Perspective: hepatocyte-directed base editing as novel treatment for human dyslipidemia-current status and remaining challenges

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REVIEW

Perspective: Hepatocyte-Directed Base Editing as Novel Treatment for Human Dyslipidemia—Current Status and Remaining Challenges

Menno Hoekstra, Miranda Van Eck, Theo J.C. Van Berkel

ABSTRACT: Hyperlipidemia is a major risk factor for the development of atherosclerotic cardiovascular disease. Lipid-lowering drug therapies therefore still form the heart of the ongoing battle against the occurrence of cardiovascular events. However, in light of the important improvements in gene interference and editing that have been made during the last 2 decades, gene therapy—the genetic modification of cells to produce a permanent therapeutic effect—is currently employed to relief hypercholesterolemic subjects from their potential (chronic) cardiovascular disease burden. In this perspective, we review the current status regarding hepatocyte-directed base editing to treat human dyslipidemia and provide suggestions for further technological improvement.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: cardiovascular disease ■ editing ■ hypercholesterolemic ■ hyperlipidemia ■ therapeutic effect

Lipid-lowering drug therapies still form the heart of the ongoing battle against the occurrence of major cardiovascular disease-related events such as a heart attack and stroke. By lowering hyperlipidemia, the major risk factor for the development of macrophage foam cells and culprit in the development of atherosclerosis is reduced. Unfortunately, current lipid-lowering interventions have their limitations. For instance, the use of cholesterol synthesis inhibiting statins has been associated with side effects such as muscle pain that can lead to reduced patient adherence or even therapy discontinuation. Moreover, the overall costs of alternative chronic lipid-lowering drug treatments puts a large burden on society, which is especially relevant for treatment with evolocumab and alirocumab, monoclonal antibody-driven PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibition therapies (Table 1). Therefore, it is not surprising that the base editing company Verve Therapeutics aims to employ gene medicines as one and done treatment to establish permanent lipid-lowering therapeutic effects via genetic modification of cells and relief hypercholesterolemic subjects from their potential (chronic) cardiovascular disease risk.

Gene therapy has already for a long time been considered a high potential approach for disease treatment. However, as nicely reviewed by Arabi et al,[6] the gene therapy field has suffered significant blows since the first successful clinical trial in 1990. The actual efficacy of gene therapy in humans has generally been low, with often only a temporary restoration of gene function. Furthermore, the fact that patients receiving gene therapy in clinical trials frequently developed pathological inflammatory conditions (ie, leukemia) or even died from the treatment almost led to a virtual stand-still of the research field as a whole by the year 2000. However, important (technological) scientific improvements in gene interference and editing have been made during the last 2 decades, already leading to approval by the FDA of gene therapy products for the treatment of several lymphoma types, retinal dystrophy, and spinal muscular atrophy. The gene therapy field is thus currently blossoming, as can also be appreciated from the observation that the number of papers published annually in PUBMED containing the term gene therapy has increased from 7.450 in the year 2000 to 36.592 in 2021.

The gene therapy approach that Verve Therapeutics is employing builds upon the principle of the revolutionary

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Nonstandard Abbreviations and Acronyms

ANGPTL3	angiopoietin-like protein 3
LNP	lipid nanoparticle
PCSK9	proprotein convertase subtilisin/kexin type 9

Highlights

- A reduction in the activity of PCSK9 (proprotein convertase subtilisin/kexin type 9) or ANGPTL3 (angiopoietin-like protein 3) is associated with reduced plasma cholesterol levels and cardiovascular disease risk.
- Base editing is currently applied as potential novel therapeutic approach to permanently lower the hypercholesterolemia extent.
- Coupling of GalNAc moieties to lipid nanoparticles will enhance the efficacy of hepatocyte-directed base editing.

CRISPR gene editing technology that uses a synthetic RNA strand to guide a Cas enzyme family member to cut the DNA at a specific location and subsequently replace a targeted nucleotide by another nucleotide, thereby inducing a permanent genetic change. According to their website, Verve is now primarily focusing on 2 therapeutic targets to treat atherosclerotic cardiovascular disease: (1) PCSK9, which prevents the LDL receptor from recycling and thereby limits cellular LDL particle uptake, and (2) ANGPTL3 (angiopoietin-like protein 3), an inhibitor of lipoprotein and endothelial lipases that increases plasma triglyceride and LDL-cholesterol levels. The choice for these targets is logical as it can rely on previous preclinical and clinical successes. Several naturally occurring variations in the genes of these 2 proteins, that is, the loss-of-function mutations PCSK9 p.C679X and ANGPTL3 S17X, are associated with significantly reduced plasma cholesterol levels.[7,8] In addition, no elevated risk for other severe pathologies such as inflammatory disease and cancer has thus far been described for PCSK9 and ANGPTL3 mutation carriers or hypercholesterolemic subjects chronically treated with PCSK9 inhibitors. When assuming that the chosen technical approach will not be associated with additional (off-target) changes within a person's DNA, it is thus likely safe to state that editing of the ANGPTL3 and PCSK9 genes will be beneficial for hypercholesterolemic as well as normolipidemic human subjects in terms of predicted long-term health outcome.

