High Content Imaging (HCI) of disease-relevant cellular models for target and phenotypic discovery

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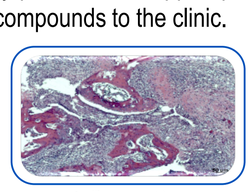
1 Introduction

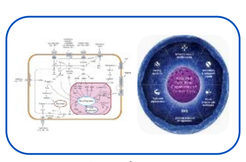
Charles River Laboratories (CRL) has developed many disease-relevant cellular models to screen small molecules and genetic modifiers for target and phenotypic based drug discovery. Our expertise and portfolio in bespoke cellular model development covers several therapeutic areas (e.g. inflammation, oncology, neurodegeneration and rare diseases) and a wide variety of target classes.

Complex cellular models derived from primary cells, patient derived embryonic stem cells and induced pluripotent stem cells have been adapted for phenotypic screening. Models have been further developed to produce physiologically relevant multicellular structures, such as 3D liver spheroids and neuronal co-cultures.

Such validated High Content (HC) assays can be applied to both target and phenotypic based discovery platforms to support preclinical drug discovery and improve translation of targets and compounds to the clinic.

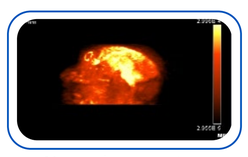


Inflammation



Oncology

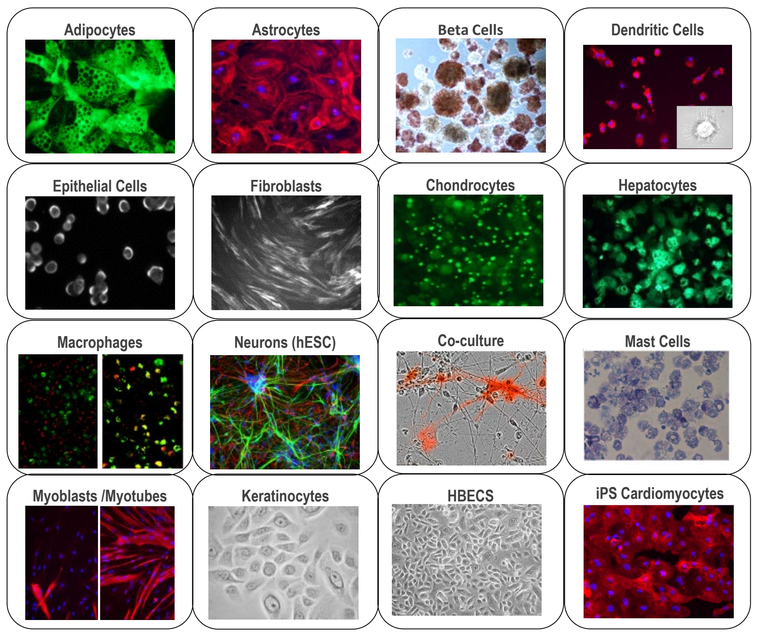
Respiratory



Neurodegeneration

Rare diseases

2 Large panel of primary cell based assays



3 Algorithms developed for numerous therapeutic areas

Formats	Assays developed	Therapeutic area(s)
Anchorage independence and colony forming assays (in 3D)	Multiple cell lines (3D), Clonogenic assays (2D)	Oncology
Apoptosis, cell death, DNA damage response, cellular stress and degeneration	Early and late stage apoptosis markers, mitochondrial function, nuclear condensation, DNA damage assay and downstream signaling assays, foci, stress granules, protein inclusions	Neurodegeneration Fibrosis, Oncology
Cell cycle	Cell cycle progression and S phase	Oncology, Obesity
Autophagy and protein aggregation	Inclusion readout, aggregates	Neurodegeneration
Cell motility and migration	Scratch wound assays	Oncology, Fibrosis
Cytoskeletal rearrangements	Changes in cell morphology (multiple markers/cell lines) hypertrophy, hyperplasia, F-actin alterations	Fibrosis, Respiratory
Post translational modification	Multiple assays for signalling events (phosphorylation and acetylation)	Neurodegenerative, Oncology, metabolism
Real time imaging	Calcium flux in neurons and cardiomyocytes, neurite outgrowth and retraction, phagocytosis	Safety toxicity Neurodegenerative disease
Marker expression	Multiple assays to assess transduction efficiency, epigenetic target and biomarker	Virus production, Neurodegeneration, Oncology
Differentiation	FISH (RNA), TG-ase Calcium deposition, myotube formation, neurite outgrowth	Fibrosis, Psoriasis Osteogenesis, Muscular dystrophy, Neurodegeneration
Neurite outgrowth	Multiple endpoint and real time formats	Neurodegeneration
Receptor internalization and degradation	Nuclear receptor degradation, receptor internalisation, ligand binding	Oncology, Neurodegeneration
Sub-cellular localization	Protein trafficking, Inclusions in cytoplasm/nucleus	Neurodegenerative disease
Translocation of transcription factors	Multiple cytosolic/nuclear translocation assays	Oncology, inflammation

