Published Studies on Curcumin with Turmeric Essential Oil

Please note: These studies may refer to either BCM-95® or Curcugreen™. Both names are used to denote the same patented blend of curcumin and turmeric essential oil.

Clinical Trials [28]

1. **Curcumin and inflammation in non-alcoholic fatty liver disease: a randomized, placebo controlled clinical trial.** BACKGROUND: The aim of the present study was to evaluate the effects of curcumin supplementation on inflammatory indices, and hepatic features in patients with non-alcoholic fatty liver disease (NAFLD). METHODS: Fifty patients with NAFLD were randomized to receive lifestyle modification advice plus either 1500 mg curcumin or the same amount of placebo for 12 weeks. RESULTS: Curcumin supplementation was associated with significant decrease in hepatic fibrosis ($p < 0.001$), and nuclear factor-kappa B activity ($p < 0.05$) as compared with the baseline. Hepatic steatosis and serum level of liver enzymes, and tumor necrosis-α (TNF-α) significantly reduced in both groups ($p < 0.05$). None of the changes were significantly different between two groups. CONCLUSION: Our results indicated that curcumin supplementation plus lifestyle modification is not superior to lifestyle modification alone in amelioration of inflammation. [Saadati S, et al. Curcumin and inflammation in non-alcoholic fatty liver disease: a randomized, placebo controlled clinical trial. *BMC Gastroenterology*. 2019 Jul 25;19(1):133.]

2. **The effect of curcumin supplementation anthropometric indices, insulin resistance and oxidative stress in patients with type 2 diabetes: a randomized, double-blind clinical trial.** Background: Diabetes mellitus is a common metabolic disorders in human and affect a lot of people around the world. Curcumin is a component of turmeric and in many studies therapeutic effects such as anti-hypertensive, anti-hyperlipidemia, anti-hyperglycemia for this substance are shown. Aim: The aim of this study was to investigate the effect of curcumin supplementation on anthropometric indices glycemic control and oxidative stress in overweight patients with type 2 diabetes. Materials and methods: In this randomized, double-blind, placebo-controlled trial, 53 participants with type 2 diabetes were divided randomly into the experimental and control groups to receive either 1500 mg curcumin or placebo capsule three times in a day for 10 weeks. Results: Supplementation with curcumin in type 2 diabetes compare to placebo causes a significant changes in mean weight (-0.64 ± 0.22 vs. 0.19 ± 0.37 $p < 0.05$), body mass index (BMI) (0.3 ± 0.03 vs. 0.1 ± 0 $p < 0.05$), waist circumference (WC) (-1.2 ± 0.4 vs. -0.43 ± 0.11 $p < 0.05$) and fasting blood sugar (FBS) (-7 ± 2 vs. 3 ± 0.2 $p < 0.05$) but did not show any difference for hemoglobin A1c (HbA1c), insulin, malondialdehyde (MDA), total antioxidant capacity (TAC), Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and pancreatic B cell function (HOMA-B) at end of study. Conclusion: This study indicated that daily administration of 1500 mg curcumin has positive effects in reducing fasting blood glucose and weight in patients with type 2 diabetes. [Hodaei H, Adibian M, Nikpayam O, Hedayati M, Sohrab G. The effect of curcumin supplementation anthropometric indices, insulin resistance and oxidative stress in patients with type 2 diabetes: a randomized, double-blind clinical trial. *Diabetol Metab Syndr*. 2019 May 27;11:41.]

3. **The effect of curcumin on high-sensitivity C-reactive protein, serum adiponectin, and lipid profiles in type 2 diabetes.** Diabetes mellitus is one of the most common and important metabolic diseases in human. Curcumin, which is a natural polyphenol found in turmeric, can be used in treatment of diabetes complications for its antidiabetic, anti-inflammatory, and antioxidant properties. In this double-blind randomized clinical trial, 44 patients with Type 2 diabetes randomly assigned to curcumin or placebo group. Patients consumed either 1,500-mg curcumin or placebo daily for 10 weeks. Anthropometric measurements were measured at baseline and at the end of the study. Serum concentrations of triglyceride (TG), total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, high-sensitivity C-reactive protein, and adiponectin were determined after 12-hr fasting at the beginning and end of study. The mean serum level of TG decreased in curcumin group compared with baseline (109 ± 36 vs. 124 ± 36; $p < 0.05$). At the end of study, the mean concentration of high-sensitivity C-reactive protein decreased in the curcumin group compared to the control (2.9 ± 2.9 vs. 3.4 ± 4.2; $p < 0.05$). The mean serum concentration of adiponectin increased (64 ± 3 vs. 63 ± 4; $p < 0.05$) in the treatment group compared with the placebo at the end of the study. The results of the current study indicate that curcumin consumption may reduce diabetes complications through decreasing TG level as well as indicators of inflammation. [Adibian M, Hodaei H, Nikpayam O, Sohrab G, Hekmatdoost A, Hedayati M. The effects of curcumin supplementation on high-sensitivity C-reactive protein, serum adiponectin, and lipid profile in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial. *Phytother Res*. 2019 May;33(5):1374-1383.]

4. **Safety and efficacy of curcumin versus diclofenac in knee osteoarthritis: a randomized open-label parallel-arm study**. BACKGROUND: The purpose of this study was to compare the efficacy and safety of curcumin with those of diclofenac in the treatment of knee osteoarthritis (OA). METHODS: In this randomized, open-label, parallel, active controlled clinical study, 139 patients with knee OA were randomly assigned to receive either a curcumin 500-mg (BCM-95®) capsule three times daily or a diclofenac 50-mg tablet two times daily for 28 days. Patients underwent assessment at baseline and days 7, 14, and 28. The main outcome measure was severity of pain using visual analogue scale score at days 14 and 28. Knee Injury and Osteoarthritis Outcome Score (KOOS) (at days 14 and 28), anti-flatulent effect (at day 7), anti-ulcer effect, weight-lowering effect, and patient's and physician's global assessment of therapy at day 28 were included as secondary outcome measures. Safety after treatment was evaluated by recording adverse events and laboratory investigation. RESULTS: At days 14 and 28, patients receiving curcumin showed similar improvement in severity of pain and KOOS scale when compared with diclofenac, and the difference was not statistically significant. At day 7, the patients who received curcumin experienced a significantly greater reduction in the number of episodes of flatulence compared with diclofenac ($P < 0.01$). At day 28, a weight-lowering effect ($P < 0.01$) and anti-ulcer effect ($P < 0.01$) of curcumin were observed. None of the patients required H2 blockers in the curcumin group, and 19 patients required H2 blockers in the diclofenac group (0% versus 28%, respectively; $P < 0.01$). Adverse effects were significantly less in the curcumin group (13% versus 38% in the diclofenac group; $P < 0.01$). Patient's and physician's global assessment of therapy was similar in the two treatment groups. CONCLUSION: Curcumin has similar efficacy

to diclofenac but demonstrated better tolerance among patients with knee OA. Curcumin can be an alternative treatment option in the patients with knee OA who are intolerant to the side effects of non-steroidal anti-inflammatory drugs. [Shep D, Khanwelkar C, Gade P, Karad S. Safety and efficacy of curcumin versus diclofenac in knee osteoarthritis: a randomized open-label parallel-arm study. *Trials*. 2019 Apr 11;20(1):214.]

5. **Role of curcumin as an adjuvant in treatment of advanced head and neck squamous cell carcinoma.** Background: Chemoradiation forms the major line of treatment in advanced head and neck squamous cell carcinoma, but the benefit of chemotherapeutic agents is at the expense of various toxicities. Curcumin has demonstrated promising results in in-vivo and in-vitro studies as a radiosensitiser. The objective of the study was to determine the role of curcumin as an adjuvant in patients undergoing chemo radiation for advanced head and neck cancers. Methods: Study involved 21 patients who underwent chemo radiotherapy for advanced head and neck cancers. They were randomized into two groups. Group A received 500 mg of curcumin while, Group B received placebo along with chemoradiation. The response was assessed using RECIST criteria at three months post treatment using contrast enhanced computerized tomography scan. Results: Overall 58.3% patients had partial response and 41.7% patients had stable disease in group A. In group B, 33.3% patients had a partial response and 66.6% patient had a stable disease. Conclusions: Patients receiving curcumin along with chemoradiation had a marginal decrease in tumour volume and 58.3% patients had partial response and 41.7% had stable disease. A statistical significance could not be achieved due to lack of stage-match controls. Further studies are required to validate the role of curcumin as an adjuvant in the treatment of head and neck squamous cell carcinomas. [Arun P, Sagayaraj A, Azeem Mohiyuddin SM, Santhosh D. Role of curcumin as an adjuvant in treatment of advanced head and neck squamous cell carcinoma. *Int J Otorhinolaryngol Head Neck Surg*. 2018 Nov;4(6):1388-1393.]

6. **Effect of Biocurcumax™ Curcumin (BCM-95) On Treatment of Moderate Chronic Periodontitis.** Background and purpose: Controlling inflammation is a major approach in periodontal treatments, but scaling and root planing are not always effective enough. Curcumin is anti-inflammatory and can adjust inflammatory reactions and its efficacy and immunity is proven. The present research aimed at evaluating the potential of Curcumin BCM-95 in treatment of patients with chronic periodontitis. Materials and methods: In a double blind clinical trial, the clinical parameters including, Gingival Sulcus Bleeding Index (GSBI), Loe and Silness Gingival Index (GI), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL) were recorded at the beginning of the study, at week 6, and month 4. In case group, patients with moderate chronic periodontitis who had no systemic disease with at least one periodontal pocket with 4-6mm depth in each quadrant and bleeding on probing were chosen. After scaling and root planing, the patients took 2 Curcumin oral capsules per day for 4 weeks. The patients in the control group were given placebos. Results: The effect of time was found to be significant in PPD, GI, CAL, and GSBI. Moreover, significant differences were seen between PPD average measurements before medication, at first follow up, and second follow-up ($P<0.05$). But, in GI, GSBI, and CAL the group effect was not significant. In other words, the reduction was seen in these parameters in both groups but they were not significant. Conclusion: The effect of Biocurcumax ^{TM} Curcumin (BCM-95) was

significant in treatment of moderate chronic periodontitis in PPD between the two groups which reduced this parameter. [Amoian B, Ehsani H, Moghadamnia A, Satari FD, Ehsani H. Effect of Biocurcumax™ Curcumin (BCM-95) On Treatment of Moderate Chronic Periodontitis. *J Mazandaran Univ Med Sci*. 2018;27(158):45-55.]

7. **A Randomized Double-Blind Placebo-Controlled Phase IIB Trial of Curcumin in Oral Leukoplakia.** Oral leukoplakia is a potentially malignant lesion of the oral cavity, for which no effective treatment is available. We investigated the effectiveness of curcumin, a potent inhibitor of NF-kB/COX-2, molecules perturbed in oral carcinogenesis, to treat leukoplakia. Subjects with oral leukoplakia (n = 223) were randomized (1:1 ratio) to receive orally, either 3.6 g/day of curcumin (n = 111) or placebo (n = 112), for 6 months. The primary endpoint was clinical response obtained by bi-dimensional measurement of leukoplakia size at recruitment and 6 months. Histologic response, combined clinical and histologic response, durability and effect of long-term therapy for an additional six months in partial responders, safety and compliance were the secondary endpoints. Clinical response was observed in 75 (67.5%) subjects [95% confidence interval (CI), 58.4–75.6] in the curcumin and 62 (55.3%; 95% CI, 46.1–64.2) in placebo arm (P = 0.03). This response was durable, with 16 of the 18 (88.9%; 95% CI, 67.2–96.9) subjects with complete response in curcumin and 7 of 8 subjects (87.5%) in placebo arm, demonstrating no relapse after 6 months followup. Difference in histologic response between curcumin andplacebo was not significant (HR, 0.88, 95% CI, 0.45–1.71; P = 0.71). Combined clinical and histologic response assessment indicated a significantly better response with curcumin (HR, 0.50; 95% CI, 0.27–0.92; P = 0.02). Continued therapy, in subjects with partial response at 6 months, did not yield additional benefit. The treatment did not raise any safety concerns. Treatment of oral leukoplakia with curcumin (3.6 g for six months), thus was well tolerated and demonstrated significant and durable clinical response for 6 months. [Kuriakose MA, et al. A Randomized Double-Blind Placebo-Controlled Phase IIB Trial of Curcumin in Oral Leukoplakia. *Cancer Prevention Research*. 2016;9:693-691.]

8. **Curcumin and cognition: a randomised, placebo-controlled, double-blind study of community-dwelling older adults.** Curcumin therapy in animals has produced positive cognitive and behavioural outcomes; results of human trials, however, have been inconsistent. In this study, we report the results of a 12-month, randomised, placebo-controlled, double-blind study that investigated the ability of a curcumin formulation to prevent cognitive decline in a population of community-dwelling older adults. Individuals (n 96) ingested either placebo or 1500 mg/d BiocurcumaxTM for 12 months. A battery of clinical and cognitive measures was administered at baseline and at the 6-month and 12-month follow-up assessments. A significant time×treatment group interaction was observed for the Montreal Cognitive Assessment (repeated-measures analysis; time×treatment; F=3·85, P<0·05). Subsequent analysis revealed that this association was driven by a decline in function of the placebo group at 6 months that was not observed in the curcumin treatment group. No differences were observed between the groups for all other clinical and cognitive measures. Our findings suggest that further longitudinal assessment is required to investigate changes in cognitive outcome measures, ideally in conjunction with biological markers of neurodegeneration. [Rainey-Smith SR, Brown BM, Sohrabi HR, Shah T, Goozee KG, Gupta VB, Martins RN.

Curcumin and cognition: a randomised, placebo-controlled, double-blind study of community-dwelling older adults. *Br J Nutr*. 2016 Apr 22:1-8 (Epub ahead of print.)]

