The tankyrase inhibitor OM153 exhibits anti-fibrotic effects in a Scar-in-a-Jar pulmonary fibrosis model

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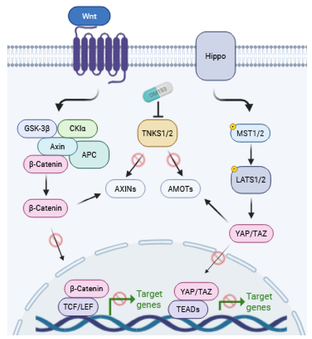
AIM

Idiopathic pulmonary fibrosis (IPF) is a progressive, chronic and fatal lung disease with limited treatment options. Fibroblast activation and ECM deposition is pivotal in development of IPF, thus inhibiting fibrogenesis and ECM deposition is crucial for anti-fibrotic approaches to treat IPF.

WNT/β-catenin and YAP signaling are significant contributors to the disease development. The post-translational modifying enzymes tankyrase 1 and 2 (TNKS1/2) are key regulators of the WNT/β-catenin and YAP signaling pathways, positioning them as promising therapeutic targets.

Novel tankyrase inhibitor OM153, stabilizes the TNKS1/2 target proteins AXIN and AMOT, reducing WNT/β-catenin and YAP signaling activities.

This study aims to prove that tankyrase inhibition reduces fibrogenesis induced by a fibrotic cocktail (FC) in primary human lung fibroblasts derived from IPF patients and decreases extracellular matrix biomarkers nordicPRO-C3™ and nordicPRO-C6™.



Wnt and Hippo pathway: The tankyrase inhibitor stabilizes AXIN and AMOT leading to reduced WNT and YAP pathway activation. Figure made using Biorender.

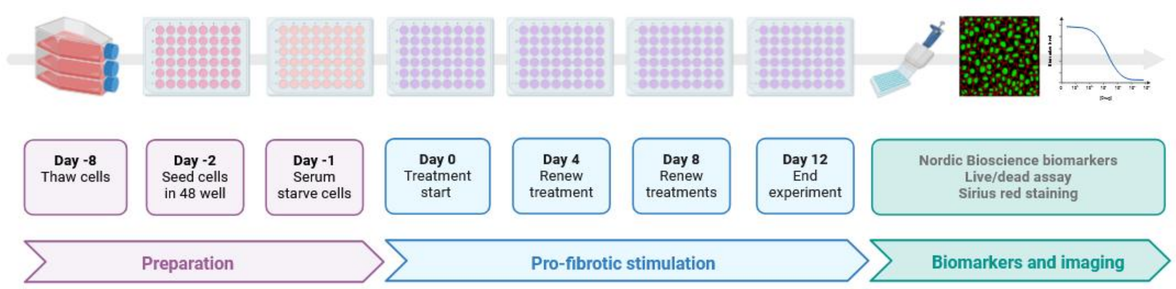
METHODS

Primary lung fibroblasts from IPF patients were cultured in the Scar-in-a-jar model, and fibroblasts were exposed to a pro-fibrotic cytokine cocktail (FC) for 12 days. Cell culture supernatants were collected at day 12 for quantification of type III and type VI collagen synthesis using the clinically relevant biomarkers nordicPRO-C3™ and nordicPRO-C6™.

Cell viability was assessed using live/dead fluorescence assay. Picrosirius red staining was used to stain the presence of fibrillar collagens at day 12.

This study examined the impact of OM153 (0.3-30 nM) on PRO-C3 and PRO-C6 levels in the presence of fibrotic cocktail compared to vehicle control.

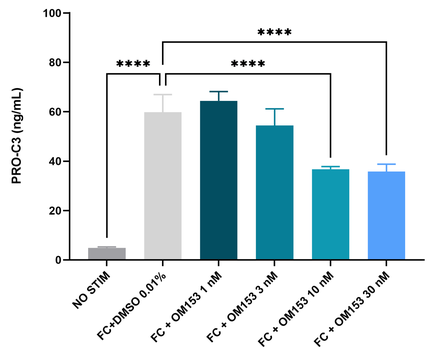
The FC, OM153 and treatment controls were administered on days 0, 4 and 8.



