Gene Editing to Treat Hypercholesterolaemia and Primary Hyperoxaluria

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THE INTERNATIONAL Liver Congress (ILC) 2021 of the The European Association for the Study of the Liver (EASL) invited three speakers to discuss their research on gene editing. The researchers discussed how to treat hypercholesterolaemia (HC) and primary hyperoxaluria Type 1 using the latest technology in gene editing.

Editing the genome was once believed to be a science-fiction scenario. However, in the past decade, developments in science and technology have led to innovative gene-editing techniques that have made repairing the function of faulty genes a reality. Pioneering scientists are continuously expanding the genetic toolbox, such as last year's Nobel prize winners, Emmanuelle Charpentier and Jennifer Doudna, who were recognised for their ground-breaking discovery: CRISPR-CAS9. These molecular scissors have the ability to accurately edit the genome. The development of CRISPR-CAS9 has now offered the exciting potential of a precise and effective solution for treating many genetic diseases in the future.

ILC 2021 invited three speakers to discuss their research in gene editing to treat liver diseases. The first of the speakers, Angelo Lombardo from HSR-TIGET, Italy, discussed current gene-editing techniques and new approaches such as epigenetic silencing. He explained that there are two main ways to edit genes: gene knock-out and gene insertion. Gene knock-out is the most common technique. This can have toxic effects as cells do not react well to an induced strand break. Therefore, scientists are researching new ways of editing genes; one of these ways is epigenetic silencing.

EPIGENETIC SILENCING TO TREAT HYPERCHOLESTEROLAEMIA

Epigenetic change is a natural process that allows genes to be switched on or off in cells without altering the genetic code. There are a few ways this can be done such as histone modification and DNA methylation. Unlike a gene knock-out, epigenetic changes are mostly transient, and the effects can be reversed.

Lombardo explained how epigenetic silencing can be used in the context of treating liver disease,

specifically HC. HC is characterised by high levels of low-density lipoprotein (LDL) in the blood. This leads to the formation of plaques in the arterial wall and the development of coronary heart disease. Patients need to adjust their lifestyle and rely on medications such as statins to improve their condition. At present there is no cure for HC, which is why there is a pressing need for novel treatment approaches.

HC can be caused by genetic mutations in the LDL receptor or the *PCSK9* gene, which codes a protein secreted by hepatocytes in the liver. This protein promotes clearance of the LDL receptor and removes the receptor from the cell membrane, thereby preventing the uptake of LDL into the cell. Normally, this is a balanced process; however, with a gain-of-function mutation in the *PCSK9* gene, more cholesterol accumulates in the blood, resulting in HC. On the contrary, loss of function of the *PCSK9* gene has been shown to protect individuals from coronary heart disease.

Ongoing clinical trials involve inhibiting the *PCSK9* gene via several techniques such as use of a monoclonal antibody. Although these trials are producing promising results, a downside to these techniques is that the therapies must be provided regularly, i.e., every 3 months. This signifies the importance of looking at alternative options. Lombardo believes gene editing is something to consider for treating HC as a 'one and done' approach.

Lombardo explained how scientists can target induced local lesions of the *PCSK9* promoter using guide RNA, specifically in the section rich in cytosine and guanines. The engineered transcriptional repressors recognise the promoter and can silence the gene. The data showed that some guide RNAs are very effective at influencing epigenetic silencing to the same degree of efficacy as gene editing. However, epigenetic silencing has the added benefit of being transient and not genotoxic. To determine the specificity of epigenetic silencing, Lombardo conducted RNA sequencing; the results showed that only the *PCSK9* gene is downregulated in gene silencing. Further to this, Lombardo noticed that there was a high level of DNA methylation at the *PCSK9* promoter, showing that epigenetic silencing is highly specific.