9. **Effect of Curcumin Supplementation During Radiotherapy on Oxidative Status of Patients with Prostate Cancer: A Double Blinded, Randomized, Placebo-Controlled Study.** Curcumin is an antioxidant agent with both radiosensitizing and radioprotective properties. The aim of the present study was to evaluate the effect of curcumin supplementation on oxidative status of patients with prostate cancer who undergo radiotherapy. Forty patients treated with radiotherapy for prostate cancer were randomized to the curcumin (CG, $n = 20$) or placebo group (PG, $n = 20$). They received curcumin (total 3g/day) or placebo during external-beam radiation therapy of up to 74 Gy. Plasma total antioxidant capacity (TAC) and activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) were measured at baseline and 3 mo after radiotherapy completion. Analysis of covariance was used to compare the variables between groups following the intervention. Serum PSA levels and MRI/MRS images were investigated. In CG, TAC significantly increased ($P < 0.001$) and the activity of SOD decreased ($P = 0.018$) after radiotherapy compared with those at baseline. In CG, however, the activity of SOD had a significant reduction ($P = 0.026$) and TAC had a significant increase ($P = 0.014$) compared with those in PG. PSA levels were reduced to below 0.2 ng/ml in both groups, 3 mo after treatment, however, no significant differences were observed between the 2 groups regarding treatment outcomes. [Hejazi J, Rastmanesh R, Taleban FA, Molana SH, Hejazi E, Ehtejab G and Hara N. Effect of Curcumin Supplementation During Radiotherapy on Oxidative Status of Patients with Prostate Cancer: A Double Blinded, Randomized, Placebo-Controlled Study. *Nutrition and Cancer*. 2016;0(0):1-9.]

10. **Impact of a 3-weeks randomized double-blind cross-over study curcuminoid supplementation on endotoxemia, inflammatory markers, and lipid profiles in healthy overweight and obese adults.** Postprandial endotoxaemia (increased bacterial lipopolysaccharide [LPS] level in the circulation) is associated to the increase of pro-inflammatory markers after intake of high-fat high-calorie meals. Endotoxaemia is a potential driver for chronic low-grade inflammation, linked to non-communicable chronic disease. Curcumin (turmeric) has potential anti-inflammatory and hypolipidemic properties; and was shown to attenuate the effect of LPS-induced endotoxaemia in rats. The aim of this study was to investigate whether curcuminoid supplementation affected postprandial endotoxaemia, inflammatory markers, and lipid profiles in humans. Healthy volunteers (n = 16, 50% men and 50% women, aged 19-43 y, BMI 25-44 kg/m^2, fat mass 19-53%) participated in a double-blinded, placebo-controlled, cross-over study (4 weeks wash-out period). Participants were randomized to 1 capsule per day, curcuminoids (380 mg) or placebo, for three weeks. Postprandial endotoxaemia was induced by single high-fat high-calorie meal intake (929 kcal, 65 g fat, 63 %E). Blood samples were collected before and after each leg of the study. Endotoxaemia markers (sCD14 & LBP) and inflammatory markers (CRP, TNF-α, IL-6, IL-1β, and IL-10) were measured with immunoassays; lipids were measure colorimetrically. Two participants dropped-out. There was no change in LBP for either trial leg. Subgroup analysis however indicated a 22% decrease in LBP in volunteers with very high fat mass (n = 5) after leg A ($p = 0.02$). No differences were seen in CRP level after either leg, with large inter-individual

variability (36.4-1028.3 ng/mL). sCD14 decreased after both legs (leg A, $p = 0.015$; leg B, $p = 0.019$). There were no effects on TNF-α, IL-6, IL-1β. HDL level (before high-fat meal) was significantly higher after leg A ($p = 0.01$) with no different after the meal. No other effects were seen on total cholesterol, LDL, and triglycerides. HDL is the main lipoprotein removing LPS from the circulation, transporting LPS to the hepatocytes for clearance. Thus increased HDL level could be of benefit to reduce LPS and inhibit subsequent inflammatory responses. Assessing LPS level in plasma will give a better understanding of this effect. [Nuraiza M, Edwards CA, Combet E. Impact of a 3-weeks randomized double-blind cross-over study curcuminoid supplementation on endotoxemia, inflammatory markers, and lipid profiles in healthy overweight and obese adults. *Proceedings of the Nutrition Society*. 2016 July;75(OCE3):E160.]

11. **Curcumin and Major Depression: A Randomised, Double-blind, Placebo Controlled Trial Investigating the Potential of Peripheral Biomarkers to Predict Treatment Response and Antidepressant Mechanisms of Change.** This trial provided partial support for the efficacy of supplementation with a patented curcumin extract (500 mg, twice daily BCM-95 Curcumin) for 8 weeks in reducing depressive symptoms in people with major depressive disorder. In the present paper, a secondary, exploratory analysis of salivary, urinary and blood biomarkers collected during this study was conducted to identify potential antidepressant mechanisms of action of curcumin. Pre and post-intervention samples were provided by 50 participants diagnosed with major depressive disorder, and the Inventory of Depressive Symptomatology self-rated version (IDS-SR30) was used as the primary depression outcome measure. Compared to placebo, 8 weeks of curcumin supplementation was associated with elevations in urinary thromboxane B2 ($p <.05$), and substance P ($p <001$); while placebo supplementation was associated with reductions in aldosterone ($p <05$) and cortisol ($p <05$). Higher baseline plasma endothelin-1 ($rs=−.587$; $p <01$) and leptin ($rs=−.470$; $p <05$) in curcumin-treated individuals was associated with greater reductions in IDS-SR30 score after 8 weeks of treatment. Our findings demonstrate that curcumin supplementation influences several biomarkers that may be associated with its antidepressant mechanisms of action. Plasma concentrations of leptin and endothelin-1 seem to have particular relevance to treatment outcome. Further investigations using larger samples sizes are required to elucidate these findings, as the multiple statistical comparisons completed in this study increased the risk of type I errors. [Lopresti AL, Maes M, Maker GL, Hood S, Drummond PD. Curcumin and major depression: A randomised, double-blind, placebo-controlled trial investigating the potential of peripheral biomarkers to predict treatment response and antidepressant mechanisms of change. *Eur Neuropsychopharmacol*. 2015;25(1):38-50.]

12. **Curcumin for the Treatment of Major Depression: A Randomised, Double-blind, Placebo Controlled Study.** In this study, 56 individuals with major depressive disorder were treated with BCM-95 curcumin (500 mg twice daily) or placebo for 8 weeks. The primary measure was the Inventory of Depressive Symptomatology self-rated version ($IDS\text{-}SR_{30}$). Secondary outcomes included $IDS\text{-}SR_{30}$ factor scores and Spielberger State-Trait Anxiety Inventory (STAI). From baseline to week 4, both BCM-95 curcumin and placebo were associated with improvements in $IDS\text{-}SR_{30}$ total score and most secondary outcome measures. From weeks 4 to 8, BCM-95 curcumin was significantly more effective than placebo in improving several mood-related symptoms, demonstrated by a

significant group x time interaction for IDS-SR_{30} total score and IDS-SR_{30} mood score, and a non-significant trend for STAI trait score. BCM-95 curcumin was shown to have antidepressant effects in people with major depressive disorder, as evidenced by benefits occurring 4 to 8 weeks after treatment. Greater efficacy from curcumin treatment was identified in a subgroup of individuals with atypical depression. [Lopresti AL, Maes M, Maker GL, Hood S, Drummond PD. Curcumin for the treatment of major depression: A randomised, double-blind, placebo controlled study. *J Affect Disord.* 2014;167:368-375.]

13. **A Pilot Clinical Trial of Radioprotective Effects of Curcumin Supplementation in Patients with Prostate Cancer.** Patients with prostate cancer receiving radiation therapy usually experience several side effects and these toxicities are sometimes dose limiting. The purpose of this investigation was to assess the radioprotective effects of BCM-95 Curcumin supplementation in patients with prostate cancer. Forty prostate cancer patients undergoing external beam radiotherapy (EBRT) were randomly assigned to curcumin group, taking 3 g/d curcumin (6 × 500 mg capsules of BCM95 n=20), or placebo group (n=20). Analysis of covariance was used to compare radiotherapy related symptoms between groups following the intervention, adjusted for baseline symptoms. The change in urinary symptoms across the 20-week period differed significantly between groups (p=0.011) and patients in the BCM-95 Curcumin group experienced much milder urinary symptoms compared with the placebo group. BCM-95 Curcumin can confer radioprotective effect in patients with prostate cancer who undergo radiation therapy through reducing the severity of radiotherapy related urinary symptoms. [Hejazi J, Rastmanesh R, Taleban F, Molana S, Ehtejab G. A pilot clinical trial of radioprotective effects of curcumin supplementation in patients with prostate cancer. *J Cancer Sci Ther*. 2013;5:320-324.]

14. **Efficacy and Safety of Curcumin in Major Depressive Disorder: A Randomized Controlled Trial.** Curcumin, an active ingredient of Curcuma longa Linn (Zingiberaceae), has shown potential antidepressant-like activity in animal studies. The objectives of this trial were to compare the efficacy and safety of curcumin with fluoxetine in patients with major depressive disorder (MDD). Herein, 60 patients diagnosed with MDD were randomized in a 1:1:1 ratio for six weeks observer-masked treatment with fluoxetine (20 mg) and curcumin (1000 mg) individually or their combination. The primary efficacy variable was response rates according to Hamilton Depression Rating Scale, 17-item version (HAM-D17). The secondary efficacy variable was the mean change in HAM-D17 score after six weeks. We observed that curcumin was well tolerated by all the patients. The proportion of responders as measured by the HAM-D17 scale was higher in the combination group (77.8%) than in the fluoxetine (64.7%) and the curcumin (62.5%) groups; however, these data were not statistically significant (P= 0.58). Interestingly, the mean change in HAM-D17 score at the end of six weeks was comparable in all three groups (P= 0.77). This study provides first clinical evidence that curcumin may be used as an effective and safe modality for treatment in patients with MDD without concurrent suicidal ideation or other psychotic disorders. [Sanmukhani J, Satodia V, Trivedi J, Patel T, Tiwari D, Panchal B, Goel A, Tripathi CB. Efficacy and safety of curcumin in major depressive disorder: a randomized controlled trial. *Phytother Res.* 2013;28(4):579-85.]

15. **A Randomized, Pilot Study to Assess the Efficacy and Safety of Curcumin in Patients with Active Rheumatoid Arthritis.** In this study, 45 patients with rheumatoid arthritis were randomized into 3 groups, with patients receiving either BCM-95 curcumin 500 mg twice daily, the prescription drug diclofenac sodium (one brand name is Voltaren®) 50 mg twice daily, or a combination of the two. The results were judged using the clinically validated Disease Activity Score (DAS) 28 and also with the American College of Rheumatology (ACR) criteria and scores for pain and swelling in joints. Patients in all 3 groups improved. The curcumin group showed the greatest improvement, and the endpoint scores were significantly better than the patients in the drug group. Using both interventions concurrently did not show any additional benefit with regards to disease scores. Curcumin was found to be safe with no adverse effects in this study. In the drug group. 14% of the patients withdrew because of adverse effects. [Chandran B, Goel A. A randomized, pilot study to assess the efficacy and safety of curcumin in patients with active rheumatoid arthritis. *Phytother Res*. 2012 Nov;26(11):1719-25.]

16. **Comparative Study of the Efficacy of Curcumin and Turmeric as Chemopreventative Agents in Oral Submucous Fibrosis: A Clinical and Histopathological Evaluation.** Oral Submucous Fibrosis (OSMF) is a chronic disease of the oral mucosa. Premalignant lesions form, with a high progression rate to oral cancer. The goal of this study was to determine if BCM-95 curcumin and turmeric essential oil could improve health of the tissue and help prevent conversion to oral cancer. Participants were randomized to 3 groups of 16 people each: Group one received 1 capsule of BCM-95 curcumin, 500 mg curcuminioids, twice daily; group 2 received 12 drops of turmeric essential oil, held in the mouth twice daily then swallowed, for an approximate dosage of 600 mg, and the last group was placebo twice daily. Both BCM-95 curcumin and turmeric essential oil reduced oral discomfort/mouth burning significantly. The study lasted 6 months, and there were significant reductions in disease scores for both group 1 and 2 at each measurement. The authors reported "remarkable improvements after only the first 15 days of use." After 6 months of use, 7 of the 16 participants in the placebo group were in the advanced disease stage (meaning closer to malignancy) compared to only 1 person in the BCM-95 curcumin group. No serious adverse effects were noted, and the authors called for more and larger trials, as this holds good promise for treatment of OSMF in the future." [Deepa Das A, Balan A, Sreelatha KT. Comparative study of the efficacy of curcumin and turmeric as chemopreventative agents in oral submucous fibrosis: a clinical and histopathological evaluation. *JIAOMR*; April-June 2010;22(2):88-92.]

17. **Human Clinical Study to Evaluate the Bioavailability of BCM-95®.** 15 healthy men and women ages 24-45; 8 assigned to plain curcumin and 7 assigned to BCM-95 curcumin. Results: overall, 7-fold increase over course of 12 hours. BCM-95 peak at 1600 ng/g; plain curcumin peak at ~230 ng/g. BCM-95 curcumin remained above 200 ng/g for 12 hours. Plain curcumin remained above 200 ng/g for less than 2 hours. Two hours after ingestion, BCM-95 levels are 10-fold over plain curcumin. [Benny M, Antony B. Bioavailability of BioCurcumax™ (BCM-095™)**.** *Spice India*. September, 2006:11-15.]

18. **A Pilot Cross-Over Study to Evaluate Human Oral Bioavailability of BCM-95®, A Novel Bioenhanced Preparation of Curcumin.** This study compared BCM-95 curcumin's absorption in human subjects to plain curcumin and also to curcumin enhanced with piperine (black pepper extract) and lecithin. The results showed that BCM-95 curcumin was absorbed 7 times (or 700%) better than plain curcumin, and at one time measure point, showed a blood level 10 times that of plain curcumin. BCM-95 was absorbed 6.3 (or 630%) better than curcumin with piperine and lecithin. [Antony B, et al. A pilot cross-over study to evaluate human oral bioavailability of BCM-95CG (Biocurcumax), a novel bioenhanced preparation of curcumin. *Ind J Pharm Sci.* 2008 Jul-Aug;70(4):445-9.]

19. **Six-Month Randomized Placebo-Controlled, Double-Blind, Pilot Clinical Trial of Curcumin in Patients with Alzheimer's Disease.** 34 participants were randomized to either 1 gram BCM-95® curcumin, 4 grams BCM-95 curcumin, or placebo. All participants were over age 50, and had a diagnosis of probable or possible Alzheimer's disease based on the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer Disease and Related Disorders Association diagnostic criteria. Some measures were serum markers of amyloid beta, plasma isoprostanes (a measure of oxidative stress) and antioxidant status. Both 1 gram and 4 grams reduced oxidative stress and improved antioxidant status. There were more adverse effects in the placebo group than in either 1 g or 4 g BCM-95 group. There was a noted increase in serum amyloid beta in both 1 g and 4 g groups, but not placebo. The authors noted this "possibly reflected an ability of curcumin to disaggregate amyloid beta deposits in the brain, releasing the amyloid beta for circulation and disposal." [Baum L, Lam CW, Cheung SK, et al. Six-month randomized placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer's Disease. *J Clin Psychopharmacol.* 2008 Feb;28(1):110-3.]

