Diindolylmethane (DIM) Information Resource Center

An Initiative of Faculty Members and Research Fellows at the University of California at Berkeley

Home / Formation / Clinical Applications / Research at Cal / References from 1975 to 2008

DIM Clinical Applications and Research

DIM is currently used to treat Recurring Respiratory Papillomatosis caused by the Human Papilloma Virus and is in Phase III clinical trials for Cervical Dysplasia. Cervical Dysplasia is a precancerous condition also caused by the Human Papilloma Virus. Until recently, DIM's biological mode of action for these conditions was not clearly understood. When scientists at UC Berkeley discovered that DIM is a potent modulator of the innate immune response system, this discovery finally shed light on DIM's global anti-viral properties, including anti-viral properties against the Human Papilloma Virus.

As a result of this discovery, DIM is currently sold as an immune enhancing supplement (**ActivaMune Diindolylmethane (DIM) Immune Support Formula**) and it is under investigation as a therapeutic for a variety of viral and bacterial infections (including HIV, HPV, Hepatitis, Influenza, the Pandemic Flu and antibiotic resistant bacteria), immune deficiency conditions and multiple forms of cancer (breast, prostate, lung, colon, skin, cervical). DIM's primary immune modulatory mode of action is the stimulation of Interferon-Gamma receptor transcription as well as the production of Interferon-Gamma. DIM has also been shown to synergize with Interferon-Gamma in the potentiation of the MHC-I Complex. The **Diindolylmethane Immune Activation Data Center** provides a more comprehensive review of DIM's immune activating properties.

Its multitude of favorable biological activities such as immune modulation, apoptosis promotion and suppression of inflammation (NFkB) are among the reasons why the National Cancer Institute has begun clinical studies of DIM for multiple forms of cancer.

Another significant discovery that has recently been made about DIM that has fueled international interest in this unique phytochemical is its synergy with the number one cancer drug worldwide (Taxol) in the promotion of apoptosis. This discovery has paved the way for the investigation of DIM as an adjuvant therapeutic to Taxol to reduce patient resistance to this important drug. (Taxol is another plant-derived chemical that was initially discovered in and extracted from the Pacific Yew Tree.)

UC Berkeley faculty members Dr. Leonard Bjeldanes, Professor and former Chairman of the Nutritional Sciences and Toxicology Department, and Dr. Gary Firestone, Director of the National Institutes of Health Cancer Biology Program and Professor of the Molecular and Cell Biology, have focused on DIM research at Berkeley for over two decades and have elucidated many of its principal molecular mechanisms of action.

In addition to elucidating DIM's molecular mechanisms of action, Dr. Bjeldanes and Dr. Firestone conducted a human clinical trial of DIM and demonstrated that it increases the 2-hydroxylation of estrogen metabolites, a study that received a lot of media attention as oncologists believe that this activity helps to reduce the risk of breast and prostate cancer. Abstracts of two papers on this subject are provided below.

