Phenotypic screening using patient-derived primary cells

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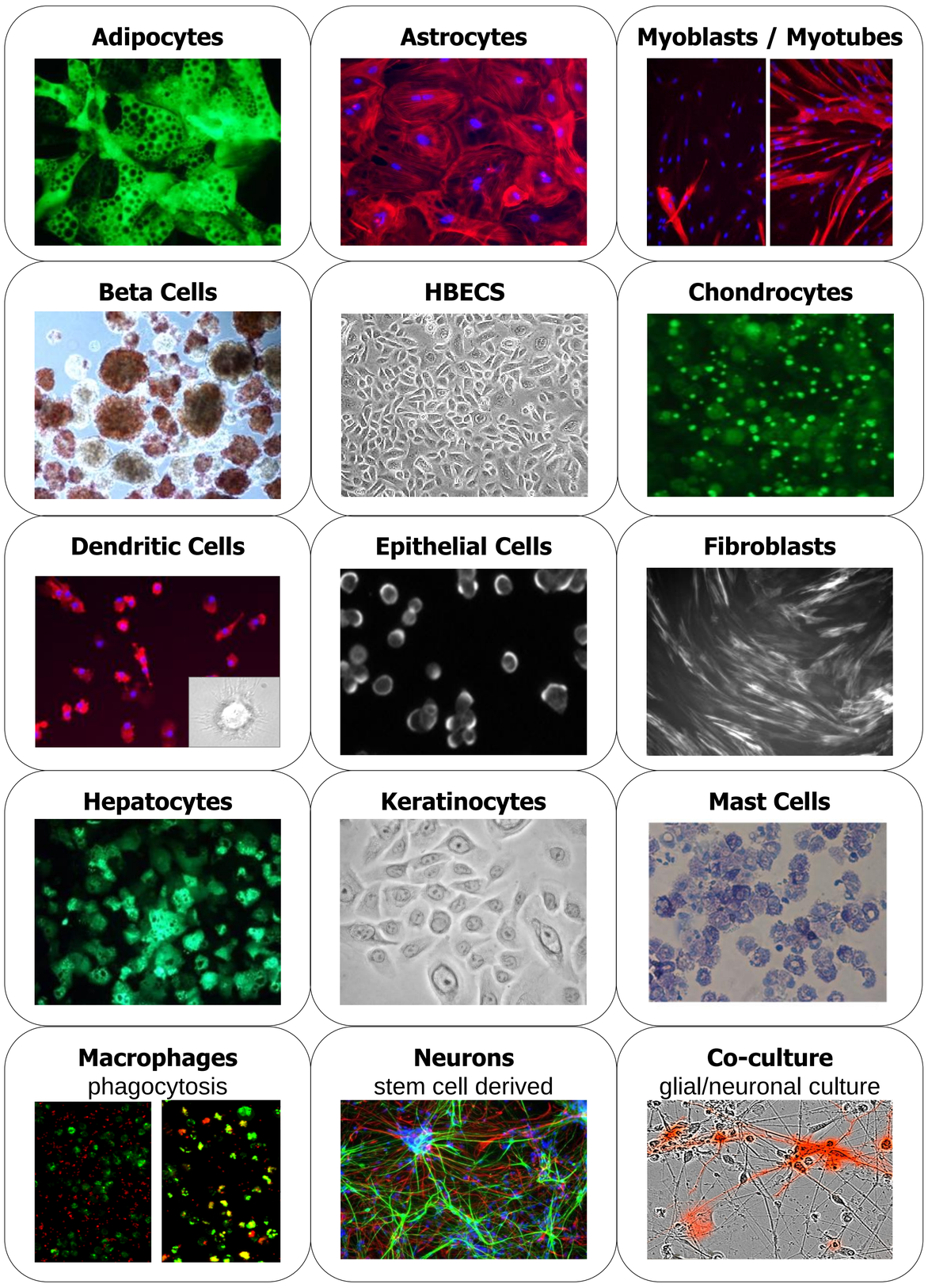
1 Introduction

- The pathophysiology of complex diseases such as metabolic syndrome, fibrosis, a variety of cancers, neurodegenerative and autoimmune diseases are often poorly understood, due to often being multifactorial, and therefore currently lack effective treatments.
- Integrated approaches measuring multiple parameters of the disease state in patient-derived primary human cell models may lead to novel therapeutic approaches for these complex diseases.

2 Primary cell assays (selection)



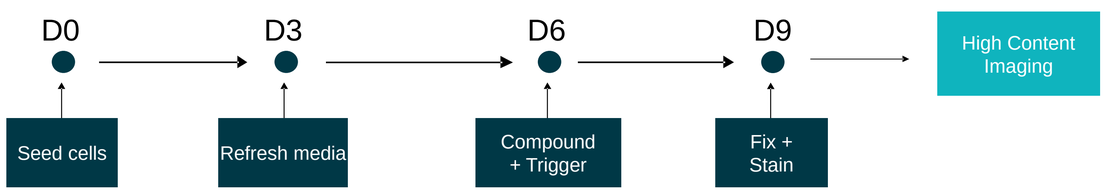
At Charles River we have an array of patient-derived primary cell lines that have been used to develop many phenotypic assays.

