Evaluation of compounds with claimed anti-fibrotic activity in an *in vitro* primary human stellate cell to myofibroblast transition assay

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1 Background and study aim

Transition of hepatic stellate cells into myofibroblasts is thought to play an essential role in liver fibrogenesis, resulting in excessive synthesis and deposition of extracellular matrix and subsequent loss of liver function.
In this study we aimed to establish and characterize a primary human cell based assay to assess the translational potential of small molecules with putative anti-fibrotic properties. Primary human hepatic stellate cells were used to establish a stellate cell to myofibroblast transition (SMT) assay in 384-well format using TGF-β1 as fibrotic stimulus. Expression of myofibroblast markers alpha-smooth muscle actin (αSMA) and the disease-relevant collagen I (Col-I) is measured by high content analysis (HCA). To validate the assay, small molecules targeting a number of molecular pathways, were tested in 8-point concentration response curves. Several compounds showed a clear dose-dependent inhibition of TGFβ-1-induced αSMA and Col-I expression.

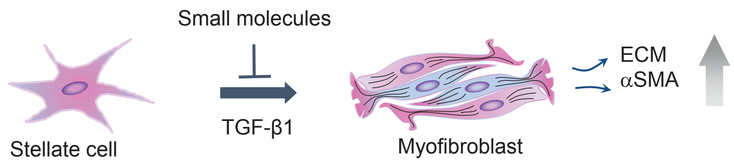

Figure 1. Schematic overview of TGF-β1-mediated trans-differentiation of hepatic stellate cells to myofibroblasts (SMT) and modulation by small molecules.

2 SMT assay

The SMT assay was established using primary human hepatic stellate cells at low passage from three non-NASH donors. The cells were plated in 384-well format and stimulated with TGF-β1 in the presence of small molecules. In the SMT assay both αSMA and Col-I were assessed as markers for trans-differentiation using high content imaging. The assays were validated with a range of small molecules in concentration response curves. The tested molecules included Nintedanib, Omipalisib, Selonsertib, Elafibranor, and the growth factor EGF. The ALK5 (TGF-β receptor I) inhibitor SB525334 served as an assay positive control.

