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

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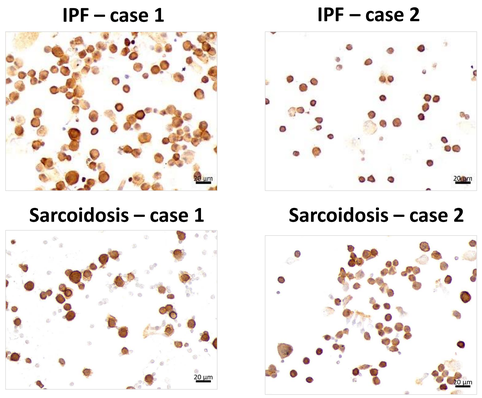
Background

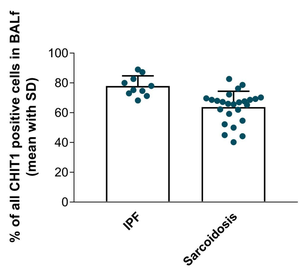
Acidic mammalian chitinase (AMCase) and chitotriosidase (CHIT1) are the enzymatically active chitinases, which have been implicated in the pathophysiology of obstructive and interstitial lung diseases. The CHIT1 activity is elevated in bronchoalveolar lavage fluid (BALf) from patients with interstitial 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.

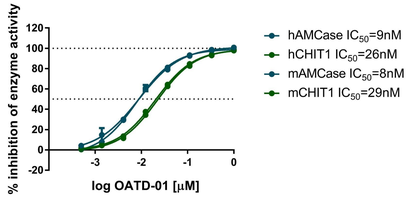


Overall, 78% and 64% of all BALf cells in IPF (n=10) and sarcoidosis (n=25), respectively, were positive for CHIT1. Cytological analysis showed that the main CHIT1-positive cell subtype in BALf are macrophages (83% positive cells in IPF and 76% in sarcoidosis), also lymphocytes stained positive (50% in IPF and 34% in sarcoidosis).



OATD-01 potently inhibited chitinolytic activity in samples from IPF patients: ex vivo assay

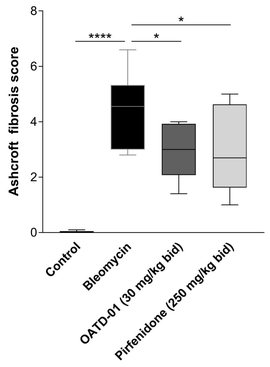
OATD-01 is a potent, dual AMCase and CHIT1 small-molecule inhibitor. OATD-01 potently inhibited elevated chitinolytic activity in serum, induced sputum and BALf collected from IPF patients.

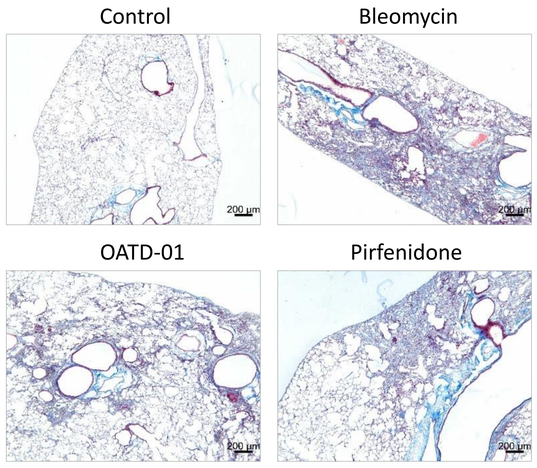


IPF samples	OATD-01 IC_{50} [nM]
Serum	16 ± 4
Induced sputum	15.5 ± 0.7
BAL fluid	11 ± 10

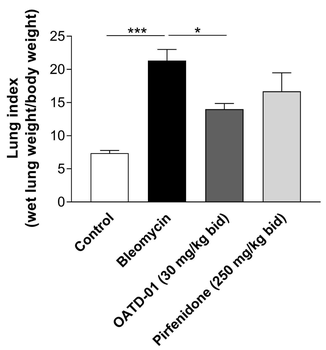
OATD-01 demonstrated anti-fibrotic efficacy in bleomycin-induced pulmonary fibrosis model

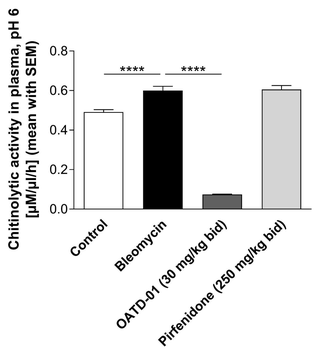
In the bleomycin-induced pulmonary fibrosis mouse model, OATD-01 administered in a therapeutic regimen from day 7, significantly reduced lung fibrosis, comparably to pirfenidone, as assessed by the modified Ashcroft scoring system.





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





Conclusions

- CHIT1 is highly expressed in BALf cells, in particular in macrophages, from IPF and sarcoidosis patients
- OATD-01 potently inhibited elevated chitinolytic activity in samples from IPF patients
- OATD-01 demonstrated significant therapeutic efficacy, comparable to pirfenidone, in the bleomycin-induced pulmonary fibrosis model
- These data support further development of OATD-01 as a first-in-class therapy for interstitial lung diseases such as IPF

Materials and Methods

IPF and sarcoidosis patients descripton, 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 ERS/ATS statement for the diagnosis and management of IPF. The sarcoidosis group included patients who presented with typical clinical and radiological features of sarcoidosis with noncaseating 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 BALf were collected, processed and stored in -80°C for subsequent analyses. BALf cells were prepared by the microscope slide smear technique.

BALf cells smears preparation and immunohistochemical stiaining for CHIT1

Smears were fixed in 10% NBF, endogenous peroxidases were inactivated by applying H_2O_2 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-postive 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.

Enzymatic 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',N''triacetylchitotrioside 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-methylumbelliferone was measured fluorometrically.

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 – Ashcroft 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 (doi: 10.2144/000112729).

Chitinolytic activity in plasma

1 μl of plasma was mixed with 96 μM 4-methylumbelliferyl B-D-N,N' diacetylchitobioside hydrate in assay buffer (0.1 M citrate, 0.2 M dibasic 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-methlyumbelliferone was measured fluorometrically using Tecan 10M microplate reader (excitation 355 nm/emission 460 nm). The chitinase activity was calculated using a standard curve of 4-methlyumbelliferone.

Lung index

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

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