Project title: Evaluation of New Anti-Prion Drugs: From Drug Design to Therapeutic Efficacy Against Human Prions In Vitro, Ex-Vivo, and In-Vivo

Principal Investigator (PI) name, direct collaborators and full affiliation:

Dr. Natalia do Carmo Ferreira de Araujo (PI) [Rocky Mountain Laboratories (RML), NIH/NIAID, USA], proposes to perform this study in collaboration with colleagues from RML (Bradley Groveman, Christina Orrù, Cathryn L. Haigh, Brent Race and Byron Caughey) and the Federal University of Rio de Janeiro, Brazil (Yraima Cordeiro, Maria Leticia de Castro Barbosa and Arthur Kummerle).

Project objective: To assess the efficacy of new anti-prion drugs which were designed based on the structure of a potential compound previously characterized by our group (Ferreira et al, 2014; Ferreira et al, 2017). These new drugs are going to be tested in laboratory grown human brain tissue (cerebral organoids) infected with sCJD and, the safety and efficacy of the most promising compounds will be evaluated in prion-infected mouse models.

Summary of accomplishments to date:

In this study we assessed the efficacy and safety of 19 drugs which were designed based on the structure of a potential compound previously characterized by our group, namely J8. The efficacy of the newly synthesized drugs was assessed in prion infected cells (ScN2a). We added the drugs to the media in which the cells were being cultivated. After that, we measured the content of infectious prions (PrP^{res}) in the cells, and we compared the untreated x drug treated cells. The figure 1 is a representation of the raw data. The black dots (untreated cells) refer to the amount of PrP^{res} in cells in the absence of drugs. The RPJs lanes correspond to the cells treated with the different drugs (RPJ8 – RPJ14), showing a less intense signal, meaning less content of PrP^{res} in the cells which were treated with the drugs.

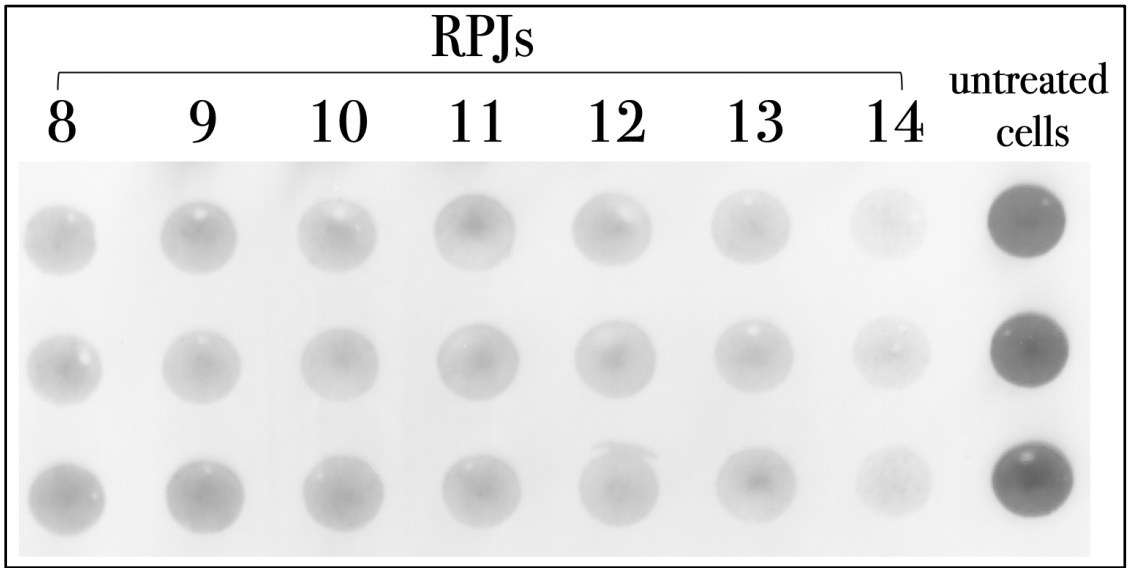

Figure 1: Representative dot blot showing infectious prions (PrP^{res}) content in prion infected cells upon treatment with drugs. ScN2a cells were cultivated for four days in the presence of the different drugs (RPJs 8-14). The amount of PrP^{res} in the treated cells was compared to the untreated cells. The drugs which inhibited the accumulation of PrP^{res} by more than 50% were further evaluated.

Then, we evaluated the cytotoxicity of the drugs, to exclude the drugs which were causing cell death. As shown in figure 2, due to the toxicity of drugs RPJ5, RPJ13, and RPJ14, they were excluded of the following analysis.

