therapy and/or CRISPR based technologies. With >1800 gene therapies in phase I/II clinical trials, development of non-invasive methods to assess gene expression is crucial to the future of the gene therapy field.

240. Inclusion of a Degron Reduces Levels of Undesired Inteins after AAV-Mediated Protein *Trans*-Splicing in the Retina

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Split intein-mediated protein *trans*-splicing expands AAV gene transfer, thus overcoming AAV limited cargo capacity. However, non-mammalian inteins persist as *trans*-splicing by-products, and this could raise safety concerns for AAV intein clinical applications. In this study, we tested the ability of several degrons to selectively decrease levels of inteins after protein *trans*-splicing, and found that a version of *E. coli* Dihydrofolate reductase, that we have shortened to better fit into the AAV vector, is the most effective. We show that subretinal administration of AAV intein armed with this short degron is both safe and effective in a mouse model of Stargardt disease (STGD1) which is the most common form of inherited macular degeneration in humans. This supports the use of optimized AAV intein for gene therapy of both STGD1 and other conditions which require transfer of large genes.

241. Drug-Regulated Splicing Switch for Gene Expression Control

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To date, gene therapies for human application rely on engineered promoters that cannot be finely controlled. Here, we report a universal switch element that allows precise control for gene silencing or gene replacement after exposure to a small molecule. Importantly, these small molecule inducers are in human use, are orally bioavailable when given to animals or humans, and can reach both peripheral tissues and the brain. Moreover, the switch system (Xon) does not require the co-expression of any regulatory proteins. Using Xon, translation of desired elements for gene knockdown or gene replacement occurs after a single oral dose, and expression levels can be controlled by drug dose or in waves with repeat drug intake. This universal switch can provide temporal control of gene editing machinery and gene addition cassettes that can be adapted to cell biology applications and animal studies. Additionally, due to the oral bioavailability and safety of the drugs employed, the Xon switch provides an unprecedented opportunity to refine gene therapies for more appropriate human application.

242. Targeted Gene Therapy with Engineered Systemic AAVs for the Central and Peripheral Nervous Systems Prevents Motor Coordination Phenotypes in a Mouse Model of Friedreich's Ataxia

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Friedreich's Ataxia (FA) is a genetic disease characterized by progressive ataxia and cardiomyopathy. In FA, insufficient expression of frataxin (FXN) increases cell susceptibility to damage from oxidative stress. Neuronal degeneration in the deep cerebellar nuclei and dorsal root ganglia (DRGs) cause loss of motor coordination, while cardiomyopathy in FA contributes to early death. Systemically delivered AAVs can non-invasively target genetic cargo to diverse sites throughout the body, with some serotypes able to reach the CNS. However, at high doses, these vectors can cause severe toxicity, emphasizing the need for targeted, efficient gene delivery vectors. In this study, we used engineered AAVs to target FXN to cell types of pathophysiologic relevance to FA, while de-targeting the liver and cell types typically spared in human disease. A DNA plasmid containing FXN and its putative gene regulatory elements and control constructs were packaged into AAV.CAP-B10[1] and AAV-PHP.PNS2, two next generation systemic AAVs that have demonstrated transduction biases toward CNS and PNS respectively. A cocktail of both capsids packaging either the FXN or control cargo were delivered intravenously to a pilot cohort of young adult FA model mice (inducible shRNA-based FXN knockdown mice (FXNiKD)[2], n=7 per group) 12 weeks prior to doxycycline-induction of the disease phenotype. Motor, sensory and cardiac function was assessed using the beam traversal test, gait analysis, weightlifting and electrocardiography before quantitative tissue analysis was performed to assess FXN levels and pathologic hallmarks. Exogenous FXN expression mimicked the endogenous patterns of non-diseased mice in the CNS and DRGs, with reduced liver expression. Importantly, prophylactic AAV-FXN expression prevented induction of motor and coordination deficits as measured by foot slips and time to traverse the narrowing beam, compared to controls, with performance resembling wildtype littermates. We are investigating whether it is also possible to reverse existing motor deficits and pathology in mice using targeted AAV-FXN intervention after induction of the disease phenotype. These findings demonstrate the utility of engineered AAVs for pre-clinical research to test precision gene therapies in neurodegenerative disease models.[1] Flytzanis, N.C., Goeden, N., Goertsen, D., Cummins, A., Pickel, J., Gradinaru, V. Broad gene expression throughout the mouse and marmoset brain after intravenous delivery of engineered AAV capsids. bioRxiv 2020.06.16.152975; doi: https://doi.org/10.1101/2020.06.16.152975 [2] Chandran V, Gao K, Swarup V, Versano R, Dong H, Jordan MC, Geschwind DH. Inducible and reversible phenotypes in a novel mouse model of Friedreich's Ataxia. Elife. 2017 Dec 19;6:e30054. doi: 10.7554/eLife.30054. PMID: 29257745; PMCID: PMC5736353.