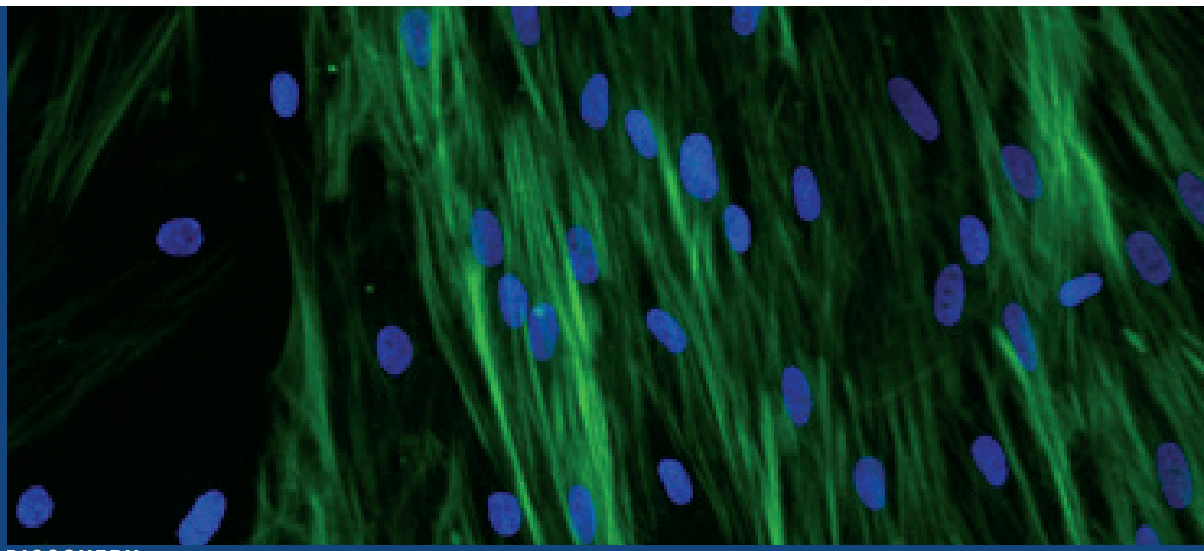
charles river

**Overview**

Fibrosis results from misregulated complex pathways involving multiple cell types such as epithelial cells and fibroblasts. We've developed an optimized, off-the-shelf panel of *in vitro* fibrosis assays using our own patient-derived donor cells to assess the translational potential of small molecules as novel therapies.



DISCOVERY

# Complex Biology *In Vitro* Assays: Fibrosis

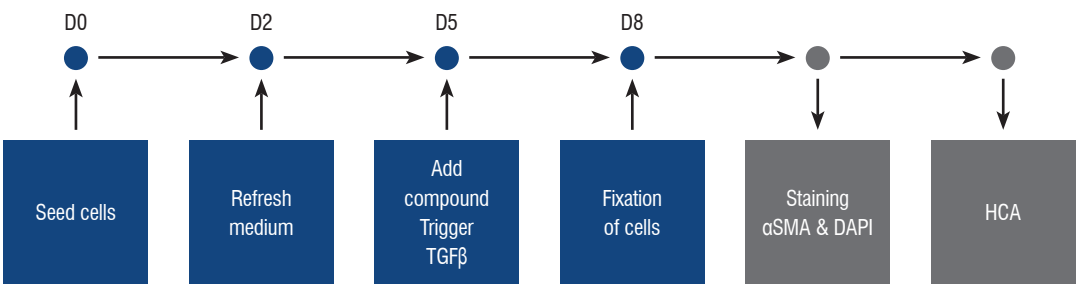
## Fibroblast-to-Myofibroblast Transition (FMT) Assay

### Fibroblast-to-Myofibroblast Transition (FMT) Assay in Human Lung Cells Derived from Idiopathic Pulmonary Fibrosis (IPF) Patients and Healthy Donors

A well-characterized hallmark of pathologic FMT is *de novo* formation of alpha-smooth muscle actin (αSMA) stress fibers. Since myofibroblasts localize at sites undergoing active matrix deposition and display elevated collagen synthetic capacity, myofibroblasts are considered to play a major role in the pathology of idiopathic pulmonary fibrosis (IPF). The well-established key fibrogenic mediator, transforming growth factor TGF-β1, induces FMT. In cells that have undergone FMT, increased expression of αSMA is observed. *In vitro*, increased αSMA expression positively correlates with contraction of myofibroblast populated collagen gels, indicating that αSMA is a strong marker of myofibroblast differentiation and hence, a relevant readout for lung fibrosis. A validated, robust TGF-β1-induced FMT assay has been developed in IPF-derived fibroblasts to evaluate therapeutic candidates with various modes-of-action in this disease area.

### FTM Assay Principle

Patient-derived primary human bronchial fibroblasts cells are seeded then refreshed in preparation for addition of small molecule compounds and the TGF-β1 trigger. After 3 days, the cells are fixed, then stained using DAPI-labeled αSMA and imaged via HCA.




