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Genome editing's potential target diseases in the cardiovascular field with potential influence on the ophthalmological pressure

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Abstract
Through editing DNA for therapeutic purposes in the heart, it is essential to achieve a high editing efficiency at a specific genomic site in the tissue that is being targeted. Using more recent cell-based methods, off-target editing can be tested across the entire genome in an objective manner. It is essential to have carefully designed gRNAs in order to achieve low off-target effects. The significant challenge that stands in the way of clinical application is the distribution of genome editors to the tissues and cells of interest. Genome editing holds the potential to either cure or prevent two different types of cardiovascular diseases. When it comes to clinical genome editing for cardiovascular diseases, the liver is the organ that has received the most attention. This is because the liver is a target organ. Any type of genome editing raises the possibility of unintended mutations, known as off-target mutagenesis. Mutations in target cells or tissues that are not intended to occur can cause off-target effects, which can include the development of cancer.

Genome editing may be used to cure or prevent several forms of cardiovascular disorders (Ates *et al.*, 2020). Cardiovascular disorders affected by genetic regions in the heart or arteries that are responsible for the disease (Mishchenko, Mishchenko and Ivanisenko, 2021). This category includes a wide range of hereditary and acquired genetic diseases in cardiovascular tissues (Domanski, Mehra and Pfeffer, 2016). Hypertrophic cardiomyopathy (HCM) (Chou and Chin, 2021), Dilated cardiomyopathy (DCM) (Yamamoto *et al.*, 2021), Long QT syndrome (LQTS) (Ergül *et al.*, 2021), and muscular dystrophies that induce cardiac dysfunction are examples of this form of heart disease (Elangkovan and Dickson, 2021). Marfan syndrome and familial pulmonary hypertension are two examples of vascular disorders (Feather, Randall and Waterhouse, 2020; Elangkovan and Dickson, 2021). While these diseases are potential therapeutic genome editing candidates in theory, existing genome editing technology makes it difficult to target primary cardiovascular tissues due to the inefficiency of in vivo editing and the lack of effective delivery methods, which we will explore later (Musunuru, 2021). While germline editing could be more beneficial for these diseases than in vivo editing, it raises ethical concerns (The Royal Society *et al.*, 2021). Another category includes those who are linked to cardiovascular risk factors, including dyslipidemia (Santulli, 2021). Non-cardiovascular tissues , such as the liver or blood cells, are targets of genome editing in this form of disease (Musunuru, 2021). Because of its feasibility, the liver is currently the organ that has been researched the most for therapeutic genome editing of cardiovascular diseases (Musunuru, 2021). An ex vivo technique for blood cells may be applied to immune cells to increase systemic inflammation and avoid atherosclerosis (Musunuru, 2021).

Non-homologous end-joining (NHEJ) and homology-directed repair (HDR), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALEN), clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), and other genome editing tools rely on endogenous DNA repair processes (Elacqua *et al.*, 2021). When genome editors insert an initial double-strand break (DSB), the host cell triggers endogenous DNA repair pathways to repair the damage. NHEJ and HDR are the two major forms of repair processes (Elacqua *et al.*, 2021).

DSBs are restored by the NHEJ mechanism in the absence of a repair DNA template (Craig *et al.*, 2021). Endogenous repair machinery specifically ligates the ends of DSBs in NHEJ (Coleman and Tsongalis, 2019; Craig *et al.*, 2021). NHEJ, on the other hand, is vulnerable to errors and often results

in spontaneous insertion/deletion (indel) mutations at the junction site (Elacqua *et al.*, 2021). If an indel is found inside a gene's coding sequence, it may trigger a frameshift or a premature stop codon, leading to gene knockout (Musunuru, 2021). NHEJ is a common repair mechanism that occurs during the cell cycle in most mammalian cell types. As a result, NHEJ genome editing may be used to knock out genes in a variety of cell types (Elacqua *et al.*, 2021).

