Banach M. Lipid-lowering nutraceuticals in clinical practice: position paper from an International Lipid Expert Panel. *Nutr Rev* 2017;**75**:731–767.
5. Banach M, Patti AM, Giglio RV, Cicero AFG, Atanasov AG, Bajraktari G, Bruckert E, Descamps O, Djuric DM, Ezhov M, Fras Z, von Haehling S, Katsiki N, Langlois M, Latkovskis G, Mancini GBJ, Mikhailidis DP, Mitchenko O, Moriarty PM, Muntner P, Nikolic D, Panagiotakos DB, Paragh G, Paulweber B, Pella D, Pitsavos C, Reiner Ž, Rosano GMC, Rosenson RS, Rysz J, Sahebkar A, Serban MC, Vinereanu D, Vrablík M, Watts GF, Wong ND, Rizzo M; International Lipid Expert Panel (ILEP). The role of nutraceuticals in statin intolerant patients. *J Am Coll Cardiol* 2018;**72**:96–118.
6. Cicero AFG, Colletti A, von Haehling S, Vinereanu D, Bielecka-Dabrowa A, Sahebkar A, Toth PP, Reiner Ž, Wong ND, Mikhailidis DP, Ferri C, Banach M; International Lipid Expert Panel. Nutraceutical support in heart failure: a position paper of the International Lipid Expert Panel (ILEP). *Nutr Res Rev* 2020;**33**:155–179.
7. Penson PE, Mancini GBJ, Toth PP, Martin SS, Watts GF, Sahebkar A, Mikhailidis DP, Banach M; Lipid and Blood Pressure Meta-Analysis Collaboration (LBPMC) Group & International Lipid Expert Panel (ILEP). Introducing the 'Drucebo' effect in statin therapy: a systematic review of studies comparing reported rates of statin-associated muscle symptoms, under blinded and open-label conditions. *J Cachexia Sarcopenia Muscle* 2018;**9**:1023–1033.
8. Zhubi-Bakija F, Bajraktari G, Bytyçi I, Mikhailidis DP, Henein MY, Latkovskis G, Rexhaj Z, Zhubi E, Banach M; International Lipid Expert Panel (ILEP). The impact of type of dietary protein, animal versus vegetable, in modifying cardiometabolic risk factors: A position paper from the International Lipid Expert Panel (ILEP). *Clin Nutr* 2021;**40**:255–276.
9. Katsiki N, Mikhailidis DP, Bajraktari G, Miserez AR, Cicero AFG, Bruckert E, Serban MC, Mirrakhimov E, Alnouri F, Reiner Ž, Paragh G, Sahebkar A, Banach M; International Lipid Expert Panel (ILEP). Statin therapy in athletes and patients performing regular intense exercise—position paper from the International Lipid Expert Panel (ILEP). *Pharmacol Res* 2020;**155**:104719.
10. Banach M, Penson PE, Fras Z, Vrablik M, Pella D, Reiner Ž, Nabavi SM, Sahebkar A, Kayikcioglu M, Daccord M; FH Europe and the International Lipid Expert Panel (ILEP). Brief recommendations on the management of adult patients with familial hypercholesterolemia during the COVID-19 pandemic. *Pharmacol Res* 2020;**158**:104891.

doi:10.1093/eurheartj/ehab478

Weekly Journal Scan

A 'Once-and-Done' Approach to the Lifelong Reduction of Elevated Cholesterol

Francesco Paneni [1]* and Massimo Volpe [2]*

[1]Center for Molecular Cardiology, University of Zürich, Switzerland and University Heart Center, University Hospital Zurich, Zürich, Switzerland; and [2]Department of Clinical and Molecular Medicine, Sapienza University of Rome, Sant'Andrea Hospital, Rome, Italy

Comment on 'In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates', which was published in *Nature*, doi:10.1038/s41586-021-03534-y.

