# MBT2, a Novel Analog of a Mitochondrially Encoded Peptide, Inhibits Fibrogenesis in Cultured Human Lung Cells and is Effective in Mouse Models of Idiopathic Pulmonary Fibrosis (IPF).

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## RATIONALE

- IPF is an age-related disease associated with a decrease in abundance and function of mitochondria in lung tissue.
- Mitochondrial dysfunction can also result in decreased levels of mitochondrially encoded peptides, some of which are secreted and regulate cellular metabolic processes in animals, ranging from energy homeostasis to cytoprotection.
- MBT2 (CB5138-1) is an improved analog of a newly discovered mitochondrially encoded peptide, CB5138.
- MBT2 (CB5138-1) and additional CB5138 analogs were evaluated for anti-fibrotic effects *in vitro* in cultured cells and *in vivo* in preclinical models of idiopathic pulmonary fibrosis.

## METHODS

**BioMAP Fibrosis Assay:** Effect of MBT2 (CB5138-1) on expression of fibrosis biomarkers was examined in co-culture of primary human small airway epithelial cells/lung fibroblasts (SAEMyoF system, DiscoverX, Fremont, CA). Cells were stimulated with TNFα and TGF-β1 and treated with MBT2 (0.3 – 10 μM). Expression of Alpha Smooth Muscle Actin (αSMA), Collagen I, and Collagen III were quantified by ELISA.

**Fibroblast-to-Myofibroblast Transition (FMT) Assays:**
**αSMA Expression:** Effect of MBT2 (CB5138-1) on expression of αSMA was determined in primary human lung fibroblasts from healthy donors (Charles River Discovery Research Services, Essex, UK). Five days post-seeding, medium was refreshed, and MBT2 (0.003 - 10 μM) added one hour before addition of TGF-β1 (1.25 ng/ml) to induce FMT. Expression of αSMA was measured at 72 hours by immunostaining and high content imaging.
**Pro-Collagen I α 1 Expression:** Effect of MBT2 (CB5138-1) on intracellular pro-collagen I α 1 was measured in WI-38 cells (ATCC; Manassas, VA). Medium replaced 24 h after seeding with serum restricted (SR) medium (0.2% FBS). After 24 h, medium replaced with fresh SR medium containing TGF-β1 (5 ng/ml) alone or with nintedanib or MBT2. After 48 h incubation, cells were lysed and pro-collagen I α 1 quantified by ELISA (Abcam; Cambridge, MA).

**Mouse IPF Models:** Studies conducted at SMC Laboratories (Tokyo, Japan) and Aragen Bioscience (Morgan Hill, CA). Lung fibrosis induced in C57BL/6 mice (n = 8-10/group) with intratracheal or oropharyngeal bleomycin; control animals received saline. In the prophylactic IPF model, MBT2 (CB5138-1) (5 mg/kg) was administered intraperitoneally for 21 days. In the therapeutic model, peptides (5 or 15 mg/kg/day) were administered for 14 days beginning 7 days after bleomycin and compared to oral nintedanib (60 mg/kg/day). Assessments included body weight, lung weight, soluble collagen in bronchoalveolar lavage fluid (BALF), differential cells count in BALF, H&E and Masson's trichrome (MT) staining and histological scoring of lung fibrosis by modified Ashcroft Score (Hubner, R-H et al. BioTechniques 2008. 44: 507-17), and inflammatory cytokine expression by MSD multiplex assay.

## RESULTS

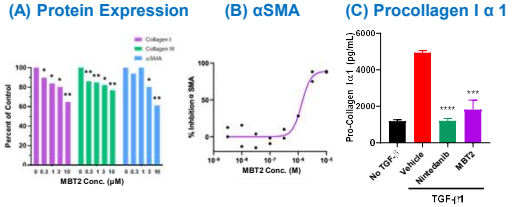



Figure 1: Antifibrotic effects of MBT2 (CB5138-1) in cultured human lung cells: (A) reduced expression of biomarkers of fibrosis in SAEMyoF system. Blocking fibroblast to myofibroblast transformation induced by TGF-β1 in (B) primary human lung fibroblasts: αSMA and (C) WI-38 cells: procollagen I α 1.

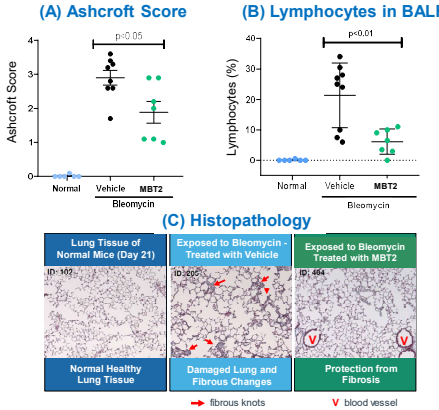



Figure 2: Efficacy of MBT2 (CB5138-1) in the mouse prophylactic IPF model: (A) reduced fibrosis, (B) reduced inflammation, (C) reduced lung damage: MT staining of lung tissue. Data are mean (SEM) for 8 animals.

