

## TITLE

### TARGETED THERAPY FOR TREATMENT OF LUNG CANCER

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We examined the cytotoxic effects of a peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) agonist, 1,1-bis (3' indolyl)-1-(p-biphenyl) methane (DIM-C-pPhC<sub>6</sub>H<sub>5</sub>) alone and in combination with docetaxel (doc) in vitro in A549 lung cancer cells. Further, the feasibility of oral and aerosolized delivery of DIM-C-pPhC<sub>6</sub>H<sub>5</sub> in combination with i.docetaxel (doc) in vivo against orthotopic lung tumor mice model was also investigated. A549 and H460 cells were treated with different doses of doc (0.00012-0.23  $\mu$ M) alone and in combination with each methylene or C-substituted DIM (C-DIM), DIM-C-pPhC<sub>6</sub>H<sub>5</sub> (3, 5, and 7  $\mu$ M), DIM-C-pPhtBu (3, 5, and 7  $\mu$ M), and DIM-C-pPhCF<sub>3</sub> (3, 5, and 7  $\mu$ M) for 72 h. Isobolographic methods were used to calculate combination index (CI) values from the cell viability data. Apoptosis was evaluated by TUNEL and cleaved PARP assays after treatment of cells for 48 and 72 h with 0.01  $\mu$ M doc and 7.5  $\mu$ M DIM-C-pPhC<sub>6</sub>H<sub>5</sub> alone and in combination. A549 cells ( $1 \times 10^6$ ) were injected into lungs of 6-week old female athymic nu/nu mice, then treated with doc (10 mg/kg iv on days 14, 18, and 22), DIM-C-pPhC<sub>6</sub>H<sub>5</sub> (40 mg/kg 3 times a week by oral gavage), and combination until the end of the study (28 days). For inhalation delivery, DIM-C-pPhC<sub>6</sub>H<sub>5</sub> solution was formulated utilizing  $\alpha$ -tocopherol polyethylene glycol succinate (TPGS). The solution was characterized for its aerodynamic properties. Applying nose-only exposure technique, tumor-bearing mice were exposed to aerosolized DIM-C-pPhC<sub>6</sub>H<sub>5</sub> solution (0.2%, three times a week, i.v. doc (10 mg/kg i.v. on days 14, 18, 22, and 29), and doc + DIM-C-pPhC<sub>6</sub>H<sub>5</sub> until the end of the study (35 days). Lung weights and tumor volumes were obtained to assess therapeutic effects of treatments. Expression of various proteins such as NF-kB, IKK $\alpha$ , P-IkK $\alpha$ , VEGF, survivin, BAD, AKT, Mcl-1, JNK, and cyclinD1 proteins by Western blotting was performed on whole lysates from control and treated tumors. DNA fragmentation and cleaved caspase-3 expression in the harvested lung tissues were studied by TUNEL and immunohistochemistry (IHC) respectively. DIM-C-pPhC<sub>6</sub>H<sub>5</sub>, DIM-C-pPhtBu, and DIM-C-pPhCF<sub>3</sub> inhibited the proliferation of A549 and H460 cells in a dose-dependent manner. In A549 and H460 cells, the IC<sub>50</sub> values varied between 8 and 12  $\mu$ M with the relative potency in the order of DIM-C-pPhtBu > DIM-C-pPhC<sub>6</sub>H<sub>5</sub> > DIM-C-pPhCF<sub>3</sub>. The CI values for the interaction between doc and C-DIMs ranged from 0.36 to 0.98 for 50 % cell kill suggesting synergistic to additive effects in both cell lines. Combination treatment led to the highest increase in a) percentage of apoptotic cells (TUNEL assay), b) levels of cleaved PARP (ELISA), and c) expression of cleaved PARP and Bax (WB) compared to treatment with doc or DIM-C-pPhC<sub>6</sub>H<sub>5</sub> alone. In vivo, doc + oral DIM-C-pPhC<sub>6</sub>H<sub>5</sub> a) reduced lung weights by 57% compared to 39 % by doc, or 22% by oral DIM-C-pPhC<sub>6</sub>H<sub>5</sub> alone b) induced apoptosis in 43 % of the tumor cells compared to 29 % and 22 % in doc and oral DIM-C-pPhC<sub>6</sub>H<sub>5</sub>, respectively, and c) increased procaspase-3 cleavage compared to doc or oral DIM-C-pPhC<sub>6</sub>H<sub>5</sub> alone. The aerodynamic properties of DIM-C-pPhC<sub>6</sub>H<sub>5</sub> solution included mass median aerodynamic diameter (MMAD) of  $1.780 \pm 0.34$ , geometric standard deviation (GSD) of  $2.31 \pm 0.02$ , and a respirable fraction of  $59.47 \% \pm 0.09$  /5-minute nebulization.

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Lung weight reduction in mice treated with combination of doc + aerosolized DIM-C-pPhC<sub>6</sub>H<sub>5</sub> was 63.71% as compared to 40.22% and 47.01% in mice treated with aerosolized DIM-C-pPhC<sub>6</sub>H<sub>5</sub> and doc respectively. DNA fragmentation was most induced in combination compared to doc or aerosolized DIM-C-pPhC<sub>6</sub>H<sub>5</sub>. WB analysis indicated that simultaneous co-treatment of doc and aerosolized DIM-C-pPhC<sub>6</sub>H<sub>5</sub> decreased expression of NF-κB, IκBα, P-IκBα, VEGF, AKT, cyclinD1, survivin, Mcl-1 and increased expression of JNK and BAD compared to tumors collected from single-agent treatment and control groups. Results from the present study suggest that the inhalation delivery of DIM-C-pPhC<sub>6</sub>H<sub>5</sub> potentiated the anti-cancer activity of doc in lung tumors by enhancing apoptosis.