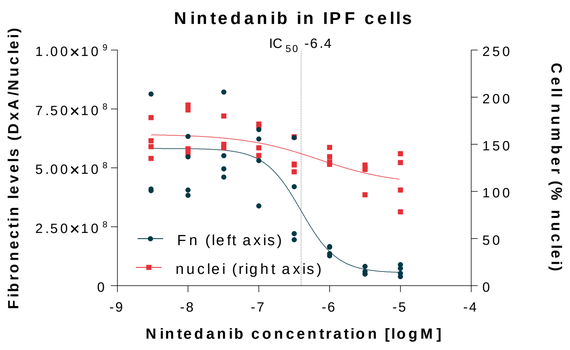
3 Fibrosis

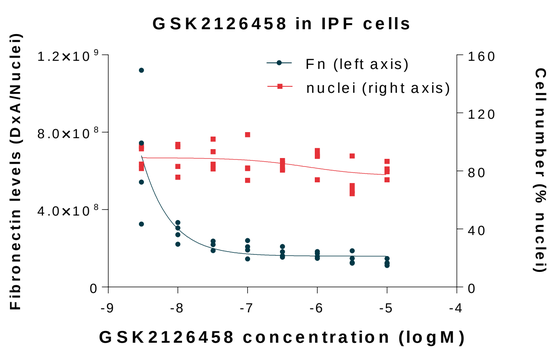
Our fibrosis assays allow the study of inflammatory (macrophages), structural (extracellular matrix deposition), as well as trans-differentiation (epithelial to mesenchymal transition and fibroblast to myofibroblasts transition) processes in idiopathic pulmonary fibrosis (IPF) patient-derived cells

Epithelial -> Mesenchymal transition

Cells IPF, COPD or healthy HBEC at 2,500 cells / well
Trigger TGFβ1
Readout Fibronectin / DAPI staining



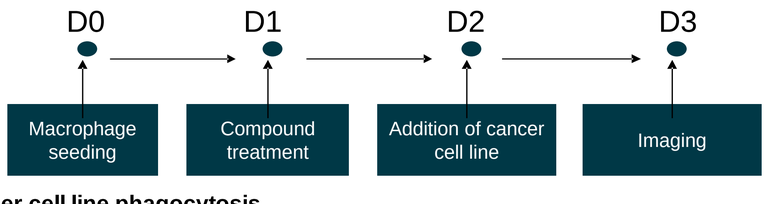


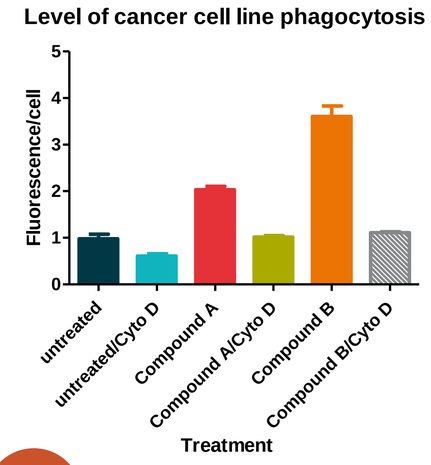


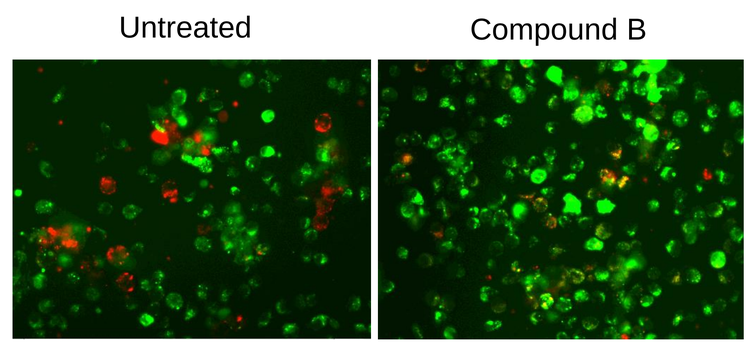
4 Phagocytosis

Our phagocytosis assay combines the use of human macrophage and human cancer cell lines to determine the effects of compounds and modulators on the levels of phagocytosis of cancer cells via fluorescent labelling and high content analysis.

