Pharmacological inhibition of chitotriosidase (CHIT1) as a novel therapeutic approach for sarcoidosis

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Introduction

Sarcoidosis is a complex, multisystem granulomatous disease of unknown etiology that affects individuals worldwide [1]. Most of the patients develop intrathoracic disease with lymph node or lung involvement. A histological hallmark of this disease is formation of non-caseating granulomas, compose mainly of macrophages (epithelioid and multinucleated giant macrophages MNGMa) and CD4+ T cells [2,3]. Still there is no specific targeted treatment for sarcoidosis, and current therapeutic approaches (corticosteroids) is often associated with serious side effects [3,4]. Novel therapies are needed. OATD-01 is a potent chitotriosidase CHIT1 inhibitor entering phase II of clinical trials this year. It has proven efficacy demonstrated in animal models of IPF and other inflammatory lung disorders [5,6]. In humans, CHIT is a sarcoidosis marker, since its activity in patients' serum is highly correlating with disease severity [7,8]. CHIT1 is expressed in lung macrophages and its expression is induced further upon their activation [5]. Specifically activated macrophages are at the core of sarcoidosis pathogenesis. With OATD-01 treatment we inhibit chitotriosidase and therefore change macrophage pro-inflammatory phenotype, including reduction in chemokines needed for recruitment of CD4+ T cells. This effects consequently reduces granulomas formation – a hallmark of sarcoidosis. Taken together, OATD-01 is a safe and promising compound with proven efficacy in murine models of sarcoidosis.

AIM: To evaluate the CHIT1 as a novel molecular target in therapy of sarcoidosis

Human samples

CHIT1 is elevated in sarcoidosis patients

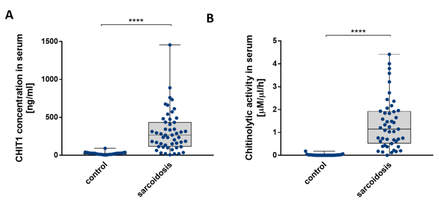


Fig.1. CHIT1 protein concentration and chitinolytic activity in serum from sarcoidosis patients and healthy volontieers.

CHIT1 is in centers of granulomas, in epithelioid macrophages and MNGMas

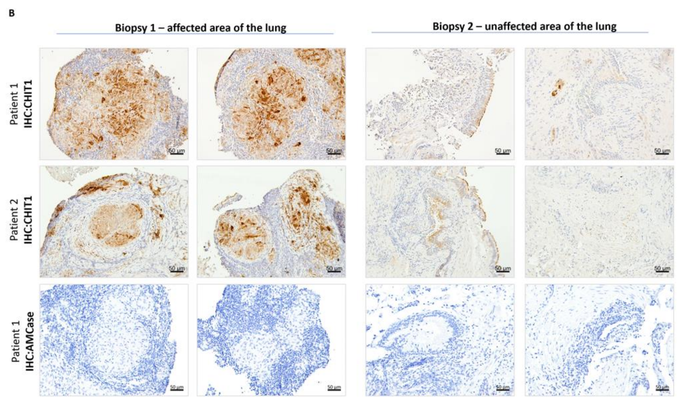


Fig.2. Immunohistochemical staining for CHIT1 and AMCase on lung biopsies from patients with sarcoidosis (in confirmed „affected" and „unaffected" areas). CHIT1 signal localization to epithelioid macrophages and MNGMa was also confirmed by independent pathologist

BALf macrophages are producing CHIT1

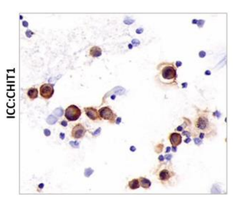


Fig.3. Immunocytochemistry for CHIT1 on smears of BALf collected from patients with sarcoidosis. Localization to macrophages was confirmed by pulmonary physician with experience in analyses of such samples.

CHIT1 inhibitor reduces pathologic chitinolytic activity in samples from patients (ex vivo)

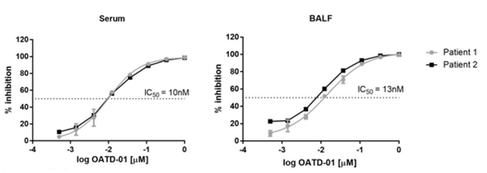
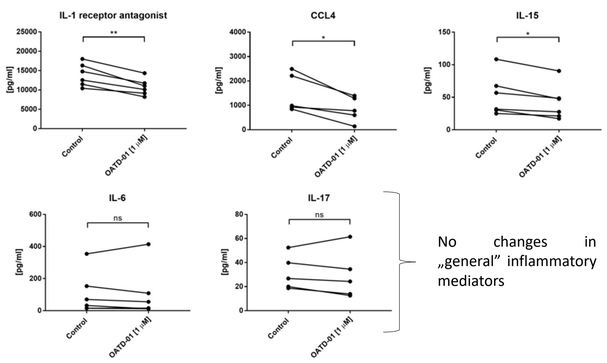


