

Key Peer-Reviewed Abstracts Supporting Efficacy of BioResponse DIM® (BR-DIM)®:

Breast Cancer Res Treat. 2017 Aug;165(1):97-107. doi: 10.1007/s10549-017-4292-7. Epub 2017 May 30.

A randomized, placebo-controlled trial of diindolylmethane for breast cancer biomarker modulation in patients taking tamoxifen.

Thomson CA, Chow HHS3, Wertheim BC, Roe DJ, Stopeck A, Maskarinec G, Altbach M, Chalasani P, Huang C, Strom MB, Galons JP, Thompson PA

Diindolylmethane (DIM), a bioactive metabolite of indole-3-carbinol found in cruciferous vegetables, has proposed cancer chemoprevention activity in the breast. There is limited evidence of clinically relevant activity of DIM or long-term safety data of its regular use. A randomized, double-blind, placebo-controlled trial was conducted to determine the activity and safety of combined use of BioResponse DIM® (BR-DIM) with tamoxifen. METHODS: Women prescribed tamoxifen (n = 130) were randomly assigned oral BR-DIM at 150 mg twice daily or placebo, for 12 months. The primary study endpoint was change in urinary 2/16 α -hydroxyestrone (2/16 α -OHE1) ratio. Changes in 4-hydroxyestrone (4-OHE1), serum estrogens, sex hormone-binding globulin (SHBG), breast density, and tamoxifen metabolites were assessed.

RESULTS: Ninety-eight women (51 placebo, 47 DIM) completed intervention; compliance with treatment was >91%. BR-DIM increased the 2/16 α -OHE1 ratio (+3.2 [0.8, 8.4]) compared to placebo (-0.7 [-1.7, 0.8], P < 0.001). Serum SHBG increased with BR-DIM compared to placebo (+25 \pm 22 and +1.1 \pm 19 nmol/L, respectively). No change in breast density measured by mammography or by MRI was observed. Plasma tamoxifen metabolites (endoxifen, 4-OH tamoxifen, and N-desmethyl-tamoxifen) were reduced in women receiving BR-DIM versus placebo (P < 0.001). Minimal adverse events were reported and did not differ by treatment arm.

CONCLUSION: **In patients taking tamoxifen for breast cancer, daily BR-DIM promoted favorable changes in estrogen metabolism and circulating levels of SHBG.** Further research is warranted to determine whether BR-DIM associated decreases in tamoxifen metabolites, including effects on endoxifen levels, attenuates the clinical benefit of tamoxifen.

JANA. 2005 8(1): 5-15

Managing Cyclical Mastalgia with Absorbable Diindolylmethane: A Randomized, Placebo-controlled Trial.

Zeligs MA., Brownstone PK, Sharp, ME, Westerlind KC, Wilson, SM, and Johs SM.

This intervention study investigated the efficacy and safety of absorbable diindolylmethane (BioResponse-DIM®) in cyclical mastalgia (recurrent breast pain). Otherwise healthy, pre-menopausal women with cyclical mastalgia were given absorbable diindolylmethane (DIM) or placebo for consecutive three month periods in a randomized, double-blind, crossover study. Breast symptoms were monitored using daily entries on a "breast pain diary" as the assessment tool. Urine and blood samples were collected to confirm safety and the impact of absorbable DIM on estrogen metabolism. Results showed clinical improvement with absorbable DIM. Improvement was not seen with placebo. A statistically significant reduction in duration of breast pain, severity of pain, swelling, and soreness accompanied absorbable DIM use, based on comparison of visual analog pain scores from treatment and placebo periods (p=.001-.03). In addition, absorbable DIM was shown to increase the ratio of 2-hydroxy to 16-hydroxy metabolites in urine (p<.05). **Supplementation with absorbable DIM was found to be an effective intervention for cyclical mastalgia. Its use as a dietary supplement deserves further investigation in conditions where modifying estrogen metabolism may be of benefit.**

Br J Cancer. 2014 Sep 23;111(7):1269-74. doi: 10.1038/bjc.2014.391. Epub 2014 Jul 15.

BRCA1 mRNA levels following a 4-6-week intervention with oral 3,3'-diindolylmethane.

Kotsopoulos J, Zhang S, Akbari M, Salmena L, Llacuachaqui M, Zeligs M, Sun P, Narod SA.

Haploinsufficiency may contribute to the development of breast cancer among women with a BRCA1 mutation. Thus, interventions that enhance BRCA1 expression may represent avenues for prevention. Studies have shown that 3,3'-diindolylmethane (DIM) can upregulate BRCA1 expression in breast cancer cells. This has yet to be demonstrated in vivo.

