2024 IMPACT CIRCLE

Project Title: Reprogramming the Proteostasis Network to Sustain Brain Function During Aging and Extend Human Healthspan.

Investigator(s) and collaborations: Claudio Hetz, PhD, Professor in Residence. Collaborators: Birgit Schilling, PhDg, Simon Melov, PhD, and Lisa Ellerby, PhD (Buck Institute), and Luke Wiseman, PhD (Scripps 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 to develop mild cognitive impairment and neurodegenerative diseases, associated with the abnormal deposition of protein aggregates in the brain. Thus, the pathways that sustain the health of the proteome (termed "proteostasis") are substantially altered in the elderly (3). Proteostasis requires the complex and dynamic coordination between folding, quality control and degradation mechanisms to reduce the load of abnormal and noxious proteins (4). One of the main nodules of the proteostasis network altered during aging involves the function of the endoplasmic reticulum, a central organelle for protein production in the cell. When misfolded proteins accumulate inside the ER, a dynamic signaling network known as the unfolded protein response (UPR) is activated, which aims to balance protein production and sustain cell function (5, 6). Adaptation and resilience against ER stress requires the reprograming of gene expression to stablish repair responses that improve the activity of multiple processes involved in proteostasis control. In fact, the UPR is governed by the concert action of three main transcription factors, ATF6, XBP1 and ATF4, regulating distinct subsets of target genes. Our laboratory has been pioneering in defining the role of the UPR in brain diseases, normal brain function and more recently brain aging. **We developed a successful gene therapy to artificially boost the proteostatic capacity of the brain by delivering an active form of the transcription factor XBP1** (technology patented and liscenced to UCB, Belgium), which restored synaptic function and the cognitive capacity of aged animals (7). We recently used the same technology in models of Alzheimer's disease, and obtained outstanding results in improving memory capacity (18). However, ATF6 has remained completely unstudied in aging and brain function. Upon ER stress, ATF6 is cleaved and activated as a potent transcription factor that translocates to the nucleus to engage gene expression programs involved in folding, quality control and protein degradation pathways (**Figure 1**, annex) (8, 9). The role of ATF6 in human diseases has been poorly explored until recently, where targeting this pathway is beneficial in different context (10), including retinal degeneration (11), autoimmunity (12), heart disease (13, 14) and stroke (15). Our collaborator Luke Wiseman (Scripps Institute) discovered compound 147, a selective molecule that activates ATF6 to artificially boost the UPR, that protects the heart and brain from

stroke (14). We have also developed a different approach to artificially deliver the processed active from of ATF6 into the brain using a gene therapy approach (17), showing outstanding effects that are superior activity to XBP1 in different diseases (unpublished). Thus, ATF6 represent a completely unexplored new pathway that could be intervened to improve brain healthspan. We have obtained preliminary evidence indicating that the administration of adeno-associated viruses (AAV) to express active ATF6 into the hippocampus of mice recovers the defects in memory and synaptic plasticity naturally occurring in aged animals.

Novel Hypothesis: The pathways that determine the decay of brain function as we age are poorly understood. Thus, it is necessary to evaluate new concepts and identify novel targets. Because proteostasis failure is a central pillar of the aging process, and ATF6 is a master regulator of the UPR, we propose that the artificial activation of the ATF6 pathway may delay normal brain aging. Here we propose to uncover the possible contribution of the ATF6 signaling branch to brain aging and develop a proof-of-concept study aiming to globally improve neuronal proteostasis to extend brain healthspan.

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 and external collaborators to test a pharmacological strategy and a gene transfer approach to artificially engage the ATF6 pathway in the brain of aging mice, followed by a functional analysis of cognition, proteomic changes and histopathological alterations including the content of senescent cells. We aim to deliver AAVs into the ventricle of aged mice (18 months) to express ATF6 in neurons or administrate intravenously compound 147 and assess cognitive and motor tests followed by electrophysiological (LTP) and detailed morphological characterization of dendritic spines, senescence, inflammation and neuronal health. Finally, we aim to determine the global impact of ATF6 expression on the proteomic changes observed in the hippocampus during aging as we recently reported on a large brain aging study (7).