DSBs can be repaired using the HDR method in the presence of a repair DNA template (Salgado, 2020). About the fact that HDR happens at a lower frequency than NHEJ, it can result in specific modifications that can be described by an exogenously inserted repair prototype (Sansbury and Kmiec, 2021). The repair prototype may be single-stranded DNA oligonucleotides or double-stranded DNA oligonucleotides with homology arms flanking the insertion sequence, as in traditional homologous recombination approaches (ssODNs) (Sansbury and Kmiec, 2021). Unlike NHEJ, HDR is only involved in the S and G2 phases of the cell cycle, and its performance varies depending on the cell type and condition (Sansbury and Kmiec, 2021). Thus, HDR can be used to trigger knock-in of a particular DNA sequence in dividing cells, but it is rarely used in non-dividing cells like cardiomyocytes, where it is frequently outcompeted by alternate repair pathways like NHEJ (Sansbury and Kmiec, 2021).

There are three major techniques for altering genomic DNA sequences to prevent or cure disease based on these two key forms of repair processes: destruction of a gene, deletion of a particular genomic region, and modification of a gene (Suckow *et al.*, 2019).

The most straightforward approach to using genome editing to prevent or cure disease is to disrupt a gene that is unhealthy or whose suppression is beneficial (Carroll, 2021). NHEJ may be used to cause disruption within a gene for this reason. Targeting PCSK9 to lower blood cholesterol levels is a promising example of this strategy. PCSK9-targeting therapies have been designed to reduce LDL cholesterol levels in patients with persistently elevated LDL cholesterol levels even on statins, based on the finding that loss-of-function mutations in PCSK9 were associated with 15–28 percent lower LDL cholesterol levels and 47–88 percent lower risk of coronary heart disease (Shantha and Robinson, 2016). Monoclonal antibodies against PCSK9, which have recently been licensed and can reduce LDL cholesterol significantly when used alone or in combination with a statin, must be taken on a regular basis. Since loss-of-function mutations in PCSK9 do not cause any obvious phenotypes, permanent disruption of PCSK9 via genome editing will be a promising strategy. Several studies show that mouse PCSK9 can be attacked by NHEJ-mediated disruption to reduce LDL cholesterol using CRISPR/Cas9 that is virally transmitted to the liver with high efficiency (Dowaidar, no date).

NHEJ-mediated genome editing with two guide RNAs (gRNAs) flanking the locus to be deleted can also be used to remove a particular genomic region (Zhang *et al.*, 2019). If a disease-causing mutation causes a frameshift, a premature stop codon, or an ectopic splicing site, deleting the genomic regions surrounding the mutation can restore the gene's protein function. The "exon skipping" technique for Duchenne muscular dystrophy (DMD) (Nelson and Gersbach, 2019) is a clear example of this approach. One of the most common lethal genetic disorders is DMD, which is an X-linked recessive muscle wasting disorder (1:5000 male births). Dystrophin, a large cytoskeletal structural protein required for muscle membrane stabilization, is the gene responsible for DMD. The mutations are often exon deletions, which interrupt the dystrophin gene's reading frame and result in the complete loss of functional dystrophin expression. DMD patients are normally diagnosed in infancy, and irregular heart function is discovered in early adolescence (Xu *et al.*, 2021). Heart disease or respiratory failure commonly kills patients in their twenties (Fayssoil *et al.*, 2021). Despite the fact that gene therapy is a potential treatment choice for DMD, gene delivery of intact dystrophin is difficult due to the large size of the dystrophin coding sequence. Fortunately, the exon-skipping technique against truncated dystrophin has been shown to repair the reading frame and recover much of the functional protein (Matsuo, 2021). Exon skipping has been suggested as a treatment choice for DMD patients (Li *et al.*, 2021). The FDA has also approved oligonucleotide-mediated exon-skipping drugs for the most frequent form of DMD with exon 51 mutation (Lin *et al.*, 2021). Since genome editing can potentially target any exon, irreversible exon-skipping via genome editing may be a potential treatment for extreme DMD patients. Exon skipping was shown to restore functional dystrophin protein in a mouse model by in vivo genome editing (Hwang and Yokota, 2019).