METHODS: We conducted a study to evaluate the ability of oral DIM to upregulate BRCA1 mRNA expression in white blood cells. A total of 18 women were enrolled in the study, including 13 BRCA1 mutation carriers who received 300 mg per day of Rx Balance BioResponse DIM for 4-6 weeks (intervention group) and 5 BRCA1 mutation carriers who did not take DIM (control group). BRCA1 mRNA expression was assessed at baseline and at 4-6 weeks by real-time, quantitative PCR and the relative change in BRCA1 mRNA expression (that is, $2(-\Delta\Delta CT)$) was calculated.

RESULTS: The relative change in BRCA1 mRNA expression among women in the intervention group achieved borderline significance (P paired t-test=0.05). In the intervention group, BRCA1 mRNA expression increased in 10 of the participants, decreased in 2 and remained unchanged in 1 of the participants following DIM intervention (P sign test=0.02). On average, women in the intervention group experienced a 34% increase in BRCA1 mRNA expression (range -24 to 194%). There was no significant difference in the relative change in BRCA1 mRNA expression among women in the control group (P paired t-test=0.45).

CONCLUSIONS: **Under the tested conditions, oral DIM was associated with an increase in BRCA1 mRNA expression in women with a BRCA1 mutation. The possibility of mitigating the effect of an inherited deleterious BRCA1 mutation by increasing the physiologic expression of the gene and normalising protein levels represents a clinically important paradigm shift in the prevention strategies available to these high-risk women.** Future studies with a larger sample size and higher doses of DIM are warranted.

Am J Transl Res. 2016 Jan 15;8(1):166-76. eCollection 2016.

Anti-androgenic activity of absorption-enhanced 3, 3'-diindolylmethane in prostatectomy patients.

Hwang C, Sethi S, Heilbrun LK, Gupta NS, Chitale DA, Sakr WA, Menon M, Peabody JO, Smith DW, Sarkar FH, Heath E.

Consumption of cruciferous vegetables is associated with a decreased risk of developing prostate cancer. Antineoplastic effects of cruciferous vegetables are attributable to bioactive indoles, most prominently, 3, 3'-diindolylmethane (DIM). In addition to effects on proliferation and apoptosis, DIM acts as an antiandrogen in prostate cancer cell lines. This study characterized the effects of prostatic DIM on the androgen receptor (AR) in patients with prostate cancer. Men with localized prostate cancer were treated with a specially formulated DIM capsule designed for enhanced bioavailability (BR-DIM) at a dose of 225 mg orally twice daily for a minimum of 14 days. DIM levels and AR activity were assessed at the time of prostatectomy. Out of 28 evaluable patients, 26 (93%) had detectable prostatic DIM levels, with a mean concentration of 14.2 ng/gm. The mean DIM plasma level on BR-DIM therapy was 9.0 ng/mL; levels were undetectable at baseline and in follow-up samples. AR localization in the prostate was assessed with immunohistochemistry. After BR-DIM therapy, 96% of patients exhibited exclusion of the AR from the cell nucleus. In contrast, in prostate biopsy samples obtained prior to BR-DIM therapy, no patient exhibited AR nuclear exclusion. Declines in PSA were observed in a majority of patients (71%). Compliance was excellent and toxicity was minimal. **In summary, BR-DIM treatment resulted in reliable prostatic DIM levels and anti-androgenic biologic effects at well tolerated doses. These results support further investigation of BR-DIM as a chemopreventive and therapeutic agent in prostate cancer.**

Cancer Res. 2017 Feb 15;77(4):918-925. doi: 10.1158/0008-5472.CAN-16-1717.

Association of Estrogen Metabolism with Breast Cancer Risk in Different Cohorts of Postmenopausal Women.

Sampson JN, Falk RT, Schairer C, Moore SC, Fuhrman BJ, Dallal CM, Bauer DC, Dorgan JF, Shu XO, Zheng W, Brinton LA, Gail MH, Ziegler RG, Xu X, Hoover RN, Gierach GL.