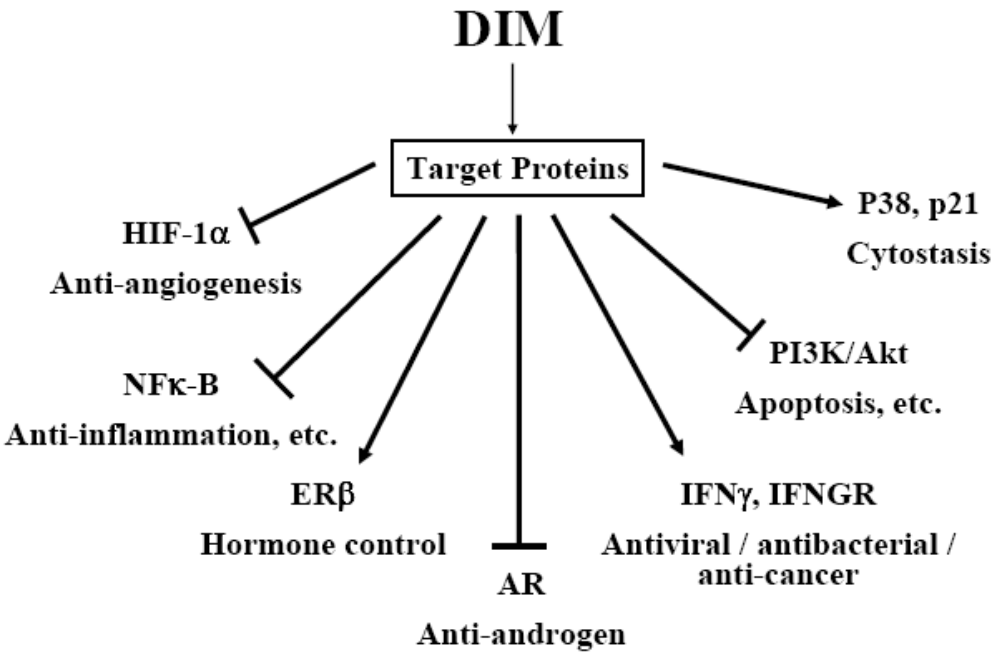
Pilot study: effect of 3,3'-diindolylmethane supplements on urinary hormone metabolites in postmenopausal women with a history of early-stage breast cancer. Journal of Nutrition and Cancer. 2004;50(2):161-7. Dalessandri KM, Firestone GL, Fitch MD, Bradlow HL, Bjeldanes LF Department of Molecular and Cell Biology, University of California, Berkeley, 94720-3200, USA.

Dietary indoles, present in Brassica plants such as cabbage, broccoli, and Brussels sprouts, have been shown to provide potential protection against hormone-dependent cancers. 3,3'-Diindolylmethane (DIM) is under study as one of the main protective indole metabolites. Postmenopausal women aged 50-70 yr from Marin County, California, with a history of early-stage breast cancer, were screened for interest and eligibility in this pilot study on the effect of DIM supplements on urinary hormone metabolites. The treatment group received daily DIM (108 mg DIM/day) supplements for 30 days, and the control group received a placebo capsule daily for 30 days. Urinary metabolite analysis included 2-hydroxyestrone (2-OHE1), 16-alpha hydroxyestrone (16alpha-OHE1), DIM, estrone (El), estradiol(E2), estriol (E3), 6beta-hydroxycortisol (6beta-OHC), and cortisol in the first morning urine sample before intervention and 31 days after intervention. Nineteen women completed the study,for a total of 10 in the treatment group and 9 in the placebo group. DIM-treated subjects, relative to placebo, showed a significant increase in levels of 2-OHE1 (P=0. 020), DIM (P =0. 045), and cortisol (P = 0.039), and an increase of 47% in the 2-OHE1/16alpha-OHE1 ratio from 1.46 to 2.14 (P=0.059). In this pilot study, DIM increased the 2-hydroxylation of estrogen urinary metabolites.

Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16alpha-hydroxyestrone ratio in premenopausal and postmenopausal women. Epidemiology. 2000 Nov;11(6):635-40. Muti P, Bradlow HL, Micheli A, Krogh V, Freudenheim JL, Schunemann HJ, Stanulla M, Yang J, Sepkovic DW, Trevisan M, Berrino F. Department of Social and Preventive Medicine, University at Buffalo, State University of New York at Buffalo, Buffalo, NY, USA, Epidemiology Division of the National Cancer Intitute (Istituto Nazionale Tumori), Milan, Italy, Department of Pediatric Hematology and Oncology, Medical School of Hannover, Hannover, Germany.