CRISPR/Cas9 was delivered to the skeletal muscle of mdx mice with a premature stop codon in exon 23 using an AAV virus. Despite the fact that the editing efficiency in skeletal muscle was only 2–3%, they demonstrated functional recovery in muscle strength after genome editing. In a canine model, the same technique was seen to work (Amoasii *et al.*, 2018). The precise effectiveness of genome editing in the heart in these models, however, is still unknown. It remains to be seen if exon skipping via genome editing will prevent heart failure, which is the most common cause of death in DMD patients.
Since there are many genetic disorders in the cardiovascular field, HDR gene correction has a lot of promise for therapeutic genome editing (Elacqua *et al.*, 2021). However, due to the low efficiency of HDR in non-dividing somatic cells, especially cardiomyocytes, it is currently difficult to extend this approach to in vivo models.
Gene correction by HDR is currently primarily used in in vitro platforms where successfully edited cells can be filtered and amplified (Karapurkar *et al.*, 2021). The phenotypes of monogenic diseases can be saved after gene correction, according to studies using patient-derived iPSCs (Turksen, 2020). This method can not be extended to in vivo genome editing because these disease-modeling experiments of iPSCs use genome editing in the stem cell stage and then pick up edited iPSC clones and differentiate them into cell types responsible for the disease (cardiomyocytes, endothelial cells, etc.). However, if gene correction can be performed with high efficiency in somatic cells and 3D organs, these studies indicate that gene correction may be a possible therapeutic alternative. Given the poor efficiency of HDR in current CRISPR/Cas9 technology, diseases in which even a small percentage of corrected cells can at least partially restore function or diseases that can be handled by ex vivo editing, whether in somatic cells or iPSCs, are viable targets (Turksen, 2020).
After the invention of CRISPR/Cas9 systems, a growing range of novel CRISPR/Cas9-based methods have emerged, with applications in gene control, epigenome editing, genomic imaging, and other areas (Turksen, 2020). The laws of NHEJ or HDR are not followed by these non-traditional instruments. Several cases of possible biomedical uses are briefly summarized here.
The homology-independent targeted integration (HITI) strategy is one approach with potential for cardiac genome editing. Since HDR's gene correction technique is ineffective in non-dividing cells, this new method was created to accomplish gene correction without the use of HDR. DNA knock-in is possible with HITI also in non-dividing cells. Since NHEJ is involved in both dividing and non-dividing cells, HITI uses modified NHEJ instead of HDR for gene correction (Bloomer *et al.*, 2021). Importantly, using AAVs, this procedure was shown to be capable of knocking in a reporter gene into the mouse heart. SATI, a new approach created by the same community, was recently published (intercellular linearized Single homology Arm donor mediated intron-Targeting Integration). SATI is a hybrid of single homology arm-mediated HDR and HITI that allows for greater target versatility (Suzuki *et al.*, 2019).
Base editors may be a good substitute for HDR-mediated gene correction (Shen *et al.*, 2018). Since base editing uses cytidine deaminases rather than DSBs to recruit enzymes, it has the potential to boost efficiency while limiting DSB damage (Villiger *et al.*, 2021). The detailed translation of C to T has enormous therapeutic potential for point mutation repair (Villiger *et al.*, 2021). Although targetable mutations are constrained by the PAM sequences of base editing tools (Shen *et al.*, 2018), it is estimated that 3000 genetic variants in ClinVar could be corrected by C > T substitution. It has been shown that base editors may be used to correct therapeutic genes in mouse models (Shen *et al.*, 2018; Yeh *et al.*, 2020). However, similar to the NHEJ gene disruption technique, research into base-editing therapy for cardiovascular disorders has been restricted to PCSK9 in the liver, with no reports of base-editing in the heart (Chadwick, Wang and Musunuru, 2017). Despite the fact that there are several disorders that can be handled through C > T replacement (e.g., hereditary cardiomyopathy, congenital cardiac diseases, LQTS, etc.), delivering base editors to the heart is also difficult due to the fact that base editors are massive fusion proteins that surpass the packing capability of AAVs, so other nonviral vectors are promising in achieving the delivery (Chadwick, Wang and Musunuru, 2017; Dowaidar, Abdelhamid, Hällbrink, Freimann, *et al.*, 2017; Dowaidar, Abdelhamid, Hällbrink, Zou, *et al.*, 2017; Dowaidar *et al.*, 2018).
A strong new method known as "prime editing" was recently announced. It works by combining a Cas9 H840A nickase with a reverse transcriptase. The prime editing guide RNAs (pegRNAs) contain