20. **Curcumin Effects on Blood Lipid profile in a 6-month Human Study**. No significant cholesterol lowering effects found, though authors speculate curcumin has other cardioprotective physiological effects. [Baum L, Cheung SK, Mok VC, et al. Curcumin effects on blood lipid profile in a 6-month human study. *Pharmacol Res.* 2007 Dec;56(6):509-14.]

Clinical Studies Using Curcumin Combinations

21. **Efficacy and safety of combination of curcuminoid complex and diclofenac versus diclofenac in knee osteoarthritis.** Background: To compare the efficacy and safety of combination of curcuminoid complex and diclofenac vs diclofenac alone in the treatment of knee osteoarthritis (OA). Methods: In this randomized trial, 140 patients of knee OA received either curcuminoid complex 500mg (BCM-95) with diclofenac 50mg 2 times daily or diclofenac 50 mg alone 2 times daily for 28 days. Patients were assessed at baseline, day 14 and day 28. Primary efficacy measures were Knee injury and OA outcome score (KOOS) subscale at day 14 and day 28. Anti-ulcer effect and patient-physician's global assessment of therapy at day 28 were included as secondary endpoints. Safety after treatment was evaluated by recording adverse events and laboratory

investigations. Results: Both treatment groups showed improvement in primary endpoints at each evaluation visit. Patients receiving curcuminoid complex plus diclofenac showed significantly superior improvement in KOOS subscales, viz.pain and quality of life at each study visit *(P>.001)* when compared to diclofenac. Less number of patients required rescue analgesics in curcuminoid complex plus diclofenac group (3%) compared to diclofenac group (17%). The number of patients who required histamine 2 (H2) blockers was significantly less in curcuminoid complex plus diclofenac group compared to diclofenac group (6% vs 28%, respectively; *P<.001)*. Adverse effects were significantly less in curcumoid complex plus diclofenac group (13% vs 38% in diclofenac group; *P<.001)*. Patient's and physician's global assessment of therapy favored curcuminoid complex plus diclofenac. Conclusion: Combination of curcuminoid complex and diclofenac showed a greater improvement in pain and functional capacity with better tolerability and could be a better alternative treatment option in symptomatic management of knee OA. [Shep D, Khanwelkar C, Gade P, Karad S. Efficacy and safety of combinations of curcuminoid complex and diclofenac versus diclofenac in knee osteoarthritis:a randomized trial. *Medicine* 2020 99:16]

22. **Efficacy and safety of curcumin and its combination with boswellic acid in osteoarthritis: a comparative, randomized, double-blind, placebo-controlled study.** BACKGROUND: The aim of this clinical trial was to assess the efficacy and safety of curcuminoid complex extract from turmeric rhizome with turmeric volatile oil (CuraMed®) and its combination with boswellic acid extract from Indian frankincense root (Curamin® [Swedish formula]) vs placebo for the treatment of 40- to 70-year-old patients with osteoarthritis (OA). METHODS: The effects of CuraMed® 500-mg capsules (333 mg curcuminoids) and Curamin® 500-mg capsules (350 mg curcuminoids and 150 mg boswellic acid) taken orally three times a day for 12 weeks in 201 patients was investigated in a three-arm, parallel-group, randomized, double-blinded, placebo-controlled trial. Primary outcome efficacy measures included OA physical function performance-based tests, the WOMAC recommended index of joint pain, morning stiffness, limitations of physical function, and the patients' global assessment of disease severity. RESULTS: Favorable effects of both preparations compared to placebo were observed after only 3 months of continuous treatment. A significant effect of Curamin® compared to placebo was observed both in physical performance tests and the WOMAC joint pain index, while superior efficacy of CuraMed vs placebo was observed only in physical performance tests. The effect size compared to placebo was comparable for both treatment groups but was superior in the Curamin® group. The treatments were well tolerated. CONCLUSIONS: Twelve-week use of curcumin complex or its combination with boswellic acid reduces pain-related symptoms in patients with OA. Curcumin in combination with boswellic acid is more effective. Combining Curcuma longa and Boswellia serrata extracts in Curamin® increases the efficacy of OA treatment presumably due to synergistic effects of curcumin and boswellic acid. [Haroyan A, et al. Efficacy and safety of curcumin and its combination with boswellic acid in osteoarthritis: a comparative, randomized, double-blind, placebo-controlled study. *BMC Complement Altern Med*. 2018 Jan 9;18(1):7.]

23. **Efficacy of curcumin, and a saffron/curcumin combination for the treatment of major depression: A randomised, double-blind, placebo-controlled study.**

Background: Several studies have supported the antidepressant effects of curcumin (from the spice turmeric) and saffron for people with major depressive disorder. However, these studies have been hampered by poor designs, small sample sizes, short treatment duration, and similar intervention dosages. Furthermore, the antidepressant effects of combined curcumin and saffron administration are unknown. Methods: In a randomised, double-blind, placebo-controlled study, 123 individuals with major depressive disorder were allocated to one of four treatment conditions, comprising placebo, low-dose curcumin extract (250 mg b.i.d.), high-dose curcumin extract (500 mg b.i.d.), or combined low-dose curcumin extract plus saffron (15 mg b.i.d.) for 12 weeks. The outcome measures were the Inventory of Depressive Symptomatology self-rated version (IDS-SR30) and Spielberger State-Trait Anxiety Inventory (STAI). Results: The active drug treatments (combined) were associated with significantly greater improvements in depressive symptoms compared to placebo ($p=.031$), and superior improvements in STAI-state ($p < .001$) and STAI-trait scores ($p=.001$). Active drug treatments also had greater efficacy in people with atypical depression compared to the remainder of patients (response rates of 65% versus 35% respectively, $p=.012$). No differences were found between the differing doses of curcumin or the curcumin/saffron combination. Limitations: Investigations with larger sample sizes are required to examine the efficacy of differing doses of curcumin and saffron/curcumin combination. Its effects in people with atypical depression also require examination in larger scale studies. Conclusions: Active drug treatments comprising differing doses of curcumin and combined curcumin/saffron were effective in reducing depressive and anxiolytic symptoms in people with major depressive disorder. [Lopresti AL, Drummond PD. Efficacy of curcumin, and a saffron/curcumin combination for the treatment of major depression: A randomised, double-blind, placebo-controlled study. *Journal of Affective Disorders*. 2017;207:188-196.]

24. **Effect of Infla-Kine supplementation on gene expression of inflammatory markers in peripheral mononuclear cells and on C-reaction protein in blood.** Background: Chronic inflammation is a predisposing factor to numerous degenerative diseases including cancer, heart failure and Alzheimer's disease. Infla-Kine is a natural supplement comprised of a proprietary blend of *Lactobacillus* fermentum extract, burdock seed (arctigenin), zinc, alpha lipoic acid, papaya enzyme and an enhanced absorption bio-curcumin complex (BCM-95®). Methods: Infla-Kine was administered twice daily to 24 health volunteers for 4 weeks. Quantitative RT-PCR was used to assess mRNA transcripts of IL-1b, IL8, IL-6, NF-κB, and TNF-α from peripheral blood mononuclear cells (PBMC). C-reactive protein (CRP) was measured from serum. Additionally, quality of life questionnaires were employed to assess general feeling of well-being. Assessments were made before treatment and at conclusion of treatment (4 weeks). Results: As compared to pre-treatment, after 4 weeks, a statistically significant reduction of IL8, IL-6, NF-κB, and TNF-α transcripts was observed in PBMC. Furthermore, reduction of IL-1b transcript and serum CRP was observed but did not reach statistical significance. Quality of life improvements were most prevalent in muscle and joint pains. Conclusions: Overall, our data demonstrate that twice daily administration of Infla-Kine for 4 weeks reduces inflammatory markers and quality of life in healthy volunteers. [Mikirova NA, Kesari S, Ichim TE, Riordan NH. Effect of

Infla-Kine supplementation on the gene expression of inflammatory markers in peripheral mononuclear cells and on C-reactive protein in blood. *J Transl Med*. 2017;15(1):213.]

25. **The efficacy and safety of a combination of glucosamine hydrochloride, chondroitin sulfate and bio-curcumin with exercise in the treatment of knee osteoarthritis: a randomized, double-blind, placebo-controlled study.** BACKGROUND: Knee osteoarthritis (OA) conservative treatment aims to delay cartilage degeneration; chondroprotective agents are a valid approach in this sense. A commercially available dietary supplement, CartiJoint Forte, containing glucosamine hydrochloride (GH), chondroitin sulfate (CS) and Bio-Curcumin BCM-95®, was used in this trial. AIM: The aim of this study was to assess the efficacy and safety of CartiJoint Forte combined with physical therapy in treating subjects with knee OA. DESIGN: A multicenter, prospective, randomized, double blind, placebo-controlled clinical trial. SETTING: Outpatients referred to the Rehabilitation Departments of two University Hospitals. POPULATION: Firty-three patients were randomly assigned to an experimental group (N=26) or a control group (N=27). Experimental subjects received two tablets of CartiJoint Forte each day for 8 weeks, while those in the control group were provided with a placebo. Three subjects dropped out during the course of the study. METHODS: The two groups both received 20 sessions of physical therapy during the course of the trial. Primary outcome was pain intensity, measured both at motion and at rest, using the Visual Analogue Scale (VAS). A secondary outcome was an assessment of knee function by Western Ontario and McMaster Universities Arthritis Index and Lequesne Index, knee ROM, and two inflammation markers (C-reactive protein and erythrocyte sedimentation rate). Each assessment was carried out at baseline (T0) at 8 weeks (T1) and at 12 weeks (T2). RESULTS: VAS at rest was found to be reduced between T0 and T1, as well as between T0 and T2 (F=13.712; P=0.0001), with no differences between groups (F=1.724; P=0.191). VAS at motion revealed a significant “group x time-check” interaction (F=2.491; P=0.032), with increasing effect of time on VAS reduction (F=17.748; P=0.0001). This was most pronounced in the experimental gropu at 8 weeks (F=3.437; P=0.045). The Lequesne Index showed reductions at T1 and T2 compared to T0 (F=9.535; P=0.0001), along with group effect, since the experimental group presented a lower score at T2 (F=7.091; P=0.009). No significant changes were found in the knee ROM and inflammation markers. CONCLUSION: CartiJoint Forte, added to physical therapy, may ameliorate pain and help to improve algofunctional score in knee OA patients. CLINICAL REHABILITATION IMPACT: Treatment of knee OA with curcuminoids plus glycosaminoglycans, added to physical therapy, improves VAS at motion and Lequesne Index scores. [Sterze S, et al. The efficacy and safety of a combination of glucosamine hydrochloride, chondroitin sulfate and bio-curcumin with exercise in the treatment of knee osteoarthritis: a randomized, double-blind, placebo-controlled study. *Eur J of Physical and Rehab Med*. June 2016;52(3):321-330.]

26. **Short Report of a Preliminary Open Study of *Synofit-Containing Bio-Curcumin, Greenlipped Mussel and Blackcurrant Leaf Extract* in Arthritis.** To evaluate the potential benefit of Synofit—an association of Curcumin, *Perna canaliculus* green-mussels and blackcurrant leaf extracts, a real life open study was performed among 86 adult out patients suffering from Fibromyalgia (n = 22), low back pain (n = 33) or knee osteoarthritis (n =31) who accepted to take 3 tablets a day during 1 week then 2 capsules

of Synofit during 2 months in addition to their conventional therapy (mainly analgesics and anti-inflammatory) and then to report their evaluation of this complementary treatment. Statistical analysis included paired t test and when possible Wilcoxon signed rank test. Accordingly, the intermediate analysis showed that already within 4 weeks of treatment, an improvement quoted as "light" was statistically reported in patients with low back pain and knee osteoarthritis but not among those with fibromyalgia on pain, physical condition, global assessment of a benefit, quality of life but not on joint stiffness (although joint stiffness considered for the whole group was statistically improved). The limited number of patients and time duration of the study and the absence of double blind controlled study do not allow concluding on the efficacy but these preliminary analyses obtained from an intermediate analysis are encouraging for further studies. [Qu J, Melot C, Appelboom T. Short Report of a Preliminary Open Study of *Synofit-Containing Bio-Curcumin, Greenlipped Mussel and Blackcurrant Leaf Extract* in Arthritis. *Open Journal of Rheumatology and Autoimmune Diseases*. 2015;5:113-117.]

27. **The use of an anti-inflammatory supplement in patients with chronic kidney disease.** Chronic kidney disease (CKD) is characterized by a continuous reduction in kidney function, increased inflammation, and reduced antioxidant capacity. The objective of this study was to assess the effects of a herbal supplement on systemic inflammation and antioxidant status in non-dialysis CKD patients. Sixteen patients with CKD (56.0±16.0 yrs, 171.4±11.9 cm, 99.3±20.2 kg) were randomly chosen to receive a herbal supplement composed of Curcuma longa and Boswellia serrata, or placebo. Plasma levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), glutathione peroxidase (GPx), and serum C-reactive protein (CRP) were measured at baseline and 8 weeks. Baseline data demonstrated elevated inflammation and low antioxidant levels. A significant time effect (p=0.03) and time x compliance interaction effect (p=0.04) were observed for IL-6. No significant differences were observed for any other variables. This study demonstrates that mild and moderate CKD is associated with chronic inflammation and low antioxidant activity. Systemic inflammation and impaired antioxidant status may be greater in CKD populations with multiple comorbidities. Curcumin and Boswellia serrata are safe and tolerable and helped to improve the levels of an inflammatory cytokine. [Moreillon JJ, et al. The use of an anti-inflammatory supplement in patients with chronic kidney disease. *J Complement Integr Med*. 2013 Jul 1;10.]

28. **Randomized, Controlled Human Clinical Study to Assess the Efficacy and Safety of BCM-95® & Bospure® Compared to Celecoxib in the Management of Knee Osteoarthritis.** Originally presented at the Osteoarthritis Research Symposium Internationale (OARSI) Annual World Congress on Osteoarthritis, September 15-18, 2011. San Diego, CA. 28 subjects with diagnosed osteoarthritis of the knee were randomized to a 500 mg blend BCM-95 curcumin and Bospure® Boswellia twice a day or to the prescription drug celecoxib (one brand name is Celebrex®) 100 mg twice a day. Symptom scoring and clinical evaluation yielded superior results on pain relief and distance walked for the BCM-95 and Bospure blend compared to celecoxib. BCM-95 and Bospure equaled celecoxib on joint flexibility. No serious adverse effects noted. [Kizhakedath R, Antony B, Benny M, Kuruvilla BT. Clinical evaluation of a herbal product (Rhulief™) in the management of knee osteoarthritis. Abstract 316. *Osteoarthritis Cartilage.* 2011;19(S1):S145-S146.]