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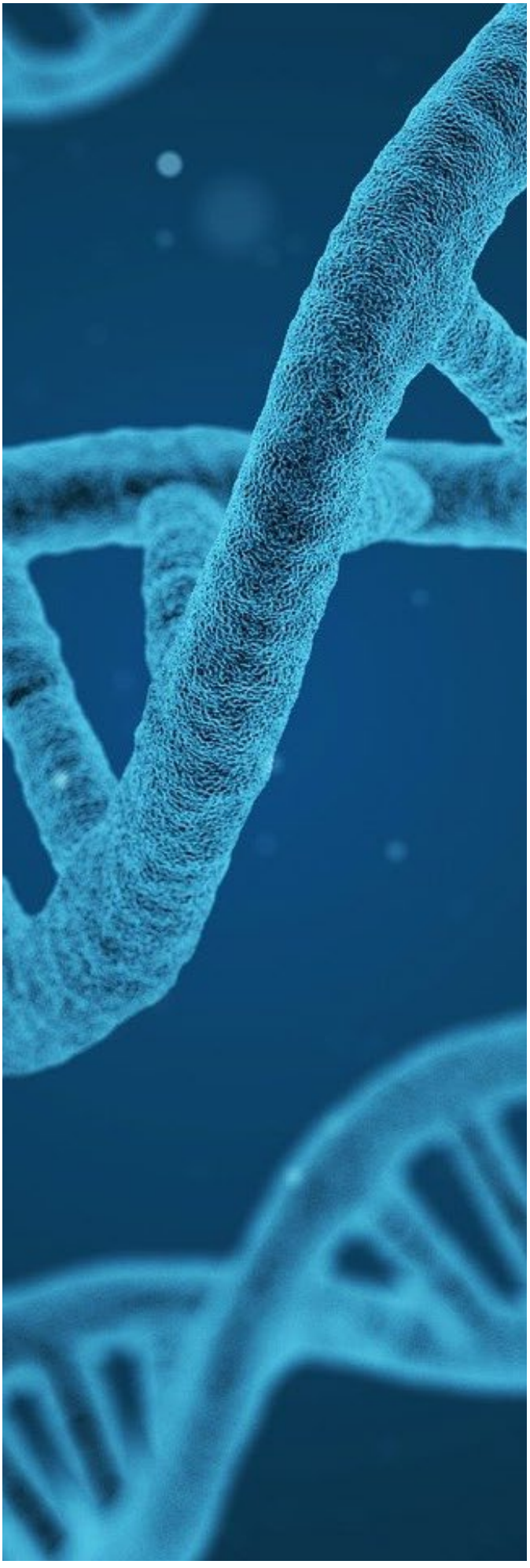

GENE EDITING TO TREAT PRIMARY HYPEROXALURIA TYPE 1

Gloria Gonzalez from the University of Navara, Spain, shared her exciting study in which she and her group used CRISPR-CAS9 to delete glycolate oxidase to treat primary hyperoxaluria Type 1. Hyperoxaluria is a serious metabolic condition that causes recurring kidney and bladder stones and, if left untreated, can lead to end-stage renal disease. Treatment involves a less than ideal liver–kidney transplant. The condition is an autosomal recessive disorder caused by a mutation in the enzyme mainly expressed in the liver: alanine-glyoxylate aminotransferase (AGT).

To understand how a mutation in AGT can lead to hyperoxaluria, Gonzalez explained the molecular biology of this metabolic pathway. In the liver cells, ethylene glycol is converted to glycolate, which in turn is converted to glyoxylate by the enzyme glycolate oxidase (GO). Finally, glyoxylate is converted to glycine by AGT and glycine is expelled through the urine. However, a mutation in the *AGT* gene prevents the final step from occurring and results in a build-up of the precursor molecule, glyoxylate. Glyoxylate cannot be secreted via the urine and is instead converted to oxalate. High levels of oxalate result in the formation of painful kidney stones.

