PHENOVISTA

PHENOTYPIC STUDIES TO DE-RISK ALZHEIMER'S DISEASE R&D

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia. Despite significant investment in R&D, only a few therapeutics for AD have been approved, and they offer only marginal improvement in cognitive ability. The complicated, multifaceted nature of AD has limited the understanding of its causes and progression, as well as the ability to replicate its biology in the lab.

We are well acquainted with the challenges associated with AD research and can work with you to develop unique, scalable, *in vitro* models for screening and characterizing your therapeutic candidates. Our assay models range from relatively simple, 2D mono-cultures to 3D neurospheres, and we provide you with a level of assay customizability unparalleled by other service providers.

Here is a selection of AD-relevant assays that may help inform your R&D decisions.

Characterize the Effect of a Drug Candidate on Neurite Networks and Mitochondrial Health

In this experiment, we measured the differential effects of an AD drug candidate that was abandoned following a Phase 3 trial on wild-type and APP-mutant neurons on neurite network and mitochondrial function.

Cells: iPSC-derived glutamatergic neurons – wild-type, A673T, A673V

Candidate AD drug: Semagacestat

Markers: Hoechst (nuclei), MitoTracker (mitochondrial membrane potential), Tuj1 (neurites)

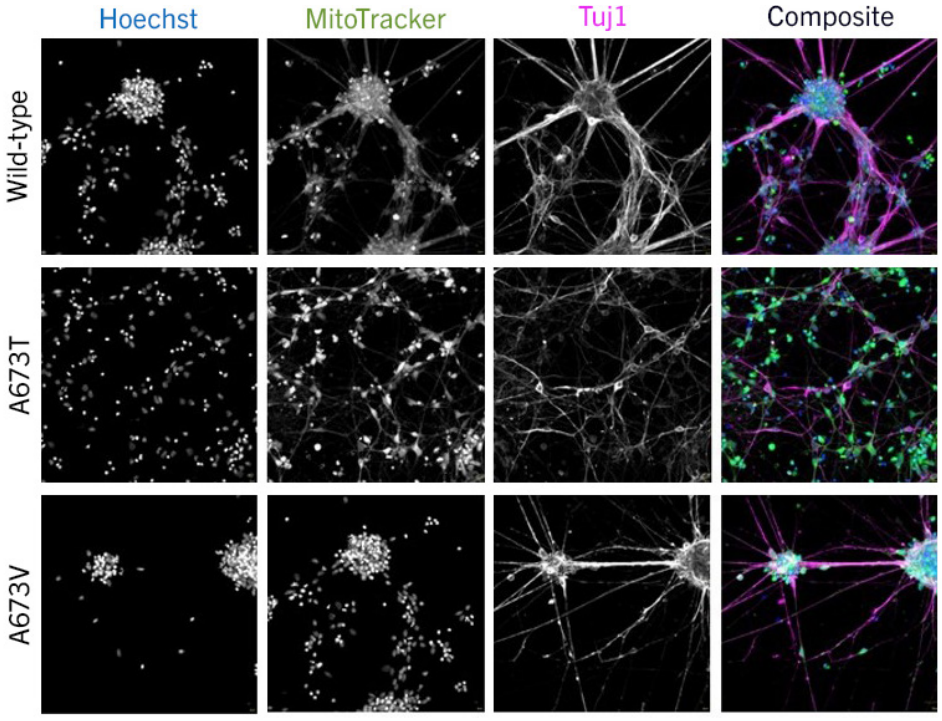


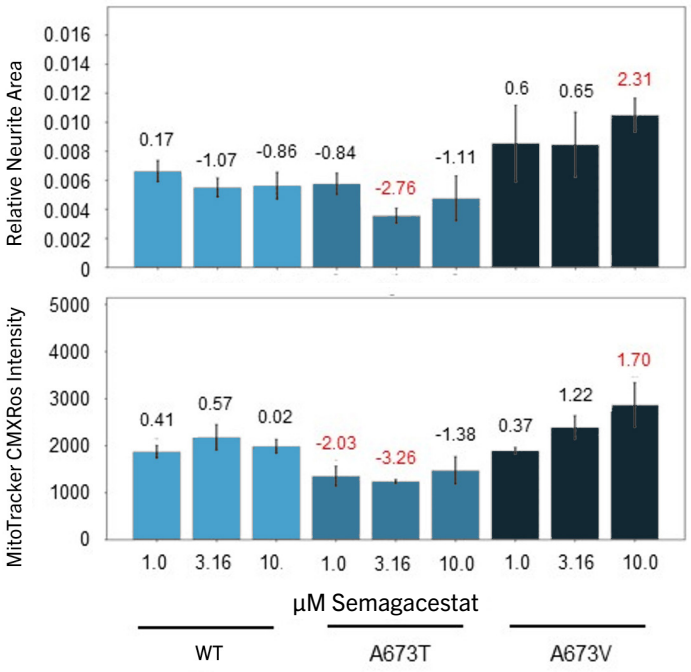
Figure 1A. *Representative images of wild-type and mutant, human, iPSC-neurons stained for nuclei (Hoechst), mitochondrial activity (MitoTracker), and neurites (Tuj1) after treatment with semagacestat.*

Assay Results:

- Semagacestat's efficacy varies with cell source (wild-type vs mutants), indicating it may have greatly varying results in patients.
- Semegacestat's effect on the measured, neuronal-health metrics suggest potential safety concerns from neurotoxicity.