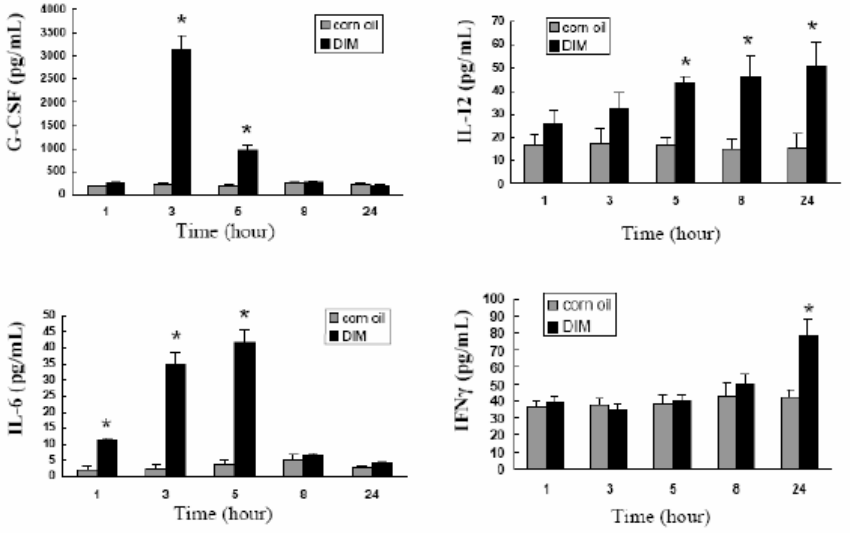
Experimental and clinical evidence suggests that 16alpha-hydroxylated estrogen metabolites, biologically strong estrogens, are associated with breast cancer risk, while 2-hydroxylated metabolites, with lower estrogenic activity, are weakly related to this disease. This study analyzes the association 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.

Overview of DIM's Anti-Cancer and Immune Modulating Properties



Sampling of Scientific Data

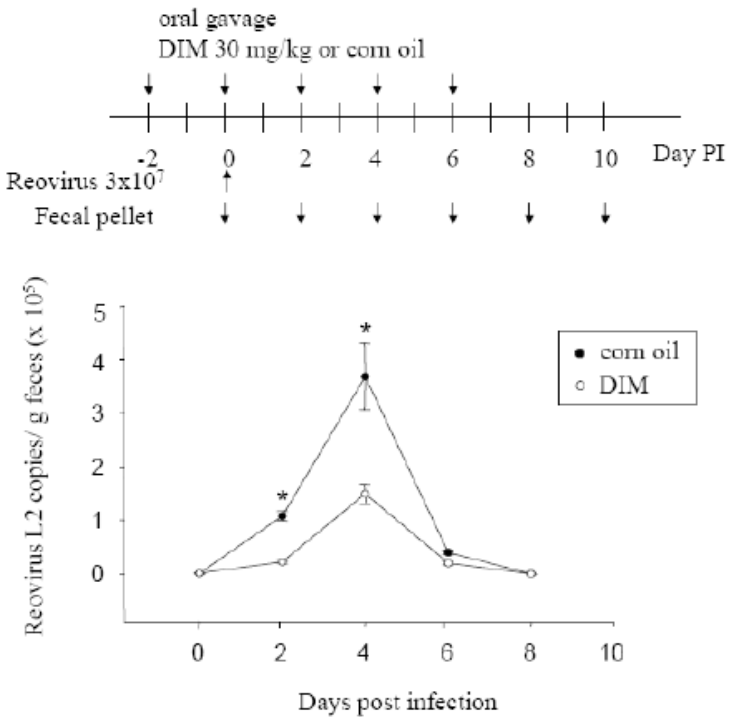
DIM In-Vivo Affect on Key Immune Modulating Cytokines



G-CSF promotes White Blood Cell formation.
IL-6 has anti-bacterial properties.
IL-12 stimulates the growth and function of T-Cells – defense against pathogens.
IFN-G is the modulator of the entire innate immune response system.
(Note: Amgen's blockbuster drugs Neupogen™ and Neulasta™ are recombinant G-CSF proteins.)

In this study DIM was administered orally at 30mg/kg of body weight.

DIM In-Vivo Anti-Viral Activity Through Immune Modulation



(Reference: Xue L, Pestka J, Maoxiang L, Firestone GL, Bjeldanes LF, *3,3'-Diindolylmethane stimulates murine immune function in vitro and in vivo,* Journal of Nutritional Biochemistry, published online, 8-20-07.)

