2025 IMPACT CIRCLE

Project Title: Gene therapy to boost proteostasis and extend brain healthspan

Investigator(s) and Collaborations: Claudio Hetz PhD, Professor in Residence. CoPI: Lisa Ellerby (Buck Institute). Collaborators: Tara Tracy and David Furman (Buck Institute).

Unmet Need/Primary Question: Decades of research have defined the hallmarks of aging, biological processes that determine when and how organisms age (1,2). Brain aging is the main risk factor for developing mild cognitive impairment and neurodegenerative diseases. At the root of the aging process, it is proposed that the pathways that sustain the health of the proteome (termed "proteostasis") are substantially altered in the elderly (3). Proteostasis requires complex and dynamic coordination between all molecular processes involved in the production of functional proteins (4). One of the main modules of the proteostasis network altered during aging involves the function of a cellular organelle known as the endoplasmic reticulum (ER), the "protein factory" of the cell. When abnormal proteins accumulate inside the ER, a repair and adaptive pathway is activated known as the unfolded protein response (UPR) (5, 6). Adaptation and resilience against proteotoxic stress requires the reprogramming of gene expression to stablish efficient repair responses that improve the activity of multiple processes involved in proteostasis. In fact, the UPR is governed by the concert action of three main transcription factors (regulators of gene expression), known as ATF6, XBP1 and ATF4. Our laboratory has been pioneering in defining the contribution of the UPR to brain diseases, normal brain function and more recently brain aging. **We have developed different technologies of gene therapy to artificially boost the proteostatic capacity of the brain by delivering active components of the UPR** (4 patents licensed to UCB, Belgium). Our strategy has been successful in restoring synaptic function and the cognitive capacity of aged animals, correcting around 70% of the natural proteomic alterations observed in the aged hippocampus (7). This technology also alleviated age-related diseases in experimental models of models of Alzheimer's disease, ALS, and frontotemporal dementia (18,19). We are currently exploring a new component of the UPR, ATF6, which has remained completely unexplored in aging and brain function (**Figure 1**, annex) (8, 9). The contribution of ATF6 to human diseases has been explored only recently, showing beneficial roles in different contexts (10), including retinal degeneration (11), autoimmunity (12), heart disease (13, 14) and stroke (15). We obtained important preliminary evidence indicating that the artificial delivery of the active form of ATF6 into the brain using a gene therapy (17) improves memory of ages animals, associated with enhanced synaptic activity (unpublished). In these experiments, we administrated genetically modified adeno-associated viruses (AAV) to carry the ATF6 gene by direct injection into the brain using stereotaxis surgery. In the last 6 months we have advanced in developing an advanced technology, where we use a new viral serotype that can be administrated intravenously, reaching the brain with high efficacy. Using this new approach, we were able to demonstrate that a single injection of this virus results in the detectable expression of ATF6 in the brain and the orchestration of repair programs. These results demonstrate that we have developed a feasible approach to artificially activate the UPR in the brain using a non-invasive gene therapy. In this project, we aim to test the capacity of our UPR-based gene therapy to improve normal brain aging at the level of cognition, synaptic function, and neuronal connectivity.

Novel Hypothesis: The pathways that determine the decay of brain function as we age are not well understood. Thus, it is necessary to evaluate new concepts and identify novel targets to extend and improve our quality of life. Because proteostasis failure is a central pillar of the aging process, and ATF6 is a master regulator of the UPR, we hypothesize that the artificial activation of the ATF6 pathway may delay normal brain aging, having enormous consequences to global body physiology. Here we propose to develop a gene therapy using state-of-the-art technologies to modify the proteostasis capacity of the brain with a single injection of a gene therapy aiming to extend brain healthspan and reduce the risk of developing disease.

Project Proposal: We plan to determine the significance of the ATF6 pathway as a central component antagonizing brain aging (see **Figure 1**). Our supporting data indicates that the delivery of active ATF6 into the hippocampus of mice using AAVs improves cognition and synaptic plasticity of aged animals. Our experimental strategy will include the establishment of a collaborative network with Buck researchers to test the efficacy of a gene transfer approach to artificially engage the ATF6 pathway in the brain of aging mice by the intravenous administration of recombinant AAVs. Our study will include the determination of the cognitive capacity of aged mice (Lisa Ellerby lab), synaptic activity (collaboration Tara Tracy), in addition to assessing the "biological age (clock)" of the brain tissue using gene expression profiling (collaboration David Furman) and canonical markers reflecting the quality of the aging process (senescence and proteostasis).

Description of Potential Impact: The brain is a complex organ that controls thought, memory, emotion, motor skills, vision, and every process that regulates our body. As we age, the function of our brain decays, having enormous consequences to our quality of life. The progressive loss of the proteostasis capacity contributes to neuronal dysfunction and may represent one of the main risk factors to accumulate abnormal proteins involved in neurodegenerative diseases (3). We have undertaken a **non-conventional approach** to address this fundamental question where instead of targeting one gene (the classical approach of pharmaceuticals), we plan to engage a full network of processes that as a whole will improve neuronal proteostasis and cell function (holistic view). We believe that the concept behind our possible findings is "**rejuvenation"**: we aim to artificially engage a repair capacity that young cells naturally have and that is lost as we age. We predict that ATF6-dependent transcriptional programming will enforce the production of healthy proteins (i.e. synaptic proteins, connectivity factors, etc.). Gene therapy is one of the most exciting areas under development in biomedicine, receiving most of the current investments. Gene therapy is simple, safe, and feasibly demonstrated by the constant approval of technologies based on the use of recombinant AAVs by the FDA. Outstanding examples are available where multiple genetic diseases were corrected, and a lethal disease such as spinal muscular atrophy (SMA) was cured by a single injection of AAVs gene. Now the challenge is to use these new technologies to modify the normal course of aging with the aim of extending our healthspan and improving our quality of life to avoid disease. This concept represents a new frontier of science that we are currently pursuing at the Buck.