reverse-transcribed sequences that would be inserted to the intended locus, as well as sequences that would determine the target loci. Without DSBs or donor DNA templates, prime editing allows for targeted insertions, deletions, and all 12 possible base-to-base transformations. In theory, this new method could correct up to 89 percent of identified genetic variants associated with human diseases, making it a promising candidate for therapeutic genome editing (Hakim, 2020).

CRISPR/Cas9-based gene regulation tools, in addition to genome editing tools, may be useful for therapeutic applications. CRISPR interfe(Hilton *et al.*, 2015)rence (CRISPRi) and CRISPR activation (CRISPRa) systems have been developed using nuclease-deactivated Cas9 to control gene expression rather than editing genomic DNA (dCas9) (Misa and Schwartz, 2021). Since dCas9 retains its ability to bind gRNAs and targeted DNA but lacks nuclease function, it can be used to recruit protein and RNA factors to a specific DNA site without cleaving genomic DNA. For CRISPRi and CRISPRa, transcriptional repressors (e.g., KRAB) and activators (e.g., VP64) are fused to dCas9 to achieve gene repression and activation, respectively. Histone acetylation (e.g., H3K27Ac using p300) (Hilton *et al.*, 2015), histone demethylation (e.g., H3K9me3 using KRAB) (Kearns *et al.*, 2015), DNA methylation (using DNMT3A/L) (Vojta *et al.*, 2016), and DNA demethylation (using TET1) (Xu *et al.*, 2016) have all been produced using dCas9 in addition to CRISPRi/a. In terms of biomedical applications, no trials of in vivo gene therapy using CRISPRi/a or other epigenome editors have been published to date. While a CRISPRi method may potentially be used to treat diseases that are targets for antibody drugs, CRISPRa could be more appealing because gene activation with current molecular selective therapy is difficult.

The flexibility of target sites is determined by how genome editors recognize the target location (Strong and Musunuru, 2017; Musunuru, 2018). Each genome editing tool binds to a different DNA chain. CRISPR/Cas9-based systems have a simpler and more versatile target-site selection rule than ZFN or TALEN-based systems. In a nutshell, CRISPR/Cas9 genome editors bind to a 20-bp protospacer sequence identified by a gRNA, which must be accompanied by a Protospacer Adjacent Motif (PAM) sequence. A PAM series is unique to each Cas9 variant. SpCas9 and SaCas9, for example, have PAM sequences of 5'-NGG and 5'-NNGRRT, respectively. The target site versatility is reduced for Cas9 variants with more complex PAM sequences. However, the increasing number of Cas9 models that are becoming available quickly will soon overcome this restriction (Strong and Musunuru, 2017; Musunuru, 2018).

Non-unique genomic sequences around the target sites may restrict their availability in certain cases. Designing suitable gRNAs to carry Cas9 to a target site that is within repeat sequences or has no adjacent sequences that are unusual relative to the rest of the genome can be challenging. As a result, genes in these locations will be poor candidates for genome editing treatment (Strong and Musunuru, 2017; Musunuru, 2018).

It is important to achieve high editing efficiency at a desired genomic site in a desired tissue for therapeutic genome editing. Despite the development of a variety of new approaches to increase on-target editing performance, achieving highly effective in vivo editing in many types of solid tissues, including the heart (Johansen *et al.*, 2017), remains a challenge.

