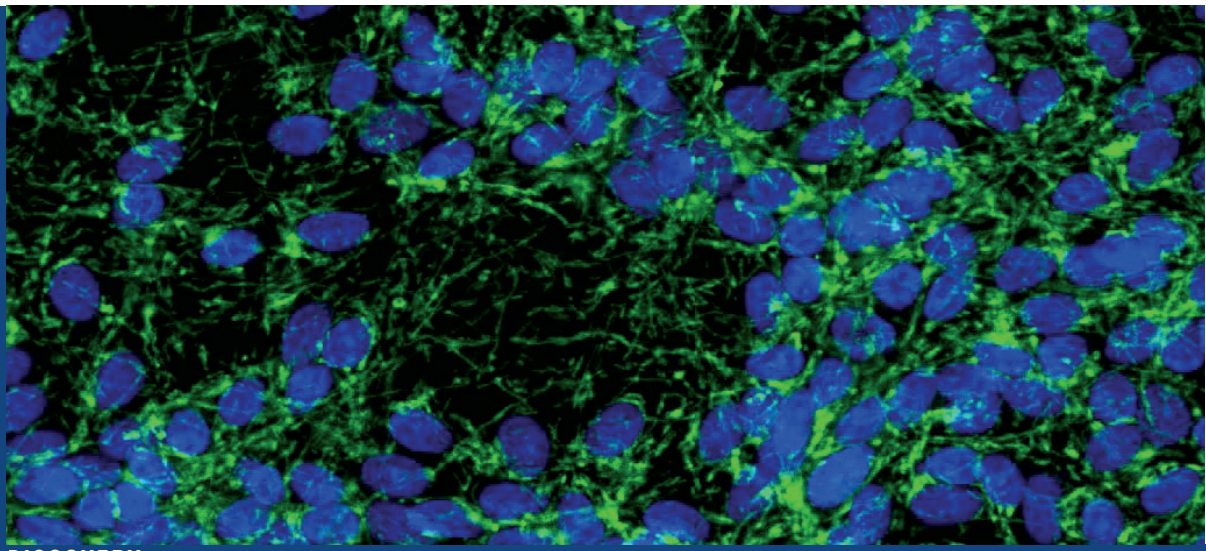
charles river

Summary
Defective mitochondrial clearance (mitophagy) in neurons is believed to contribute to Parkinson's disease and ALS. Our scientists have developed an assay to quantify expression of the mitochondrial protein TOM20 in a human in vitro neuronal model in a format compatible with drug discovery.



DISCOVERY

Off-the-Shelf Assay:

Complex Biology *In Vitro* Assays: Neuroscience

Mitophagy TOM20 Loss Assay

Click to learn more

Parkinson's disease (PD) pathogenesis has been linked to mitochondrial dysfunction through several lines of research, starting with the finding that the mitochondrial complex I inhibitor rotenone induces parkinsonism. In addition, mutations in genes encoding proteins involved in the selective clearance of dysfunctional and redundant mitochondria (mitophagy), such as PARK2 and PINK1, are present in most autosomal recessive cases of PD. Likewise, a growing body of evidence implicated mitochondrial dysfunction in other neurodegenerative disorders such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). For instance, genetic perturbations in genes involved in mitophagy have been demonstrated to underlie ALS, whereas amyloid beta (Aβ) and hyperphosphorylated Tau-induced defective mitophagy has been shown to contribute to AD pathology. Phenotypic readouts to measure mitochondrial (dys) function in disease-relevant cellular backgrounds are therefore thought to represent powerful predictive tools to probe neurodegenerative pathobiology and identify potential therapeutics that can augment mitophagy.

TOM20 is a subunit of the mitochondrial translocase of the outer membrane (TOM) complex and represents a biomarker for mitochondrial abundance. By profiling therapeutic candidates in absence (mono-treatment) and presence (co-treatment) of an established mitophagy-inducing trigger, candidate molecules that enhance trigger-induced mitochondrial clearance without damaging mitochondria directly may be selected.

The Mitophagy TOM20 loss assay represents a scalable and fast neuroscience *in vitro* assay to potentially screen large compound sets for their ability to augment mitophagy in a neuronal background. The assay combines data-rich high content analyses with high-throughput image acquisition. The off-the-shelf format for this assay, and a complimentary α-synuclein assay, is intended to evaluate small sets of potential lead series prior to larger lead-optimization endeavors and *in vivo* translation.