	Effect Relative to Wild-type Neurons	
	A673T APP	**A673V APP**
Neurite Area	Decreased	Increased
Mitochondrial Function	Decreased	Increased

Figure 1B. *Treatment of A673T-mutant neurons with 3.16μM semagacestat significantly decreased neurite area. Treatment of A673V-mutant neurons with 10μM semagacestat significantly increased neurite area. Treatment with 1-3.16μM semagacestat significantly decreased mitochondria activity in A673T-mutant neurons, while treatment with 10μM significantly increased mitochondria activity in A673V-mutant neurons.*



Characterize the Effect of a Clinical Candidate on Microglia Phagocytosis

The role of microglia in maintaining neuronal health has been well defined. In AD, prolonged microglia activation may contribute to neuronal death. In this assay, we compared the phagocytotic activation of wild-type and TREM2-mutant microglia through their ability to take up a fluorescent bioparticle.

Cells: Human, iPSC-derived microglia – wild-type, TREM2 homozygous, TREM2 heterozygous

Test Articles: IL-10, IFN-γ, cytochalasin D

Marker: pH-sensitive, fluorescent bioparticle

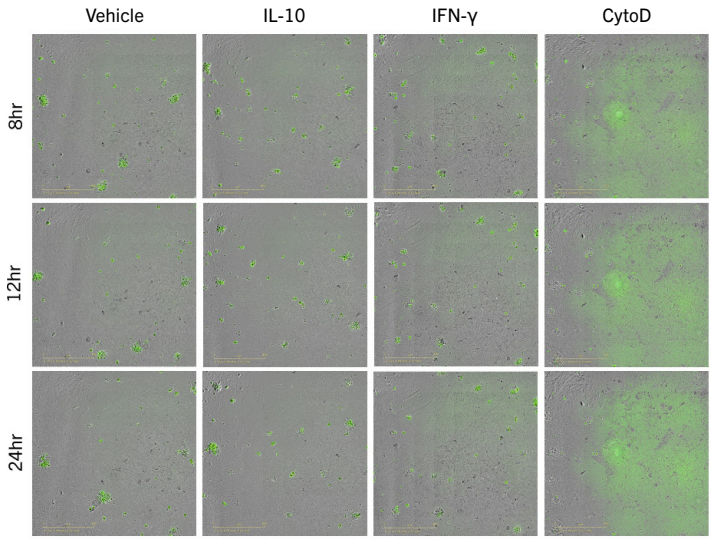


Figure 2A. *Representative images of phagocytosis of a fluorescent bioparticle by wild-type, human, iPSC-derived microglia treated with CytoD, IFN-gamma, IL-10, or vehicle at 8, 12, and 24 hours post-treatment.*

Assay Results:

- Phagocytosis activity can be modulated differently with various stimuli: IL-10 increased activity, IFN-γ and cytochalasin D decreased activity.
- The degree of modulation of microglia phagocytosis activity varied with wild-type versus mutant neurons, which indicates potentially varying abilities to alter activity in patients.

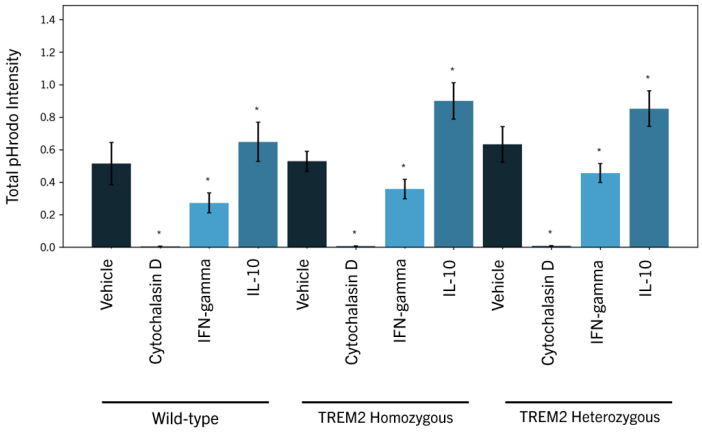


Figure 2B. *Treatment of wild-type or TREM2-mutant, human, iPSC-derived microglia with cytochalasin D or IFN-gamma decreases phagocytosis activity relative to vehicle, while treatment with IL-10 increases phagocytosis.*

Characterize AAV Gene Delivery to Neuronal Cell Types

As research reveals more about the contributions of genetics to AD, interest in using gene therapy as a potential treatment has increased significantly, particularly because some AAV serotypes can cross the blood-brain barrier. A major limitation of current AAV-based therapies, however, is the promiscuity of AAV serotypes; extremely high doses of vector must be injected to reach an effective dose in the cell type of interest.

