Profiling of drug response mechanisms and patient sub-populations through readouts from a biobank of patient-derived neurons

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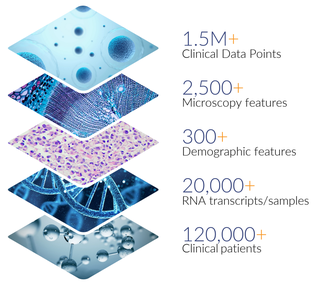
Introduction

- The success rate for drug development for central nervous system (CNS) disorders, such as major depression, is one of the lowest, with estimates of around 6-7% of drugs entering clinical trials reaching approval.
- Biomarkers assessed in iPSC-derived neurons from individual well-characterized depressed patients can serve as a precise and personalized model for drug development.
- Leveraging a biobank of hundreds of patient samples and millions of clinical data points enables the characterization of drug activity and associated response mechanisms in specific, pre-defined, patient populations.
- In a collaboration between NeuroKaire and Clexio Biosciences this approach is utilized to profile the response to Esketamine ,its major metabolites (S-Norketamine and Hydroxy-norketamine) alongside CLE-901-M, a Clexio pipeline compound, in depressed patients with a known response profile to citalopram.



Objectives

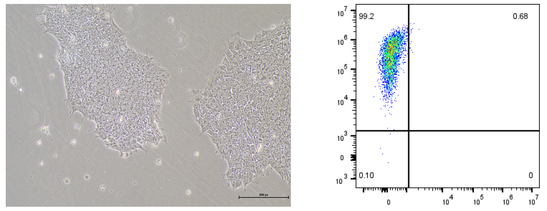
- To profile the antidepressant response induced in-vitro by esketamine in iPSC-derived neurons from depressed patients who are known to respond or not respond to citalopram.
- To determine the role of esketamine metabolites in inducing a response profile in iPSC-derived neurons.
- To compare between the effects of CLE-901-M and esketamine in terms of neuronal response and synaptic plasticity effects.

Design

- In this study, induced pluripotent stem cell (iPSC) -derived neurons from 8 depressed patients with known response to Citalopram (3 responders and 5 non-responders) were used to investigate the molecular effects of esketamine.
- Lymphoblastoid Cell Lines (LCL) from individuals participating in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study were reprogrammed into iPSCs and then differentiated into cortical neurons.
- Derived-neurons were exposed to esketamine with and without its two major metabolites (Hydroxy-norketamine and norketamine) for 8 hours.
- A multitude of imaging-based features reflecting various aspects of neuronal plasticity and synaptic connectivity, were captured and an AI-model for antidepressant response applied.

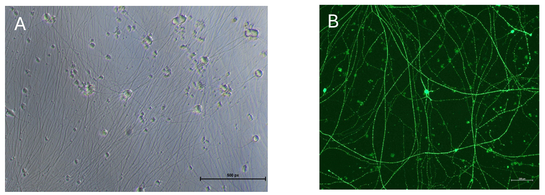
Results

1. Reprogram LCL to iPSC



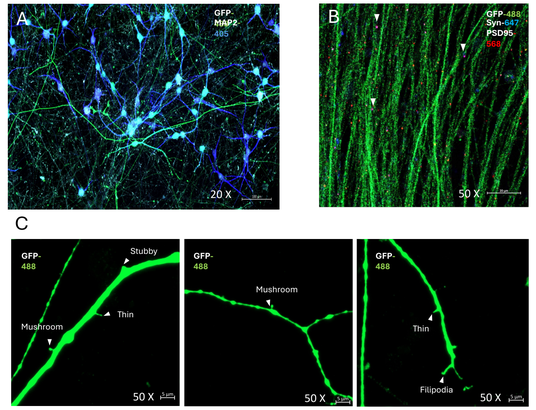
Patient LCL samples successfully reprogrammed into stable iPSC lines. A) LCL-derived iPSCs colony formation. B) FACS analysis shows 99.2% of the population expressing pluripotency marker Tra-160.

2. Differentiate iPSCs into cortical neurons



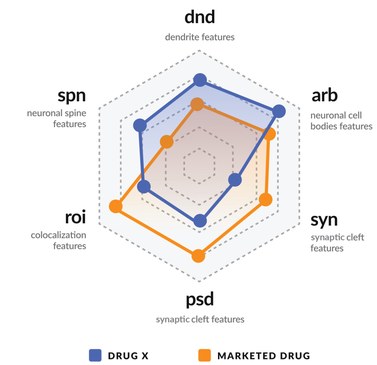
iPSCs derived from patient samples differentiate into cortical neurons. A) Brightfield image of neurons produced by iPSCs after 34 days in differentiation media. B) Neurons expressing GFP on day 34, following LV-GFP-Syn transduction at day 10 of differentiation.

3. Immunofluorescence



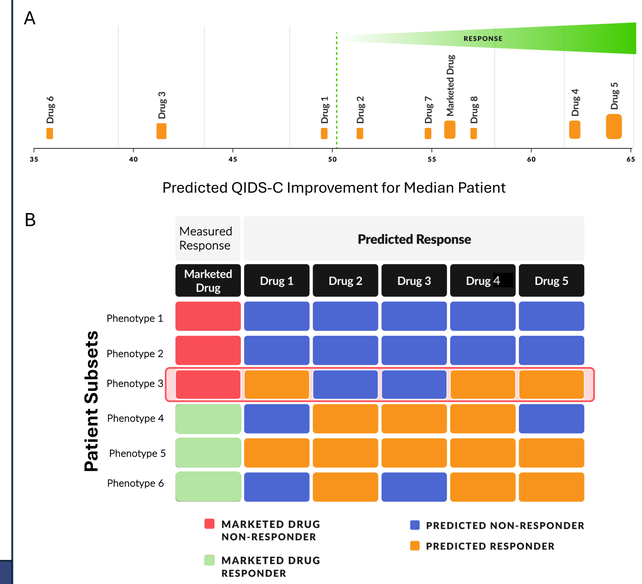
Immunofluorescent imaging at d34 of differentiation shows expression of mature neuronal markers and the presence of dendritic spines. A) Neurons stained with MAP2 and GFP show the formation of dendritic arbors of the patient derived neurons. B) PSD95 (red) and Synapsin (blue) are colocalized (pink) on the spines of GFP expressing neurons. C) Stubby, mushroom, filipodia, and thin spines are detected on neurons.

4. Mechanistic Drug Differentiation



Comparing drugs in development with marketed antidepressants reveals distinct impacts on neuronal features, including dendrites, neuronal spines, and synaptic clefts. Features are grouped and quantified into composite scores for visualization, highlighting the potential to target specific neurobiological pathways for enhanced therapeutic efficacy.

5. Response Prediction



Predicted and measured responses for marketed and investigational drugs highlight responder and non-responder profiles across patient samples.

A) Average predicted response

B) Individual patient phenotype responses

Conclusions

- Esketamine induced an antidepressant profile in derived neurons from responders and non-responders to citalopram. Esketamine's major metabolites significantly contributed to the response.
- CLE-901-M induced a synaptic plasticity profile higher than citalopram and similar to esketamine with metabolites, demonstrating its potential for further development in depression treatment.
- This study demonstrates the utility of iPSC-derived neurons from clinically characterized patients for profiling the efficacy of antidepressant compounds in various patient populations.
- The potential of both esketamine and CLE-901-M to induce an anti-depressant effect that is stronger than the citalopram effect, even in patients that are citalopram responders, alongside the importance of the major esketamine metabolites for drug efficacy, can be used to drive the clinical development strategy for the drug. Further confirmation with clinical samples and data is warranted.