Animal Studies [13]

29. **Effect of curcumin supplementation on serum expression of select cytokines and chemokines in a female rat model of nonalcoholic steatohepatitis.** OBJECTIVE: We recently reported that curcumin supplementation in a metabolically (i.e., Western diet [WD]) and chemically (i.e., CCl4) induced female rat model of non-alcoholic steatohepatitis (NASH) was associated with lower liver pathology scores and molecular markers of inflammation. This occurred when curcumin was given during induction of disease (preventative arm; 8-week WD with or without curcumin [8WD + C vs. 8WD]) as well as when given after disease development (treatment arm; 12-week WD with or without curcumin during weeks 9-12 [12WD + C vs. 12WD]). Herein, we sought to extend our findings from that study by determining the effects of curcumin supplementation on cytokine/chemokine expression in serum collected from these same rats. RESULTS: 24 cytokines/chemokines were assayed. IL-2 (+ 80%) and IL-13 (+ 83%) were greater with curcumin supplementation in the prevention arm. IL-2 (+ 192%), IL-13 (+ 87%), IL-17A (+ 81%) and fractalkine (+ 121%) were higher while RANTES was lower (- 22%) with curcumin supplementation in the treatment arm ($p < 0.05$ for all). RANTES concentrations also correlated significantly with hepatic pathology scores of inflammation ($r = 0.417$, $p = 0.008$). Select serum cytokines/chemokines were affected with curcumin supplementation in this female rat model of NASH. Moreover, curcumin's effect(s) on RANTES and its association with liver disease pathogenesis and progression may warrant further investigation. [Pickich MB, et al. Effect of curcumin supplementation on serum expression of select cytokines and chemokines in a female rat model of nonalcoholic steatohepatitis *BMC Res Notes*. 2019 Aug 9;12(1):496.]

30. **Curcumin supplementation mitigates NASH development and progression in female Wistar rats.** Curcumin, a naturally occurring plant polyphenolic compound, may have beneficial effects in nonalcoholic steatohepatitis (NASH) development. We examined whether curcumin supplementation could be used in both prevention and treatment of NASH with fibrosis. Female Wistar rats were provided ad libitum access to a "western diet" (WD) high in fat (43% kcal), sucrose (29% kcal), and cholesterol (2% w/v), as well as 15% fructose drinking water. Intraperitoneal CC14 injections (0.5 mL/kg) were also administered at weeks 1, 2, 4, and 6 to accelerate development of a NASH with fibrosis phenotype. Rats were randomized to four groups (n = 9-12/group) and fed ad libitum: (1) WD for 8-weeks (8WD), (2) WD enriched with curcumin for 8-weeks (8WD+C; 0.2% curcumin, BCM-95, DolCas Biotech) to assess prevention, (3) WD for 12-weeks (12WD), (4) WD for 8-weeks followed by 4-weeks WD+C (12WD+C) to assess treatment. Curcumin prevention (8WD vs. 8WD+C) attenuated ($P < 0.05$) histological liver inflammation, molecular markers of fibrosis (Col1a1 mRNA) and a serum marker of liver injury (AST). Curcumin treatment (12WD vs. 12WD+C) reduced ($P < 0.05$) hepatocellular inflammation, steatosis, NAFLD Activity Scores, and serum markers of liver injury (AST, ALP). Moreover, curcumin treatment also increased hepatic pACC/ACC, ApoB100, and SOD1 protein, and decreased hepatic FGF-21 levels; whereas, curcumin prevention increased hepatic glutathione levels. Both curcumin prevention and treatment reduced molecular markers of hepatic fibrosis (Col1a1 mRNA) and inflammation (TNF-α, SPP1 mRNA). Curcumin supplementation beneficially altered the NASH phenotype in female Wistar rats, particularly the reversal of hepatocellular

inflammation. [Cunningham RP, et al. Curcumin supplementation mitigates NASH development and progression in female Wistar rats. *Physiol Rep*. 2018 Jul;6(14):e13789]

31. **Risperidone-induced metabolic dysfunction is attenuated by *Curcuma longa* extract administration in mice.** Antipsychotics, such as risperidone, increase food intake and induce alteration in glucose and lipid metabolism concomitantly with overweight and body fat increase, these biological abnormalities belong to the metabolic syndrome definition (high visceral adiposity, hypertriglyceridemia, hyperglycemia, low HDL-cholesterol and high blood pressure). Curcumin is a major component of traditional turmeric (*Curcuma longa*) which has been reported to improve lipid and glucose metabolism and to decrease weight in obese mice. We questioned the potential capacity of curcumin, contained in *Curcuma longa* extract (Biocurcuma™), to attenuate the risperidone-induced metabolic dysfunction. Two groups of mice were treated once a week, for 22 weeks, with intraperitoneal injection of risperidone (Risperdal) at a dose 12.5 mpk. Two other groups received intraperitoneal injection of the vehicle of Risperdal following the same schedule. Mice of one risperidone-treated groups and of one of vehicle-treated groups were fed a diet with 0.05% Biocurcuma™ (curcumin), while mice of the two other groups received the standard diet. Curcumin limited the capacity of risperidone to reduce spontaneous motricity, but failed to impede risperidone-induced increase in food intake. Curcumin did not reduce the capacity of risperidone to induce weight gain, but decreased visceral adiposity and decreased the risperidone-induced hepatomegaly, but not steatosis. Furthermore, curcumin repressed the capacity of risperidone to induce the hepatic over expression of enzymes involved in lipid metabolism (LXRα, FAS, ACC1, LPL, PPARγ, ACO, SREBP2) and decreased risperidone-induced glucose intolerance and hypertriglyceridemia. Curcumin decreased risperidone-induced increases in serum markers of hepatotoxicity (ALAT, ASAT), as well as of one major hepatic pro-inflammatory transcription factor (NFκB: p105 mRNA and p65 protein). These findings support that nutritional doses of curcumin contained in *Curcuma longa* extract are able to partially counteract the risperidoneinduced metabolic dysfunction in mice, suggesting that curcumin ought to be tested to reduce the capacity of risperidone to induce the metabolic syndrome in human. [Auger F, et al. Risperidone-induced metabolic dysfunction is attenuated by *Curcuma longa* extract administration in mice. *Metab Brain Dis*. Feb 2018;33(1):63-77.]

32. **Curcumin and metformin-mediated chemoprevention of oral cancer is associated with inhibition of cancer stem cells.** Effective chemoprevention is critical for improving outcomes of oral cancer. As single agents, curcumin and metformin are reported to exhibit chemopreventive properties, in vitro as well as in patients with oral cancer. In this study, the chemopreventive efficacy of this drug combination was tested in a 4-nitro quinoline-1-oxide (4NQO) induced mice oral carcinogenesis model. Molecular analysis revealed a cancer stem cell (CSC)-driven oral carcinogenic progression in this model, wherein a progressive increase in the expression of CSC-specific markers (CD44 and CD133) was observed from 8th to 25th week, at transcript (40-100-fold) and protein levels ($P \leq 0.0001$). Chemopreventive treatment of the animals at 17th week with curcumin and metformin indicated that the combination regimen decreased tumor volume when compared to the control arm (0.69+0.03 vs 6.66+2.4 mm3; $P = 0.04$) and improved overall survival of the animals ($P = 0.03$). Assessment of the molecular status showed an

overall downregulation of CSC markers in the treatment arms as compared to the untreated control. Further, in vitro assessment of the treatment on the primary cells generated from progressive stages of 4NQO-induced mice tissue showed a concordant and consistent downregulation of the CSC markers following combination treatment ($P < 0.05$). The treatment also inhibited the migratory and self-renewal properties of these cells; the effect of which was prominent in the cultures of early dysplastic tissue ($P < 0.002$). Collectively, our observations suggest that the combination of curcumin and metformin may improve chemopreventive efficacy against oral squamous cell carcinoma through a CSC-associated mechanism. [Siddappa G, et al. Curcumin and metformin-mediated chemoprevention of oral cancer is associated with inhibition of cancer stem cells. *Molecular Carcinogenesis*. 2017 November;56(11):2446-2460.]

33. **Curcumin sensitizes pancreatic cancer cells to gemcitabine by attenuating PRC2 subunit EZH2, and the lncRNA PVT1 expression.** Development of resistance to chemotherapeutic drugs is a major challenge in the care of patients with pancreatic ductal adenocarcinoma (PDAC). Acquired resistance to chemotherapeutic agents in PDAC has been linked to a subset of cancer cells termed 'cancerstem cells' (CSCs). Therefore, an improved understanding of the molecular events underlying the development of pancreatic CSCs is required to identify new therapeutic targets to overcome chemoresistance. Accumulating evidence indicates that curcumin, a phenolic compound extracted from turmeric, can overcome de novo chemoresistance and re-sensitize tumors to various chemotherapeutic agents. However, the underlying mechanisms for curcumin-mediated chemosensitization remain unclear. The Enhancer of Zeste Homolog-2 (EZH2) subunit of Polycomb Repressive Complex 2 (PRC2) was recently identified as a key player regulating drug resistance. EZH2 mediates interaction with several long non-coding RNAs (lncRNAs) to modulate epithelial-mesenchymal transition and cancer stemness, phenomena commonly associated with drug resistance. Here, we report the re-sensitization of chemoresistant PDAC cells by curcumin through the inhibition of the PRC2-PVT1-c-Myc axis. Using gemcitabine-resistant PDAC cell lines, we found that curcumin sensitized chemoresistant cancer cells by inhibiting the expression of the PRC2 subunit EZH2 and its related lncRNA PVT1. Curcumin was also found to prevent the formation of spheroids, a hallmark of CSCs, and to down-regulate several self-renewal driving genes. In addition, we confirmed our in vitro findings in a xenograft mouse model where curcumin inhibited gemcitabine-resistant tumor growth. Overall, this study indicates clinical relevance for combining curcumin with chemotherapy to overcome chemoresistance in PDAC. [Yoshida K, Toden S, Ravindranathan P, Han H, Goel A. Curcumin sensitizes pancreatic cancer cells to gemcitabine by attenuating PRC2 subunit EZH2, and the lncRNA PVT1 expression. *Carcinogenesis*. 2017 Oct 1;38(10):1036-1046.]

34. **Essential turmeric oils enhance anti-inflammatory efficacy of curcumin in dextran sulfate sodium-induced colitis.** Turmeric has been used as a medicinal herb for thousands of years for treatment of various disorders. Although curcumin is the most studied active constituents of turmeric, accumulating evidence suggests that other components of turmeric have additional anti-inflammatory and anti-tumorigenic properties. Herein, we investigated anti-inflammatory efficacy and associated gene expression alterations of a specific, curcumin preparation containing essential turmeric

oils (ETO-curcumin) in comparison to standard curcumin at three specific doses (0, 5, 25 or 50 mg/kg), in an animal model of dextran sodium sulfate (DSS)-induced colitis. The present study showed that both ETO and standard curcumin treatments provided protection against DSS-induced inflammation. However, ETO-curcumin improved disease activity index (DAI) dose-dependently, while the anti-inflammatory efficacy of standard curcumin remained constant, suggesting that ETO-curcumin may provide superior anti-inflammatory efficacy compared to standard curcumin. Gene expression analysis revealed that anti-inflammatory cytokines including IL-10 and IL-11 as well as FOXP3 were upregulated in the colon by ETO-curcumin. Collectively, these findings suggest that the combined treatment of curcumin and essential turmeric oils provides superior protection from DSS-induced colitis than curcumin alone, highlighting the anti-inflammatory potential of turmeric. [Toden S, Theiss AL, Wang X, Goel A. Essential turmeric oils enhance anti-inflammatory efficacy of curcumin in dextran sulfate sodium-induced colitis. *Sci Rep*. 2017 Apr 11;7(1):814.]

35. **Systematic and comprehensive investigation of the toxicity of curcuminoid-essential oil complex: A bioavailable turmeric formulation**. Curcumin, the active component present in Curcuma longa of the family Zingiberaceae, has a number of pharmacological effects, including potential anti‑inflammatory activity. One of the major limitations of curcumin/turmeric extract is its poor absorption through the gastrointestinal tract. Several approaches have been adopted to increase the bioavailability of curcumin, including loading curcumin into liposomes or nanoparticles, complexation with phospholipids, addition of essential oils and synthesizing structural analogues of curcumin. In the present study, the toxicity and safety of one such bioavailable turmeric formulation, curcuminoid‑essential oil complex (CEC), the toxicity profile of which has not been reported, were examined using in vivo and in vitro models, as per the guidelines of the Organisation for Economic Co-operation and Development. Investigations of acute toxicity study were performed in rats and mice, and the results revealed no signs and symptoms or toxicity or mortality in any of the animals at the maximum recommended dose level of 5,000 mg/kg body weight. The repeated administration of CEC for 90 days in Wistar rats at a dose of 1,000 mg/kg body weight did not induce any observable toxic effects, compared with corresponding control animals. Mutagenicity/genotoxicity investigations were also performed using a bacterial reverse mutation assay (Ames test), a mammalian bone marrow chromosome aberration test and a mammalian erythrocyte micronucleus test in mice. CEC was found to be non‑mutagenic in all three mutagenic investigations. Consequently, the present study indicated that CEC elicited no toxic effects in animals or in vitro. Therefore, following investigations of acute toxicity, repeated dose toxicity and mutagenicity, CEC was deemed a safe, non‑toxic pharmacological formulation. [Aggarwal ML, Chacko KM, Kuruvilla BT. Systematic and comprehensive investigation of the toxicity of curcuminoid-essential oil complex: A bioavailable turmeric formulation. *Molecular Medicine Reports*. 2016;13:592-604. DOI: 10.3892/mmr.2015.4579.]