Phagocytosis
Cells Human macrophage and cancer cell line
Readout Phagocytosis of cancer cells, Fluorescent imaging





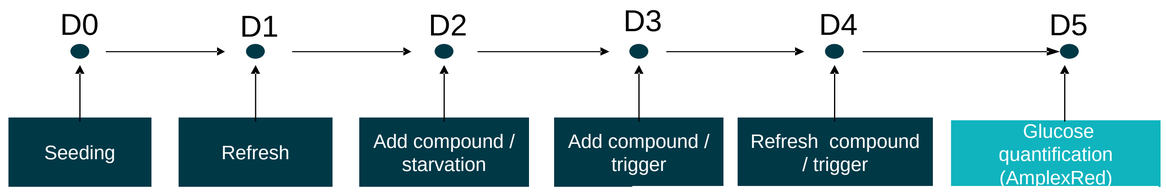


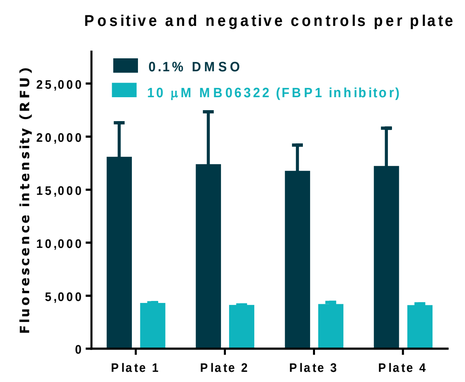
Green:macrophage;Red:Cancer cell line

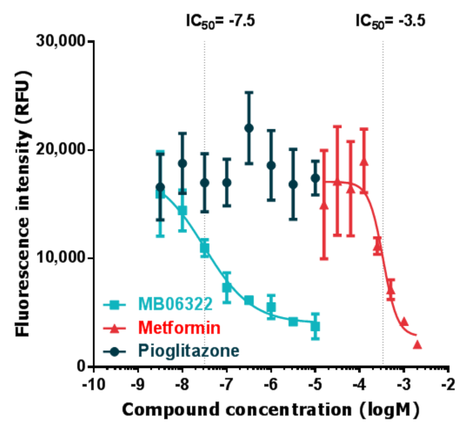
5 Metabolic Syndrome

The metabolic syndrome platform enables interrogation of the metabolic and endocrine state of patient-derived primary cells, including type II diabetes (T2D) hepatocytes, T2D adipocytes and pancreatic islets.

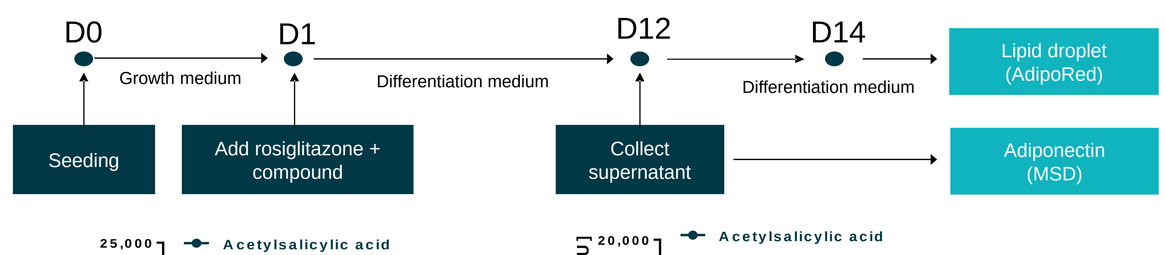
Hepatocyte gluconeogenesis
Cells T2D human primary hepatocytes at 50,000 cells / well
Trigger Gluconeogenesis substrates, cAMP, dexamethasone
Readout Glucose quantification by AmplexRed™

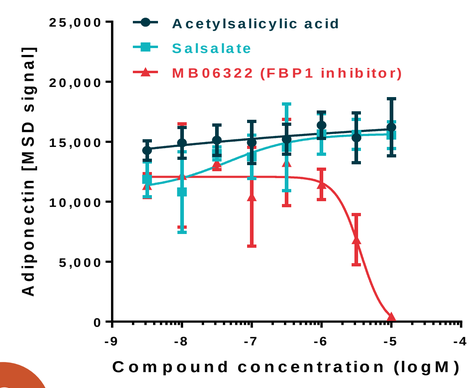


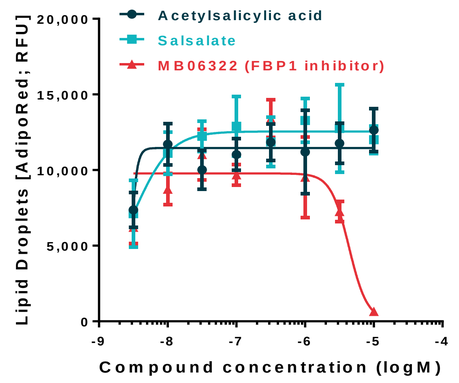




Adipocyte biology
Cells T2D human primary adipocytes at 4,000 cells / well
Trigger Rosiglitazone
Readout Lipid droplet formation (AdipoRed HCA)







6 Conclusions

High content imaging can be a powerful tool enabling the quantification of numerous events in different cellular populations at the subcellular level. Multiple measurements can be multiplexed in the same well facilitating the identification and separation of toxicity and on-target pharmacology as well as following cellular differentiation using multiple biomarkers. Here we have described many exemplar assays showing how, in combination with access to primary human cells, the high content imaging approach can be used to profile compounds across a range of cell types relevant to a broad spectrum of therapeutic areas.

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