls.[36]

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Quercetin

- Anti-inflammatory
- Anticancer and chemo-preventive effects including:
  - Growth inhibition
  - Promotion of apoptosis
  - Action through multiple novel signaling pathways
  - Favorable modulation of estrogen metabolism
  - Antagonism of estrogen receptors
- Estrogen metabolism plays an important role in estrogen-induced breast cancer. Quercetin treatment to a non-neoplastic breast cell line was shown to dramatically increase

cytochrome P450 enzyme CYP1A1, thereby increasing the more favorable estrogen metabolite 2-OHE(2) one of the mechanisms by which quercetin may provide protection against breast cancer.[37]

- Quercetin treatment significantly inhibited cell proliferation in estrogen and progesterone receptor positive breast cancer cell lines, with induction of apoptosis involving the activation of both the intrinsic and extrinsic apoptotic pathways, via modulation of a number of cell signaling pathways. Significant elevation of ERβ expression was noted.[38]
- Quercetin was shown to inhibit growth of breast cancer cells and promoted apoptosis by inducing G_0/G_1 phase arrest as well as regulating the expression of survivin mRNA.[39]
- Quercetin was found to exert anticancer activity through the downregulation of Wnt/β-catechin signaling activity, a signaling pathway whose abnormal activation has recently been implicated in mammary tumorigenisis.[40]
- Quercetin was found to inhibit glucose uptake, reduce cell viability and proliferation in both estrogen receptor positive and negative breast cancer cells by affecting multiple cell signaling pathways independent of estrogen signaling, showing potential for ER negative breast tumors.[41]
- Quercetin was shown to have potential for treatment and prevention of cancer via upregulation of a novel pathway, nonsteroidal antiinflammatoy drug (NSAID) activated gene-1 (NAG-1), a member of the transforming growth factor superfamily, showing potential as a target of action against cancers like colorectal, pancreatic, prostate and breast.[42]
- Quercetin significantly inhibited cell proliferation induced by 17-beta-estradiol in human breast cancer cells, indicating it may act as an estrogen receptor antagonist.[43]
- Quercetin possesses a broad range of biological properties and can be considered the prototype of a naturally occurring chemopreventive agent.[44]

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Diindolymethane (DIM)

- The most physiologically active metabolite of glucosinolates found in cruciferous vegetables. DIM has multiple roles in promoting breast tissue health:
  - More physiologically active than I3C
  - Chemoprevention
  - Modulation of estrogen metabolism to more favorable metabolites
  - Anticancer via multiple mechanisms

- Brassica vegetable consumption may confer a protective effect against cancer, possibly attributed to their glucosinolates of which glucobrassicin is predominant and a precursor of indole-3-carbinol (I3C). DIM is the primary metabolite of I-3C. While I3C did not appear in urine after ingestion of brassica family vegetables, urinary excretion of DIM was highly correlated with the levels of glucobrassicin/I3C in the ingested food.[45]
- A Phase I trial of women given I3C demonstrated that the primary metabolite is DIM and that after oral dosing of I3C, it was not detectable in the plasma. DIM however was detectable and increased with increased I3C dosing.[46]
- *In vitro* addition of I3C to human breast cancer cells revealed that IC3 did not accumulate in the cells; however DIM was detected in cells and appeared to accumulate in the nucleus, present even after 72 hours post treatment, suggesting that DIM is active inside the cell nucleus.[47]
- DIM was shown to stop cell cycle progression of human breast cancer cells regardless of their estrogen dependence and p53 status, via modulation of specific cell cycle regulatory pathways.
- An *in vivo* xenograft model showed DIM strongly inhibited development of breast tumors.[48]
- Studies with I3C and DIM have indicated efficacy against a number of human cancers and have demonstrated chemo-sensitization activity wherein they help reduce the toxicity and resistance against conventional chemotherapeutic drugs.[49]
- Molecular targets modulated by these compounds include survivin, uPA/uPAR and NF-*k*β, which are important for the apoptosis-inducing and chemo-sensitizing properties of these compounds.[49]
- Epidemiological studies have shown that consumption of cabbage and sauerkraut is connected with a significant reduction in breast cancer incidence, with reduction in unfavorable estrogen metabolites that substantially contributing to carcinogenic activity via P450 enzyme modulation.[50]
- Treatment with cabbage and sauerkraut juices, I3C and DIM induced CYP1A1 preferentially to CY1B1 supporting the chemo-preventive activity of these compounds.[50]
- DIM was found to differentially modulate cell-cycle signaling pathways in both estrogen-dependent and estrogen receptor negative p53 mutant human breast cancer cells. DIM inhibited growth *in vitro* and *in vivo*, causing cell-cycle arrest by down-regulating protein levels of cell-cycle related kinases CDK 1, CDK 2, CDK 4 and CDK 6 as well as Cyclin B1 and Cdc25a. DIM increased miR-21 expression and down-regulated Cdc25A.[51]
- I3C and DIM induced overlapping and unique responses in multiple cancer cell lines and tumors; these included growth inhibition, apoptosis and antiangiogenic activities. The mechanisms of these responses are complex and dependent on cell context. I3C and/or DIM activate or inactivate multiple nuclear receptors, induce endoplasmic reticulum stress, decrease mitochondrial membrane potential, and modulate multiple signaling pathways including kinases.[52]

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