



Exploring Nonsense Suppression as a Treatment for Rett Syndrome

Lucas Pozzo-Miller, PhD

The University of Alabama at Birmingham

\$150,000.00

Rett syndrome (RTT) is a genetic disorder that is a common cause of intellectual disability in females. RTT results mainly from mutations on the X chromosome in the MECP2 gene that disrupt MECP2 protein function, which regulates the expression of multiple genes. Presently, there are no treatments available for RTT. Several drugs that have previously been approved by the U.S. Food and Drug Administration for other disorders are in early-phase clinical trials to determine whether they can be repurposed to treat certain aspects of RTT. However, these drugs are expected to treat only a subset of RTT clinical features due to the complex disease etiology. New therapeutic approaches are needed to effectively treat all aspects of the RTT phenotype.

In this grant application, our team proposes to determine whether recently-identified novel small molecules are potential candidates for nonsense suppression therapy in RTT. Nonsense suppression therapy acts at the level of protein synthesis to restore MECP2 function directly. We hypothesize that nonsense suppression therapy may be able to restore enough MECP2 protein function to alleviate all (or nearly all) phenotypes associated with RTT, as opposed to only targeting a subset of phenotypes. Since more than one-third of RTT patients carry nonsense mutations, nonsense suppression therapy could potentially alleviate RTT in a significant number of patients.

As part of a collaboration with the Cystic Fibrosis Foundation, we developed and carried out a NanoLuc luciferase reporter assay that would detect compounds affecting both premature termination codon (PTC) readthrough activation and nonsense-mediated mRNA decay (NMD) inhibition. A total of 771,345 samples were screened/tested at a single concentration, followed by confirmation of concentration-dependent activity. Confirmed hits were then repurchased for subsequent confirmatory and cytotoxicity testing. A total of 157 compounds from the entire high-throughput screen (HTS) were confirmed as hits exhibiting a concentration-dependent response with a maximum % activation ($\geq 20\%$ of the positive control) when retested in the primary assay. The compounds consist of both readthrough inducers and NMD inhibitors and thus have the potential to counteract the effects of nonsense mutations in RTT.

The project proposed here would screen this collection of new, validated nonsense suppression agents for their ability to promote readthrough of four common RTT nonsense mutations in luciferase reporter assays (Aim 1) and restore full-length MECP2 protein levels and function (Aim 2). These studies lay the groundwork for identifying multiple compounds for future applications (ANGEL award) and for ultimate advancement to IND status.