ICOC Board Meeting Written Comments by Applicant: November 30, 2017

PI: Vicki Nienaber, Ph.D. President and CSO, Zenobia Therapeutics, Inc.

Re Grant DISC1-106: A new phenotypic screening platform that identifies biologically-relevant targets and lead compounds for the treatment of Parkinson's disease

We request consideration of our proposal for funding as it meets the DISC1 award objective *"to test new**, potentially transformational ideas** that … **require the generation of additional data** to be competitive for larger funding opportunities …"* and addresses a **significant unmet medical need**.

> There is ***no disease modifying treatment for Parkinson's disease***. The most common therapy, L-dopa, has been in use since the 1960s! Modern therapies have been confounded by ***PD likely being a family of diseases rather than resulting from one specific cause***. Our technology allows not only identification of potential therapies and their targets but determination of their ***relevance across broad patient populations using patient-derived iPSC-neurons***. Immediately, compounds and targets identified in this proposal will progress into a program to identify new treatments for PD and the data used to obtain additional funding. ***Long-term, the technology and potential treatments will be broadened to other neurodegenerative diseases such as Alzheimer's, Huntington's, ALS and brain injury. Currently there are no treatments for this class of debilitating conditions.***

Reviewers found our proposal "*potentially transformative*" as evidenced by the following "Strengths":

> "***The envisioned work has a high chance of identifying a novel, candidate drug for PD*** and could fulfill an ***unmet need*** for a therapeutic agent for PD and ***new models for the quick screening of potential drugs using patient specific iPSCs.***"
>
> **"Innovative"** and **"Solid scientific premise"**

The primary ***"Concern"*** of reviewers that this is an "***untested technology***" and requires a ***positive control*** in a "well-studied system" (omitted in proposal due to page constraints).

> The technology ***has been tested and validated in HeLa cells in the laboratory of our collaborators at Scripps (Dr. K Barry Sharpless, Nobel prize in chemistry 2001)*** and published in the Journal of the American Chemical Society in 2016 (details next page).
>
> The chemistries ***that allow us to identify targets responsible for the phenotypic change*** (which ***differentiates us*** from other high-throughput screening approaches) were ***invented by our collaborators (Dr. K Barry Sharpless and Dr. John Moses)***. This de-risks the technology since we understand the parameters to transition from HeLa to iPSc derived neurons.
>
> We have demonstrated activity for our parkin inhibitors in iPSc-derived dopaminergic neurons ***validating that we can conduct these assays***

In closing, a quote that is true for most truly transformative ideas:

> **"Everyone thinks things are hard or cannot be done until someone does it, then they think it is easy!"** - K. Barry Sharpless

We hope you will consider funding this application and thank you for your consideration.

Appendix with more detailed answers to primary reviewer "Concerns":

1. "It is unclear how this is different from other high-throughput small molecule screening strategies and platforms."
    - ***Existing high-throughput screening campaigns*** are plagued with the task of connecting phenotypic changes with the target (or targets) of the ligand
        - ***Targets are identified indirectly***
            - Cells are engineered (artificial systems) to bias one pathway
            - Libraries are biased to known chemical classes
            - The target-ligand complex is not directly observed in the cell
            - ***Difficult to apply to patient derived iPSc and directly identify the target of the ligand responsible for a shift in phenotype***
    - ***Our screening strategy*** not only results in ligand-induced phenotypic changes but ***allows us to fish the target of the ligand from the cellular milieu*** directly pairing target and phenotype in a highly relevant cell assay (patient derived cells).
        - A unique chemical handle forms a highly specific covalent linkage with the target active site and a second chemical handle facilitates "fishing" the target from the cell milieu.
        - This handle is activated by inherent properties of ligand binding and requires no outside activation such as UV light which could be damaging to neurons.

2. Concern about using an "***unvalidated technology***" and need for a positive control in a "***well-studied system***."
    - Initial proof of concept in a "easy" system has been completed and published although details were not included in the original proposal due to page constraints. The chemistries we are using were developed in the laboratory of Dr. K. Barry Sharpless (our collaborator) and proof-of-concept published out of his laboratory in 2016 in JACS. Here, they showed that the chemistry works in HeLa cells (a well-studied system) and they "fished out" a set of intracellular lipid binding proteins as the targets. They validated binding of ligands to these targets in cell-free assays and eventually by completing x-ray co-crystal structures.
    - Our application expands upon these POC studies by both increasing the size and diversity of the chemical library and moving to a "real-world" unmet scientific need, PD
    - Because our screening approach invokes phenotypic changes and allows target identification in the same step, we think it is sensible to conduct our screening in patient derived neurons. Model systems can be very misleading for homogeneous diseases such as PD. Identification of a potential therapeutic in iPSC-derived neurons puts us much close to the clinic than if we used a model system.
    - We do choose engineered A53T-synuclien iPSC-derived neurons as the model system for this proof-of-concept grant as the biology is better understood but can be easily translated to idiopathic patient derived cells as the technology matures. It may also translate to other neuronal diseases such as AD, HD and brain injury. This partially addresses the reviewer's concern given that POC already exists in the simple HeLa cell model.