One method to treat this condition is to inhibit GO, as this reduces the production of glyoxylate and consequently oxalate. Inhibiting GO leads to a build-up of glycolate; however, this is harmless as it can easily be removed through the urine. A successful clinical trial showed the efficacy and safety of using small interfering RNA bound to a linker to reversibly silence GO expression in hepatocytes. This inspired Gonzalez and her team to design a system to permanently reduce the expression of the GO protein using CRISPR-CAS9. The group designed two guide molecules to target the murine *hydroxyacid oxidase 1 (Hao1)* gene and used a viral vector to deliver the *Hao1* gene along with the *Staphylococcus aureus* Cas9 enzyme. Gonzalez treated primary hyperoxaluria mice with either a vector carrying guide 1 or a vector carrying guide

2. The team used saline and a Cas9 vector as a control. The mice were sacrificed, and the GO mRNA was analysed using reverse transcription polymerase chain reaction. There was a significant reduction in the expression of GO mRNA in the mice that had been given the guides.

Furthermore, primary hyperoxaluria mice were challenged with ethylene glycol, a molecule that increases oxalate production, for 7 days. The urine of the mice was analysed, and the results showed oxalate was significantly reduced in treated mice compared to wild-type and control mice. More importantly, the scientists discovered that only 1 out of 8 mice developed mild kidney stones, reinforcing the efficacy of mice treated using CRISPR-CAS9.

Finally, Gonzalez and colleagues conducted a molecular analysis of the CRISPR-CAS9 deletion and observed a high editing frequency in both guides. Analysing the pattern of the indel size, the researchers found that each guide had a different effect: Guide 1 resulted in the insertion of 1 nucleotide whereas guide 2 resulted in the deletion of 2 nucleotides. This difference prompted scientists to consider introducing both guides at the same time. The results demonstrated a clear decrease in GO when the two guides were given together with CRISPR-CAS9. Interestingly, at the genome level in >98% of cases, Gonzalez observed a precise deletion of 64 base pairs.

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LIPID NANOPARTICLES TO DELIVER GENE-EDITING TOOLS

Gonzalez and Lombardo both highlighted how gene editing can be used to treat liver diseases in the future. It is equally important to consider how these genetic tools can be accurately and safely delivered to the correct cell. The final speaker, Roy Van Meel from Eindhoven University of Technology, the Netherlands, discussed recent research in this field and how lipid nanoparticle technology can be used to deliver the genetic cargo. Lipid nanoparticles (LNP) are composed of four molecules: phospholipid, cholesterol, polyethylene glycol (PEG)-lipid, and ionisable cationic lipid. These lipids create a protective barrier around the gene-editing tools. Van Meel explained the mechanism by which LNP is taken up into the hepatocytes. Upon injection, the PEG lipids dissociate, and endogenous apolipoprotein-E is recruited to the LNP. The LNP can then pass through the epithelium and bind to the LDL receptor, which allows uptake of the LNP. Endosomal escape is facilitated by the charged lipids, which release the gene-editing contents into the cell cytoplasm.

A study from 2021 evaluated the efficacy of LNPs in delivering CRISPR-based gene editing in primates. The results showed that LNPs allowed a precise introduction of *PCSK9* loss-of-function mutation, which resulted in a significant decrease in LDL cholesterol; thereby proving that LNPs are an effective delivery mechanism. Van Meel further showed how LNPs are versatile and can be used in all types of liver cells. He presented the results of another study that involved using Cre-mRNA inside LNPs and measured cell fluorescence. The authors showed that by increasing the amount of PEG-lipids, the LNPs reduced in size and the smaller particles passed through the endothelium more easily. However, excessively increasing the pegylation prevented uptake entirely. Van Meel concluded that LNPs are successful at delivering gene-editing tools and have a lot of versatility.

This session presented exciting and fresh ideas for treating liver diseases, such as epigenetic silencing. The research presented by Gonzalez provides hope for treating otherwise incurable primary hyperoxaluria using CRISPR-CAS9. This nicely tied in with the valuable insight from Van Meel into the use of LNPs for delivering gene therapy. One exciting prospect discussed was the possibility of using the LNP approach to deliver CRISPR-CAS9 for the treatment of primary hyperoxaluria. Overall, there is a great deal of potential for using gene editing to treat liver diseases, and scientists can learn from each other to optimise the approach. Hopefully, new therapies will come to fruition very soon. ■