Regenerative Medicine Minnesota
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Principal Investigator; Jonathan Marchant, PhD
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NAADP Signaling: novel pharmacotherapy for neuronal regeneration and repair

This document provides a 1st year progress report for the initial 12 month funding period of this project, as requested in grant award correspondence.

Project Abstract. There is a key need to generate new drug treatments for neurodegenerative diseases that stop disease progression rather than just mask symptoms. In this regard, our team has recently discovered that analogs of NAADP - a potent second messenger - are able to reverse cellular defects seen in cells isolated from patients with Parkinson's disease. To understand whether this therapy has clinical potential, we need to optimize the properties of these NAADP analogs for use *in vivo* and assess the beneficial effects of newly generated lead compounds in assays of neuronal regeneration and viability.

Reporting to All Minnesotans. The estimated costs of Parkinson's disease are estimated at ~$15 billion/year in the US. This cost burden will rise substantially as baby-boomers age. PD will place an increasing economic burden on the state of Minnesota in terms of nursing home costs for people living with PD, their families and local communities as it is estimated the number of patients will double by 2030. Developing new treatments is of particular relevance for Minnesota health outcomes as Minnesota is one of the 'Heartland Hub' states (NE, ND, SD, IA) which exhibit the highest incidence rates for PD in the nation. Death rates in Minnesota from PD are higher than the US average, contrasting with the outcome statistics for all other prevalent diseases in our state. Therefore, there is an urgent need for new drugs for treating PD: especially for identifying novel therapies that promote neuronal regeneration, rather than focusing on alleviating symptoms. Our work explores the druggability of a novel target pathway to yield therapies designed to prevent or reverse neuronal cell loss in Parkinson's disease patients.

Progress to date: Year 1. In our original grant, we proposed several milestones for completion in Year 1. We have made good progress with each of these goals as detailed below.

1. *Synthesis of novel analogs to manipulate the NAADP signaling system via modulation of TPC channels*. TPCs are intracellular calcium channels that are activated by NAADP. Owing to their intracellular localization in lysosomes it is difficult to screen activity of analogs. We have circumvented this barrier by optimizing single cell microinjection and genetic reporter imaging methods to deliver ligands and resolve their activity at lysosomal calcium release. We have tested the activity of over 20 small molecule ligands based around the NAADP pharmacophore for activity against the TPC channel, and found novel analogs that block TPC activity that merit testing to correct NAADP/TPC over activity phenotypes seen in our cellular models of Parkinson's disease. Figure 1 depicts our hypothesis that inhibition of NAADP-evoked Ca2+ signals,

or blockade of TPCs will reverse lysosomal abonormalities seen in Parkinson's disease afflicted patients.

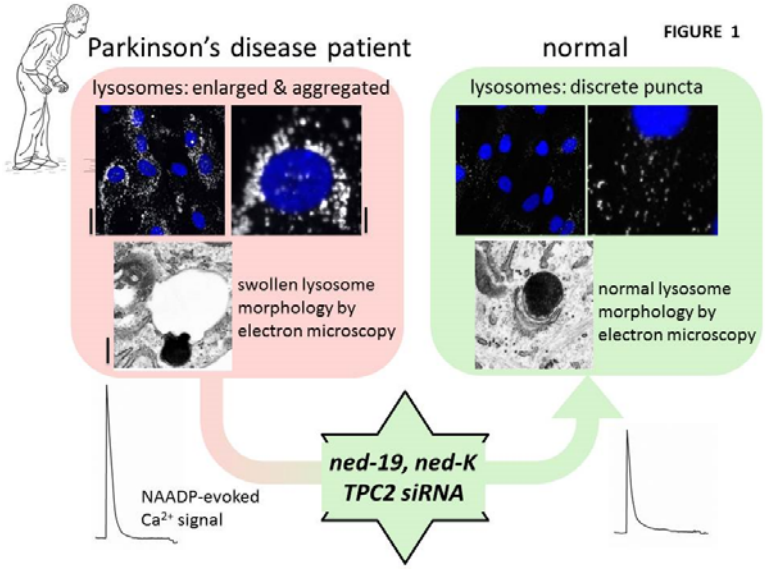

FIGURE 1. Examination of lysosomal structure and function in primary cultured fibroblasts . In healthy control fibroblasts, lysosomes were well resolved as puncta dispersed throughout the cell (right). Left, in contrast lysosomes appeared enlarged and clustered in age-matched fibroblasts derived from PD patients harboring the G2019S mutation in LRRK2. NAADP-evoked Ca^{2+} signals were also potentiated compared to control cells (bottom). These changes in PD fibroblasts were reversed by knockdown of TPC2 or by using NAADP blockers.

During Year One – as proposed - we have screened for small molecules of TPC/NAADP activity with properties suitable as lead compounds for clinical application. Figure 2 shows that we now have in hand candidate molecules that selectively block NAADP-evoked Ca^{2+} release pathways ((Figure 2A) and that these molecules are able to reverse the Parkinsonian phenotype in a manner proportional to their efficacy at blocking this target (Figure 2B).