This assay assessed the tropism of AAV constructs and compared it to other AAV serotypes in various CNS-associated cells – human, iPSC-derived glutamatergic neurons, GABAnergic neurons, and astrocytes.

These data reveal:

- A measurable and comparable difference in the levels of transduced cells across AAV serotypes, suggesting that some cell types may be more susceptible to transduction than others.
- A clear difference in the selectivity of some AAV serotypes for individual cell types, indicating the potential to develop cell-specific gene therapy.

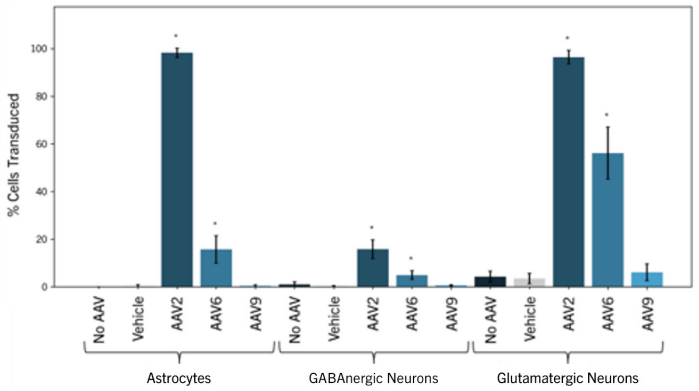


Figure 3B. *AAV2, AAV6, and AAV9 packaged with a gene for GFP have differential uptake and transduction levels in human, iPSC-derived astrocytes, GABAnergic neurons, and glutamatergic neurons.*

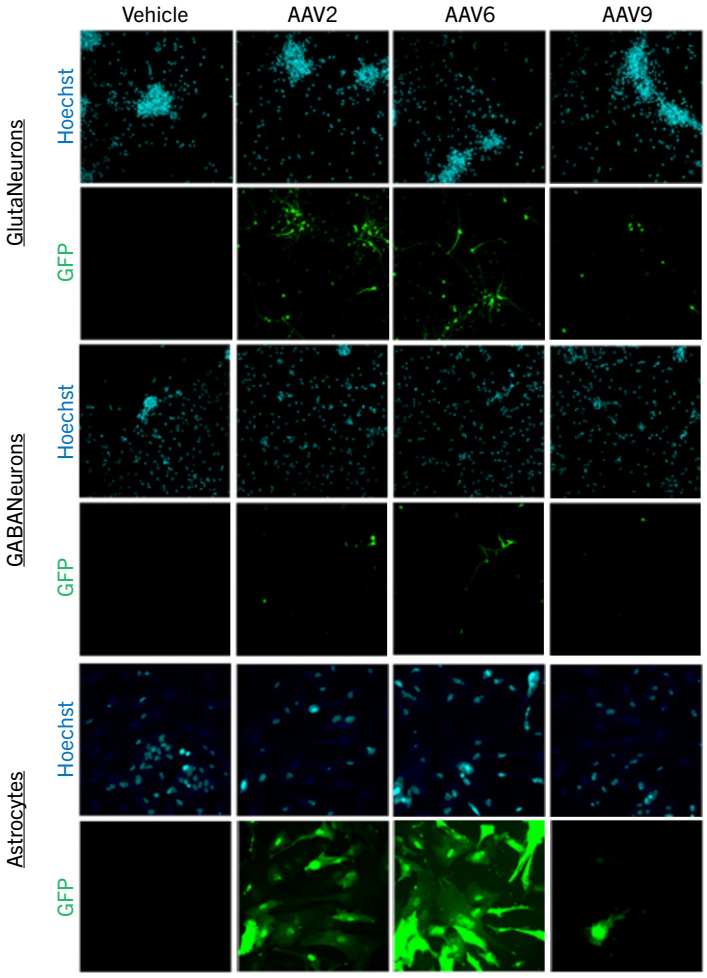


Figure 3A. *Representative images of human, iPSC-derived, glutamatergic neurons, GABAnergic neurons, and astrocytes transduced with AAV2, AAV6, or AAV9.*

PHENOVISTA SERVICES

As demonstrated by these examples, we design phenotypic studies to measure diverse aspects of AD-relevant biology, from neuronal morphology and organelle dysfunction to glial-cell activity and therapeutic uptake.

We develop assays in close collaboration with our clients to ensure that your specific questions will be answered. You can choose from a range of services to select the best fit for your needs.

Custom Assay Services
Custom assays to answer your specific, complex biological questions.

Ready-2-Go Assay Services
Defined assay offerings across a range of disease and therapeutic areas.

Cell Painting
Compare your compounds' effects against those of reference compounds.

Imaging & Analysis
Send us plates of fixed & stained cells, and we'll send you data.