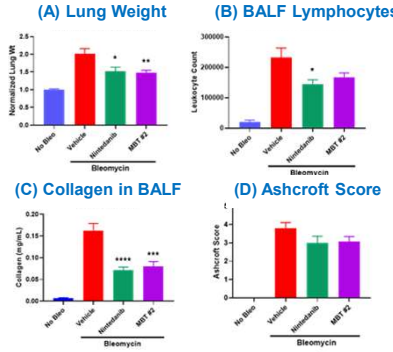
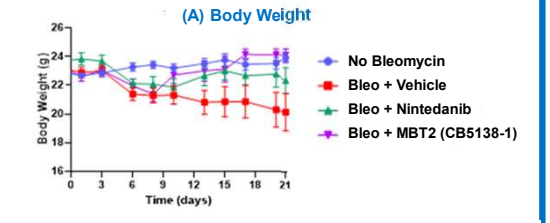



Figure 3: Efficacy of MBT2 (CB5138-1) in the mouse therapeutic IPF model: (A) reduced lung weight, (B) reduced inflammation, (C) reduced collagen deposition and (D) reduced fibrosis. Data are mean (SEM) for 10 animals.

## RESULTS



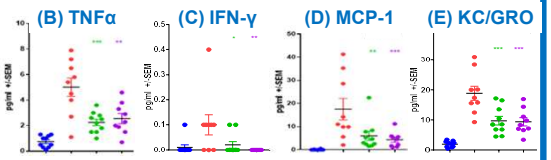




Figure 4: Efficacy of MBT2 (CB5138-1) in the mouse therapeutic IPF model: (A) reduced body weight loss and (B-E) significantly reduced levels of key secreted proinflammatory cytokines in BALF. Data are mean (SEM) for 10 animals.

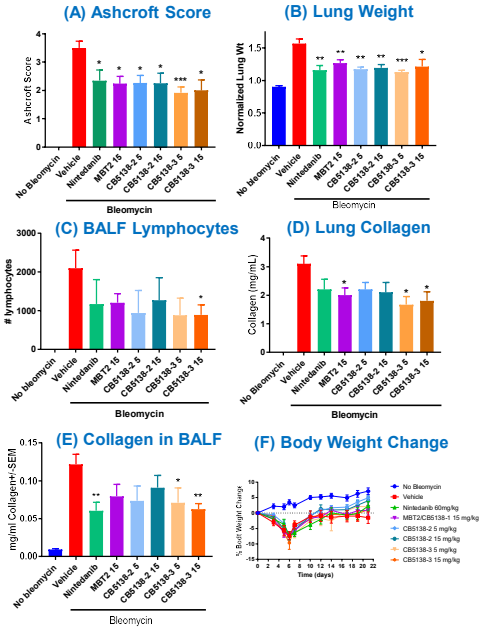



Figure 5: Efficacy of additional CB5138 analogs in the mouse therapeutic IPF model: (A) reduced fibrosis; (B) reduced lung weight; (C) reduced inflammation; (D and E) reduced collagen; (F) reduced body weight loss. Data are mean (SEM) for 10 animals.
*p<0.05; **p<0.01, ***p<0.001; ****p<0.0001 compared to vehicle control.

## RESULTS

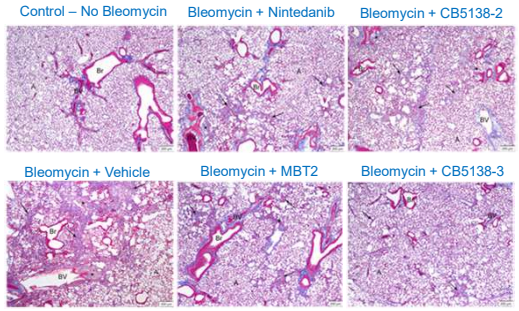



Figure 6: MBT2 (CB5138-1) and additional analogs reduced fibrosis and inflammation in the mouse therapeutic IPF model. Representative photomicrographs from MT staining of lung tissue.
A - alveoli; Br - bronchiolus, BV - blood vessel; arrows – fibrosis; asterisk - lymphocytic infiltrate.

## CONCLUSIONS

- MBT2 (CB5138-1) demonstrated consistent antifibrotic effects in cultured human lung cells, decreasing expression of collagen I, collagen III, and αSMA and inhibiting fibroblast to myofibroblast transition.
- MBT2 (CB5138-1) was effective in prophylactic and therapeutic models of IPF in mice, reducing fibrosis, inflammation, lung weight, collagen deposition, and key proinflammatory cytokines in BALF.
- Additional novel analogs of CB5138 showed antifibrotic effects in the therapeutic IPF model, significantly reducing Ashcroft score, lung weight, collagen deposition, and lymphocytes in BALF.
- CB5138 analogs represent a novel class of antifibrotic molecules based on a natural, mitochondrially encoded peptide, with potential for treatment of IPF and other fibrotic diseases.

## DISCLOSURES

Kenneth C. Cundy, Lindsay Stark, Annie Lennek, Tracy Yu, Kent Grindstaff: Employees and shareholders of CohBar, Inc.