Fig.4. OATD-01 (specific and potent CHIT1 inhibitor, currently in clinical phases for idiopathic pulmonary fibrosis), administered to samples of serum and BALf collected from patients with sarcoidosis (see: Fig.1.) was able to efficiently and dose-dependently reduce the elevated chitinolytic activity levels (IC50 – 10 nM and 13 nM respectively)

OATD-01 inhibits sarcoidosis-specific inflammatory profile of macrophages isolated from patients (ex vivo)

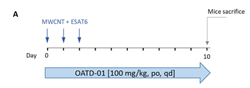


No changes in „general" inflammatory mediators

Fig.5. Bio-plex analysis of the levels of inflammatory mediators in the supernatants from sarcoidosis patients' BALf macrophages, performed in scheme „before-after" administration of OATD-01

Mouse model: orotracheal administration of multiwalled carbon nanotubes (MWCNT)+ ESAT6

1) 10-day-long „acute" model of granulomatous inflammation (inflammatory responce)



Diseased mice have elevated chitinolytic activity, manageable by OATD-01

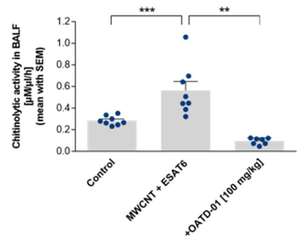


Fig.6. Chitinolytic activity measured in BALf from mice in the study. BALf was collected 24h post last dose

OATD-01 treatment reduces pathological levels of sarcoidosis specific cytokines (in BALf)

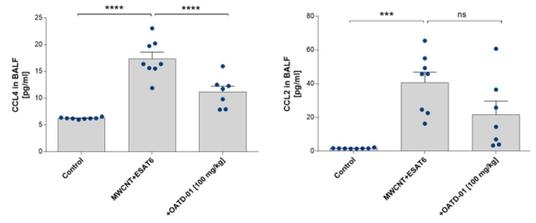


Fig.7. levels of granulomatous inflammation-associated cytokines in BALf collected from mice in the study.

OATD-01 has anti-inflammatory effect on BALf cells

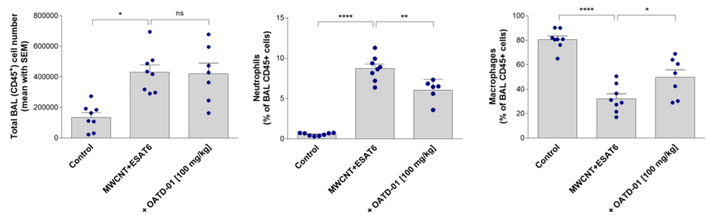
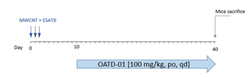


Fig.8. cytometric analyses of immune cells populations in BALf collected from mice in the study

2) 40-day-long „chronic" model of persistent granulomatous inflammation (with granulomas)



OATD-01 treatment reduces expression of markers of macrophages activation

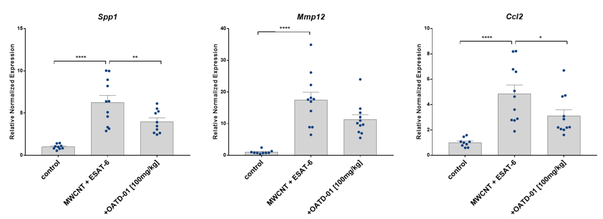


Fig.9. Expression of genes associated with sarcoidosis in lungs from mice from the study

Inhibition of CHIT1 reduses the numer of the most advanced granulomas

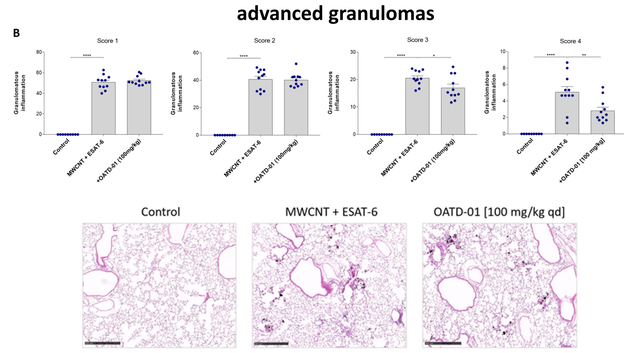
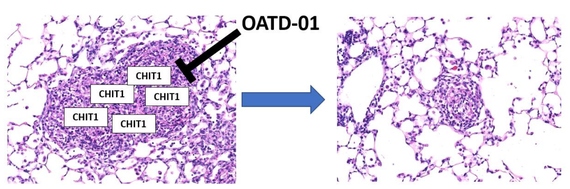