Description of Potential Impact: The brain is a complex organ that controls thought, memory, emotion, touch, motor skills, vision, breathing, temperature, hunger and every process that regulates our body. As we age, the function of our brain decays, having enormous consequences in our quality of life and our families. The progressive loss of the proteostasis capacity contributes to neuronal dysfunction and may represent one of the main risk factors to accumulate abnormal damaging proteins that trigger neurodegenerative diseases (3). Understanding the pathways that mediate brain aging are fundamental to intervene the process to sustain health. We have undertaking a **non-conventional approach** to address this fundamental question where instead of targeting one gene (the classical approach of pharmaceuticals and research labs), 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"**: <u>we aim to artificially engage a repair capacity that young cells naturally have and that is loss as we age</u>. We predict that ATF6-deppedent transcriptional programs will enforce folding, quality control, protein degradation pathways, and other unknown processes to be uncovered, that altogether will improve neuronal function and produce healthy proteins (i.e. synaptic proteins). Since gene therapy with

AAVs is a reality in the US due the recent approval of several technologies, if this proposal is successful (and follow-up initiatives), this knowledge may translate into the development of cutting-age approaches to maintain brain function as we age.

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

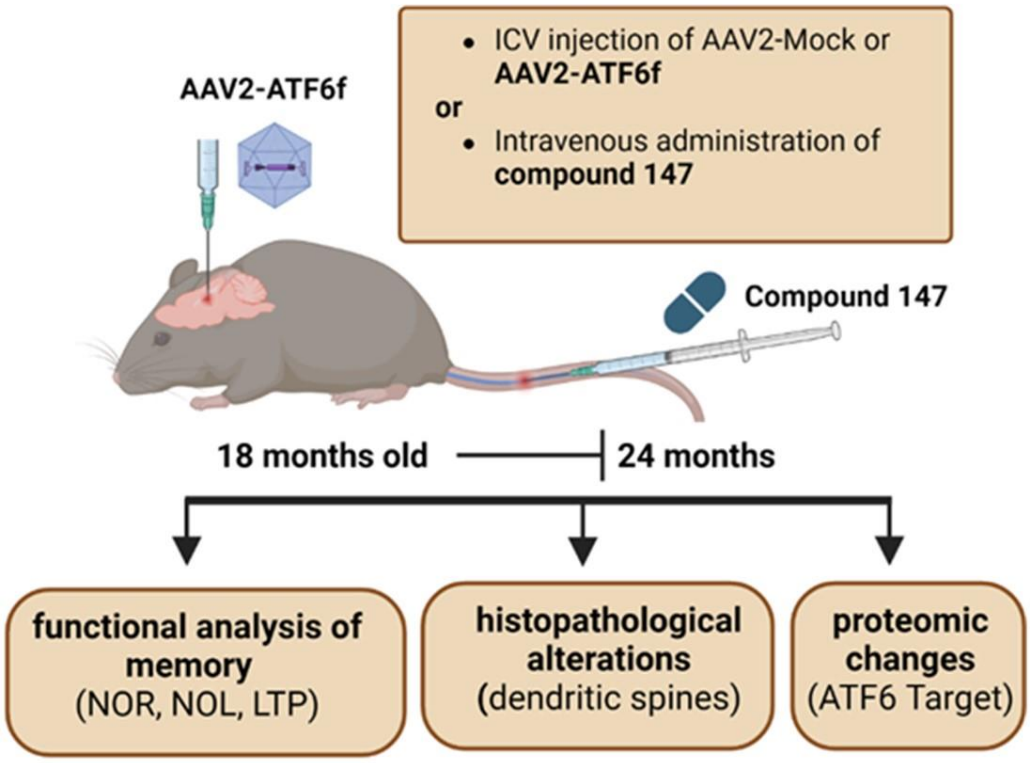

Figure. Idea and workflow: We plan to define the efficacy of targeting the UPR transcription factor ATF6 as a strategy to improve brain function during normal aging and how it determines the functional decay on its function. We plan to develop a 1-year project (proof-of-concept) to test strategies to artificially engage the UPR using pharmacological and gene therapy strategies and restore the buffering capacity of the proteostasis network to restore neuronal function. Animals of 12 months of age that already show cognitive impairment, synaptic alterations and accumulation of senescent cells (see our characterization in (7)) will be manipulated with two approaches to artificially engage the ATF6 pathway and improve proteostasis: a gene therapy (stereotaxis injection of AAV2-ATF6f into the hippocampus) and a pharmacological strategy (IV delivery 3 times per week of compound 147) to either deliver active ATF6 or induce the activation of the pathway. 6 months later, a 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), and protein expression profiling (proteomics, collaboration with B. Schilling).