Since local chromatin structure and epigenetic status influence genome editor accessibility to the target site, editing efficiency varies across loci, cell cycle stages, cell types, and tissue types (Klaver-Flores *et al.*, 2020; Pavani and Amendola, 2020). For in vivo genome editing, the difference in editing efficiency across tissues is particularly significant. Several groups succeeded in in vivo genome editing of PCSK9 in mouse liver through NHEJ-mediated gene disruption as a possible trigger of dyslipidemia (Ding *et al.*, 2014; Carreras *et al.*, 2019; Musunuru *et al.*, 2021; Wang *et al.*, 2021). These experiments show that the editing performance in the mouse liver is sufficient to achieve the functional phenotype. CRISPR/Cas9 was delivered to the mouse liver using viral vectors, and more than half of the cells were knocked out, resulting in lower LDL cholesterol levels (Musunuru *et al.*, 2021; Rothgangl *et al.*, 2021; Wang *et al.*, 2021). In vivo genome editing in the heart, on the other hand, has a much lower performance (Johansen *et al.*, 2017). After systematic administration of viral vectors to postnatal mice, the efficiency in cardiomyocytes was just 2–10%. While the efficiency of delivery systems affects genome editing, tissue (or cell) type appears to be another significant factor that influences genome editor operation. Except in a Cas9-transgenic mouse model, where distribution

of the big Cas9 protein would not be a challenge, editing performance in the heart was still poor (Carroll *et al.*, 2016). Another interesting finding was that when genome editing was done in neonatal mice, the editing quality of the heart was higher (Nelson *et al.*, 2019). When AAV8 was systemically administered to neonatal (P2) mdx mice, approximately 9% of gene alteration was observed in the heart up to one year after genome editing (Nelson *et al.*, 2019). The variations in editing performance between the liver and the heart, as well as between the neonatal and adult heart, are most likely due to differences in epigenetic status, such as chromatin accessibility, DNA repair machinery function, and cell cycle phases. In the cardiovascular region, genome editing in the liver has been studied more thoroughly than genome editing in the heart due to the adult heart's poor editing ability. Currently, using NHEJ to reduce the risk of atherosclerosis in the liver is a more viable approach than using it to treat inherited cardiac conditions in the heart.

While there are many genetic disorders for which gene repair of the heart might theoretically be a possible therapeutic alternative (e.g., HCM, DCM, LQTS, etc.) in the cardiovascular area, the heart's poor editing efficiency will be a major barrier (Musunuru, 2017). HDR-mediated gene repair is far more difficult than NHEJ-mediated gene destruction or deletion, as discussed previously. Methods to increase the efficacy of HDR must be built in order to realize in vivo genome editing of the heart as a cure for inherited heart disorders. Inhibiting NHEJ, activating HDR, or modifying cell cycle status are all possible options (Lin *et al.*, 2014). Another strategy is to create gene correction methods that do not rely on HDR, such as HITI or prime editing (Dowaidar, 2021).

While genome editing's on-target performance has increased, the most important problem in realizing the promise of genome-editing therapy is protection (Klaver-Flores *et al.*, 2020). In addition to the dangers associated with other gene therapy (e.g., transmission reagent toxicity, immune response, etc.), all forms of therapeutic genome editing carry the risk of off-target mutagenesis, which occurs when new mutations are added to sites in the genome other than the targeted on-target location. Off-target mutations in target cells or tissues may trigger unwanted functional phenotypes like oncogenesis (Papathanasiou *et al.*, 2021). Off-target mutagenesis makes treating the heart more difficult than other tissues in the cardiovascular region, so even uncommon off-target mutations can induce cardiac arrhythmias that can be lethal to the patient.

Off-target mutations vary between people because each patient has a particular genetic history that can not be replicated using model organisms (Xu *et al.*, 2019; Murugan *et al.*, 2020). As a result, prior to therapeutic implementation, each patient's off-target mutations must be assessed. Off-target mutations should be checked before ex vivo genome editing cells or tissues are implanted into the original patients. However, assessing off-target results prior to in vivo genome editing will be more difficult. Patients' primary cells or iPSC-derived cells may be used to monitor genome editing and anticipate off-target results in patients.

A variety of methods for detecting off-target mutations have been developed, but they are still in the early stages of development, and no systematic methods for therapeutic genome editing in patients have been developed (Kempton and Qi, 2019). Off-target mutations can be assessed using three different methods of assays. To begin, numerous in silico methods for predicting off-target sites have been developed and are now widely used in gRNA design.These tools are focused on the idea that a locus's off-target potential is determined by how close its sequence is to the on-target site (Mali *et al.*, 2013; Kempton and Qi, 2019).