Key points

- CRISPR (clustered regularly interspaced short palindromic repeats)-related technologies are emerging therapeutic strategies to induce DNA modifications in humans. In this regard, gene editing of proprotein convertase subtilisin/kexin type 9 (PCSK9) might represent a promising approach for the prevention of coronary heart disease (CHD). The present study[1] investigates the impact of a single-nucleotide PCSK9 loss-of-function mutation by CRISPR adenine base editors (ABE) on low-density lipoprotein cholesterol (LDL-C) levels in non-human primates.
- To introduce a precise single-nucleotide PCSK9 loss-of-function mutation, a CRISPR ABE was delivered in macaques using lipid nanoparticles (LNPs). Adenine base editors of PCSK9 was confirmed in primary human hepatocytes, primary monkey hepatocytes, and mice.
- *In vivo* CRISPR ABE delivery led to a near-complete knockdown of PCSK9 in the liver after a single infusion of LNPs, with concomitant reductions in blood levels of PCSK9 and LDL-C of ∼90% and 60%, respectively. These changes were sustained for at least 8 months after a single-dose treatment. No relevant side effects were observed in the animals treated with a CRISPR editor-based strategy.
- Off-target gene editing was found at only one site in macaque liver, whereas no off-target editing was found in human hepatocytes.

Comment

Individuals with spontaneous loss-of-function PCSK9 mutations experience a significant reduction of both LDL-C levels (∼30–40%) as well as CHD risk (88%), and appear free from adverse clinical consequences.[2] Gene-editing technologies, which include the CRISPR–Cas nucleases and CRISPR base editor, have the potential to permanently modify disease-causing genes.[3] The demonstration of durable editing of PCSK9 in target organs is a key step before *in vivo* administration of specific gene editors in clinical trials. In this experimental proof-of-concept study, genetic inactivation of PCSK9 gene by CRISPR editors was associated with a substantial and sustained lowering of LDL-C levels in non-human

*Corresponding authors. Tel: +41446355096, Email: francesco.paneni@uzh.ch (F.P.); Tel: +39633775654, Email: massimo.volpe@uniroma1.it (M.V.)

primates.[1] Although current PCSK9 targeting approaches are highly effective in reducing LDL-C levels, their effects on LDL-C are short-lived, and patients need to receive regular shots every few weeks.[4] The recent development of RNA-based approaches blocking PCSK9 transcription (e.g. Inclisiran) has provided a significant progress by reducing the administrations to twice a year. Yet, a 'single shot' therapy would definitely solve the issue of poor treatment adherence, the latter being a key predictor of unsatisfactory LDL-C targets (only 20% of high-risk patients are on target) and cardiovascular mortality in statin-treated patients.[4] Unlike currently available PCSK9-targeting drugs, gene-editing approaches offer the potential for a 'once-and-done' therapy. Although the long-term permanence of CRISPR-based liver editing remains to be established, no signs of attenuation of the pharmacodynamic effects of liver editing were shown after 8 months of follow-up.[1]

A central aspect to consider when developing safe and effective genome editing therapies is the minimization of off-target editing and mutations.[3] In this respect, our patients would likely ask a reasonable question: 'how precise is the gene editing and what are the possible side effects after a "once-and-done" approach has been delivered'? The authors[1] show that the use of a base editor resulted in the alteration of a single base pair as the predominant editing event and had no risk of vector sequence integration. Notably, no off-target editing was observed in human hepatocytes, which is reassuring in terms of safety concerns. In contrast, a recent study using adeno-associated virus (AAV)-delivered meganuclease—defined as engineered variants of homing endonucleases that can be redirected to DNA sequences—showed off-target editing at numerous genomic sites in the non-human primate liver and in human hepatocytes.[5] Hence, the use of mRNA (CRISPR ABE) rather than a DNA vector (meganucleases) may confer a more precise editing, mainly due to a larger degree of integration of the AAV vector sequence into the genome at the site of the break.[3] The precise correction of disease-causing single-nucleotide mutations by ABE had already been shown in mouse models of genetic disorders such as phenylketonuria (through the correction of *Pah* mutations by a cytosine base editor) or Hutchinson–Gilford progeria syndrome (through the correction of *LMNA* transgene mutations by an ABE).[3] Moreover, a couple of considerations suggest that off-target editing might not be associated with major risks: (i) human cells (e.g. hepatocytes) can tolerate a large number (>1000) of spontaneous point mutations during the life course and (ii) continuous expression of ABE over the period of 1 year did not increase hepatocellular carcinoma formation in Trp53 mutant mice.[6] Lipid nanoparticle-delivered base editor was also associated with a more reproducible and pronounced editing (over 50%) as compared with meganucleases-based editing.[1] Furthermore, long-term follow-up of monkeys receiving CRISPR ABE showed only transient and moderate rises in liver enzymes (AST and ALT) that were entirely resolved after 2 weeks, with no adverse health effects. Although base editors might induce an adaptive immune response, the absence of late increases in AST and ALT observed in the present study suggests that such a response (if any) does not adversely affect the efficacy of the treatment.