References: (1) Nature. 571:183-192. **(2)** Cell. 184:1929-1939. **(3)** Cell. 184:1545-1560. **(4)** Science. 319(5865):916-9. **(5)** Science, 334: p. 1081-6. **(6)** Nat Rev Mol Cell Biol, 21: p. 421-438. **(7)** EMBO J. 41:e111952. (8) Cell structure and function 33: p. 75-89. **(9)** Mol Biol Cell 10: p. 3787- 99. **(10)** Trends Mol Med, 25: p. 538-550. **(11)** Invest Ophthalmol Vis Sci, 53(: p. 7159-66. **(12)** PNSA 111: p. 13046-51. **(13)** Circ Res, 98: p. 1186-93. **(14)** Nat Commun 10: p. 187. **(15)** J Cereb Blood Flow Metab 37: p. 1069-1079. **(16)** Elife, 2016. 5. **(17)** Mol Ther, 29: 1862-1882. **(18)** Mol Ther. 31:2240-2256. **(19)** Mol Ther. 2025 33(3):1226-1245.

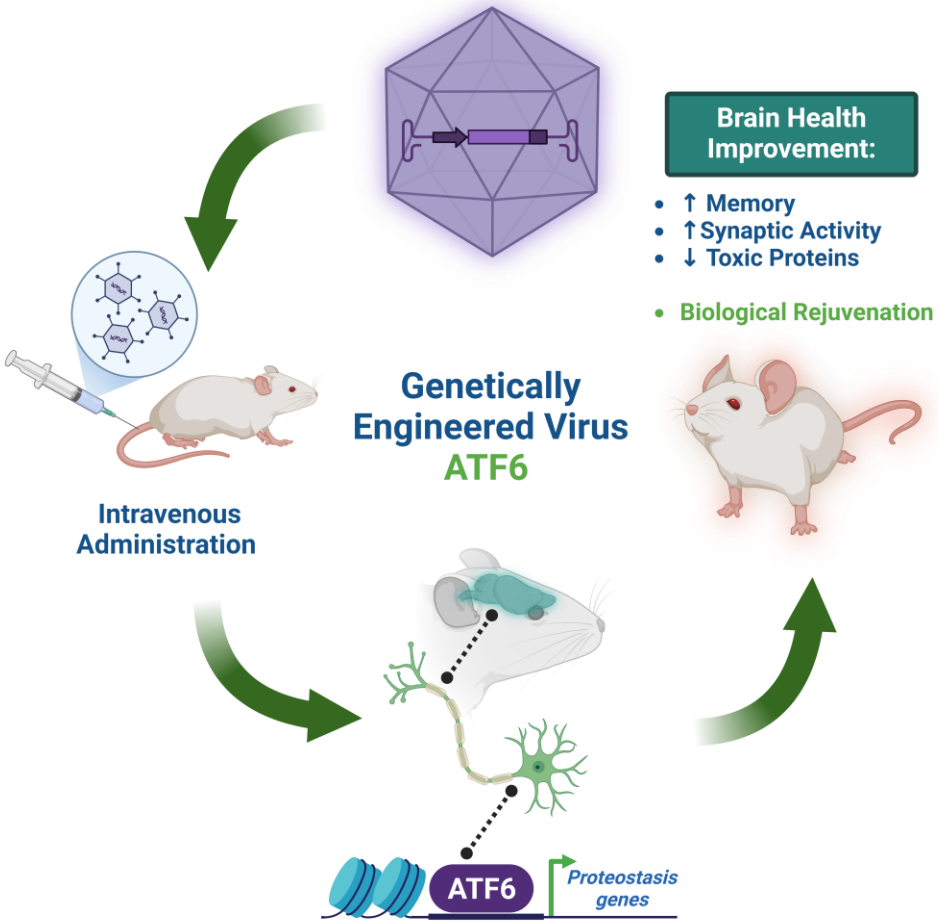

Figure 1. Idea and workflow: We plan to define the efficacy of administrating a gene therapy based on the use of recombinant AAV (AAVPHP.eB serotype, the ATF6f transgene controlled with the neuronal synapsin promoter for safety and specificity). We plan to determine the consequences of inducing ATF6-deppedent gene expression programs in the aging brain by the intravenous injection of a single dose of AAVs. We already demonstrated that this virus reaches the brain and engages UPR repair programs. We plan to develop a 10-month project (proof-of-concept) to test strategies to artificially engage the UPR and restore the buffering capacity of the proteostasis network to restore neuronal function. Animals of 18 months of age that already show cognitive impairment, synaptic alterations and accumulation of senescent cells (see our characterization in (7)) will be treated with AAV-ATF6 or control virus. 3 and 6 months later, cognitive assessment will be performed (new object location (NOL) and new object recognition (NOR)), in addition to electrophysiological measurements in hippocampal slices (long term potentiation (LTP)) and morphological characterization (dendritic spines, senescent cell content), and gene expression profiling to determine aging clocks and the proteostasis status.