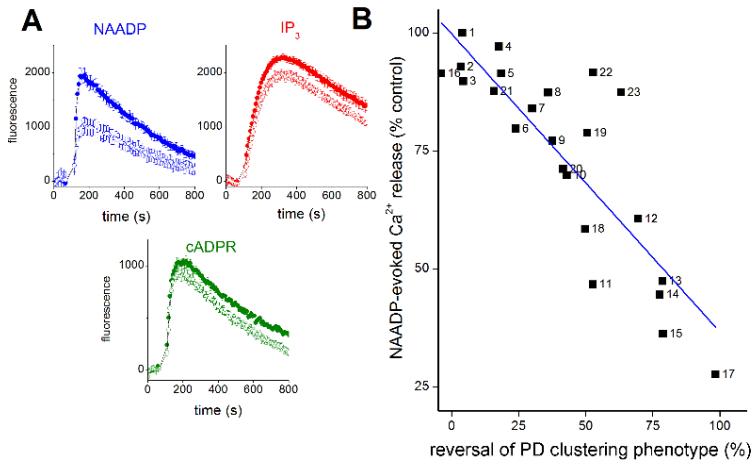

FIGURE 2. Drugs that inhibit NAADP-evoked Ca^{2+} release block PD phenotypes. Ca^{2+} release as resolved by fluo-3 fluorescence measurements. Ca^{2+} liberation was measured in the absence (solid circles) or presence of a lead compound (open circles, 10µM) in response to NAADP (blue, 70nM), IP_3 (red, 200nM) or cADPR (green, 100nM). Data represent values from a minimum of 3 independent experiments and are expressed as mean ± SEM. **B**, Correlation plots comparing the extent of inhibition of NAADP-evoked Ca^{2+} release observed with different ligands (10µM) and the inhibition of morphological phenotypes evoked by the same ligands (10µM). None of the tested ligands evoked Ca^{2+} release by themselves.

This milestone has been met and delivered ligands for testing in Year 2 activities.

2. *Optimization of methods for generating hiPSC-derived neurons for testing defined LRRK2 mutations from patient-derived fibroblasts.*

The next part of our analyses is to test whether these analogs reverse PD phenotypes in patient cells. To do this we are attempting to derive neurons from hIPSC cells created from patient fibroblasts. We have no prior experience in doing this, but we have hired appropriate expertise and are able now to reproducibly derive neurons from hIPSC cell lines. Progress was slower than expected owing to a delay in hiring personnel with appropriate expertise to perform these analyses. However, we have been highly fortunate to be able to recruit an experienced post-doctoral stem cell researcher (Mohammed Rashid) to this project, who was previously trained in the study of neurogenesis of embryonic neuronal stem cells at NIH. This took some time (1st October 2016, hire date) but this was an important hire (Researcher 6) to expand our skillset to perform required analyses which will pay dividends in Year 2 of this project.

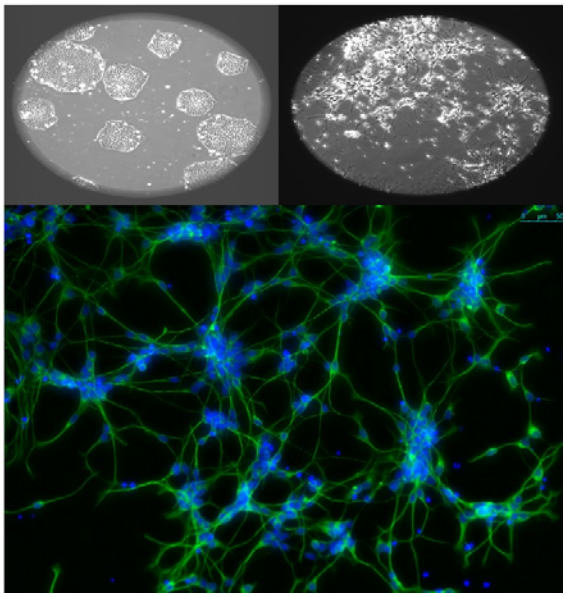

Figure 3 shows our ability to derive hiPSC derived neuronal cultures. These cultures are NAADP-sensitive and TPC1/2 positive. Over expression of TPCs replicates a lysosomal mis-trafficking phenotype, providing an avenue for testing our ligands in a neuronal, and eventually patient, background. Optimization of this technology with patient derived cells will allow us to test the efficacy of our new ligands.

FIGURE 3. **Neuronal differentiation of hiPSC cell lines**. *Top*, low magnification views of hiPSC colonies at day 4 (left) at day 10 (right) of small molecule based differentiation protocol. *Bottom*, immunostaining of differentiation neurons - Images courtesy of P. Walsh - (blue, DAPI; green, TUJ1).

Budget Update: The direct cost balance as of 1/28/17 was $11,000 remaining (last available report) within the allocated budget for Year 1. This grant provided support for

Milestones Year 2: We intend to submit a NIH grant and manuscript for peer-review in year 2, based upon the data generated during this award. Disclosures/patents: We are meeting with Karen Ohlfest (OTC, U of MN) in April to discuss any potential use patentability of several of our analogs pending further biological validation in other endolysosomal functional assays (neurogenesis, viral trafficking, anti-mycobacterial). Data will be presented at the Gordon Research Conferences for organelle transport and calcium signaling this summer, and hopefully in time for the Society for Neuroscience in Fall 2017.