That having said, further preclinical studies evaluating the long-term safety of base editing *in vivo* are needed before moving on with first in-human studies. Adjustment of dose levels and dosing schedule(s) could also contribute to increasing editing rates *in vivo*. A recent study[7]—which was published simultaneously with the present work—showed that LPNs–based delivery of ABE targeting PCSK9 induced up to 67% editing in mice and up to 34% editing in macaques, with LDL-C reductions by 58% in mice and 14% in macaques. Moreover, no off-target mutations in genomic DNA were observed.[7] In conclusion, the landmark study by Musunuru *et al.*[1] paves the way for a broad application of CRISPR editors delivery for the treatment of dyslipidaemias as well as a wide panel of monogenic human diseases. The potential risks of this approach must be carefully weighed against their benefits.

Conflict of interest: F.P. is the recipient of an H.H. Sheikh Khalifa bin Hamad Al Thani Foundation Assistant Professorship at the Faculty of Medicine, University of Zürich. M.V. reports personal fees for speaker bureau and/or consulting in Advisory Board from Amgen, Astra Zeneca, Daiichi-Sankyo, Menarini Int, MSD, Novartis Pharma, Novo Nordisk, outside the submitted work.

References

1. Musunuru K, Chadwick AC, Mizoguchi T, Garcia SP, DeNizio JE, Reiss CW, Wang K, Iyer S, Dutta C, Clendaniel V, Amaonye M, Beach A, Berth K, Biswas S, Braun MC, Chen HM, Colace TV, Ganey JD, Gangopadhyay SA, Garrity R, Kasiewicz LN, Lavoie J, Madsen JA, Matsumoto Y, Mazzola AM, Nasrullah YS, Nneji J, Ren H, Sanjeev A, Shay M, Stahley MR, Fan SHY, Tam YK, Gaudelli NM, Ciaramella G, Stolz LE, Malyala P, Cheng CJ, Rajeev KG, Rohde E, Bellinger AM, Kathiresan S. In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. *Nature* 2021;**593**:429–434.
2. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006;**354**:1264–1272.
3. Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nat Biotechnol* 2020;**38**:824–844.
4. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, De Backer GG, Delgado V, Ference BA, Graham IM, Halliday A, Landmesser U, Mihaylova B, Pedersen TR, Riccardi G, Richter DJ, Sabatine MS, Taskinen MR, Tokgozoglu L, Wiklund O; ESC Scientific Document Group. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J* 2020;**41**:111–188.
5. Wang L, Breton C, Warzecha CC, Bell P, Yan H, He Z, White J, Zhu Y, Li M, Buza EL, Jantz D, Wilson JM. Long-term stable reduction of low-density lipoprotein in nonhuman primates following in vivo genome editing of PCSK9. *Mol Ther* 2021;**29**:2019–2029.
6. Blokzijl F, de Ligt J, Jager M, Sasselli V, Roerink S, Sasaki N, Huch M, Boymans S, Kuijk E, Prins P, Nijman IJ, Martincorena I, Mokry M, Wiegerinck CL, Middendorp S, Sato T, Schwank G, Nieuwenhuis EES, Verstegen MMA, van der Laan LJW, de Jonge J, IJzermans JNM, Vries RG, van de Wetering M, Stratton MR, Clevers H, Cuppen E, van Boxtel R. Tissue-specific mutation accumulation in human adult stem cells during life. *Nature* 2016;**538**:260–264.
7. Rothgangl T, Dennis MK, Lin PJC, Oka R, Witzigmann D, Villiger L, Qi W, Hruzova M, Kissling L, Lenggenhager D, Borrelli C, Egli S, Frey N, Bakker N, Walker JA 2nd, Kadina AP, Victorov DV, Pacesa M, Kreutzer S, Kontarakis Z, Moor A, Jinek M, Weissman D, Stoffel M, van Boxtel R, Holden K, Pardi N, Thony B, Haberle J, Tam YK, Semple SC, Schwank G. In vivo adenine base editing of PCSK9 in macaques reduces LDL cholesterol levels. *Nat Biotechnol* 2021; doi:10.1038/s41587-021-00933-4.