DIM Stimulates Transcript Expression of Interferon-γ and Related Genes in Human Breast Cancer Cells

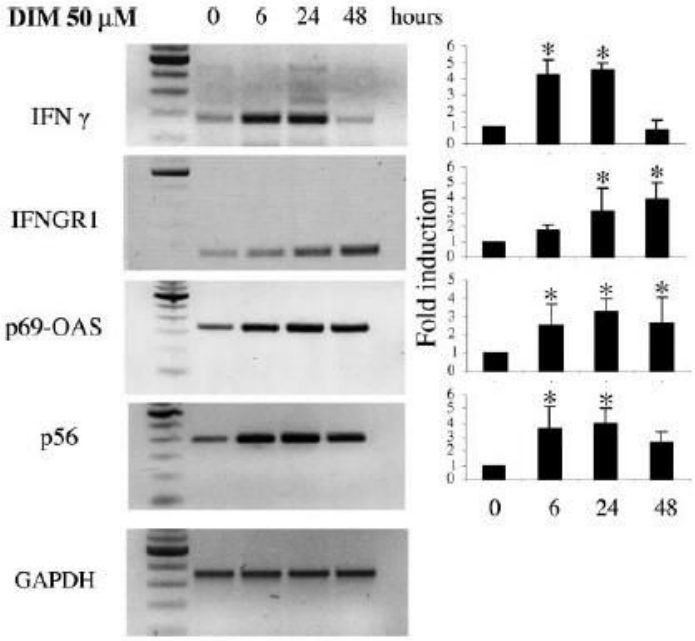


Fig. 1. Transcriptional activation of IFNγ and related genes by DIM. Cells were treated with 50 μM DIM for the indicated times. The mRNA levels were measured by RT-PCR. Quantitative detection of amplicons required 40 PCR cycles for IFNγ and 17 cycles for the other genes. Images of the ethidium bromide-stained gels were inverted (negative black/white) for presentation. Molecular weights are compared with a 100-bp ladder (darker band is 600 bp) to confirm identity of the amplicons. GAPDH was used as the control. Densitometry results are presented as -fold induction over the 0-h treatment after correction for GAPDH. Results are presented as the mean ± S.D. of three separate experiments. *, statistically significant difference with time 0 control ($p < 0.05$).

Activation and Potentiation of Interferon-γ by DIM: Stimulation of Interferon-γ Receptor 1 Production

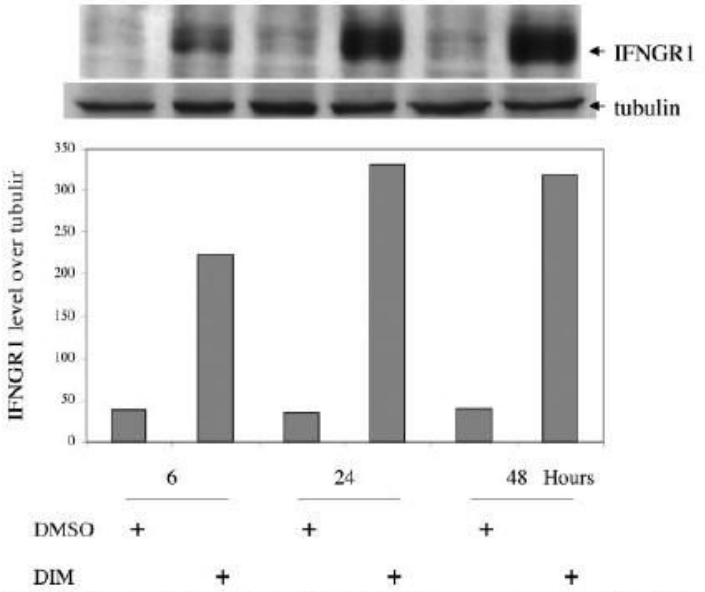


Fig. 2. Western blot analysis of INFGR1. Cells were treated with DMSO (control) or 50 μM DIM for 6, 24, or 48 h. The relative abundance of IFNGR1 was corrected for variations in tubulin, as a loading control between samples. A representative Western blot from three separate experiments is shown.