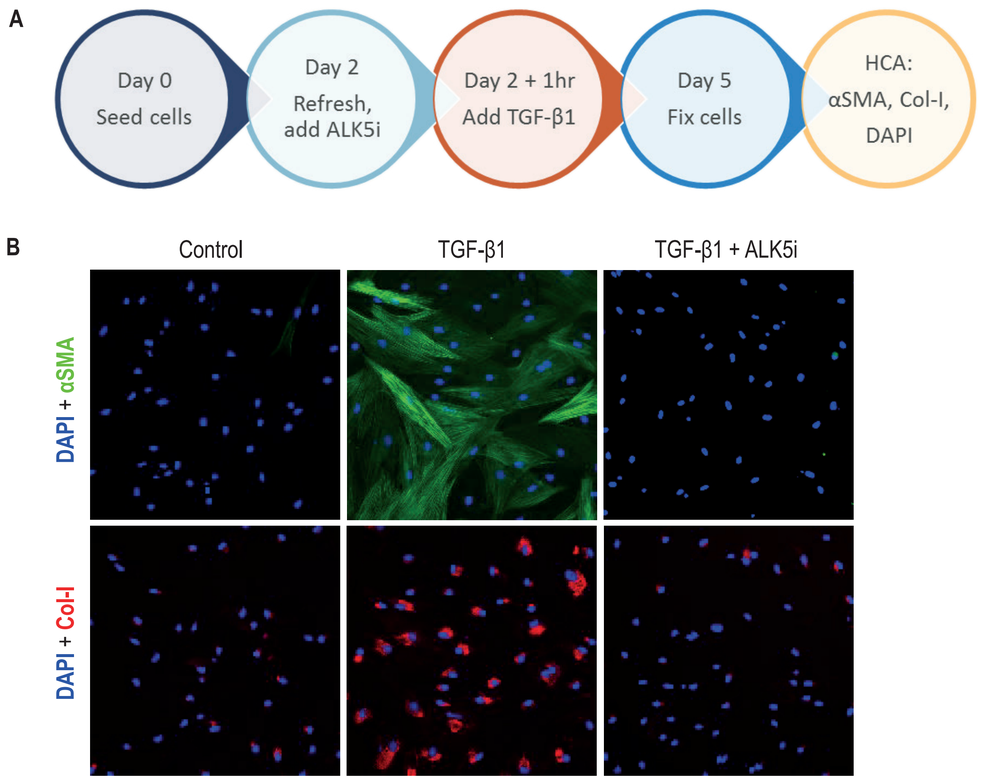

Figure 2. (A) Outline and timelines of the optimized SMT assay. **(B)** High content imaging of αSMA and Col-I with an IN Cell Analyzer 2200. Exposure to TGF-β1 stimulates expression of αSMA and Col-I in activated stellate cells demonstrating cell transition. Increased αSMA was detected in all three tested donors, although levels of basal expression and induction vary between donors. TGF-β1-dependent increase of Col-I was detected in one of three donors. Marker expression is quantified using in-house developed image analysis algorithms.

3 Assay performance

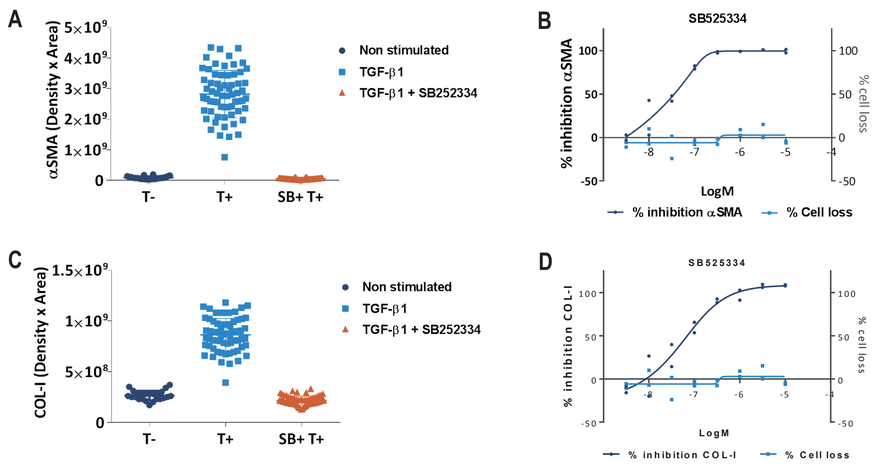

Figure 3. Performance of the αSMA **(A, B)** and Col-I **(C, D)** markers in the SMT assay as measured by the reference compound SB525334 (ALK5 inhibitor). **(A, C)** αSMA and Col-I signals were quantified for non-stimulated, TGF-β1 and TGF-β1 in combination with SB525334 treated cells. Exposure to the ALK5 inhibitor completely inhibited TGF-β1-mediated marker expression. For αSMA an assay window [signal TGFβ1/(signal TGFβ1 + SB525334)] of 63 was observed with a replicate Pearson correlation of 0.89 and a Z-factor of 0.21. An assay window of 4.0 was observed for Col-I with a replicate Pearson correlation of 0.92 and a Z-factor of 0.18. **(B, D)** An 8-point SB525334 concentration response curve demonstrates submicromolar potency in the αSMA (B) and Col-I (D) assays.