4 Examples of high content based assays

PRIMARY RAT NEURON CORTICO-STRIATAL CO-CULTURES

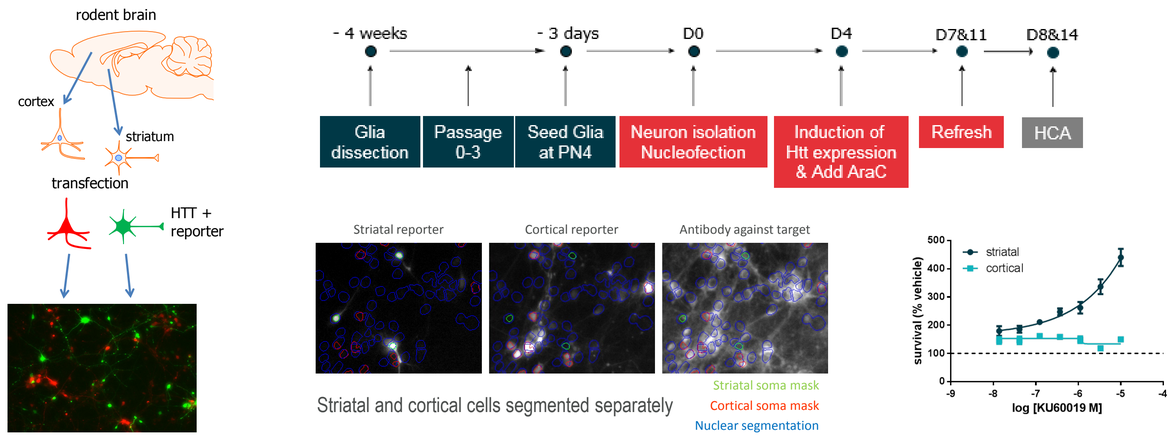


Figure 1. Cell survival assay in rat cortico-striatal co-cultures
The assay measures the survival of cortical and striatal neurons isolated from rodent brain after transfection with mutant Huntingtin (HTT) fragments. Cortical and striatal neurons are transfected separately with different fluorescent reporter alongside with a mutant HTT fragment expressing plasmid. Striatal and cortical cell survival is measured by high content analysis of the number of fluorescent cells remaining after a certain time window, and compared to cells transfected with an empty vector plasmid (instead of the mutant HTT vector). Compounds demonstrating a neuroprotective effect in this assay will result in an increase in the number of fluorescent striatal and/or cortical cells (ex: KU60019).

MYOFIBROBLAST TO FIBROBLAST TRANSITION (MFT)