Potentiation of Interferon-γ Mediated Cell Cycle Arrest by DIM in Human Breast Cancer Cells

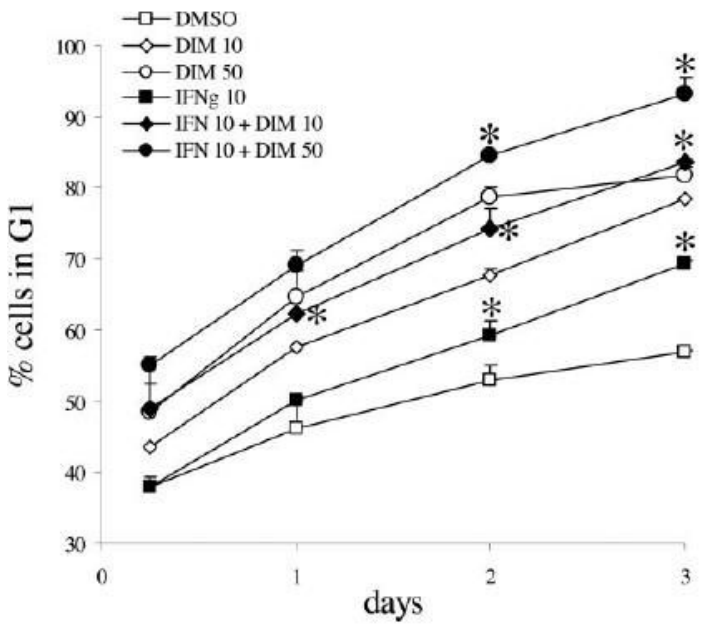


Fig. 7. Flow cytometry. The percentage of cells in the G_1 phase of the cell cycle was measured in cells treated with DIM, IFNγ, or combinations of the two for 6 h or 1, 2, or 3 days. Results are presented as the mean ± S.D. of three separate experiments. *, statistically significant difference with the corresponding DIM treatment without IFNγ at the same time point is noted ($p < 0.05$).

DIM Enhances Interferon-γ Induced Expression of MHC-I Complex in Human Breast Cancer Cells

(Please Note Section D of this figure. DIM may be used as an adjuvant to IFN-γ treatment models because of this unique synergistic effect on the MHC-I Complex. This synergy is currently under investigation for HIV, HPV, Hepatitis and multiple forms of cancer.)

