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Breathing new life into in vivo lung editing

New lipid nanoparticle designs make targeted in vivo lung editing possible in mice and may lead to new treatment options for cystic fibrosis.

Casgevy, the world's first approved CRISPR therapy for sickle cell disease and β-thalassemia, is an ex vivo therapy – hematopoietic stem cells are isolated from the patient, edited, quality controlled and then put back in the body after the patient has undergone chemotherapy. Ex vivo therapy is able to treat these diseases because the target cells can be isolated and returned safely to the body. However, this is not possible for the majority of our tissues, such as the lung. The continued success of CRISPR therapies relies on developing efficient and safe delivery systems that can enable CRISPR to perform editing in vivo, within targeted tissues. A paper published in *Science* from Sun and colleagues enables an important step forward in the in vivo space, delivering CRISPR base editors into the mouse lung, successfully opening up new avenues for treating cystic fibrosis[1].

The first wave of gene therapies to gain regulatory approvals used adeno-associated virus (AAV) vectors for delivery[2]. These protein shells contain about 4.8 kb of DNA and show high infectivity, as well as long-term gene expression, and they naturally target specific tissues in the body: the liver, lungs and central nervous system. However, early clinical trials using AAV for gene therapy showed dose-limiting liver toxicity[3]. Long-term expression of Cas9 may also result in genome integration and off-target activity. Also, CRISPR components can be large, and AAVs are limited in what they can carry.

An alternative to AAVs are assemblies of viral proteins called virus-like particles (VLPs), which contain ribonucleoproteins and can infect cells, but lack viral genetic material, avoiding any risks of viral genome integration. Engineered VLPs (eVLPs) that contain different envelope glycoproteins have been shown to deliver Cas9, CRISPR base editors[4] and prime editors[5] to organs such as the brain, liver and retina. Nvelop Therapeutics launched in 2022, aiming to continue the work on targeting eVLPs to more tissues, although it is not clear whether they will target the lung.

The third option for delivery is engineered lipid nanoparticles (LNPs), which avoid the technical limitations of viral vectors – they can contain larger cargos and are less immunogenic. LNPs are typically made up of an ionizable lipid, a helper lipid, a polyethylene glycol lipid and cholesterol, and the ratio of these components affects the size, shape, charge and stability of the particle. The COVID mRNA vaccine proved LNPs to be safe and effective through repeatable dosing. LNPs have their own set of limitations – their transfection efficiency is lower than an AAV's, and when injected intravenously, they are sequestered and accumulate in the liver.

While there are promising clinical trials that take advantage of the natural hepatic targeting of LNPs, targeting other cell types within the body has proven difficult. The fastest way to target the lung is through inhalation, and gene therapy is no exception. However, any delivery vehicle must bypass a barrier made up of mucus secretions, tightly bound epithelial cells and other factors to reach the target stem cells that are located at the epithelium base. The resident macrophages in the airway lumen make delivery more difficult, and the lung epithelial cells contain oscillating cilia that help to trap and clear foreign particles.

A recent study published in *Nature Biotechnology* tested 720 new lipids in LNP formulations and showed that certain cationic LNP lipids could increase the transfection efficacy of LNPs in the lung following intratracheal dosing[6]. This study also showed that LNPs are amenable to repeat dosing, further improving gene editing efficiency – a substantial advantage compared with viral vectors. Inhalation of LNPs may be the quickest route of delivery to the lung epithelium, but in many diseases, such as cystic fibrosis, the mucus layer of the lung is thick and sticky and blocks effective drug delivery. More than 27 clinical trials for cystic fibrosis gene therapies have failed to get past the epithelial barrier.

Sun and colleagues in *Science* engineered their gene therapy with LNPs that are delivered intravenously, avoiding the mucus barrier entirely. The LNP formulation has optimized membranes for targeting specific organs, similarly to eVLPs[7]. In this case, the particles are targeted to the lung by a protein corona highly enriched in vitronectin[1], a receptor expressed by a high percentage of lung cells. As less than 4% of cells throughout the rest of the body express this receptor, it minimizes off-target effects. The researchers use the SORT LNP delivery system to deliver a CRISPR base editor to a mouse model of cystic fibrosis, correcting a targeted pathogenic mutation in lung stem cells in a single treatment and restoring cystic fibrosis transmembrane regulator (CFTR) function. Editing was maintained for 22 months as the stem cells differentiated and matured. This study has been performed only in mice, but ReCode Therapeutics is collaborating with Intellia Therapeutics to move the SORT LNP system forward in the clinic.

Today, patients with cystic fibrosis have better lung function and live longer compared to 20 years ago. This is because 90% of people with cystic fibrosis carry at least one copy of a common mutation in *CFTR* and respond well to small molecule drugs that boost and modulate the functionality of the mutated protein. Vertex Pharmaceuticals won the 2024 Breakthrough Prize in Life Sciences for the development of these small molecule drugs, and the company is seeking approval for a triple-combination CFTR modulator therapy that has recently been shown to outperform the current triple-combination and widely approved therapy Trikafta (elexacaftor/tezacaftor/ivacaftor). Other treatments are also available to mitigate the symptoms of the disease – antibiotics, anti-inflammatory medicines and mucus thinners.

Still, the 10% of people with other *CFTR* mutations are not helped by these medications. Some of these rarer mutations could be corrected using base editor or prime editor technologies – if efficient, targeted delivery was possible. A CRISPR therapy would be a single-dose treatment, compared to the twice-daily administration of Trikafta. If proven to work in humans, the gene editing therapy would certainly be expensive (Casgevy is priced at $2.2 million). Trikafta costs about $300,000 annually in the United States, although much of this is covered by insurance, and patients pay on average $1,700 a year. Other medications that alleviate the symptoms of cystic fibrosis are more affordable,

and these treatments are the primary options for patients in lower-income countries.

Vertex is also looking to develop alternative therapies for this 10% of patients — it has launched early-stage clinical trials for an inhalable mRNA therapy with Moderna that would deliver full-length *CFTR* mRNA directly to cells in the lung, also using an LNP. Arcturus Therapeutics has a similar phase 1 study in the pipeline. Neither of these treatments would be a complete genetic correction, but from a patient's perspective, the more options, the better.

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