# Researchers develop 'dimmer switch' to help control gene therapy

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In a major advancement in the field of gene therapy for rare and devastating diseases, researchers at Children's Hospital of Philadelphia (CHOP) have developed a "dimmer switch" system that can control levels of proteins expressed from gene therapy vectors. The system is based on alternative RNA splicing using an orally available small molecule and works effectively in tissues throughout the body, including the brain. The first research regarding this innovation was published today in the journal *Nature*.

"We're taking the field of gene therapy to an entirely new level where fine-tuned dosing is required for safety, utility and success," said senior study author Beverly L. Davidson, Ph.D., Director of the Raymond G. Perelman Center for Cellular and Molecular Therapeutics and Chief Scientific Strategy Officer at Children's Hospital of Philadelphia. "This study shows that by using a splicing modulator in combination with gene therapy tools, the dose of protein expressed from gene therapy vectors can be controlled for maximum therapeutic benefit."

Many advancements in gene therapy have involved its delivery system, in the form of engineered viral vectors or lipid nanoparticles, but while improvements in these vehicles have delivered treatments to tissues more effectively, the cargo being delivered and elements controlling the resulting gene expression have not received the same amount of attention. Once gene therapy has been successfully delivered into the tissue, it is difficult to regulate the levels of expression. Too much expression may have toxic effects on the patient, and too little expression may mean that the patient does not receive the intended benefits of the therapy.

To address this problem, CHOP researchers developed a delivery system called the $X^{on}$ system, which can finely control protein translation by using a "dimmer switch" to adjust the levels of expression up or down as needed. This method employs alternative RNA splicing, a process that allows a single gene to code for multiple proteins, depending on how the RNA is spliced. Using the $X^{on}$ system, a gene therapy vector's cargo is inactive until the oral drug is used, which then drives the splicing of the desired corrective gene into its active form.

"The newly developed switch not only controls protein levels, but if needed, those proteins can be induced again and again by the simple ingestion of an orally bioavailable drug," said Alex Mas Monteys, Ph.D., a research assistant professor in Davidson's lab at CHOP and co-lead author of the study.

In one example reported in this paper, the researchers used the $X^{on}$ system in mice to adjust levels of erythropoietin (Epo), which is used to treat anemia associated with kidney disease. The researchers found that their delivery system induced hematocrit levels to 60 to 70% above baseline levels depending on the dose, and once levels slowly dropped to base levels, the system could be used again to safely re-induce the levels as would be needed for patients with chronic kidney disease.

The research was conducted as part of a multi-year collaboration with scientists at the Novartis Institutes for BioMedical Research (NIBR). CHOP and NIBR are collaborating to develop next-generation small molecule splicing modulators and the $X^{on}$ system to achieve fine-tuned gene regulation across multiple clinical applications. The team has also shown that the $X^{on}$ system can be used to control expression of gene products that are toxic to the brain when expressed at high levels.

"The dose of a drug can determine how high you want expression to be, and then the system can automatically 'dim down' at a rate related to the

half-life of the protein," Davidson said. "We can envision scenarios where a drug would be given only once, such as for controlling the expression of foreign proteins needed for gene editing, or with limited frequency. Since the splicing modulators we have tested are given orally, compliance to control protein expression from viral vectors employing $X^{on}$-based cassettes should be high."

Although the paper focuses on using $X^{on}$ with gene therapy delivered via viral vectors, the researchers note it could also be engineered for use in cell engineering for CAR-T cell therapy. Here, the $X^{on}$ system could be used to pause therapy if needed to give T-cells a rest.

**More information:** Regulated control of gene therapies by drug-induced splicing, *Nature* (2021). DOI: 10.1038/s41586-021-03770-2 , www.nature.com/articles/s41586-021-03770-2

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