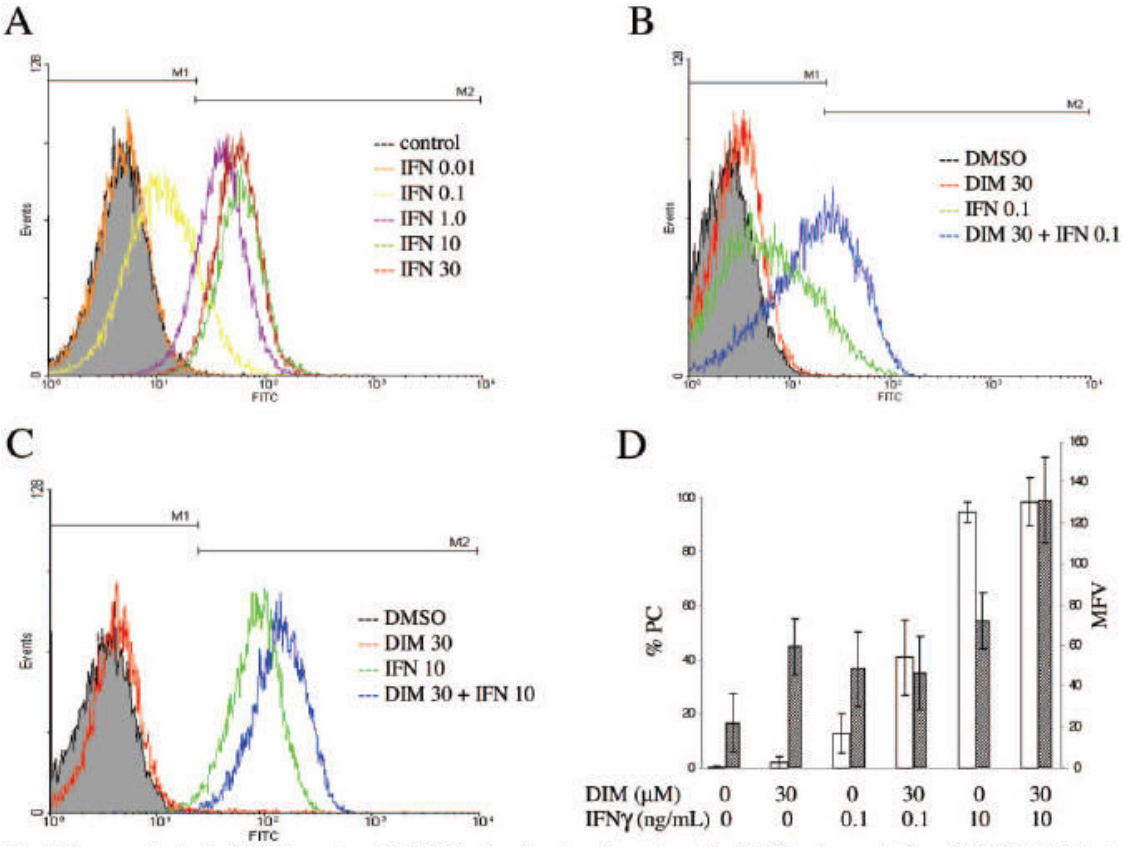


Fig. 8. The expression level of MHC-I complex on the MCF-7 cell surface. A, cells were treated with different concentrations of IFNγ for 16 h. B and C, cells were pretreated with DMSO (control) or 30 μM DIM for 48 h, followed by 0.1 ng/ml of IFNγ (B) or 10 ng/ml of IFNγ (C) for another 16 h. One million cells were incubated in 90 μl of PBS with 10 μl of FITC-conjugated anti-HLA-ABC mouse monoclonal antibody. The fluorescence intensity was measured by flow cytometry using a Coulter Elite instrument and analyzed by WinMDI 2.8 software provided by Duke University. D, the expression of HLA class I complex was calculated as %PC (open bars) and MFV (cross-hatched bars).

(Reference: Riby JE, Xue L, Chatterji U, Bjeldanes EL, Firestone GL, Bjeldanes LF, *Activation and Potentiation of Interferon-(gamma) Signaling by 3,3'-Diindolylmethane in MCF-7 Breast Cancer Cells*, Molecular Pharmacology, Nov. 2, 2005)

DIM and Taxol Synergize to Promote Apoptosis

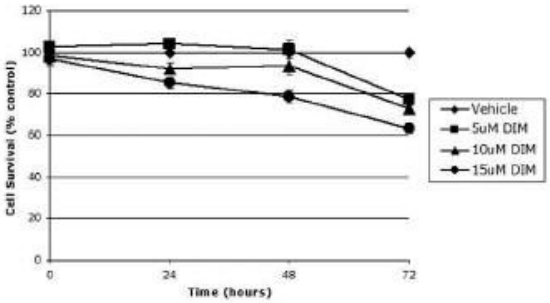


FIG. 1. Increasing concentrations of DIM inhibit cell growth in human breast carcinoma 435 eb1 cells (Her2/neu positive). Cells were plated in 96-well plates, incubated for 24 h and then treated with 0, 5, 10, and 15 uM DIM for 0, 24, 48, and 72 h. The number of viable cells was determined with ProCheck cell viability assay. Values represent mean ± SEM.