An important feature to take into account when considering the potential efficacy and safety of gene editing approaches is efficient delivery to the desired tissue. Plasma levels of PCSK9 and ANGPTL3 are predominantly controlled by the production and subsequent secretion of these proteins by parenchymal liver cells. To achieve a clinical relevant effect, an efficient delivery of the gene editing moieties to the specifically these cells in the liver is therefore highly desirable. Notably, selective delivery of the guide RNA and Cas protein to the liver may also help to overcome potential RNA uptake-associated pro-inflammatory polarization of monocytes and macrophages and the occurrence of a life-threatening cytokine storm.[9] In this context, it is of importance to acknowledge that Novartis has successfully entered the pharmaceutical market at the end of 2020 with Inclisiran (brand name: Leqvio; Table 1), a hepatocyte-directed oligonucleotide-based RNA interference therapy for adults with primary hypercholesterolemia (heterozygous familial and nonfamilial) or mixed dyslipidemia that markedly lowers plasma PCSK9 levels.[10] Following the hepatic uptake of Inclisiran, Argonoute-mediated breakdown of PCSK9 mRNA rapidly occurs (thereby eliminating protein translation) due to incorporation into the multiprotein RNA-induced silencing complex of the antisense strand corresponding to human PCSK9 mRNA, eventually lowering PCSK9 protein levels and reducing LDL receptor degradation.

The hepatocyte-specific effect of Inclisiran can be attributed to the fact that the small interfering oligonucleotides

Table 1. Comparison of Treatment Frequencies and Lipid-Lowering Efficacies of PCSK9-Centered Cardiovascular Drug Therapies

Therapy	Type	Dosing	Frequency	Estimated annual costs	LDL-C reduction	CVD risk reduction	References
Alirocumab	PCSK9 monoclonal antibody	75 mg/300 mg (SC injection)	Every 2/4 wk	$14 600	50%–80%	25%	1,2
Evolocumab	PCSK9 monoclonal antibody	420 mg (SC injection)	Every month	$6000	50%–60%	Unknown	3
Inclisiran (Leqvio)	PCSK9 siRNA	284 mg (SC injection)	Every 6 mo	$6500	40%–50%	30%	4
Lipid nanoparticle containing PCSK9 base editor (ie, Verve-101)		SC injection	Once	Unknown	70%*	Unknown	5

PCSK9 indicates proprotein convertase subtilisin/kexin type 9.
*As expected from studies in monkeys.

are covalently coupled to triantennary N-acetylgalactosamine carbohydrates (GalNAc). GalNAc is a specific ligand for the asialoglycoprotein receptors that are solely located on parenchymal liver cells, enabling targeted delivery of the oligonucleotide complex. As initially shown by our laboratory already at the end of the 20th century, conjugation of an antisense oligonucleotide to a high-affinity asialoglycoprotein receptor ligand increases the binding efficiency to parenchymal liver cells in vitro and greatly improves the hepatic accumulation of the oligonucleotides after their administration into rats and mice.[11–13] The commercial application of oligonucleotide/GalNAc coupling for use in humans has been initialized by Alnylam Pharmaceuticals at the beginning of the last decade and is currently also already employed to treat other pathological conditions, that is, hereditary transthyretin amyloidosis (Patisiran) and hepatic porphyria (Givosiran) by targeting the hepatic enzymes transthyretin and delta-aminolevulinic acid synthase 1, respectively.

The Verve Therapeutics PCSK9 gene editing product VERVE-101 that is currently being tested in clinical trials uses lipid nanoparticles (LNPs) as transport vehicles to simultaneously deliver the Cas protein and gene targeting oligonucleotide to parenchymal liver cells. Upon injection into the blood circulation, the LNPs acquire apolipoprotein E. As a result, the LNPs are subject to plasma clearance through whole particle uptake via LDL receptors present on parenchymal liver cells[14] (Figure). Since the Verve Therapeutics approach is developed for use in heterozygous familial hypercholesterolemia patients, it can be assumed that LNP uptake will be significantly hampered by the genetically reduced LDL receptor expression (theoretically also limiting the gene editing efficiency). It therefore seems favorable to make use of an alternative delivery method that is centralized around the interaction between GalNAc-coupled LNPs and the hepatocyte asialoglycoprotein receptor (Figure). According to the website of Verve Therapeutics, the company is already aware of this possibility as it plans to utilize GalNAc-coated LNPs for their second base editing therapy product that intends to lower ANGPTL3 expression and is currently optimized for potential clinical application. GalNAc-coupled LNPs are relatively easy to reproducibly synthesize, efficiently protect the base editor from enzymatic degradation, and stimulate the release of the targeting mRNA into the cytoplasm, and are generally regarded safe for use in clinical applications. As such, it is conceivable that this gene editing method can be introduced into the human setting without considerable complications. However, critical care should be taken during the manufacturing process of the lipid nanoparticles with regard to the obtained particle size, given that our previous studies have identified 70 nm as maximal particle diameter for recognition and subsequent cellular delivery of liposomes to parenchymal liver cells via asialoglycoprotein receptor-mediated uptake.[15]

