

Published Studies on BCM-95[®] Curcumin

With Study Results Summary

- 1. 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.]
- 2. 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. *European Neuropsychopharmacology*. Dec. 5, 2014]

3. **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-SR₃₀). Secondary outcomes included IDS-SR₃₀ 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-SR₃₀ 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-SR₃₀ total score and IDS-SR₃₀ 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.]
4. **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.]
5. **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.]

6. **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.]
7. **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.]
8. **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 curcuminoids, 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.]

9. **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.]
10. **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.]
11. **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.]
12. **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.]

13. **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.]
14. **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 (α 1-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.]
15. **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.]
16. **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.]

17. **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 propionyl-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.]
18. **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.]
19. **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 Pentylentetrazole (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.]

20. **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.]
21. **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 curcumin-mediated 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 chemosensitization to 5-fluorouracil through miRNA-induced suppression of epithelial-to-mesenchymal transition in chemoresistant colorectal cancer. *Carcinogenesis*. 2015 Feb 4 (Epub ahead of print).]

22. **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).]

23. **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- β signaling molecules (TGF- β 3, 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- κ B, MMP-13), TGF- β 3, 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- β 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.]

24. **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).]

25. **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- κ B) 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 μ M 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- κ B activation and NF- κ B-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-fluorouracil in a 3D alginate tumor microenvironment of colorectal cancer. *BMC Cancer*. 2015 Apr 10;15:250.]
26. **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.]

27. **Curcumin inhibits cancer-associated fibroblast-driven prostate cancer invasion through MAOA/mTOR/HIF-1 α 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-1 α (HIF-1 α) 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 CXCR4 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-1 α 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-1 α signaling. *International Journal of Oncology*. 2015;1899:0-0.]

Additional Supporting Studies

28. **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).]