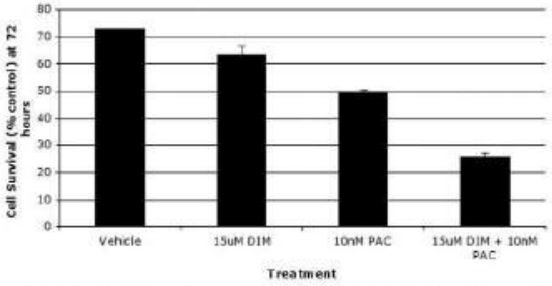


FIG. 2. DIM in combination with paclitaxel inhibits cell growth in human breast carcinoma 435 eb1 cells (Her2/neu positive). Cells were plated in 96-well plates, incubated for 24 h and then treated with vehicle, 10 uM DIM, 15 uM DIM, 10 nM paclitaxel, 10 uM DIM +10 nM paclitaxel, and 15 uM DIM +10 nM paclitaxel for 0, 24, 48, and 72 h. The number of viable cells was determined with ProCheck cell viability assay. Values represent mean ± SEM.

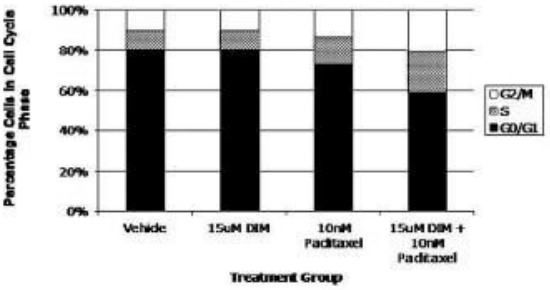


FIG. 3. DIM in combination with paclitaxel induces G2/M cell cycle phase arrest. Cells were plated in T-175 flasks, incubated for 24 h and then treated with vehicle, 15 uM DIM, 10 nM paclitaxel, and 15 uM DIM +10 nM paclitaxel and incubated for an additional 72 h. Cells were then harvested and underwent cell cycle analysis by flow cytometry.

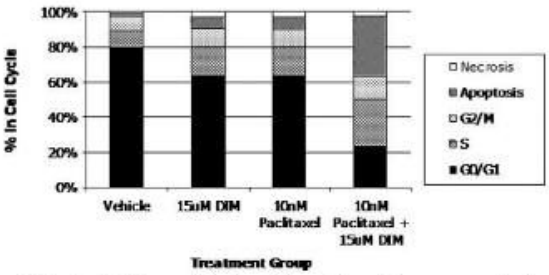


FIG. 4. DIM in combination with paclitaxel increases apoptosis. Cells were plated in T-175 flasks, incubated for 24 h and then treated with vehicle, 15 uM DIM, 10 nM paclitaxel, and 15 uM DIM +10 nM paclitaxel and incubated for an additional 72 h. Cells were then harvested and underwent TUNEL analysis.

(Reference: K. McGuire, N. Ngoubilly, M. Neavyn, S. Lanza-Jacoby. *3,3'-Diindolylmethane and Paclitaxel Act Synergistically to Promote Apoptosis in HER2/Neu Human Breast Cancer Cells.* Journal of Surgical Research, 2006 May 15;132(2):208-13. Department of Surgery, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107)

