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Competing interests
The authors declare no competing interests.

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GENOME EDITING

CRISPR base editing lowers cholesterol in monkeys

Targeting a gene linked to cardiovascular disease shows therapeutic promise.

Sebastiaan Johannes van Kampen and Eva van Rooij

CRISPR base editors are advancing rapidly toward the clinic with the publication of two studies demonstrating successful reduction of blood cholesterol in monkeys. The papers, by Rothgangl et al.[1] in *Nature Biotechnology* and Musunuru et al.[2] in *Nature*, show that transient expression of base editors to knock out a gene associated with cardiovascular disease is effective and safe in non-human primates. Delivery of the base editors to the liver in lipid nanoparticles led to efficient knockout of the target gene—proprotein convertase subtilisin/kexin type 9 (*PCSK9*)—and significant reductions of blood cholesterol, with no substantial off-target editing or serious immune responses. These impressive results suggest that the first cardiovascular gene-editing therapy could rapidly become a reality once the approach is fine-tuned for human testing.

The secreted enzyme PCSK9 is highly expressed in the liver and is important for cholesterol homeostasis. Binding of PCSK9 to the low-density lipoprotein (LDL) receptor results in endocytosis and degradation of the receptor, reducing the uptake of LDL cholesterol particles from the blood[3]. Gain-of-function variants in *PCSK9* have been linked to familial hypercholesterolemia, a condition marked by high circulating LDL cholesterol and high mortality[4]. In contrast, some naturally occurring mutations or knockouts of *PCSK9* lower the risk of cardiovascular disease[5].

Existing treatments for patients with familial hypercholesterolemia include statins, RNA interference (RNAi) therapeutics, and antibodies targeting PCSK9. However, these transient therapies must be taken frequently throughout life and have a high incidence of drug intolerance. Hepatocytes, the main cell type of the liver, are marked by a slow turnover rate and the ability to divide. A one-time administration of gene editing components to permanently inhibit PCSK9 function in the liver could therefore be effective for decades, improving quality of life and reducing healthcare costs.

Gene editing first entered the clinic with zinc finger nucleases[6]. But unlike zinc finger nucleases, CRISPR–Cas9 can be easily reprogrammed to target genomic loci of interest, enabling many academic and commercial laboratories to develop gene-editing therapies. Fusions of cytidine

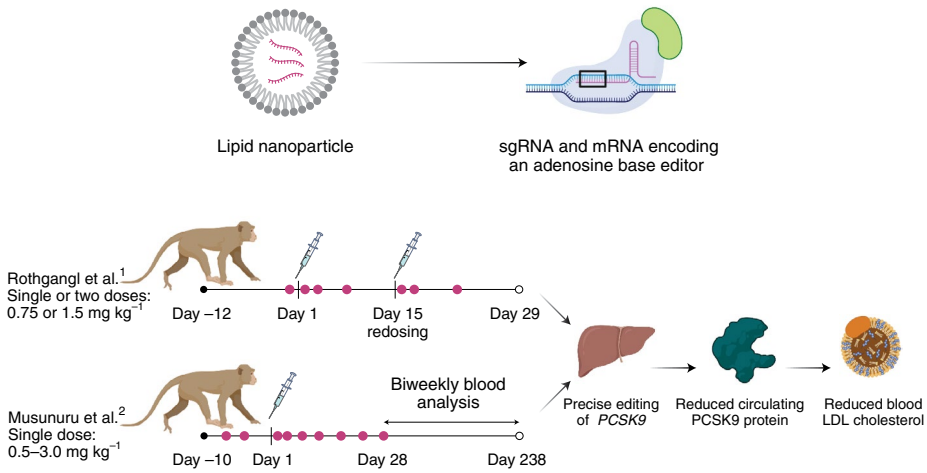

Fig. 1 | Delivery of CRISPR base editing components in lipid nanoparticles for precise genome editing. A single-guide RNA and mRNA encoding a CRISPR base editor are packaged into lipid nanoparticles and administered to cynomolgus macaques. After uptake by hepatocytes, the CRISPR base editor introduces a loss-of-function mutation in the disease-causing gene *PCSK9*, reducing PCSK9 protein levels and LDL cholesterol particles in the blood. In vivo base editing of *PCSK9* in monkeys[1,2] paves the way for precise genome editing in patients. Each dot in magenta represents a time point for sample collection and analysis. LNP, lipid nanoparticle; sgRNA, single-guide RNA; mRNA, messenger RNA; PCSK9, proprotein convertase subtilisin/kexin type 9; LDL, low-density lipoprotein. (Portions created with BioRender.com.)

and adenosine deaminases to Cas9 or to the safer nickase variants of Cas9 catalyze specific C-to-T and A-to-G base conversion in vitro and in vivo[7]. Adenine base editors, in particular, induce few discernible off-target events[8,9]. The ability to introduce specific base changes without making double-strand DNA breaks opens a wealth of opportunities for correcting pathogenic mutations or modulating gene expression[7].