Endogenous estradiol and estrone are linked causally to increased risks of breast cancer. In this study, we evaluated multiple competing hypotheses for how metabolism of these parent estrogens may influence risk. Prediagnostic concentrations of estradiol, estrone, and 13 metabolites were measured in **1,298 postmenopausal cases** of breast cancer and **1,524 matched controls** in four separate patient cohorts. The median time between sample collection and diagnosis was 4.4 to 12.7 years across the cohorts. Estrogen analytes were measured in serum or urine by liquid chromatography-tandem mass spectrometry. Total estrogen levels (summing all 15 estrogens/estrogen metabolites) were associated strongly and positively with breast cancer risk. **Normalizing total estrogen levels, we also found that a relative increase in levels of 2-hydroxylation pathway metabolites, or in the ratio of 2-hydroxylation:16-hydroxylation pathway metabolites, were associated inversely with breast cancer risk. These associations varied by total estrogen levels, with the largest risk reductions occurring in women in the highest tertile. With appropriate validation, these findings suggest opportunities for breast cancer prevention by modifying individual estrogen metabolism profiles through either lifestyle alterations or chemopreventive strategies.** Cancer Res; 77(4); 918-25.

Epidemiology. 2000 Nov;11(6):635-40.

Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16alpha-hydroxyestrone ratio in premenopausal and postmenopausal women.

Muti P, Bradlow HL, Micheli A, Krogh V, Freudenheim JL, Schünemann HJ, Stanulla M, Yang J, Sepkovic DW, Trevisan M, Berrino F.

Experimental and clinical evidence suggests that 16alpha-hydroxylated estrogen metabolites, biologically strong estrogens, are associated with breast cancer risk, while 2-hydroxylated metabolites, with lower estrogenic activity, are weakly related to this disease. This study analyzes the association of breast cancer risk with estrogen metabolism, expressed as the ratio of 2-hydroxyestrone to 16alpha-hydroxyestrone, in a prospective nested case-control study. Between 1987 and 1992, 10,786 women (ages 35-69 years) were recruited to a prospective study on breast cancer in Italy, the "Hormones and Diet in the Etiology of Breast Cancer" (ORDET) study. Women with a history of cancer and women on hormone therapy were excluded at baseline. At recruitment, overnight urine was collected from all participants and stored at -80 degrees C. After an average of 5.5 years of follow-up, 144 breast cancer cases and four matched controls for each case were identified among the participants of the cohort. Among premenopausal women, a higher ratio of 2-hydroxyestrone to 16alpha-hydroxyestrone at baseline was associated with a reduced risk of breast cancer: women in the highest quintile of the ratio had an adjusted odds ratio (OR) for breast cancer of 0.58 [95% confidence interval (CI) = 0.25-1.34]. The corresponding adjusted OR in postmenopausal women was 1.29 (95% CI = 0.53-3.10). **Results of this prospective study support the hypothesis that the estrogen metabolism pathway favoring 2-hydroxylation over 16alpha-hydroxylation is associated with a reduced risk of invasive breast cancer risk in premenopausal women.**

Ann Hepatol. 2017 May - Jun;16(3):382-394. doi: 10.5604/16652681.1235481.

Testosterone, Sex Hormone-Binding Globulin and Nonalcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis.

Jaruvongvanich V Sanguankeo A, Riangwiwat T, Upala S.

Endogenous sex hormones are associated with the risk of diabetes and metabolic syndrome. Recent studies suggested the role of these hormones in nonalcoholic fatty liver disease (NAFLD). We conducted a systematic review and meta-analysis of observational studies investigating the association between sex hormones and NAFLD. MATERIAL AND METHODS: A comprehensive search of the databases of the MEDLINE and EMBASE was performed from inception through April 2016. The inclusion criterion was the observational studies that assessed the association of serum total testosterone (TT) and sex-hormone binding globulin (SHBG) and NAFLD. We calculated pooled effect estimates of TT and SHBG with 95% confidence intervals (CI) comparing between subjects with and without NAFLD by using random-effects model. RESULTS: Sixteen trials comprising 13,721 men and 5,840 women met the inclusion criteria. TT levels were lower in men with NAFLD (MD = -2.78 nmol/l, 95%CI -3.40 to -2.15, I² = 99%) than in those without. Men with higher TT levels had lower odds of NAFLD whereas higher TT levels increased the odds of NAFLD in women. In both sexes, SHBG levels were lower in patients with NAFLD than controls and this inverse association was stronger in women than men and higher SHBG levels were associated with reduced odds of NAFLD. CONCLUSION: Our meta-analysis demonstrated a sex-dependent association between TT and NAFLD. Lower TT levels are associated with men with NAFLD and inversely associated with women with NAFLD, **whereas higher SHBG levels are associated with lower NAFLD odds in both men and women.**