INTRODUCTION

Persistent and progressive fibrosis is a characteristic of many chronic diseases in which the accumulation of pro-fibrogenic myofibroblasts is a cardinal feature. Following liver injury, activated fibroblasts produce in an uncontrolled manner the extracellular matrix (ECM) components such as fibrillar collagen. This will progressively lead to liver tissue deposition, changes in organ architecture and its functional impairment. The initial phase of this pathologic response can be reproduced in a primary human cell model by activating the quiescent hepatic stellate cells with TGFβ to differentiate into myofibroblasts, whose expression 6-Month Mucin 4 (MUC4) and fibroblast collagen.

Our approach to target fibrosis consists of the identification of FDA-approved drugs with unexpected anti-fibrotic properties as based on their capacity to inhibit the phenotypic transformation of the quiescent human primary HSC into pro-fibrogenic myofibroblasts.

This process of finding new uses outside the scope of the original medical indication is particularly appealing since approved drugs offer a shorter route to the clinic for the new indication [1,2].

AIMS

- Discover novel anti-fibrotic properties in FDA-approved drugs through a phenotypic screening approach in primary human stellate cells.
- Demonstrate the anti-fibrotic activity of prioritized candidates in fibroblasts from different organs and in preclinical models of liver fibrosis.

STUDY DESIGN & SELECTION PROCESS

DRUG REPOSITIONING SCREEN (2467 COMPOUNDS)

HIT IDENTIFICATION BY PHENOTYPIC SCREENING

Compound inhibition of α-SMA in TGFβ-induced HSC

HIT SELECTION BY LITERATURE MINING

Investigation of drug profiles: Compounds chemistry; pharmacology; toxicity, function, known mechanism of action & therapeutic target; compatibility with Intellectual Property

HIT CONFIRMATION (PRIORITIZED CANDIDATES)

α-SMA

REVIEW OF EXISTING DATA PACKAGE

FDA-approved drugs

PROOF OF CONCEPT IN MURINE MODELS OF LIVER INJURY

Chronic CDAA/c or CCl4

SELECTED DRUGS

CONCLUSIONS

A. MTS screening with a repositioning library containing 2467 APIs allowed the identification of several approved drugs with unexpected anti-fibrotic properties.

Nitazoxanide, as well as its active metabolite Tizoxanide, exerted anti-fibrotic activity in TGFβ-stimulated HSCs, as well as in TGFβ-induced fibroblasts derived from other organs such as the lung, heart and intestine. Altogether, these data indicate that nitazoxanide could have beneficial effects on various fibrotic diseases affecting different organs.

In a proof-of-concept studies in 2 murine models of liver fibrosis we confirmed, confirming the nitazoxanide anti-fibrotic properties.

Our phenotypic screening approach combined with the use of FDA-approved drug libraries allowed the identification of nitazoxanide and other promising drug candidates suitable for drug repositioning in the field of fibrotic diseases and rapid advancement into clinical trials.

NTZ administration significantly attenuates hepatic fibrosis development in 2 murine disease models.

(A) Liver fibrosis was induced in C57BL/6J male mice by gavage of CCI4 emulsified in olive oil. The CCI4 was given 3 times a week for 6 weeks. Nitazoxanide was incorporated into the diet at a percentage corresponding to an estimated dose of 10, 30 and 100 mg/kg/day. Hepatic collagen content was significantly reduced at a dose of 100 mg/kg/day.

(B) The murine choline-deficient L-amino Acid defined diet supplemented with 1% cholesterol (CDAA/c) was used as a NASH model [3, 4]. Six weeks old C57BL/6 mice were fed a control (CDAA/c), CDAA/c + CDAA/c-diet supplemented with NTZ at equivalent doses of 10, 30, and 100 mg/kg/day for 12 weeks. Hepatic total collagen content was significantly reduced in a dose-response manner.

(C) Representative images of Picrosirius Red/Fast Green staining of liver sections derived from the murine NASH CDAA/c model.

The anti-fibrotic properties of NTZ & TZ were also demonstrated in fibroblasts derived from diverse other organs.

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