**THE IDENTIFICATION OF NOVEL SMALL MOLECULE COMPOUNDS WITH POTENT ANTI-FIBROTIC PROPERTIES BY PHOTONIC SCREENING OF PRIMARY HUMAN STELLATE CELLS**

Carole BELANGER 1, Mathieu DUBERNET 1, Emilie NEGRO 1, Raphaël DARTELL 1, Dean W. HUM 1, Bart STAELS 1, Robert WALCZAK 1

1 GENFIT SA, Loos, France 2 INSERM UMR1011, Univ. Lille, Institut Pasteur de Lille, Lille, France

**INTRODUCTION**

Fibrosis is a devastating outcome of many chronic liver diseases, where an excess deposition of extracellular matrix proteins impairs liver function. There is presently no approved therapeutic approach for fibrosis in humans. Although a number of small molecules and biologics have shown efficacy in preclinical models of liver fibrosis, there is presently no approved effective therapy for liver fibrosis in humans. In a growing effort to fulfill this unmet medical need, GENFIT used an unbiased phenotypic screening approach to identify novel anti-fibrotic molecules. A phenotypic screening method was implemented to search for small molecule compounds that have the ability to reduce the expression of α-SMA in TGF-β stimulated HSC. This screening of a library of 70,000 small molecules, including both new chemical entities and FDA-approved drugs, led to the identification of structurally diverse compounds with potential anti-fibrotic properties. Combined with good efficacy and no toxicity, these hit compounds were further prioritized with respect to their pro-fibrogenic and drug properties. Accordingly, the anti-fibrotic efficacy of novel test compounds was assessed in a newly optimized model of advanced fibrosis of the severe fibrotic mouse.

**AIMS**

- To identify new drug candidates with potential anti-fibrotic properties by using a relevant and unbiased phenotypic screening approach in a library that contains both new chemical entities (NCEs) and known FDA-approved drugs.
- To confirm the efficacy of the prioritized candidates in animal models of liver fibrosis.

**METHODS**

- **PHOTONIC MTS-SET UP AND VALIDATION**
  - Photonic screening approach was implemented to identify small molecules with anti-fibrotic properties in hepatic stellate cells.
  - (A) General outline of the semi-robotic photonic screening assay. The HSC were preincubated for 3hr with the compounds followed by TGF-β stimulation. The measure of α-SMA was performed by a home-made sandwich ELISA on the HSC lysate.
  - (B) A TGF-β inhibitor (SMAF2137) served as a positive control for inhibition of α-SMA production in TGF-β stimulated HSC. The factor of 0.5 was obtained on the master screen test, thus confirming the validity of the method.
  - (C) The equation used to measure the inhibition percentage of α-SMA:
    \[ \text{Normalized percent inhibition} = \left( \frac{\text{Absorbance} \text{ of sample} - \text{Absorbance of SMAF2137}}{\text{Absorbance} \text{ of TGF-β control} - \text{Absorbance of SMAF2137}} \right) \times 100 \]

- **STATISTICAL ANALYSIS**
  - Data is shown as Mean ± SEM.
  - 5 x 10^5 cells were seeded in 384-well plates in 100 μL of DMEM + 10% FCS. The cells were treated with a panel of hit compounds for 72hrs. The data is represented as mean ± SEM.

- **STATISTICAL ANALYSIS**
  - Data is shown as Mean ± SEM.
  - 5 x 10^5 cells were seeded in 384-well plates in 100 μL of DMEM + 10% FCS. The cells were treated with a panel of hit compounds for 72hrs. The data is represented as mean ± SEM.

**CONCLUSION**

- A MTS-compatible phenotypic assay in hepatic stellate cells was successfully used to identify structurally diverse compounds that confer strong anti-fibrotic properties.
- Several candidates that obey to classical drug-likeness properties were identified among the hit compounds with strong anti-fibrotic activity in HSCs. Anti-fibrotic properties of a prioritized hit compound (GFE2137) were confirmed in the liver fibrosis model in vivo.
- Other promising lead candidates will be further investigated in animal models of fibrosis. As well, complementary studies aiming to define the mechanism of action and molecular targets will be undertaken.
- Drug repositioning is becoming a popular alternative to classical drug discovery processes. Our phenotypic screening approach has led to the discovery of known FDA-approved drugs with unsuspected anti-fibrotic properties.

**REFERENCES**

1. Carole Belanger, Mathieu Dubernet, Emilie Negro, Raphaël Darreell, Dean W. Hum, Bart Staels, Robert Walczak. Corresponding author’s email: robert.walczak@genfit.com

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