In conclusion, the potential successful application of base editing to cure hypercholesterolemia may be regarded a key milestone on the rocky road that the gene therapy field has taken so far. When taking the current scientific evidence into account, all tools and technical considerations appear to be readily available to pursue this approach with enthusiasm. However, it should not be

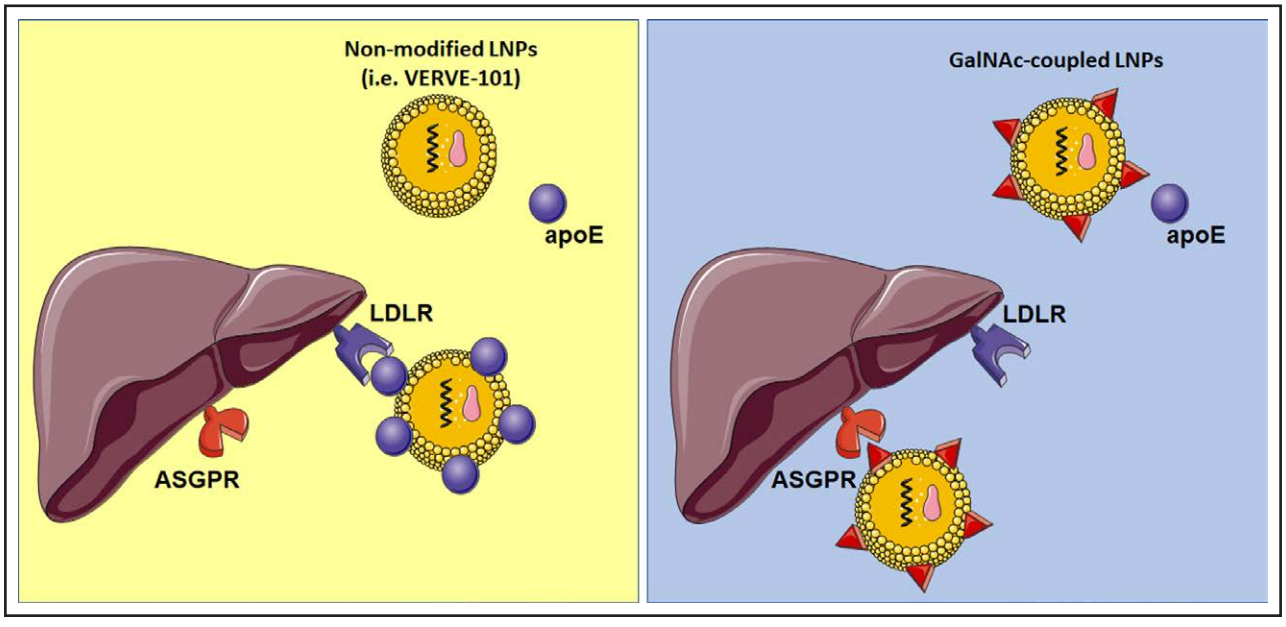

Figure. Comparison between the delivery routes of nonmodified and GalNAc-coupled lipid nanoparticles (LNPs). Nonmodified LNPs carrying the gene targeting oligonucleotide as well as the Cas protein rapidly acquire apoE (apolipoprotein E) after injection into the blood circulation, resulting in plasma clearance via the LDL receptor (LDLR) present on parenchymal liver cells (left), while GalNAc-coupled LNPs do not incorporate apoE and rather act as high-affinity ligands for uptake through the hepatic asialoglycoprotein receptor (ASGPR; right).

Table 2. Pros and Cons of Using Gene Editing to Battle Cardiovascular Disease

Pros	Cons
Only once in a lifetime treatment needed	Hepatocyte-directed gene editing approach needs to be optimized
Germline cure (chronic benefit for society)	Ethical dilemma/therapy skepticism
Unlimited lipid gene targets available (ie, PCSK9/ANGPTL3)	Safety risks due to potential off-target effect or imprecise edits

ANGPTL3 indicates angiopoietin-like protein 3.

neglected that—even if possible technical issues can be effectively dealt with—one of the hardest things to achieve may be to convince the general public that permanently changing DNA to treat cardiovascular disease is morally acceptable. As highlighted by the review of Brokowski and Adli,[16] the application of gene therapy approaches such as the CRISPR/Cas technology can lead to significant benefits for society but may also be subject to misuse or lead to damage to the ecosystem. Also in light of the recent global resistance from specific subgroups in society to receive a RNA-based vaccination against severe acute respiratory syndrome coronavirus 2, we assume that significant effort from Verve Therapeutics will be required to also take this last gene therapy-associated hurdle and shift the balance to favor the pros of gene editing to battle cardiovascular disease over the associated cons (see Table 2 for a pros and cons summary).

ARTICLE INFORMATION

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Disclosures

None.

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