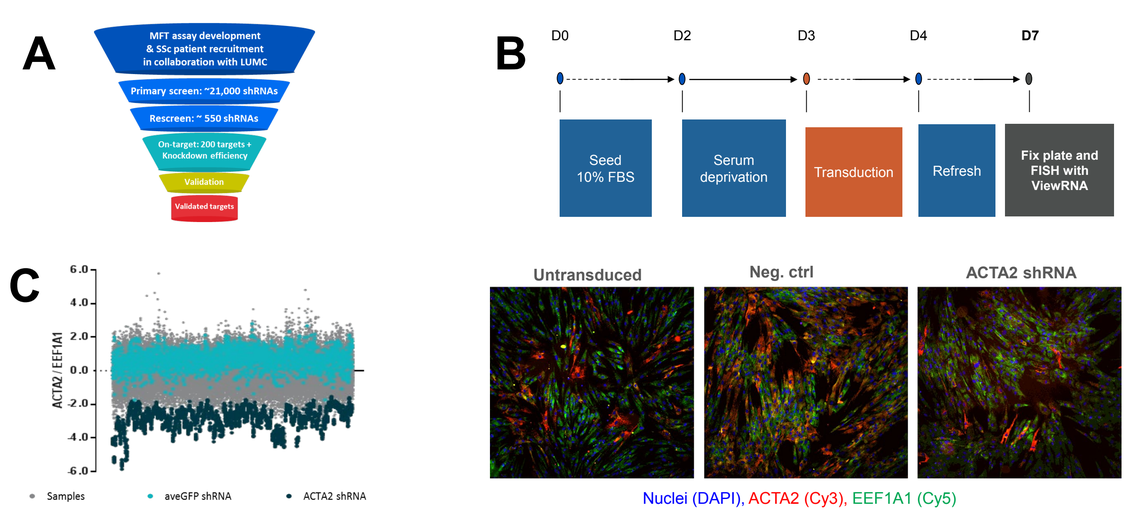


Figure 2: (A) Schematic representation target discovery for Systemic Sclerosis using ViewRNA (B) Primary patient derived human myofibroblasts were seeded and transduced with AAV encoding shRNA prior to fixation and fluorescence in situ hybridization (FISH) for quantification of ACTA2 and EEF1A1 mRNA expression levels by high content analysis. The assay was optimized for a high content screen with 21,000 shRNAs. (C) Scatter plot for the normalized expression levels of ACTA2 for of all shRNAs screened. 542 shRNA were identified as potential modifiers of ACTA2 expression levels which could be further validate