EVERY STEP OF THE WAY

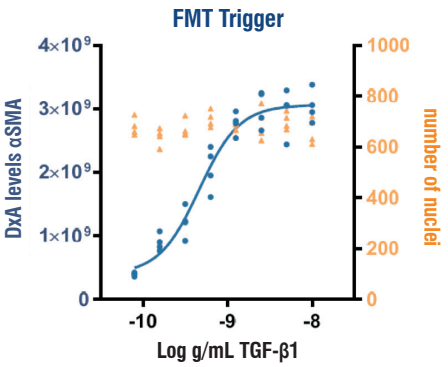
## FTM Assay Setup

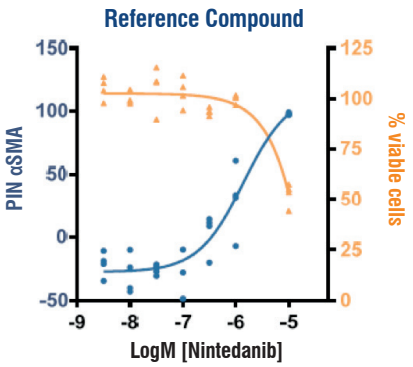
FTM protocol has been developed for optimum analysis of trans-differentiation of fibroblasts to myofibroblasts. Marker expression is quantified using in-house developed algorithms.

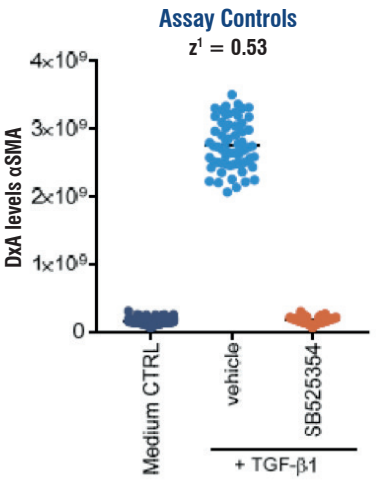
- Cells → lung fibroblasts from IPF donors or 3 healthy donors
- Seeding density → 3,000 cells/well in 96-well plates
- Trigger → 1.25 ng/mL TGF-β1
- Assay controls → 0.1% DMSO (negative control) and 1 µM SB525334 (positive control)
- Compounds → 8-point concentration response curves (in biological duplicate)
- Fix → 72 hours post-trigger
- Readout → αSMA and DAPI staining (high-content analysis)

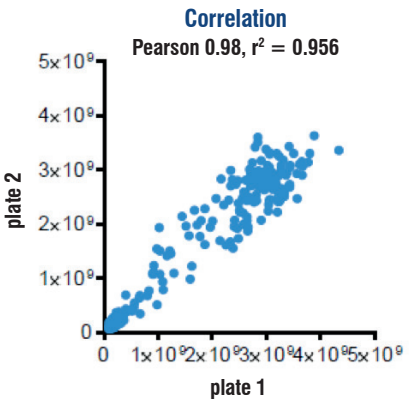
## Assay Performance

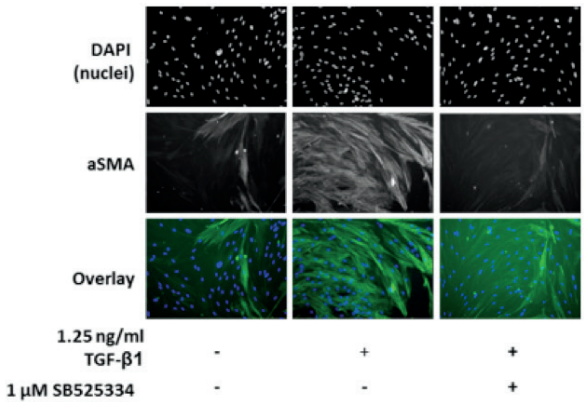
Representative dose response data shown below from patient-derived donor, 72 hours post TGF-β1 trigger.






## Summary

Lung-derived fibroblasts stimulated with TGFβ1 demonstrated a clear dose-dependent increase in αSMA levels, while increasing the TGFβ1 stimuli showed no effect of on the number of nuclei, indicative of no cytotoxic events. TGFβ1 trigger could be inhibited by treatment with an ALK-5, showing full inhibition of αSMA regardless of the presence of TGFβ1. IC50 values were consistent between donor samples, and strong Pearson correlation denotes consistency between biological replicates. Using these fibrosis assays, trans-differentiation FMT can be monitored to evaluate therapeutic candidates.

The therapeutic candidates can be evaluated in 8-step CRC for their effect on αSMA modulation. In addition, potential cytotoxic side-effects of the tested therapeutic candidate will be assessed by monitoring the loss of nuclei as a measure for cell death. Results will be provided as percentage inhibition (PIN values) and % viable cells.

For our clients' scheduling convenience, we perform FMT assays on a routine bi-monthly basis. Results are issued within 4 – 6 weeks of receipt due date.

## FMT Assay – Compound Receipt Due Date

| JAN | March | May | July | September | November |
|---|---|---|---|---|---|
| 10 | 7 | 9 | 11 | 5 | 7 |

## Assay Reference codes

Fibroblast-to-Myofibroblast Transition (FMT) Assay – IPF Human-Derived Donor Cells

**Assay reference code: OTS101-FMT-LUNG-IPF**

Fibroblast-to-Myofibroblast Transition (FMT) Assay – Healthy Human-Derived Donor Cells

**Assay reference code: OTS102-FMT-LUNG-HEALTHY**