Scar-in-a-Jar fibrogenesis model:
Primary fibroblasts from IPF donors were seeded in 48 well plates with 25.000 cells/well. The cells were cultivated in DMEM with 0.4% FBS, ascorbic acid and ficoll from day -1 to day 12. Growth medium containing vehicle or treatments were replenished at day 0, 4 and 8 and the supernatant was saved from day 4, 8 and 12 for later biomarker analysis. At the end of the experiment the biomarkers were quantified, a live/dead cell viability assay was performed followed by ECM decellularization and staining using picrosirius red. Figure made using Biorender.

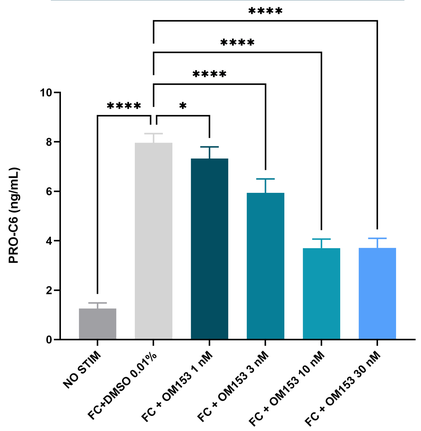
RESULTS

Type III collagen formation



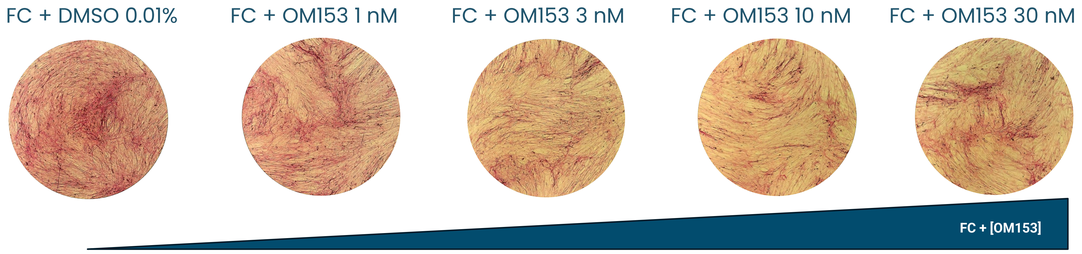
OM153 reduces PRO-C3: OM153 reduces PRO-C3 in a concentration dependent manner at day 12 of the Scar-in-a-Jar. Error bars represents mean SEM, one-way ANOVA, n=2, 4 technical replicates each. Alpha threshold 0.05

Type VI collagen formation



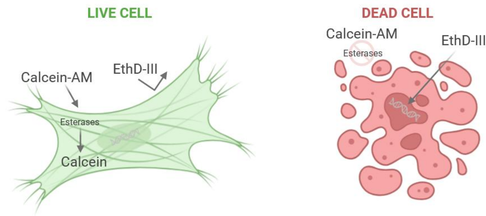
OM153 reduces PRO-C6: OM153 reduces PRO-C6 in a concentration dependent manner at day 12 of the Scar-in-a-Jar. Error bars represents mean SEM, one-way ANOVA, n=2, 4 technical replicates each. Alpha threshold 0.05

Picrosirius red staining

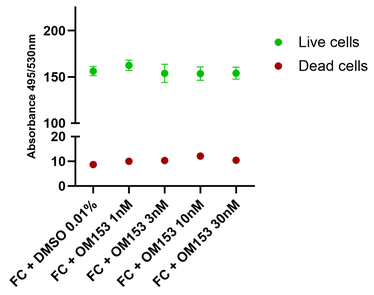


OM153 reduces picrosirius red staining: Representative pictures of OM153 reducing the picrosirius red staining in decellularized ECM from day 12 in Scar-in-a-Jar. Imaged at 4x.

Cell viability



Live/dead assay principle: Non-fluorescent calcein-AM is converted to fluorescent green calcein by intracellular esterases in living cells. EthD-III is impermeable to intact plasma membrane in living cells. When plasma membrane integrity is compromised, EthD-III binds to nuclei DNA and stains dead cells red. Figure made using Biorender.



OM153 does not affect the cell viability: 12 days of exposure to FC + OM153 did not affect the cell viability when using the live/dead assay. Absorbance measured using SpectraMax M5 plate reader. Error bars represents mean SEM, n=1, 4 technical replicates.

CONCLUSION

These findings highlight the potential of tankyrase inhibiton as a therapeutic target for IPF and support the use of the Scar–in-a-Jar model as an effective tool for IPF drug screening.

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Disclosures: MK, SJ, MS are employed at Nordic Bioscience and are shareholders