In-Vivo Animal Study: DIM Inhibits DMBA-induced Mammary Tumor Growth

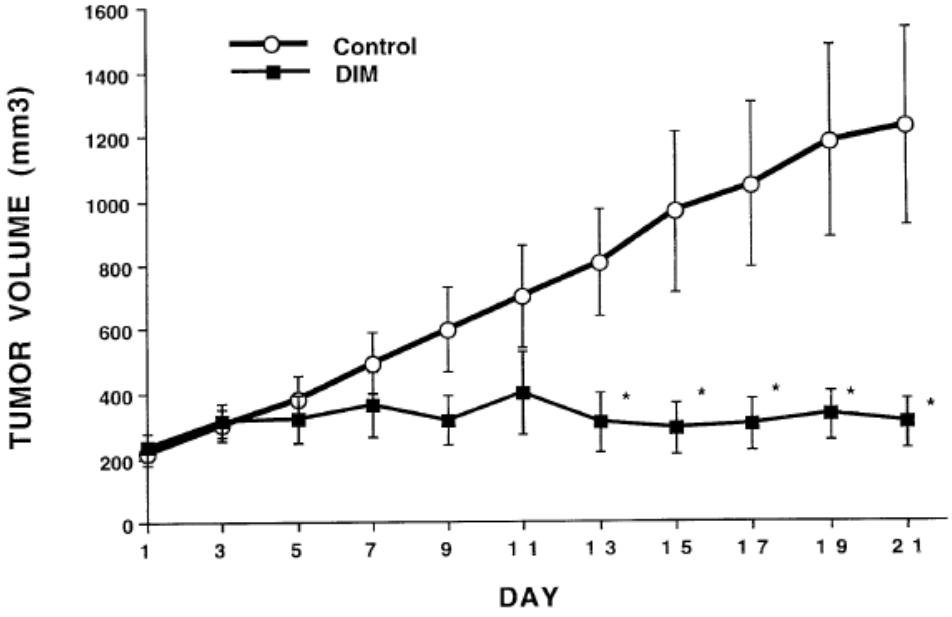


Fig. 6. Time course inhibition of mammary tumor growth by DIM (5 mg/kg). Animals were treated orally every other day with DIM or corn oil (control) and tumor volumes/mass were determined as described in Materials and methods.

(Reference: Chen I, McDougal A, Wang F, Safe S, Carcinogenesis, vol. 19, no.9. pp.1631. 1998.)

In-Vivo Animal Study: DIM Inhibits Growth of Human Mammary Tumor Cells in Athymic Mice

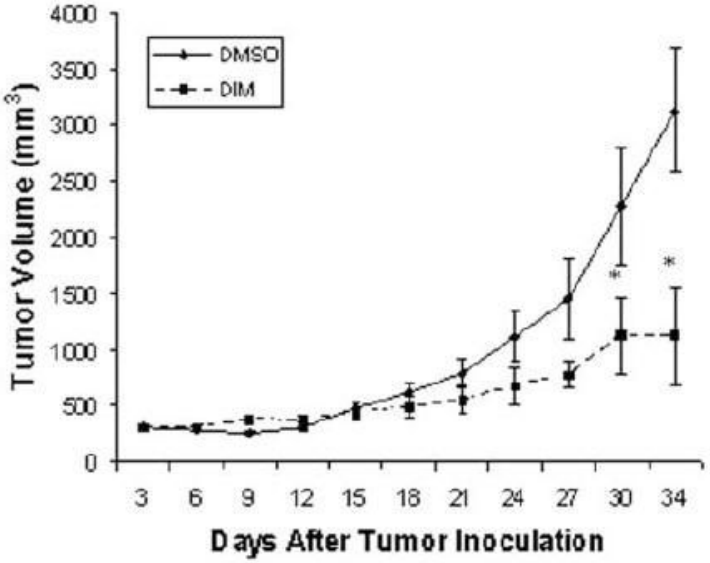


Fig. 8. The effect of DIM on growth of transplantable human breast carcinoma in athymic mice. The tumor growth curves of transplantable MCF-7 human breast carcinoma in female athymic (nu/nu) mice. Mice were inoculated subcutaneously in the bilateral flanks with 0.1 ml Matrigel containing 3×10^6 human breast cancer cells MCF-7. DMSO or 5 mg/kg DIM were injected subcutaneously five times weekly. Tumor sizes were measured twice per week using a caliper and calculated as $(\pi/6) \times$ [length (mm) $\times$ width^2 (mm^2)]. The experiment was terminated on day 34. Values are mean $\pm$ SE, $n = 10$. * indicates significant difference from control at a level of $P \leq 0.05$.

(Reference: Chang X., Tou J.C., Hong C., Kim H.A., Riby J.E., Firestone G.L., Bjeldanes L.F., Diindolylmethane inhibits angiogenesis and the growth of transplantable human breast carcinoma in athymic mice, Carcinogenesis, Janurary 2005, pp.771-778.)