36. **Anti-inflammatory activity of BCM-95 (bio-enhanced formulation of turmeric with increased bioavailability) compared to Curcumin in Wistar rats.** Objective: To evaluate anti-inflammatory activity of bioenhanced turmeric formulation (BCM-95)

compared to commercial Curcumin formulation (Curcuminoids 95%) in Carrageenan-induced acute inflammatory model. Materials and Methods: Thirty six Wistar rats were divided into six groups-Normal control (2 ml of vehicle), Standard control (Indomethacin 10 mg/kg), 2 doses of BCM 95 (10 and 20 mg/kg) and Curcuminoids 95% (10 and 20 mg/kg). Paw volume was measured using a digital plethysmometer. Vehicle or test drugs were given to rats 30 min before carrageenan administration. Baseline paw volume reading (V_0) was noted just prior to administration of 0.1 ml of 1% carrageenan to right hind paw of the rat. Test paw volume readings (V_t) were measured at 30, 60, 120, 180, 240, 300 and 360 min, after carrageenan injection. Oedema expressed as increased paw volume (v_t-v_0) was noted and percentage inhibition of oedema was calculated for all treatment groups. Statistical analysis: Difference between groups were analyzed with ANOVA followed by Tukey test. Results: All treatment groups demonstrated significant ($p<0.05$) anti-inflammatory activity (oedema suppression) compared to normal control. Anti-inflammatory activity of BCM 95 treated groups were comparable to standard control group except at certain time points, whereas the same activity at all-time points with Curcuminoid 95% treated groups were significantly less than standard control group. Percentage inhibition of paw oedema was maximum with standard control group followed by BCM 95 treated groups followed by Curcuminoid 95% treated groups. Conclusion: BCM 95 treated groups showed significant anti-inflammatory activity compared to Curcuminoid 95% treated groups. [Vinaykumar S, et al. Anti-inflammatory activity of BCM-95 (bio-enhanced formulation of turmeric with increase bioavailability) compared to Curcumin in Wistar rats. *Pharmacogn. J.* July-Aug 2016;8(4):380-383.]

37. **Evaluation of Antiepileptic and Memory Retention Activity of Curcumin Per SE and in Combination with Antiepileptic Drugs.** Antiepileptic activity of curcumin and its combination with phenytoin and sodium valproate were studied in chronic model (14 days) of Maximal Electroshock Seizure (MES) and Pentylenetetrazole (PTZ) induced seizure respectively. Elevated plus maze test was used to study effect of drugs and/or seizures on memory retention in MES and PTZ groups. Curcumin in both doses did not show any significant effect ($P = 0.33$) on tonic extension, while curcumin 100 mg/kg significantly ($P < 0.01$) reduced clonic phase compared to vehicle control. Curcumin in 100 mg/kg dose significantly ($P < 0.001$) inhibited PTZ induced seizure. Addition of curcumin to sub therapeutic dose of sodium valproate showed synergistic effect. Curcumin did not show any effect on memory retention. Inhibition of PTZ induced seizure by curcumin could be due to effect on γ-amino butyric acid receptor (GABA) pathway and its antioxidant property. Curcumin can be effective in absence seizure alone and as add on with sodium valproate. [Anovadiya AP, Sanmukhani JJ, Vadgama VK, Tripathi CB. *Asian J Pharm Clin Res*. 2013;6(2):145-148.]

38. **Chemoprevention and Treatment Efficacy of Curcumin in Combination with Metformin in an in Vivo Oral Carcinogenesis Model.** Evaluation of the efficacy of [BCM-95] Curcumin and Metformin in prevention of oral pre-malignant lesion (PML) progression. The animal model was established using 4-6 weeks C57BI/6 mice (N=60); the mice were divided into control arm (N=10) with plain drinking water and the treatment arm (N=50) which received the cancer causing 4-nitroquinoline-oxide. After 17 weeks, the mice were taken off the carcinogen and divided into 4 groups: arm 1 with plain water, arm 2 with curcumin, arm 3 with the drug metformin, and arm 4 with a

combination of both curcumin and metformin. The mice were examined at 17th week as well as at the end of the 25 week study period, and samples collected for molecular analysis. The average tumor volume was reduced in the combination arm (0.693±0.034) and the individual arms (curcumin 2.45; metformin 1.45±0.33) as compared to the 4NQO arm (6.65±2.37). The average number of lesions (malignant tumors) per mouse was also reduced in the combination arm (Avg 0.375) and the curcumin arm (Avg 0.25) as compared to the 4NQO arm (Avg 0.8). The overall survival of the combination arm was better when compared to individual treatment (p= 0.0006). The animals in the water control arm remained healthy. Curcumin reduced tumor formation both on its own and in conjunction with metformin. Conclusion: The clinical results suggest that the combination arm is more efficient in chemoprevention. Further studies using the molecular markers and subsequent functional studies are currently ongoing. [Siddappa G, Ravindra D, Kulsum S, et al. Chemoprevention and Treatment Efficacy of Curcumin in Combination with Metformin in an in Vivo Oral Carcinogenesis Model. 5th International Federation of Head and Neck Oncologic Societies (IFHNOS). July 26th-30th 2014, New York, NY. Also presented at the 13th National Conference of Foundation for Head and Neck Oncology (FHNO). September 27th – 29th 2013, JAIPUR.]

39. **Evaluation of Antidepressant Like Activity of Curcumin and its Combination with Fluoxetine and Imipramine: an Acute and Chronic Study.** In animal model of depression, BCM-95 curcumin is compared to generic fluoxetine (one brand name is Prozac®) and imipramine (one brand name is Tofranil®). BCM-95 curcumin performed as well as either prescription anti-depressant drug on all measures of depression. However, adding BCM-95 curcumin to the prescription drugs did not increase antidepressant effects. [Sanmukhani J, et al. Evaluation of antidepressant like activity of curcumin and its combination with fluoxetine and imipramine: an acute and chronic study. *Acta Pol Pharm.* 2011 Sep-Oct;68(5):769-75.]

40. **Oral Bioavailability of BCM-95® in Dogs**. This study looked specifically at bioavailability in dogs for veterinary purposes. Six healthy adult male and female dogs were divided between plain curcumin and BCM-95 curcumin (reported as the veterinary NMXCC-95 designation). No adverse effects reported. The BCM-95 group had approximately 7-fold increase in absorption over plain curcumin over 8 hours and approximately 9-fold increase over plain curcumin when measured for 12 hours. [Antony B, Butchin RK, Griffin DW. Bioavailability of a novel, bioenhanced preparation in dogs. Poster Presentation. 2009 ACVIM Forum/Canadian VMA Convention: June 3-6, 2009; Montréal, Québec, Canada.]

41. **Enhancing the Absorption of Curcuminoids.** Turmeric (*Curcuma longa*), one of the familiar spice has got number of medicinal properties such as anti-septic, anti-inflammatory, wound healing, anti-oxidant, anti-tumor etc. These properties of turmeric are attributed to the active principle, curcumin and essential oil present in the rhizome. But it is suggested and proved that only 50 – 60 percent of total curcumin is absorbed by animal system. Studies conducted on albino rats and results described in this paper reveal that 96 – 97 percent absorption of curcuminoids by mixing curcumin and standardized essential oil of turmeric. [Antony B, Benny M, Rao SB. Enhancing the Absorption of Curcuminoids. *Spice India*. July 2005;23-26.]

Animal Studies Using Curcumin Combinations [6]

42. **The Effect of Exercise and Nutritional Supplementation on Proinflammatory Cytokine Expression in Young Racehorses During Training.** The inflammatory response to vigorous exercise ranges from the mild symptoms of delayed-onset muscle soreness to debilitating injuries affecting soft tissue, joint, and bone. Although there is a great deal of information available on the inflammatory response to exercise in human athletes, less information is available regarding the inflammatory response to exercise in young horses undergoing training for racing careers. Here, we assessed the cytokine response to exercise in a group of young Thoroughbred racehorses during their initial training. Because there is interest in nonpharmacologic approaches to control or ameliorate exercise-induced inflammation, we also examined the anti-inflammatory effect of a nutritional supplement [containing BCM-95® curcumin, BosPure® boswellia, coenzyme Q10, glycine proprionyl-L-carnitine HCl, and D-ribose] fed to half of the horses undergoing training. Twenty-five Thoroughbred horses aged 2 years were followed through their initial race training. Peripheral blood samples were collected at various times during the exercise for the quantitation of lactic acid, oxidative stress, and inflammatory cytokine gene expression. There was an intensity-dependent effect of exercise on lactate, malondialdehyde, and proinflammatory cytokine gene expression. Although training itself was associated with an overall reduction in inflammatory markers, horses receiving the supplement exhibited further reductions in their indicators of inflammation. As such, this study provides novel evidence of nutritional supplementation reducing postexercise inflammation. [Horohov DW, Sinatra ST, Chopra RK, Jankowitz S, Betancourt A, Bloomer RJ. The effect of exercise and nutritional supplementation on proinflammatory cytokine expression in young racehorses during training. *J Equine Vet Sci*. 2012 December;32(12):805-15.]

43. **BreastDefend™ prevents breast-to-lung cancer metastases in an orthotopic animal model of triple-negative human breast cancer.** We have recently demonstrated that a natural dietary supplement BreastDefend (BD), which contains extracts from medicinal mushrooms (Coriolus versicolor, Ganoderma lucidum, Phellinus linteus), medicinal herbs (Scutellaria barbata, Astragalus membranaceus, Curcuma longa), and purified biologically active nutritional compounds (diindolylmethane and quercetin), inhibits proliferation and metastatic behavior of MDA-MB-231
invasive human breast cancer cells in vitro. In the present study, we evaluated whether BD suppresses growth and breast-to lung cancer metastasis in
an orthotopic model of human breast cancer cells implanted in mice. Oral application of BD (100 mg/kg of body weight for 4 weeks) by intragastric gavage did not affect body weight or activity of liver enzymes and did not show any sign of toxicity in liver, spleen, kidney, lung and heart tissues in mice. Moreover, BD significantly decreased the change in tumor volume over time compared to the control group ($p=0.002$). BD treatment also markedly decreased the incidence of breast-to-lung cancer metastasis from 67% (control) to 20% (BD) ($p<0.05$) and the number of metastases from 2.8 (0.0, 48.0) in the control group to 0.0 (0.0, 14.2) in the BD treatment group ($p<0.05$). Finally, anti-metastatic activity of BD in vivo was further confirmed by the downregulation of expression of PLAU (urokinase plasminogen activator, uPA) and CXCR4 (C-X-C chemokine receptor-4) genes in breast tumors. In conclusion, BD may be considered as a biological therapeutic agent against invasive breast cancers. [Jiang J, et al. BreastDefend™ prevents

breast-to-lung cancer metastases in an orthotopic animal model of triple-negative human breast cancer. *Oncol Rep*. 2012 Oct;28(4):1139-1145.]

44. **Evaluation of hepatoprotective activity of combination of *Phyllanthus niruri* and *Curcuma longa* extracts in Wistar rats.** Hepatoprotective activity of combination of *Phyllanthus niruri(PN)* and *Curcuma longa(CL)* extract was evaluated against carbon tetrachloride(CCl4) induced liver damage. Combination of *PN+CL* extract at a dose of 400mg/kg, orally was coadministered with CCl4 (0.5 mg/kg i.p) to rats for 7 days. On 8th day serum enzyme levels such as AST, ALT, ALP, TB were determined. Thiopentone induced sleeping time is estimated as an indirect index of functionality of liver. Liver tissue was used to estimate antioxidants such as MDA, GST levels and for histopathological assessment. There was a significant increase in serum enzyme levels and duration of thiopentone induced sleep time in CCl4 treated rats. Coadministration of *PN+CL* extract combination with CCl4 significantly prevented the rise in serum enzyme levels and normalize the duration of thiopentone induced sleep time. Combination of *PN +CL* produced significant reduction and increase in MDA & GST liver levels respectively. Histological section of liver in animals treated with CCl4 showed centrilobular area of necrosis with derangement in hepatic architecture. *PN+CL* administration prevented these deleterious changes, histological section of liver in rats treated with *PN+CL* showed normal hepatic parenchyma. Combination of *PN+CL* extract showed significant hepatoprotection against CCl4 induced liver damage. [Adiga S, et al. Evaluation of hepatoprotective activity of combination of *Phyllanthus niruri* and *Curcuma longa* extracts in Wistar rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* 2012 July – September; 3(3):1260.

45. **ProstaCaid™ inhibits tumor growth in a xenograft model of human prostate cancer.** We have recently demonstrated that the dietary supplement ProstaCaid™ (PC) inhibits growth and invasive behavior of PC-3 human prostate cancer cells in vitro. In the present study, we evaluated toxicity and whether PC suppresses growth of prostate cancer in a xenograft model of human prostate cancer cells implanted in mice. Here, we show that an oral administration of PC (100, 200 and 400 mg/kg) did not affect body weight or activity of liver enzymes (ALT, AST) and did not show any sign of toxicity in liver, spleen, kidney, lung and heart tissues in mice. In addition, PC treatment resulted in the inhibition of tumor volumes (1024.6±378.6 vs. 749.3±234.3, $P<0.001$) in a xenograft model of prostate cancer with human hormone refractory (independent) PC-3 prostate cancer cells. Moreover, qRT-PCR analysis demonstrated significant upregulation of expression of CDKN1A (p21) and inhibition of expression of IGF2, NR2F2 and PLAU (uPA) genes by an oral administration of PC in prostate cancer xenografts. Our study demonstrates that the concentrations of the dietary supplement ProstaCaid tested did not show signs of toxicity, and its oral application has significant anticancer activity in vivo and can be considered as an alternative treatment for prostate cancer patients. [Jiang J, et al. ProstaCaid™ inhibits tumor growth in a xenograft model of human prostate cancer. *Int J Oncol*. 2012 May;40(5):1339-1344.

46. **Effect of Citrus Polyphenol- and Curcumin-supplemented Diet on Inflammatory State in Obese Cats.** Veterinary study looking at obesity-induced pro-inflammatory state in cats and impact of BCM-95 on liver and inflammatory markers. Showed safe use in cats, and significant impact on interleukin 2 (IL-2) and reduction of AGP (a1-acid

glycoprotein) which shows that curcumin impacts hepatocytes (liver cells) to reduce AGP, illustrating that BCM-95 is helping liver cells to behave more like liver cells in non-obese cats. [Leray V, Freuchet B, Le Bloc'h J, Jeusette I, Torre C, Nguyen P. Effect of citrus polyphenol- and curcumin-supplemented diet on inflammatory state in obese cats. *Br J Nutr.* 2011 Oct;106 Suppl 1:S198-201.]