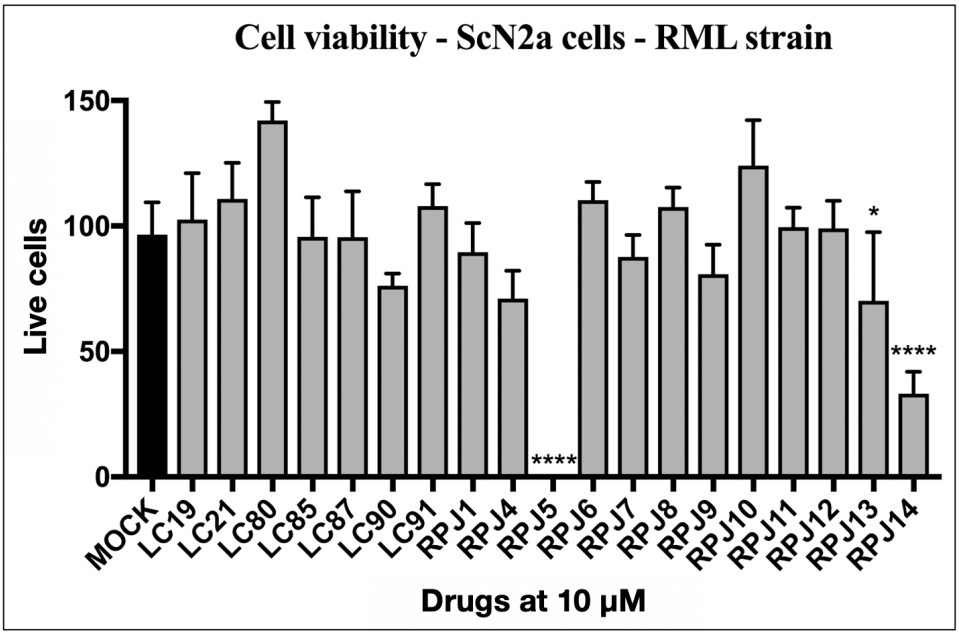

Figure 2: Assessment of cellular viability of prion infected cells upon treatment with drugs. Cells were cultivated in the presence of the drugs for four days. Then, the percentage of live cells was measured, and the drugs which caused a significative effect on cell death (e.g., RPJ5, RPJ13, and RPJ14) were no longer analyzed on this study.

As the main event in prion diseases is the conformational change of PrP^{C} to PrP^{res}, we assessed the ability of the drugs to prevent this conversion in a cell-free assay. In this assay we incubated a human brain homogenate infected with prions (sCJD) with normal prion protein (recombinant PrP^{C}). In the absence of an inhibitor, the normal prion protein is converted in PrP^{res} by the infectious prions seeds present in the brain homogenate. After 18 hours incubation, we added proteinase K (PK), an enzyme that is able to eliminate normal prion protein. As shown in figure 3, in the reaction where a brain homogenate from a healthy human was used (NBH = normal brain homogenate), there is no PrP^{res} (the PK enzyme digested the normal PrP); whereas in the reactions where a sCJD brain homogenate was used, different fragments of PrP^{res} are seeing (lane sCJD +). Lanes J8 to LC91 show the final product of each reaction in the presence of the specified tested drug. Besides its effect in inhibiting PrP^{res} accumulation in prion infected cells, drugs LC19 and mainly LC87 were also able to inhibit the conversion of PrP^{C} to PrP^{res} in a cell-free assay where a brain homogenate from a sCJD patient was used as seed. Furthermore, when assessed at different concentrations, LC87 was effective even at concentrations as low as 1 μM (figure 4).