4 Compound profiling

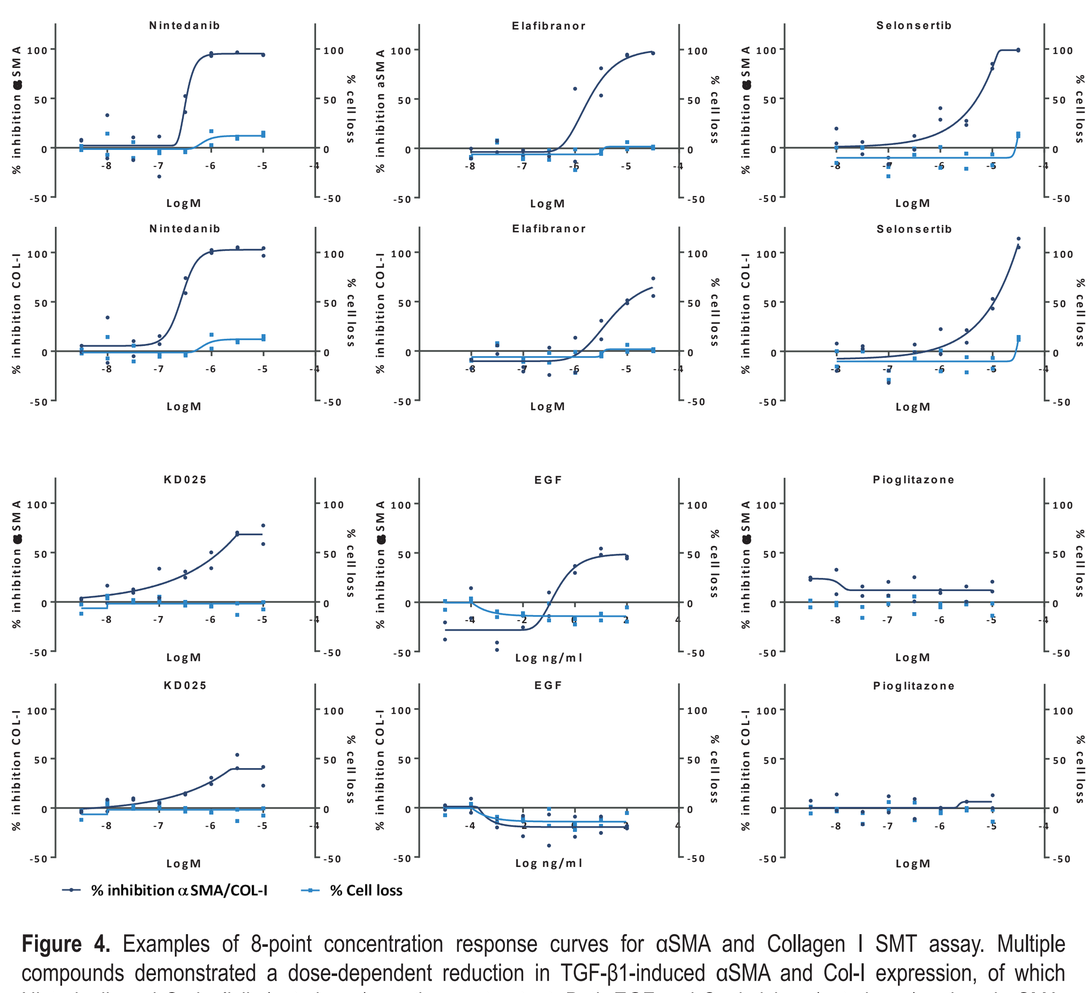

Figure 4. Examples of 8-point concentration response curves for αSMA and Collagen I SMT assay. Multiple compounds demonstrated a dose-dependent reduction in TGF-β1-induced αSMA and Col-I expression, of which Nintedanib and Omipalisib (not shown) are the most potent. Both EGF and Cenicriviroc (not shown) reduced αSMA, whereas Col-I expression was unaffected. No effect on αSMA and Col-I expression was observed with Pioglitazone and obeticholic acid (not shown).

5 Conclusions

Several compounds show clear dose-dependent inhibition of TGFβ-1-induced αSMA and Col-I in the hepatic stellate to myofibroblast transition assay. Of the compounds tested, Nintedanib and Omipalisib showed clearest efficacy and high potency with submicromolar IC50s. The PPARα/δ agonist Elafibranor, which is currently in phase 3 clinical trial for NASH,also effectively reduced αSMA and Col-I expression. KD025, a Rho-associated kinase inhibitor in clinical trial for IPF, also reduced expression of both markers (IC50 ~1 μM) although the effect was only partial. Both EGF and Cenicriviroc reduced αSMA expression without effecting Col-I, indicating differential regulation of these markers. No inhibitory effect of Pioglitazone and obeticholic acid was seen, suggesting any anti-fibrotic activity is likely to be upstream of stellate cell activation. These results suggest that a transition assay with primary human hepatic stellate cells may be a useful translatable tool to identify and/or validate potential new drugs to treat liver fibrosis.

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