47. **Effect of a topical curcumin preparation (*BIOCURCUMAX*) on burn wound healing in rats.** Background: Curcumin, a naturally occurring o-methoxyphenol derivative, has been shown to possess several biological properties including antioxidant (free radical scavenging activity), induction of detoxification enzymes and provides protection against degenerative diseases. Topical applications of compounds with free radical scavenging properties in patients have shown to improve significantly wound healing and protect tissues from oxidative damage. Objectives: To assess the effect of a topical curcumin preparation on healing of partial thickness burn wounds in rats. Methods: The rats are randomly divided into four groups, comprising of six rats in each group. Partial thickness burn wounds are created by pouring hot molten wax at 80ºC. Group I acts a control, Group 2 receives the standard silver sulphadiazine cream, Group 3 gets 20% curcumin cream, and Group 4 receives the combination of the dexamethasone and curcumin cream. Parameters observed are epithelialization period and wound contraction. Results & Discussion: The percentage of wound contraction was significantly increased in the topical curcumin preparation (20%) and silver sulfadiazine group compared to control group. The mean period of epithelization was significantly reduced in topical curcumin preparation (20%) group and silver sulfadiazine group as compared to the control. Conclusion: Topical curcumin preparation is effective in healing burn wound and the effect was comparable to that of standard drug i.e. silver sulfadiazine. [Durgaprasad S, Reetesh R, Hareesh K, Rajput R. Effect of a topical curcumin preparation (*BIOCURCUMAX*) on burn wound healing in rats. *Journal of Pharmaceutical and Biomedical Sciences*. 2011;8(23):1-3.]

Ex Vivo or Cellular Studies [7]

48. **Curcumin inhibits polycomb repressive complex 2 through lncRNA-PVT1 and enhances gemcitabine sensitivity in chemoresistant pancreatic cancer.** Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies, and a major cause of PDAC-associated mortality is acquisition of resistance to chemotherapy. Curcumin is a phenolic compound extracted from turmeric, and is known for its potent anti-inflammatory, anti-oxidative and anti-tumorigenic properties. Accumulating evidence suggests that curcumin can overcome *de-novo* chemoresistance and re-sensitize tumors to various chemotherapeutic drugs. Recently, polycomb repressive complex 2 (PRC2) was reported to be involved in drug resistant through interaction with several long non-coding RNAs (lncRNAs). Our data provide previously unrecognized evidence for curcumin-induced sensitization to gemcitabine, through co-modulation of PRC2 and PVT1 in PDAC. These data highlight the potential adjunctive therapeutic role for curcumin together with conventional chemotherapeutic drugs in patients with PDAC. [Yoshida K, Toden S, Ravindranathan P, Goel A. Curcumin sensitizes pancreatic cancer cells to gemcitabine by attenuating PRC2 subunit EZH2, and the lncRNA PVT1 expression. *Carcinogenesis*. 2017:1-11.]

49. **Curcumin mediates chemosensitization to 5-fluorouracil through miRNA-induced suppression of epithelial-to-mesenchymal transition in chemoresistant colorectal cancer.** Resistance to cytotoxic chemotherapy is a major cause of mortality in colorectal cancer (CRC) patients. Chemoresistance has been linked primarily to a subset of cancer cells undergoing epithelial-mesenchymal transition (EMT). Curcumin, a botanical with anti-tumorigenic properties, has been shown to enhance sensitivity of cancer cells to chemotherapeutic drugs, but the molecular mechanisms underlying this phenomenon remain unclear. Effects of curcumin and 5-fluorouracil (5FU) individually, and in combination, were examined in parental and 5FU resistant (5FUR) cell lines. We performed a series of growth proliferation and apoptosis assays in 2D and 3D cell cultures. Furthermore, we identified and analyzed the expression pattern of a subset of putative EMT-suppressive microRNAs (miRNAs) and their downstream target genes regulated by curcumin. Chemosensitizing effects of curcumin were validated in a xenograft mouse model. Combined treatment with curcumin and 5FU enhanced cellular apoptosis and inhibited proliferation in both parental and 5FUR cells, while 5FU alone was ineffective in 5FUR cells. A group of EMT-suppressive miRNAs were upregulated by curcumin treatment in 5FUR cells. Curcumin suppressed EMT in 5FUR cells by downregulating BMI1, SUZ12 and EZH2 transcripts, key mediators of cancer stemness-related polycomb repressive complex subunits. Using a xenograft and mathematical models we further demonstrated that curcumin sensitized 5FU to suppress tumor growth. We provide novel mechanistic evidence for curcuminmediated sensitization to 5FU-related chemoresistance through suppression of EMT in 5FUR cells via upregulation of EMT-suppressive miRNAs. This study highlights the potential therapeutic usefulness of curcumin as an adjunct in patients with chemoresistant advanced CRC. [Toden S, Okugawa Y, Jascur T, Wodarz D, Komarova NL, Buhrmann C, Shakibaei M, Boland, Goel A. Curcumin mediates chemsensitization to 5-fluorouracil through miRNA-induced suppression of epithelial-to-mesenchymal transition in chemoresistant colorectal cancer. *Carcinogenesis*. 2015 Feb 4 (Epub ahead of print).]

50. **Curcumin inhibits cancer-associated fibroblast-driven prostate cancer invasion through MAOA/mTOR/HIF-1α signaling.** Cancer-associated fibroblasts (CAFs) are key determinants in the malignant progression of cancer, supporting tumorigenesis and metastasis. CAFs also mediate epithelial to mesenchymal transition (EMT) in tumor cells and their achievement of stem cell traits. Curcumin has recently been found to possess anticancer activities via its effect on a variety of biological pathways involved in cancer progression. In this study, we found that CAFs could induce prostate cancer cell EMT and invasion through monoamine oxidase A (MAOA)/mammalian target of rapamycin (mTOR)/hypoxia-inducible factor-1α (HIF-1α) signaling pathway, which exploits reactive oxygen species (ROS) to drive a migratory and aggressive phenotype of prostate carcinoma cells. Moreover, CAFs was able to increase CXC chemokine receptor 4 (CXCR4) and interleukin-6 (IL-6) receptor expression in prostate cancer cells. However, curcumin abrogated CAF-induced invasion and EMT, and inhibited ROS production and CXCR4 and IL-6 receptor expression in prostate cancer cells through inhibiting MAOA/mTOR/HIF-1α signaling, thereby supporting the therapeutic effect of curcumin in prostate cancer. [Du Y, Long Q, Zhang L, Shi Y, Liu X, Li X, Guan B, Tian Y, Wang X, Li L, He D. Curcumin inhibits cancer-associated fibroblast-driven prostate cancer

invasion through MAOA/mTOR/HIF-1α signaling. *International Journal of Oncology*. 2015;1899:0-0.]

51. **Curcumin potentiates antitumor activity of 5-fluorouracil in a 3D alginate tumor microenvironment of colorectal cancer.** BACKGROUND: To overcome the limitations of animal-based experiments, 3D culture models mimicking the tumor microenvironment in vivo are gaining attention. Herein, we investigated an alginate-based 3D scaffold for screening of 5-fluorouracil (5-FU) or/and curcumin on malignancy of colorectal cancer cells (CRC). METHODS: The potentiation effects of curcumin on 5-FU against proliferation and metastasis of HCT116 cell and its corresponding isogenic 5-FU-chemoresistant cells (HCT116R) were examined in a 3D-alginate tumor model. RESULTS: CRC cells encapsulated in alginate were able to proliferate in 3D-colonospheres in a vivo-like phenotype and invaded from alginate. During cultivation of cells in alginate, we could isolate 3 stages of cells, (1) alginate proliferating (2) invasive and (3) adherent cells. Tumor-promoting factors (CXCR4, MMP-9, NF-κB) were significantly increased in the proliferating and invasive compared to the adherent cells, however HCT116R cells overexpressed factors in comparison to the parental HCT116, suggesting an increase in malignancy behavior. In alginate,curcumin potentiated 5-FU-induced decreased capacity for proliferation, invasion and increased more sensitivity to 5-FU of HCT116R compared to the HCT116 cells. IC50 for HCT116 to 5-FU was 8nM, but co-treatment with 5 μM curcumin significantly reduced 5-FU concentrations in HCT116 and HCT116R cells (0.8nM, 0.1nM, respectively) and these effects were accompanied by down-regulation of NF-κB activation and NF-κB-regulated gene products. CONCLUSIONS: Our results demonstrate that the alginate provides an excellent tumor microenvironment and indicate that curcumin potentiates and chemosensitizes HCT116R cells to 5-FU-based chemotherapy that may be useful for the treatment of CRC and to overcome drug resistance. [Shakibaei M, Kraehe P, Popper B, Shayan P, Goel A, Buhrmann C. Curcumin potentiates antitumor activity of 5-flurouracil in a 3D alginate tumor microenvironment of colorectal cancer. *BMC Cancer*. 2015 Apr 10;15:250.]

52. **BCM-95 Curcumin Improves Efficacy of Chemotherapy 5-Fluorouracil in Chemoresistant Colorectal Cancer.** More than 15% of colorectal cancer (CRC) patients are resistant to 5-Fluorouracil (5-FU)-based chemotherapeutic regimens, and tumor recurrence rates can be as high as 50-60%. Cancer stem cells (CSC) are capable of surviving conventional chemotherapies that permit regeneration of original tumors. This study investigated the effectiveness of 5-FU and BCM-95 Curcumin in context of DNA mismatch repair (MMR) status and CSC activity in 3D cultures of CRC cells. Pre-treatment with BCM-95 curcumin significantly enhanced the effect of 5-FU on HCT116R and HCR116+ch3R cells, in contrast to 5-FU alone as evidenced by increased disintegration of colonospheres, enhanced apoptosis and by inhibiting their growth. Curcumin and/or 5-FU strongly affected MMR-deficient CRC cells in high density cultures; however, MMR-proficient CRC cells were more sensitive. These effects of curcumin in enhancing chemosensitivity to 5-FU were further supported by its ability to effectively suppress CSC pools as evidenced by decreased number of CSC marker positive cells. The results illustrate novel and previously unrecognized effects of curcumin in enhancing chemosensitization to 5-FU-based chemotherapy on DNA MMR-

deficient and their chemo-resistant counterparts by targeting the CSC sub-population. [Shakibaei M, Buhrmann C, Kraehe P, Shayan P, Lueders C and Goel A. Curcumin chemosensitizes 5-Fluorouracil resistant MMR-deficient human colon cancer cells in high density cultures. *PLoS ONE*. 2014:9(1)**.]**

53. **Curcumin suppresses crosstalk between colon cancer stem cells and stromal fibroblasts in the tumor microenvironment: potential role of EMT.** Objective: Interaction of stromal and tumor cells plays a dynamic role in initiating and enhancing carcinogenesis. In this study, we investigated the crosstalk between colorectal cancer (CRC) cells with stromal fibroblasts and the anti-cancer effects of curcumin and 5-Fluorouracil (5-FU), especially on cancer stem cell (CSC) survival in a 3D-co-culture model that mimics in vivo tumor microenvironment. Methods: Colon carcinoma cells HCT116 and MRC-5 fibroblasts were co-cultured in a monolayer or high density tumor microenvironment model in vitro with/without curcumin and/or 5-FU. Results: Monolayer tumor microenvironment co-cultures supported intensive crosstalk between cancer cells and fibroblasts and enhanced up-regulation of metastatic active adhesion molecules (b1-integrin, ICAM-1), transforming growth factor-b signaling molecules (TGF-b3, p-Smad2), proliferation associated proteins (cyclin D1, Ki-67) and epithelial-to-mesenchymal transition (EMT) factor (vimentin) in HCT116 compared with tumor mono-cultures. High density tumor microenvironment co-cultures synergistically increased tumor-promoting factors (NF-kB, MMP-13), TGF-b3, favored CSC survival (characterized by up-regulation of CD133, CD44, ALDH1) and EMT-factors (increased vimentin and Slug, decreased E-cadherin) in HCT116 compared with high density HCT116 mono-cultures. Interestingly, this synergistic crosstalk was even more pronounced in the presence of 5-FU, but dramatically decreased in the presence of curcumin, inducing biochemical changes to mesenchymal-epithelial transition (MET), thereby sensitizing CSCs to 5-FU treatment. Conclusion: Enrichment of CSCs, remarkable activation of tumor-promoting factors and EMT in high density co-culture highlights that the crosstalk in the tumor microenvironment plays an essential role in tumor development and progression, and this interaction appears to be mediated at least in part by TGF-b and EMT. Modulation of this synergistic crosstalk by curcumin might be a potential therapy for CRC and suppress metastasis. [Buhrmann C, Kraehe P, Lueders C, Shayan P, Goel A, Shakibaei M. Curcumin suppresses crosstalk between colon cancer stem cells and stromal fibroblasts in the tumor microenvironment: potential role of EMT. *PLoS ONE*. 2014;9(9): e107514.]

54. **Comparative Bioavailability of Curcumin, Turmeric, and Biocurcumax™ in Traditional Vehicles using Non-Everted Rat Intestinal Sac Model.** The bioavailability of curcumin from turmeric, Biocurcumax and as plain curcumin was investigated using conventional vehicles by a non-everted rat intestinal model. Results of ex vivo intestinal permeability studies showed an enhancement in the permeability of curcumin with increase in lipophilicity of the vehicle used. Maximum permeability of curcumin was obtained from corn oil (13.4%) followed by clarified butter (9.82%), milk (4.24%) and aqueous suspension (1.66%) in 8 h. Another very interesting and important observation was that the permeation of curcumin was more from turmeric and Biocurcumax than from plain curcumin. These studies strongly suggest that curcumin may be consumed as turmeric/Biocurcumax in lipophilic vehicles instead of plain curcumin for maximum

beneficial effects. [Shishu MM. Comparative bioavailability of curcumin, turmeric, and Biocurcumax™ in traditional vehicles using non-everted rat intestinal sac model. *J Funct Foods*. 2010;2(1):60-65.]

