OATD-01, a Dual Chitinase Inhibitor, Significantly Ameliorates Pulmonary Fibrosis in the Bleomycin-Induced Mouse Model

Dymek B1, Sklepkiwicz P1, Młacki M2, Zagodzonz A1, Koralewski R1, Mazur M1, Paplinska-Goryca M3, Nejman-Gryz P3, Proboszcz M2, Gorska K2, Maskey-Warzechowska M2, Przysucha N1, Krenke R1, Dobrzanski P1, Golebiowski A1, Dzwonek K1

1OncoArendi Therapeutics SA, Warsaw, Poland; 2Department of Internal Medicine, Pulmonary Diseases and Allergy, Medical University of Warsaw, Poland

BACKGROUND
Acidic mammalian chitinase (AMCase) and chitotriosidase (CHIT1) are the enzymatically active chitinases, which have been implicated in the pathophysiology of obstructive and interstitial lung diseases. The CHIT1 activity is elevated in bronchoalveolar lavage fluid (BALf) from patients with interstitial lung diseases such as sarcoidosis and idiopathic pulmonary fibrosis (IPF). Moreover, significantly elevated serum chitinolytic activity in sarcoidosis patients (5- to 100-fold) correlates with the disease stage and severity and is considered to be one of the best biomarkers of disease progression. These data suggest that inhibition of the chitinolytic activity might represent a novel therapeutic approach for interstitial lung diseases.

OATD-01 is a novel, potent and selective chitinase inhibitor currently in phase I clinical trial.

CHIT1 is highly expressed in BALf macrophages from IPF and sarcoidosis patients

Immunocytological staining of BALf cells smears from IPF and sarcoidosis patients with anti-CHIT1 antibody demonstrated that majority of BALf cells are CHIT1-positive.

Overall, 78% and 64% of all BALf cells in IPF (n=10) and sarcoidosis (n=25), respectively, were positive for CHIT1. Cytological analysis also lymphocytes stained positive (50% in IPF and 34% in sarcoidosis).

In the bleomycin-induced pulmonary fibrosis mouse model, OATD-01 administered in a therapeutic regimen from day 7, significantly reduced lung fibrosis, comparable to pirfenidone, as assessed by the modified Ashcroft scoring system.

The anti-fibrotic effects of OATD-01 correlated with a significant reduction of the lung index and was associated with a strong pharmacodynamic effect: plasma chitinolytic activity in animals dosed with OATD-01 was significantly reduced in comparison to control mice, confirming the target engagement.

OATD-01 POTENTLY INHIBITED CHITINOlytic ACTIVITY IN SAMPLES FROM IPF PATIENTS: EX VIVO ASSAY

OATD-01 is a potent, dual AMCase and CHIT1 small-molecule inhibitor. OATD-01 potently inhibited elevated chitinolytic activity in serum, induced sputum and BALf collected from IPF patients.

OATD-01 demonstrated anti-fibrotic efficacy in bleomycin-induced pulmonary fibrosis model

In samples from IPF patients treated with OATD-01 for 28 days, chitinolytic activity was significantly reduced in BALf, induced sputum, and plasma.

MATERIALS AND METHODS

IPF and sarcoidosis patients description, serum, induced sputum and BALf collection

The treatment-naïve male and female patients were recruited in the Public Central Teaching Clinical Hospital of the Medical University of Warsaw, Poland. Patients were included to the IPF study group when they met the IPF diagnostic criteria according to the 2013 ATS/ERS statement for the diagnosis and management of IPF. The sarcoidosis group included patients who presented with typical clinical and radiological features of sarcoidosis with non-caseating granulomas showed in mediastinal lymph node or transbronchial lung biopsies and in whom other granulomatous diseases were excluded.

The study was approved by the Local Bioethics Committee, at the Medical University of Warsaw, Poland, No. of approval: KB/236/2015. Blood, induced sputum and BAL f were collected, processed and stored in -80°C for subsequent analysis. BAL f cells were prepared by the microscope slide smear technique.

BALF CELLS PREPARATION AND IMMUNOCYTOLOGICAL STAINING FOR CHIT1

Smears were fixed in 10% NBF, endogenous peroxidases were inactivated by applying H2O2 and non-specific binding sites were blocked with normal goat serum. CHIT1 was then detected with primary antibody (Biorbyt, orb377995), appropriate secondary antibody conjugated with HRP and visualized with DAB reaction. Cells were counterstained with hematoxylin, dehydrated in alcohols, cleared with xylene and mounted with resin-based medium. The differential cell count of CHIT1-positive cells (the percentage of eosinophils, neutrophils, macrophages and lymphocytes) in BALF was determined based on microscopic examination of the morphology of 300 cells from various fields.

DISHONOMIC ASSAYS

For determination of chitinolytic activity of recombinant enzymes (AMCase and CHIT1) 4-methylumbelliferyl (β-D-N,N'-diacetylchitobioside hydrate or 4-methylumbelliferyl (β-D-N,N'-diacetylchitotrioside substrate, enzyme and varying concentrations of compound in citrate assay buffer pH 5.2 were incubated at 37°C for 60 minutes. Substrate hydrolysis product – 4-methylumbelliferyl was measured fluorimetrically.

BLEOMYCIN-INDUCED PULMONARY FIBROSIS MOUSE MODEL

Three groups (n=8) of age-matched 8-week-old female C57BL/6 mice were instilled intranasally with bleomycin (2 u/kg) at day 0, 1 and 2. Control mice were intranasally administered with PBS at times of bleomycin instillations (n=8). OATD-01 was administered orally at dose 30 mg/kg bid in 0.5% CMC starting from day 7 onwards. Pirfenidone was administered orally at dose 250 mg/kg bid in 0.5% CMC starting from day 7 onwards similarly to OATD-01.

HISTOLOGICAL ANALYSIS – AHSCROFT SCORING

After fixation in NBF, lungs were separated into lobes, pre-embedded in agarose-gelatin medium, processed and embedded in paraffin. Blocks were cut into 5-µm thick sections, which were then stained with Masson’s trichrome for visualization of collagen. Fibrosis was evaluated blindly basing on modified Ashcroft scoring system (scale 0-8) adapted to laboratory rodents (10.2144/0931.1279).

CHITINASE ACTIVITY IN PLASMA

1 µl of plasma was mixed with 96 µM 4-methylumbelliferyl (β-D-N,N'-diacetylchitobioside hydrate in assay buffer (0.1 M choline, 0.2 M free chloride phosphate, 1 mg/ml BSA, pH 6) and incubated in a 96-well black microtiter plate with shaking in the dark, at 37°C for 60 minutes followed by addition of stop solution (0.3 M glycine/NaOH Buffer, pH 10.5). The product - 4-methylumbelliferyl was measured fluorometrically using Tecan 104A multimode reader (excitation 355 nm/emission 460 nm). The chitinase activity was calculated using a standard curve of 4-methylumbelliferyl.

LUNG INDEX

Lung index was calculated as wet lung-to-body weight ratio.

FINANCIAL SUPPORT

"Preclinical research and clinical trials of a first-in-class therapy in pulmonary fibrosis and sarcoidosis was supported by the European Commission, within the Horizon 2020 framework program under the Actiris-1 and Actiris-2 Research Projects (grant agreement no. 643064 & 665727)."