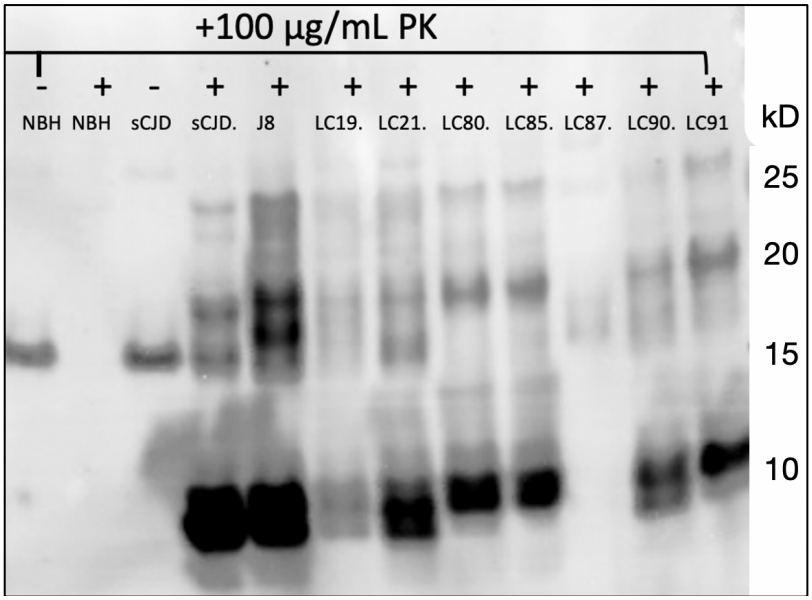

Figure 3: Assessment of PrP^{res} formation in a cell-free assay. Normal brain homogenate (NBH) or a sporadic CJD brain homogenate (sCJD) was used as seed. Each reaction consisted of normal prion protein (recombinant PrP^{C}), seed (either NBH or sCJD) and the tested drug (LC19-LC91). Reactions were incubated for 18 hours under mild agitation.

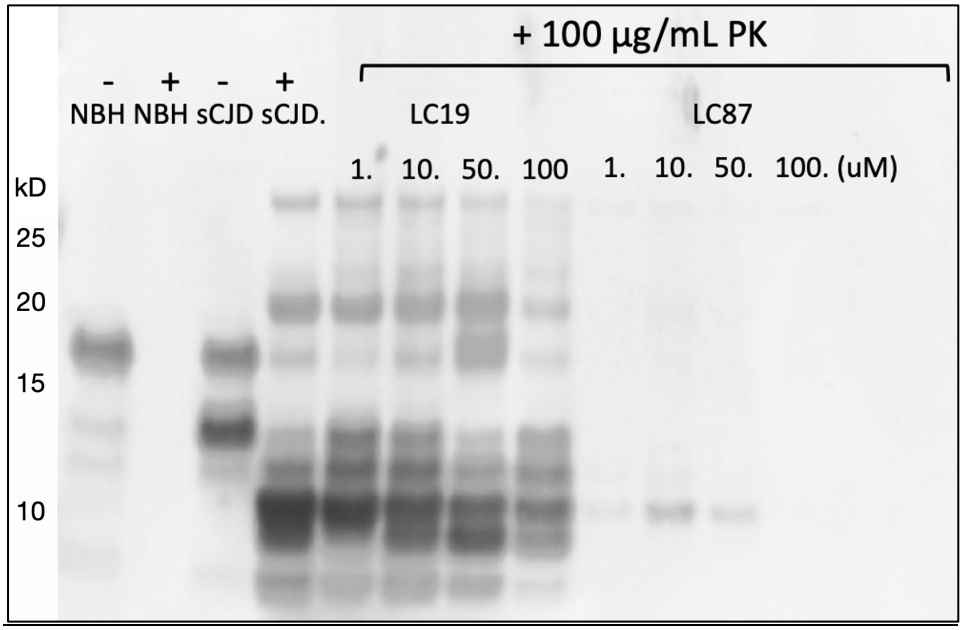

Figure 4: Evaluation of inhibitory effect of LC19 and LC87 at different concentrations in the cell-free assay. Normal brain homogenate (NBH) or a sporadic CJD brain homogenate (sCJD) was used as seed. Each reaction consisted of normal prion protein (recombinant PrP^{C}), seed (either NBH or sCJD) and the tested drug (LC19 or LC87) from concentrations ranging from 1 to 100 µM. Reactions were incubated for 18 hours under mild agitation.

Key findings and implications for the prion disease field: In this project we found 2 promising anti-prion drugs, LC19 and LC87, which were able to inhibit PrP^{res} accumulation in prion infected cells by more than 50%, as well as inhibited PrP^{res} formation in a cell-free assay where sCJD brain homogenate was used as seed.

Next steps in your work: Currently we are assessing the efficacy of LC19 and LC87 in prion infected cerebral organoids ("mini human brains"). Natalia C. Ferreira and colleagues just reported the use of the cerebral organoids model as a valuable tool to screen for anti-prion drugs (Groveman & Ferreira, 2021 – Scientific Reports) with funding provided by this grant. Next, we aim to test LC19 and LC87 in prion-infected mouse.

Due to COVID-19 the progress of this study was delayed and because of that, animal experiments were not completed yet. We appreciate the financial support and in a near future we intend to share the final results of this study.