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EVERY STEP OF THE WAY

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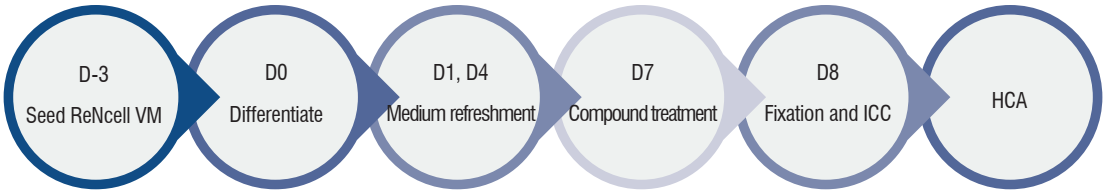
Assay Principle

Immortalized human mesencephalic progenitor cells (ReNcell VM) are differentiated by withdrawal of growth factors (bFGF, EGF) and addition of pro-differentiation factors (cAMP, GDNF) for seven days. Cells are then treated with test compounds in the absence and presence of 1 μM oligomycin/antimycin (O/A; a commonly used mitophagic trigger) for 18 hours followed by immunocytochemical staining of TOM20. Combining treatment of mitophagy-enhancing compounds with 1 μM O/A leads to a dramatic reduction in TOM20 levels on top of reductions induced by O/A treatment alone. TOM20 immunostaining intensity is quantified using in-house developed high content analysis-based (HCA) algorithms. Nuclear counts are quantified to identify potential compound-induced cytotoxicity.

Assay Setup

Cell type	ReNcell VM cells
Seeding density	50,000 cells/well in laminin-coated 96-well plates
Differentiation	Differentiation medium (-bFGF/EGF, +cAMP/GDNF) on D0, with refreshments on D1 and D4
Compound treatment	8-point CRC on D7, both in absence and presence of 1 μM O/A
Assay controls	1 μM O/A (positive control) and .1% DSMO (negative control) on D7
Immunocytochemistry	Fixation after 18h treatment, anti-TOM20 and DAPI staining on D8
Readout	HCA-based quantification of TOM20 immunoreactivity



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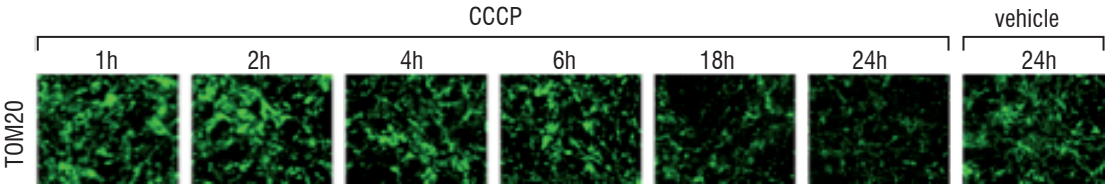
Assay Performance

Representative data are shown below.

A. Immunocytochemical staining of differentiated ReNcell VM cells using an antibody directed against human Tom20. The mitochondrial uncoupler CCCP (10 μM) or vehicle was added for the indicated treatment times followed by immunocytochemical analysis using mouse anti-TOM20.

B. Assay validation using prototypical mitophagy triggers. Differentiated ReNcell VM cells were treated with increasing concentrations of CCCP, oligomycin, valinomycin or actinonin for 18 hours followed by immunocytochemistry using mouse anti-TOM20 antibody and HCA-based quantification of TOM20 immunoreactivity.

A.



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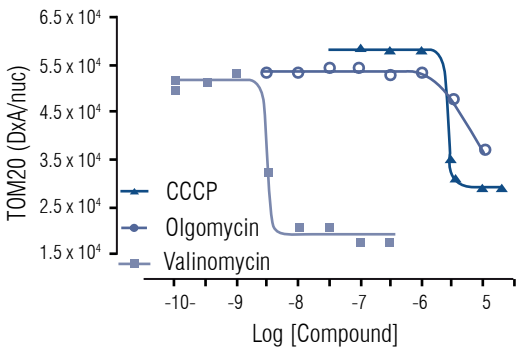

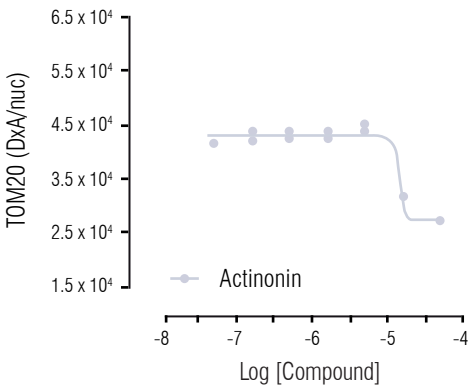

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Summary

The therapeutic candidates are evaluated in 8-point concentration response curves at 0.5Log-fold dilution steps in biological triplicates in the absence and presence of 1 μM O/A. In addition, potential cytotoxicity of the tested therapeutic candidates is assessed by HCA-based quantification of nuclei loss derived from DAPI-staining. As a positive control for Mitophagy TOM20 loss, 1 μM O/A is tested on each plate as a benchmark for assay quality. Results are provided as percentage effect (PE) and percentage remaining cells.

For your scheduling convenience, Mitophagy TOM20 loss assays will be routinely run once every three months. Please send Charles River your compounds by the due dates below. Turn-around time for data delivery will be 6-8 weeks after compound receipt due date.

Service Schedule – Compound Receipt Due Date

October 2020
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Assay Reference Code

OTS220-Mitophagy TOM20 Loss

Complementary Neuroscience Assay's

Alpha-Synuclein Expression and Aggregation Quantification