Ex Vivo or Cellular Studies Using Curcumin Combinations [10]

55. **Effects of anti-inflammatory and adaptogenic herbal extracts on gene expression of eicosanoids signaling pathways in isolated brain cells.** INTRODUCTION: The adaptogens modulate expression of genes playing key roles in development of aging-related disorders, which are considered as low-grade systemic inflammatory conditions characterized by an imbalance between pro-and anti-inflammatory eicosanoids. AIM OF THE STUDY: We compared the effects of anti-inflammatory and adaptogenic plant extracts on the expression of genes involved in biosynthesis of eicosanoids with the purpose to find those plants, which selectively upregulated the expression of anti-inflammatory lipoxins signaling pathways and inhibited pro-inflammatory signaling pathways associated with biosynthesis of leukotrienes, prostaglandins and thromboxanes. MATERIALS AND METHODS: We conducted transcriptome-wide RNA sequencing to profile gene expression alterations in T98G neuroglia cells upon treatment of plant extract and analyzed the relevance of deregulated genes to eicosanoids signaling pathways using in silico models. RESULTS: For the first time, we demonstrated that Rhodiola rosea, Withania somnifera and Eleutherococcus senticosus downregulate the expression of key genes (ALOX5AP, DPEP2, LTC4S) involved biosynthesis of leukotrienes A, B, C, D and E, resulting in inhibition of leukotriene signaling pathway suggesting their potential benefits in Alzheimer disease. The common feature for all tested anti-inflammatory plants extracts was related to downregulation of ALOX12, which was also associated with neuroprotective action of these medicinal plants as well as their potential benefits in neurodegenerative diseases. None of tested anti-inflammatory and adaptogenic plants selectively activated the ALOX15-mediated signaling pathway, which is associated with generation anti-inflammatory lipoxins. Almost all tested plants upregulated the expression of the prostaglandin E receptor 3 gene (PTGER3) suggesting their potential benefits in the treatment of cancer. CONCLUSION: Every single plant tested in this study revealed a specific "signature" on eicosanoid signaling-related gene expression, regardless of their common features as anti-inflammatory or adaptogenic activity. Further studies of the combination of Rhodiola with Withania (Adaptra) for the treatment of Alzheimer disease are required. [Panossian A, Seo EJ, Efferth T. Effects of anti-inflammatory and adaptogenic herbal extracts on gene expression of eicosanoids signaling pathways in isolated brain cells. *Phytomedicine*. 2019 Mar 10: 152881] Note: Curcugreen reviewed in this study-see full study for details.

56. **Curcumin downregulates expression of opioid-related nociception receptor gene (OPRL1) in isolated neuroglia cells.** Background: Curcumin (CC) exerts polyvalent pharmacological actions and multi-target effects, including pain relief and anti-nociceptive activity. In combination with *Boswellia serrata* extract (BS), curcumin shows greater efficacy in knee osteoarthritis management, presumably due to synergistic interaction of the ingredients. Aim: To elucidate the molecular mechanisms underlying the analgesic activity of curcumin and its synergistic interaction with BS. Methods: We performed gene expression profiling by transcriptome-wide mRNA sequencing in human

T98G neuroglia cells treated with CC (Curamed®), BS, and the combination of CC and BS (CC-BS; Curamin®), followed by interactive pathways analysis of the regulated genes. Results: Treatment with CC and with CC-BS selectively downregulated opioid-related nociceptin receptor 1 gene (*OPRL1*) expression by 5.9-fold and 7.2-fold, respectively. No changes were detected in the other canonical opioid receptor genes: *OPRK1, OPRD1*, and *OPRM1*. Nociceptin reportedly increases the sensation of pain in supra-spinal pain transduction pathways. Thus, CC and CC-BS may downregulate *OPRL1*, consequently inhibiting production of the nociception receptor NOP, leading to pain relief. In neuroglia cells, CC and CC-BS inhibited signaling pathways related to opioids, neuropathic pain, neuroinflammation, osteoarthritis, and rheumatoid diseases. CC and CC-BS also downregulated ADAM metallopeptidase gene *ADAMTS5* expression by 11.2-fold and 13.5-fold, respectively. *ADAMTS5* encodes a peptidase that plays a crucial role in osteoarthritis development via inhibition of a corresponding signaling pathway. Conclusion: Here, we report for the first time that CC and CC-BS act as nociceptin receptor antagonists, selectively downregulating opioid-related nociceptin receptor 1 gene (*OPRL1*) expression, which is associated with pain relief. BS alone did not affect *OPRL1* expression, but rather appears to potentiate the effects of CC via multiple mechanisms, including synergistic interactions of molecular networks. [Seo EJ, Efferth T, Panossian A. Curcumin downregulates expression of opioid-related nociception receptor gene (OPRL1) in isolated neuroglia cells. *Phytomedicine*. 20 September 2018; https://doi.org/10.1016/j.phymed.2018.090.202]

57. **Novel molecular mechanisms for the adaptogenic effects of herbal extracts on isolated brain cells using systems biology.** Introduction: Adaptogens are natural compounds or plant extracts that increase adaptability and survival of organisms under stress. Adaptogens stimulate cellular and organismal defense systems by activating intracellular and extracellular signaling pathways and expression of stress-activated proteins and neuropeptides. The effects adaptogens on mediators of adaptive stress response and longevity signaling pathways have been reported, but their stress-protective mechanisms are still not fully understood. Aim of the Study: The aim of this study was to identify key molecular mechanisms of adaptogenic plants traditionally used to treat stress and aging-related disorders, i.e., Rhodiola rosea, Eleutherococcus senticosus, Withania somnifera, Rhaponticum carthamoides, and Bryonia alba. Materials and Methods: To investigate the underlying molecular mechanisms of adaptogens, we conducted RNA sequencing to profile gene expression alterations in T98G neuroglia cells upon treatment of adaptogens and analyzed the relevance of deregulated genes to adaptive stress-response signaling pathways using *in silico* pathway analysis software. Results and Discussion: At least 88 of the 3516 genes regulated by adaptogens were closely associated with adaptive stress response and adaptive stress-response signaling pathways (ASRSPs), including neuronal signaling related to corticotropin-releasing hormone, cAMP-mediated, protein kinase A, and CREB; pathways related to signaling involving CXCR4, melatonin, nitric oxide synthase, GP6, Gαs, MAPK, neuroinflammation, neuropathic pain, opioids, renin–angiotensin, AMPK, calcium, and synapses; and pathways associated with dendritic cell maturation and G-coupled protein receptor–mediated nutrient sensing in enteroendocrine cells. All samples tested showed significant effects on the expression of genes encoding neurohormones CRH, GNRH, UCN, G-protein–coupled and other transmembrane receptors TLR9, PRLR, CHRNE, GP1BA,

PLXNA4, a ligand-dependent nuclear receptor RORA, transmembrane channels, transcription regulators FOS, FOXO6, SCX, STAT5A, ZFPM2, ZNF396, ZNF467, protein kinases MAPK10, MAPK13, MERTK, FLT1, PRKCH, ROS1, TTN), phosphatases PTPRD, PTPRR, peptidases, metabolic enzymes, a chaperone (HSPA6), and other proteins, all of which modulate numerous life processes, playing key roles in several canonical pathways involved in defense response and regulation of homeostasis in organisms. It is for the first time we report that the molecular mechanism of actions of melatonin and plant adaptogens are alike, all adaptogens tested activated the melatonin signaling pathway by acting through two G-protein–coupled membrane receptors MT1 and MT2 and upregulation of the ligand-specific nuclear receptor RORA, which plays a role in intellectual disability, neurological disorders, retinopathy, hypertension, dyslipidemia, and cancer, which are common in aging. Furthermore, melatonin activated adaptive signaling pathways and upregulated expression of UCN, GNRH1, TLR9, GP1BA, PLXNA4, CHRM4, GPR19, VIPR2, RORA, STAT5A, ZFPM2, ZNF396, FLT1, MAPK10, MERTK, PRKCH, and TTN, which were commonly regulated by all adaptogens tested. We conclude that melatonin is an adaptation hormone playing an important role in regulation of homeostasis. Adaptogens presumably worked as eustressors ("stress-vaccines") to activate the cellular adaptive system by inducing the expression of ASRSPs, which then reciprocally protected cells from damage caused by distress. Functional investigation by interactive pathways analysis demonstrated that adaptogens activated ASRSPs associated with stress-induced and aging-related disorders such as chronic inflammation, cardiovascular health, neurodegenerative cognitive impairment, metabolic disorders, and cancer. Conclusions: This study has elucidated the genome-wide effects of several adaptogenic herbal extracts in brain cells culture. These data highlight the consistent activation of ASRSPs by adaptogens in T98G neuroglia cells. The extracts affected many genes playing key roles in modulation of adaptive homeostasis, indicating their ability to modify gene expression to prevent stress-induced and aging-related disorders. This study provides a comprehensive look at molecular mechanisms by which adaptogens exerts stress-protective effects. [Panossian A, Seo EJ, Efferth T. Novel molecular mechanisms for the adaptogenic effects of herbal extracts on isolated brain cells using system biology. *Phytomedicine*. 17Sep 2018;50:257-284.] Note: Curcugreen reviewed in this study-see full study for details.

58. **A combination of curcumin and oligomeric proanthocyanidins offer superior anti-tumorigenic properties in colorectal cancer.** Combining anti-cancer agents in cancer therapies is becoming increasingly popular due to improved efficacy, reduced toxicity and decreased emergence of resistance. Here, we test the hypothesis that dietary agents such as oligomeric proanthocyanidins (OPCs) and curcumin cooperatively modulate cancer-associated cellular mechanisms to inhibit carcinogenesis. By a series of in vitro assays in colorectal cancer cell lines, we showed that the anti-tumorigenic properties of the OPCs-curcumin combination were superior to the effects of individual compounds. By RNA-sequencing based gene-expression profiling in six colorectal cancer cell lines, we identified the cooperative modulation of key cancer-associated pathways such as DNA replication and cell cycle pathways. Moreover, several pathways, including protein export, glutathione metabolism and porphyrin metabolism were more effectively modulated by the combination of OPCs and curcumin. We validated genes belonging to these pathways, such as HSPA5, SEC61B, G6PD, HMOX1 and PDE3B to be

cooperatively modulated by the OPCs-curcumin combination. We further confirmed that the OPCs-curcumin combination more potently suppresses colorectal carcinogenesis and modulated expression of genes identified by RNA-sequencing in mice xenografts and in colorectal cancer patient-derived organoids. Overall, by delineating the cooperative mechanisms of action of OPCs and curcumin, we make a case for the clinical co-administration of curcumin and OPCs as a treatment therapy for patients with colorectal cancer. [Ravindranathan P, et al. A combination of curcumin and oligomeric proanthocyanidins offer superior anti-tumorigenic properties in colorectal cancer. *Sci Rep*. 2018 Sep 14;8(1):13869.]

59. **BCM-95 and (2-hydroxypropyl)-β-cyclodextrin reverse autophagy dysfunction and deplete store lipids in Sap C-deficient fibroblasts.** Saposin (Sap) C deficiency is a rare variant form of Gaucher disease (GD) caused by impaired Sap C expression or accelerated degradation, and associated with accumulation of glucosylceramide (GC) and other lipids in the endo/lysosomal compartment. No effective therapies are currently available for the treatment of Sap C deficiency. We previously reported that a reduced amount and enzymatic activity of cathepsin (Cath) B and Cath D, and defective autophagy occur in Sap C-deficient fibroblasts. Here, we explored the use of two compounds, BCM-95, a curcumin derivative, and (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD), to improve lysosomal function of Sap C-deficient fibroblasts. Immunofluorescence and biochemical studies documented that each compound promotes an increase of the expression levels and activities of Cath B and Cath D, and efficient clearance of cholesterol (Chol) and ceramide (Cer) in lysosomes. We provide evidence that BCM-95 and HP-β-CD enhance lysosomal function promoting autophagic clearance capacity and lysosome reformation. Our findings suggest a novel pharmacological approach to Sap C deficiency directed to treat major secondary pathological aspects in this disorder. [Tatti M, Motta M, Scarpa S, Di Bartolomeo S, Cianfanelli V, Tartaglia M, Salvioli R. BCM-95 and (2-hydroxypropyl)-β-cyclodextrin reverse autophagy dysfunction and deplete store lipids in Sap C-deficient fibroblasts. *Hum Mol Genet.* 2015 Aug 1;24(15):4198-4211.]

60. **Synergistic and Additive Effects of Modified Novel evidence for curcumin and boswellic acid induced chemoprevention through regulation of miR-34a and miR-27a in colorectal cancer.** Colorectal cancer (CRC) is one of the most common causes of cancer-associated mortality worldwide, but it is truly a preventable disease. Both curcumin and boswellic acids are well-established dietary botanicals with potent anti-tumorigenic properties which have been shown to modulate multiple oncogenic pathways. Recent data suggest that the chemopreventive effects of these botanicals may in part be mediated through regulation of key cancer-related microRNAs (miRNAs) and their downstream gene targets. Here, we investigated the anti-tumorigenic effects of curcumin and 3 acetyl-11-keto-β-boswellic acid (AKBA) on modulation of specific cancer-related miRNAs in CRC cells and validated their protective effects *in vivo* using a xenograft mouse model. Both curcumin and AKBA inhibited cellular proliferation, induced apoptosis and cell cycle arrest in CRC cell lines, and these effects were significantly enhanced with combined treatment. Gene-expression arrays revealed that curcumin and AKBA regulated distinct cancer signaling pathways including key cell-cycle regulatory genes. Combined bioinformatics and *in-silico* analysis identified

apoptosis, proliferation and cell-cycle regulatory signaling pathways as key modulators of curcumin and AKBA-induced anti-cancer effects. We discovered that curcumin and AKBA induced upregulation of tumor-suppressive miR-34a and downregulation of miR-27a in CRC cells. Furthermore, we demonstrated in a mouse xenograft model that both curcumin and AKBA treatments suppressed tumor growth, which corresponded with alterations in the expression of miR-34a and miR-27a, consistent with our *in vitro* findings. Herein we provide novel mechanistic evidence for the chemopreventive effects of curcumin and AKBA through regulation of specific miRNAs in colorectal cancer. [Toden S, Okugawa Y, Buhrmann C, Nattamai D, Anguiano E, Baldwin N, Shakibaei M, Boland CR, Goel A. *Cancer Prev Res (Phila).* 2015 Feb 23. (Epub ahead of print).]

61. **Citrus Pectin With Two Polybotanical Compounds, in the Suppression of Invasive Behavior of Human Breast and Prostate Cancer Cells.** Aim. The objective of this study was to evaluate the combined effect of a known galectin-3 inhibitor, PectaSol-C modified citrus pectin (MCP), and 2 novel integrative polybotanical compounds for breast and prostate health, BreastDefend (BD) and ProstaCaid (PC), on invasive behavior in human breast and prostate cancer cells in vitro, respectively. Methods. The effect of MCP and BD and of MCP and PC on invasiveness was assessed by cell adhesion, cell migration, and cell invasion assays. Secretion of urokinase plasminogen activator (uPA) was determined by Western blot analysis. Results. Although low concentrations of MCP (0.25-1.0 mg/mL) do not suppress cell adhesion of breast or prostate cancer cells, the combination of MCP with BD or PC synergistically inhibits adhesion of these cells. Dose-dependent inhibition of breast and prostate cancer cell migration by MCP (0.25-1.0 mg/mL) is synergistically enhanced by BD (20 μg/mL) and PC (10 μg/mL), respectively. BD or PC did not further inhibit the invasion of breast and prostate cancer cells by MCP; however, the combination of MCP with BD or PC suppressed secretion of uPA from breast and prostate cancer cells, respectively. Conclusion. The combination of MCP with BD and of MCP with PC synergistically inhibits the metastatic phenotypes of human breast and prostate cancer cells, respectively. Further studies confirming these observations in animal models of breast and prostate cancer metastasis are warranted. [Jiang J, Eliaz I, Sliva D. Synergistic and Additive Effects of Modified Citrus Pectin With Two Polybotanical Compounds, in the Suppression of Invasive Behavior of Human Breast and Prostate Cancer Cells. *Integrative Cancer Therapies*. 2012;12(2):145-152.]