LYSOSOMAL DISRUPTION IN NEURONS DERIVED FROM IPSCS

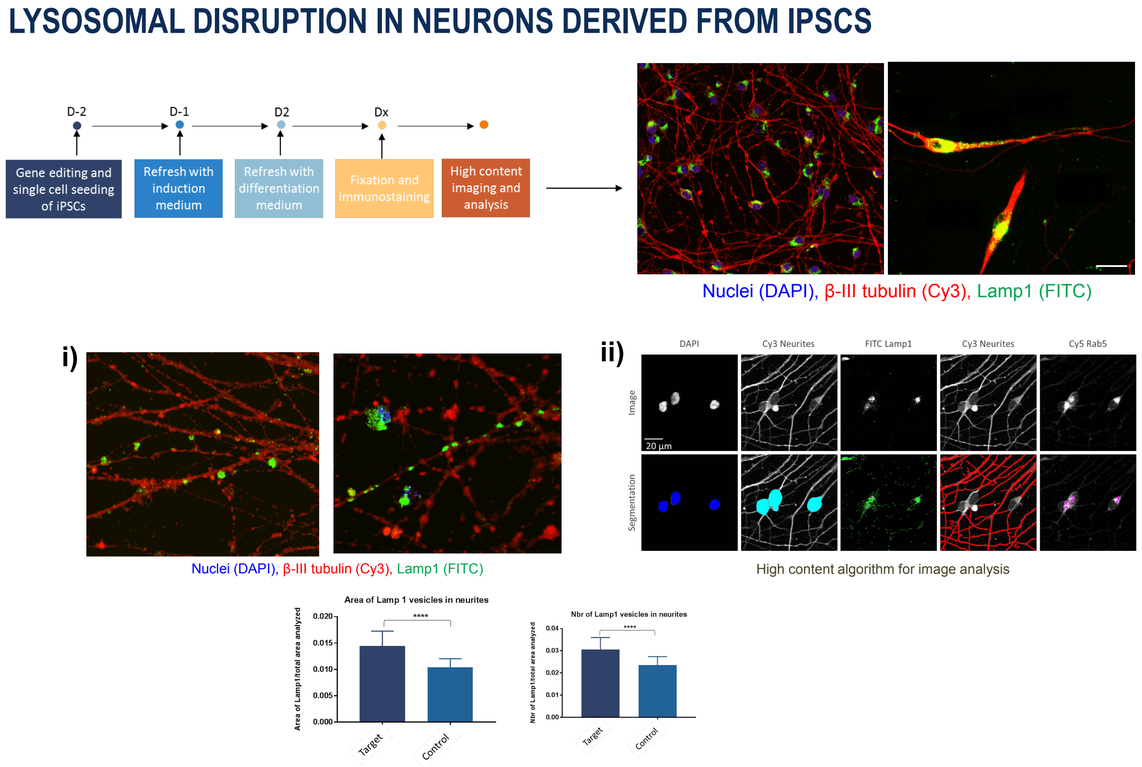


Figure 3: A) Schematic representation of CRISPR based gene editing in human iPSCs and subsequent differentiation into cortical neurons by overexpression of the NGN2 transcription factor. Neuronally differentiated iPSCs were stained for β-III tubulin to visualize neuronal structures and LAMP1 to visualize lysosomal vesicles. (B) (i) Knockout of the target gene leads to the accumulation of lamp1 vesicles in neurites and within the synapses. ii) Example of the high content algorithm used to quantify the number, size and area of Lamp1 vesicles within the neurons and soma. C) Crispr based knockout of the target gene leads to significant increases in the number and area of Lamp1 vesicles in the neurites. The total area or number of lamp1 vesicles was normalized to the total area of the neurons that was analyzed. Unpaired t-test was used to determine significance relative to unedited neurons. (**** denotes p-values < 0.0001)

IMMUNO-ONCOLOGY 3D SPHEROID T CELL CYTOTOXIC ASSAY

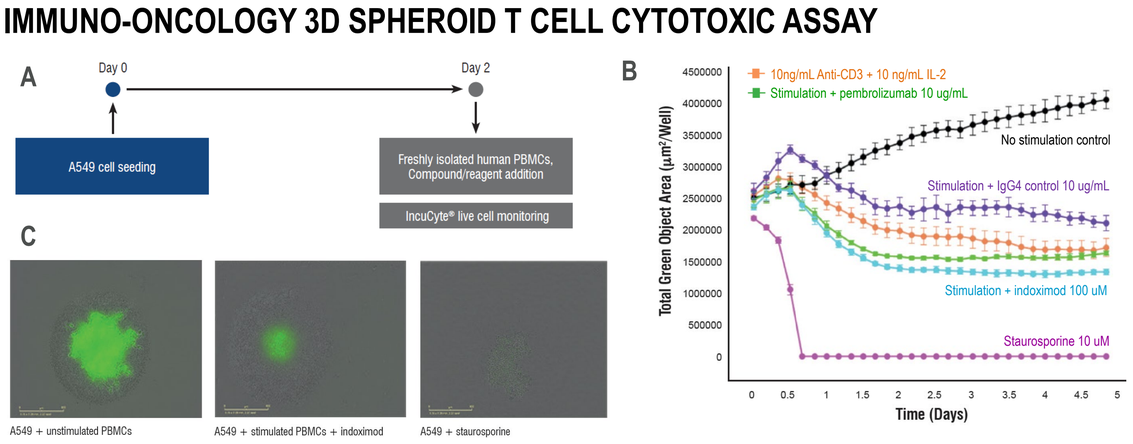


Figure 4. 3D Spheroid T cell cytotoxicity Assay. (A) A549 cells were transfected with IncuCyte® Nuclight Green lentivirus reagent to generate a stable cell line. Cells were seeded in ultra-low attachment plates to enable spheroid formation, and treated after 2 days of culture with activated T cells in the absence and presence of test compounds or therapeutic antibodies. This method uses the IncuCyte® live-cell analysis system to generate data in the form of presentation-ready and time-lapse graphs and movies. **(B)** Example of data generated with IncuCyte® over a period of 5 days after treatment. **(C)** Examples of live cell imaging acquired with IncuCyte®

6 Conclusion

Our extensive experience in the development of complex cellular assays involving co-culture, iPSC, 3D and organoid systems, execution of high-throughput phenotypic screenings involving multi-parametric readouts, design of quantitative image segmentation algorithms, and experience in a wide variety of disease models allows us to approach with confidence any preclinical drug discovery program.