In their study, Rothgangl et al. knocked out *PCSK9* by impairing a GT splice donor site in intron 1. They used lipid nanoparticles to intravenously deliver a chemically modified single-guide RNA together with an mRNA encoding an in vitro–evolved adenine base editor (ABEmax) to healthy cynomolgus macaques (Fig. 1). The target sequence for the single-guide RNA is perfectly conserved between humans and macaques, which simplifies interpretation of how their results might translate to humans.

The authors tested two concentrations of RNA (0.75 mg/kg and 1.5 mg/kg) and administered either one dose or two doses two weeks apart. Targeted amplicon sequencing of liver biopsies taken after 29 days from all four lobes showed that the desired base was edited at efficiencies of 2% for the low dose and 28% for the high dose. Analysis of on-target editing in other tissues of macaques in the high-dose group revealed low rates of editing. Animals receiving a single high-dose shot showed a 26% decrease in circulating PCSK9 and a 9% decrease in serum LDL cholesterol 29 days after drug administration. Two high-dose shots lowered circulating PCSK9 by 39% and serum cholesterol by 19% after 29 days. An in-depth analysis of off-target editing (both single-guide RNA dependent and independent) in animals treated with high doses revealed no conspicuous events. Immune responses—evidenced by elevated levels of serum transaminases, pro-inflammatory cytokines and immune-modulating chemokines—were mild and transient, and were determined to have been caused by the lipid nanoparticle formulation. In addition, *Sp*Cas9- and TadA-specific immunoglobulin G (IgG) antibodies were detected in monkeys who received two doses, which presumably affected the efficacy of the second dose.

In the related study, Musunuru et al. built on previous work in which they demonstrated CRISPR base editing of *Pcsk9* in mice[10]. Like Rothgangl et al., they studied healthy cynomolgus macaques and knocked out *PCSK9* by introducing a splice site mutation at a sequence identical in humans and non-human primates using a base editor delivered in lipid nanoparticles. The base editor was one of the latest adenine base editors (ABE8.8)[11]. A single intravenous infusion of the therapeutic induced efficient base editing in the liver, which was accompanied by persistently reduced levels of blood PCSK9 protein and LDL cholesterol. Minimal on-target editing was observed in other tissues, with the highest editing observed in the spleen. One dose (3.0 mg/kg) resulted in 66% editing of the desired base in liver biopsies collected 2 weeks after infusion, which, after 8 months, led to a 90% reduction in circulating PCSK9 and a 60% reduction in blood LDL cholesterol. Like Rothgangl et al., the authors observed a modest, transient elevation of serum transaminases induced by the lipid nanoparticle. Evaluation of undesired off-target editing in monkey livers at lower doses (1.0 and 1.5 mg/kg) revealed low-level editing (<1%) at one predicted, yet poorly conserved, off-target site, which had not been detected in in vitro studies of primary human hepatocytes.

One apparent difference between the two studies is the efficiency of on-target editing, which was higher in the work of Musunuru et al. As noted by Rothgangl et al., it is conceivable that their redosing strategy, with a second dose after two weeks, evoked an immune response, evidenced by the presence of IgG antibodies against Cas9 and ABEmax, that resulted in elimination of infected hepatocytes. This observation underscores the necessity of optimizing the dosing schedule in large mammals to maximize on-target editing before entering the clinical arena. The use of the improved ABE8.8 may also have contributed to the higher editing efficiency observed by Musunuru et al.

Both papers reported rapid clearance of the single-guide RNA, base editor mRNA, and lipid nanoparticle components from the monkeys. This is in sharp contrast to delivery of CRISPR components by adeno-associated viruses, for which permanent integration and long-lasting expression has been observed[12]. From a clinical perspective, long-term persistence of editing components is undesirable as it increases the likelihood of off-target edits and of immune responses against cells expressing the bacterial CRISPR–Cas. This suggests a clear advantage for transient delivery by lipid nanoparticles. However, lipid nanoparticles have strong tropism for hepatocytes, limiting their utility for targeting organs other than the liver. There is a need for new methods that allow transient delivery of CRISPR components to any desired organ and cell type.

The studies by Rothgangl et al. and Musunuru et al. are exciting examples of the tremendous therapeutic potential of CRISPR base editing. Of course, concerns regarding off-target mutations, immunogenicity and organ targeting must be addressed. Even so, with the rapid progress in CRISPR-based systems, it seems just a matter of time before the advantages of precise genome editing outweigh the disadvantages in moving to clinical translation. ❐

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Competing interests

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