62. **Suppression of Proliferation and Invasive Behavior of Human Metastatic Breast Cancer Cells by Dietary Supplement BreastDefend.** Aim: The study was to evaluate the effect of the dietary supplement BreastDefend (BD) on the proliferation and invasive behavior of highly metastatic human breast cancer cells in vitro. Methods: Cell proliferation and cytotoxicity of BD was evaluated in MDA-MB-231 cells treated with BD (0-40 μg/mL) by MTT assay and trypan blue staining, respectively. Expression of cell cycle regulatory genes were determined by DNA-microarray analysis. Effect of BD on invasiveness was assessed by cellular adhesion, migration, and invasion assays. Results: BD treatment of cells MDA-MB-231 resulted in the cytostatic inhibition of cell proliferation with IC_{50} 22.2, 19.1, and 17.5 μg/mL for 24, 48, and 72 hours, respectively. The inhibition of proliferation was mediated by the upregulation expression of *CCNG1, CHEK1, CDKN1C, GADD45A*, and *E2F2*, whereas BD downregulated expression of *CCNA1* and *CDK6* genes. The induction of expression of GADD45A and inhibition of

expression of cyclin A1 (gene *CCNA1*) by BD was also confirmed on the protein level. BD treatment suppressed the invasive behavior of MDA-MB-231 cells by the inhibition of cellular adhesion, migration, and invasion. This inhibition of invasiveness was mediated by the suppression of secretion of urokinase plasminogen activator (uPA), and by the downregulation of expression of CXCR4 in breast cancer cells treated with BD. Conclusion: BD inhibits proliferation and invasive behavior of the highly metastatic human breast cancer cells in vitro. BD may have a therapeutic potential for prevention or treatment of highly metastatic breast cancers. [Jiang J, Wojnowski R, Jedinak A, Sliva D. Suppression of Proliferation and Invasive Behavior of Human Metastatic Breast Cancer Cells by Dietary Supplement BreastDefend. *Integrative Cancer Therapies*. Jun 2011;10(2):192-200.]

63. **Suppression of growth and invasive behavior of human prostate cancers cells by ProstaCaid™: Mechanism of activity.** Since the use of dietary supplements as alternative treatments or adjuvant therapies in cancer treatment is growing, a scientific verification of their biological activity and the detailed mechanisms of their action are necessary for the acceptance of dietary supplements in conventional cancer treatments. In the present study we have evaluated the anti-cancer effects of dietary supplement ProstaCaid™ (PC) which contains mycelium from medicinal mushrooms (*Ganoderma lucidum, Coriolus versi-color, Phellinus linteus*), saw palmetto berry, pomegranate, pumpkin seed, green tea [40% epigallocatechin-3-gallate (EGCG)], Japanese knotweed (50% resveratrol), extracts of turmeric root (BCM-95®), grape skin, pygeum bark, sarsaparilla root, Scutellaria barbata, eleuthero root, Job's tears, astragalus root, skullcap, dandelion, coptis root, broccoli, and stinging nettle, with purified vitamin C, vitamin D3, selenium, quercetin, citrus bioflavonoid complex, β sitosterolzinc, lycopene, α lipoic acid, boron, berberine and 3.3'-diinodolymethane (DIM). We show that PC treatment resulted in the inhibition of cell proliferation of the highly invasive human hormone refractory (independent) PC-3 prostate cancer cells in a dose- and time-dependent manner with IC50 56.0, 45.6 and 39.0 μg/ml for 24, 48 and 72 h, respectively. DNA-microarray analysis demonstrated that PC inhibits proliferation through the modulation of expression of *CCND1*, *CDK4*, *CDKN1A*, *E2F1*, *MAPK6* and *PCNA* genes. In addition, PC also suppresses metastatic behavior of PC-3 by the inhibition of cell adhesion, cell migration and cell invasion, which was associated with the down-regulation of expression of *CAV1*, *IGF2*, *NR2F1*, and *PLAU* genes and suppressed secretion of the urokinase plasminogen activator (uPA) from PC-3 cells. In conclusion, the dietary supplement PC is a promising natural complex with the potency to inhibit invasive human prostate cancer. [Jiang J, Eliaz I, Sliva D. Suppression of growth and invasive behavior of human prostate cancer cells by ProstaCaid™: Mechanism of activity. *Int J Oncology*. Jun 2011;38(6):1675-1682.]

64. **ProstaCaid induces G2/M cell cycle arrest and apoptosis in human and mouse androgen-dependent and-independent prostate cancer cells.** The anticancer effects of ProstaCaid, a novel integrative blend of vitamins, minerals, multiherb extracts, and derivatives, were tested in human and mouse androgen-dependent (AD) and -independent (AI) prostate cancer cell lines. ProstaCaid shows growth inhibitory effects on both human and mouse AD prostate cancer cells (LNCaP and CASP 2.1) and AI prostate cancer cells (PC3 and CASP 1.1) in a dose-/time-dependent manner. Consistently, long-term

treatment with ProstaCaid also reduced colony formation capacities of prostate cancer cells. Flow cytometry assays revealed that ProstaCaid induces G2/M arrest and apoptosis in LNCaP and PC3 cells after 72 hours of treatment. Immunoblotting assay demonstrated that 25 microg/mL of ProstaCaid treatment resulted in (1) the reduction of cyclin D1, cyclin B1, and Cdc2 expression in a time-dependent way; (2) increase in p21(WAF1/Cip1) as early as 12 hours after the treatments in PC3 cells and reduction to base line at the 72-hour time point; and (3) repression of Bcl-2, BclxL, and induction of Bim as well as the cleavages of caspase-3 and poly(ADP-ribose) polymerase (PARP) at 72 hours of treatment, suggesting caspase-3-dependent apoptosis. Moreover, ProstaCaid suppressed activation of AKT and MAPK signaling pathways in PC3 and LNCaP cells by reducing phosphorylation levels of AKT, its downstream target S6 ribosomal protein and GSK3beta, and ERK1/2, respectively. In summary, these findings strongly suggest that ProstaCaid may be a potential chemopreventive and therapeutic agent for both AD and, more importantly, AI prostate cancer. [Yan J, Katz AE. ProstaCaid induces G2/M cell cycle arrest and apoptosis in human and mouse androgen-dependent and and-independent prostate cancer cells. *Integr Cancer Ther*. 2010 Jun;9(2):186-196.]

Additional Supporting Studies

65. **Efficacy of Curcumin and Boswellia for knee osteoarthritis: Systematic review and meta-analysis.** PURPOSE: The unfavorable safety profiles of commonly prescribed knee osteoarthritis (OA) treatments have led clinicians and patients to seek safer alternatives. Research has suggested that curcuminoid and boswellia formulations could moderate key inflammatory pathways that are associated with worsening symptoms and disease progression. We conducted a systematic review and meta-analysis to assess the efficacy and safety of these treatments vs. placebo or NSAIDs for knee OA. METHODS: We searched Medline, EMBASE, Google Scholar, Web of Science and the Cochrane database from inception to February 21, 2018. We also hand searched reference lists and reviewed conference proceedings. We included randomized clinical trials (RCTs) comparing curcuminoid or boswellia formulations with placebo or NSAIDs for knee OA. We calculated standardized mean differences (SMD) or risk ratios (RR) for all relevant outcomes. Meta-analyses were conducted using random effects models. Heterogeneity was assessed using the I2 statistic. RESULTS: Eleven RCTs (N = 1009) were eligible for analysis. Study quality was low overall, and most included RCTs were conducted on fewer than 100 participants. Both curcuminoid and boswellia formulations were statistically significantly more effective than placebo for pain relief and functional improvement. There were no significant differences between curcuminoids or boswellia and placebo in safety outcomes. Curcuminoids showed no statistically significant differences in efficacy outcomes compared to NSAIDs; patients receiving curcuminoids were significantly less likely to experience gastrointestinal adverse events. No RCTs compared boswellia against approved NSAIDs. CONCLUSIONS: The results of our study suggest that curcuminoid and boswellia formulations could be a valuable addition to the knee OA treatment regimens by relieving symptoms while reducing safety risks. The current body of evidence is not adequate in size or quality to make any meaningful clinical practice recommendations. Further research through large, high quality RCTs probably investigating the synergistic effect of these products with other OA treatments is warranted. [Bannuru RR, Osani MC, Al-Eid F, Wang C. Efficacy of Curcumin and

Boswellia for knee osteoarthritis: Systematic review and meta-analysis. *Semin Arthritis Rheum*. 2018 Dec;48(3):416-429.]

66. **The Role of Curcumin Administration in Patients with Major Depressive Disorder: Mini Meta-Analysis of Clinical Trials.** Major depression is a common, recurrent, and chronic disease that negatively affects the quality of life and increases the risk of mortality. Several studies have demonstrated that curcumin, the yellow-pigmented substance of the turmeric, possesses antidepressant properties. The aim of this review is to meta-analytically assess the antidepressant effect of curcumin in patients with major depressive disorders. We extensively searched the literature until August 2015. The random-effect model was used to calculate the pooled standardized difference of means (SMD). Subgroup analyses were also performed to examine the effect of different study characteristics on the overall model. Six clinical trials met the inclusion criteria. Overall, curcumin administration showed a significantly higher reduction in depression symptoms [SMD = -0.34; 95% confidence interval (CI) = -0.56, -0.13; p = 0.002]. Subgroup analyses showed that curcumin had the highest effect when given to middle-aged patients (SMD = -0.36; 95% CI = -0.59; -0.13; p = 0.002), for longer duration of administration (SMD = -0.40; 95% CI = -0.64, -0.16; p = 0.001), and at higher doses (SMD = -0.36; 95% CI = -0.59, -0.13; p = 0.002). The administration of new formulation of curcumin (BCM-95) had non-significantly higher effect on depression as compared with the conventional curcumin-piperine formula. We conclude that there is supporting evidence that curcumin administration reduces depressive symptoms in patients with major depression. [Al-Karawi D, Al Mamoori DA, Tayyar Y. The Role of Curcumin Administration in Patients with Major Depressive Disorder: Mini Meta-Analysis of Clinical Trials. *Phytother Res.* 2015 Nov 27. doi: 10.1002/ptr.5524. (Epub ahead of print).]

67. **Curcumin for neuropsychiatric disorders: a review of in vitro, animal and human studies.** Turmeric has been used in traditional medicine for centuries to treat a range of ailments. Its primary active constituent curcumin, can influence an array of biological activities. Many of these, such as its anti-inflammatory, antioxidant, neuroprotective, and monoaminergic effects are dysregulated in several neuropsychiatric disorders. In this systematic review, in vitro, animal, and human studies investigating the potential of curcumin as a treatment for neuropsychiatric disorders such as major depressive disorder, post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), bipolar disorder, psychotic disorders, and autism are reviewed, and directions for future research are proposed. It is concluded that curcumin is a promising, natural agent for many of these conditions, however, further research utilizing robust, clinical designs are essential. The problem associated with the poor oral bioavailability of standard curcumin also requires consideration. Currently the greatest support for the efficacy of curcumin is for the treatment of major depressive disorder. [Lopresti AL. Curcumin for neuropsychiatric disorders: a review of in vitro, animal and human studies. *Journal of Psychopharmacology*. 2017 Mar;31(3):287-302.]

68. **The Role of Turmerones on Curcumin Transportation and P-Glycoprotein Activities in Intestinal Caco-2 Cells.** The rhizome of Curcuma longa (turmeric) is often used in Asia as a spice and as a medicine. Its most well-studied component, curcumin,

has been shown to exhibit poor bioavailability in animal studies and clinical trials. We hypothesized that the presence of lipophilic components (e.g., turmerones) in turmeric extract would affect the absorption of curcumin. The effects of turmerones on curcumin transport were evaluated in human intestinal epithelial Caco-2 cells. The roles of turmerones on P-glycoprotein (P-gp) activities and mRNA expression were also evaluated. Results showed that in the presence of α- and aromatic turmerones, the amount of curcumin transported into the Caco-2 cells in 2 hours was significantly increased. α - Turmerone and verapamil (a P-gp inhibitor) significantly inhibited the efflux of rhodamine-123 and digoxin (i.e., inhibited the activity of P-gp). It is interesting that aromatic turmerone significantly increased the rhodamine-123 efflux and Pgp (MDR1 gene) mRNA expression levels. The effects of a- and aromatic turmerones on curcumin transport as well as P-gp activities were shown here for the first time. The presence of turmerones did affect the absorption of curcumin in vitro. These findings suggest the potential use of turmeric extract (including curcumin and turmerones), rather than curcumin alone, for treating diseases. [Yue GGL, et al. The Role of Turmerones on Curcumin Transportation and P-Glycoprotein Activities in Intestinal Caco-2 Cells. *Journal of Medicinal Food*. 2012;15(3):242-252.]

69. **Oat Fiber As a Carrier for Curcuminoids.** The curcuminoid-carrying potential of oat fiber was examined as a potential route to overcome the low aqueous solubility of curcuminoids. Aqueous dispersions of oat fiber were mixed with curcuminoids solubilized in ethanol to obtain curcuminoids−oat fiber (1% w/w) dispersions in aqueous ethanol (2% v/v). Centrifugation of the curcuminoids−oat fiber dispersions resulted in a supernatant (95.3% w/w: 0.11% w/w protein, 0.17% w/w β-glucan) and precipitate (4.74% w/w: 0.18% w/w protein, 0.11% w/w β-glucan) with the curcuminoids being almost equally partitioned into both fractions. Curcuminoids solubility in the supernatant was markedly greater than that in aqueous ethanol and water. The curcuminoids were in the amorphous state in the precipitated fraction and were more stable to degradation than the curcuminoids in the supernatant. These studies show the potential of oat fiber as a carrier for curcuminoids into functional foods. [Sayanjali S, Sanguansri L, Buckow R, Gras S, Augustin MA. Oat Fiber As a Carrier for Curcuminoids. *J Agric. Food Chem*. 2014;62:12172-12177.]