Fig.10. Analysis of granuloma count in histopathological samples of mouse lungs

Conclusions



- In human CHIT1 is present at the cores of sarcoid granulomas in epithelioid macrophages and MNGMa and ex vivo OATD-01 reduces its pathological level
- In mouse models of acute and chronic pulmonary granulomatous inflammation, OATD-01 treatment inhibited levels of sarcoidosis markers and reduced number mature forms of granulomas

CHIT1 is a promising novel target in sarcoidosis therapy

Materials and methods

Human samples collection: the study, procedures and analyses were approved by the Local Bioethics Committee at the Medical University of Warsaw, Poland, No. of approval: KB/236/2015. Patients were recruited in the Department of Internal Medicine, Pulmonary Diseases and Allergy of the Medical University of Warsaw, Poland between January 2016 and June 2018.
Human CHIT1 concentration: The CHIT1 concentration in human serum and BALf samples was measured using CircuLex Human Chitotriosidase ELISA Kit (CycLex) according to the manufacturer's protocol.
Chitinolytic activity: The chitinolytic activity in human serum samples was measured as previously described [7]
Animal studies: Research was approved by the Local Ethics Committee for Animal Experimentation, Warsaw, Poland (No. of approval: WAW1/798/2018). Animal models of granulomatous inflammation were obtained based on [9]. Shortly: model was induced by triple oropharyngeal administration of MWCNT (SES Research; Catalog# 900-1201) and ESAT-6 peptide (Innovagen) to 8-week-old C57Bl/6 female mice, under 5% isoflurane anesthesia. In applied timepoints, mice were euthanized, blood and BALf were collected, lungs were dissected and divided for several analyses (qPCR, histopathology)
Histopathology: severity of granulomatous inflammation in the mouse lungs was assessed basing on the numer and „maturity" of the granulomas in lung slices, on HE stained slides by blinded examiner with scoring system (0-5, where 0 – no MWCNT particles, 1 – free uninvolved MWCNT particles, 2 – MWCNT particle accompanied with few cells, 3 – 1 layer of cells around MWCNT particle, 4 – 2-3 layers of cells around MWCNT particle, 5 – small organized granulomatous structure)
Immunohistochemistry and immunocytochemistry: Stainings was performed with standards methods, using validated antibodies: anti-CHIT1 (Biorbyt, orb377995; Lot# CQ2228)
ELISA assays, qPCR, histological staining methods, cell culture were performed with standard procedures or according to vendor instructions.

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The National Centre for Research and Development

Literature

[1] Hunninghake GW, et al, Statement on Sarcoidosis. Am J Respir Crit Care Med, 160:736–755; PMID: 10560120
[2] Spagnolo P, et al, Pulmonary sarcoidosis. Lancet Respir Med, 2018, 6:389–402. DOI: 10.1016/S2213-2600(18)30064-X
[3] Zissel G, et al, Cellular Players in the Immunopathogenesis of Sarcoidosis. Clin Chest Med 2015, 36:549–560, DOI: 10.1016/j.ccm.2015.08.016
[4] Nunes H, et al, Indications for treatment of sarcoidosis. Curr Opin Pulm Med 2019, 25:505–518, DOI: 10.1097/MCP.0000000000000604
[5] Sklepkiewicz P et al, Inhibition of CHIT1 as a novel therapeutic approach in idiopathic pulmonary fibrosis. Eur J Pharmacol, 2022 Mar 15;919:174792, DOI: 10.1016/j.ejphar.2022.174792
[6] Koralewski R et al, Discovery of OATD-01, a First-in-Class Chitinase Inhibitor as Potential New Therapeutics for Idiopathic Pulmonary Fibrosis. J Med Chem 2020, 63:15527–15540, DOI: 10.1021/acs.jmedchem.0c01179
[7] Boot RG et al, Plasma chitotriosidase and CCL18 as surrogate markers for granulomatous macrophages in sarcoidosis. Clin Chim Acta ,2010, 411:31–36; DOI: 10.1016/j.cca.2009.09.034
[8] Bennett D, et al, Chitotriosidase: A biomarker of activity and severity in patients with sarcoidosis. Respir Res, 2020, 21, DOI:10.1186/s12931-019-1263-z
[9] Malur A, et al, Exposure to a Mycobacterial Antigen, ESAT-6, Exacerbates Granulomatous and Fibrotic Changes in a Multiwall Carbon Nanotube Model of Chronic Pulmonary Disease, J Nanomed Nanotechnol 2015 6(6):340, DOI: 10.4172/2157-7439.1000340.
[10] in preparation: Dymek BA, et al, Pharmacological Inhibition of Chitotriosidase (CHIT1) as a Novel Therapeutic Approach for Sarcoidosis.