Despite the fact that these methods are useful and commonly used due to their ease, recent studies have shown that off-target editing can occur at locations other than those expected by sequence similarity. Second, newer cell-based approaches such as GUIDE-Seq, BLISS, and DISCOVER-seq (Tsai *et al.*, 2015; Wienert *et al.*, 2019) can be used to test off-target editing in a genome-wide unbiased manner. GUIDE-seq, for example, tags DSBs created by genome editors with small oligonucleotides (Tsai *et al.*, 2015). The PCR-amplified genomic sites are then sequenced to trace the locus in the genome. While GUIDE-seq has been widely used in cultured cells, it would be difficult to adapt this approach to in vivo genome editing because it involves oligonucleotide transfection. Reasonable platforms to evaluate genome editing in patient cells are needed regardless of whether cell-based methods in this group are used. Third, unbiased cell-free in vitro assays including Digenome-seq, SITE-seq, and CIRCLE-seq have recently been developed (Kim *et al.*, 2015).

To detect genome-wide CRISPR/Cas9 action, these assays use cell-free genomic DNA isolated from target cells or tissues. They should prevent drawbacks associated with culture conditions and modification of living cells because genome editing is conducted in a cell-free in vitro environment using isolated genomic DNA in these assays. Cell-free methods have such a high sensitivity compared to cell-based methods that they can identify even unusual off-target sites. This benefit is critical in the clinical implementation of therapeutic genome editing, where unusual off-target mutations can trigger lethal phenotypes. One research found that by coupling cell-free CIRCLE-seq with amplicon sequencing, almost all off-target sites caused by in vivo genome editing can be identified (Kim *et al.*, 2015; Akcakaya *et al.*, 2018). There are two steps to this method: After in vivo genome editing, CIRCLE-seq was used to recognize off-target candidates using extracted genomic DNA (in vitro discovery step), and then targeted amplicon sequencing against these candidates was performed in mouse liver samples (in vivo confirmation step). Since it is a noninvasive approach that can reflect each patient's genetic history, this technique may be used for therapeutic genome editing in patients.

In terms of attempts to minimize off-target effects, it has been shown that using well-designed gRNAs is important for achieving low off-target effects. When a "promiscuous" gRNA was used against Pcsk9, for example, 19 off-target mutations were detected in the mouse liver, while no off-target mutations were detected when a well-designed gRNA was used (Akcakaya *et al.*, 2018). Other than gRNA sequences, a variety of attempts have been made to increase editing accuracy, including approaches that use shorter gRNAs (Mali *et al.*, 2013; Akcakaya *et al.*, 2018), chemically adapted gRNAs (Hendel *et al.*, 2015), Cas9 nickase variants that cause two single-strand breaks instead of DSBs (Ran *et al.*, 2013; Hendel *et al.*, 2015), and engineered new Cas9 variants (Liu *et al.*, 2019; Yamamoto *et al.*, 2019; Suh, 2020; Bharathkumar *et al.*, 2021). Although some of these non-traditional approaches seem promising, even higher fidelity Cas9 variants have lower editing performance, and delivery methods for in vivo therapeutic genome editing must be optimized.

Aside from the challenges inside genome editors, delivering genome editors to target tissues and cells is a big obstacle for clinical application (Cheng and Tsai, 2018; Hernandez-Gordillo *et al.*, 2020; Amendola *et al.*, 2021). Therapeutic genome editing, including on-target editing and off-target mutagenesis, necessitates the effective and precise distribution of genome editors to the target tissues. In vivo delivery methods for most gene therapies currently depend on AAV vectors, which have been licensed by the FDA (Riyad and Weber, 2021). Despite the fact that existing AAV systems have drawbacks such as cargo size and immunogenicity, they are highly efficient at delivering to a range of tissue types, including the eye, brain, liver, and muscle (Samulski and Muzyczka, 2014). The viral delivery system with AAV9 has already been used in clinical trials of cardiac gene therapies in the cardiovascular region (Ishikawa, Weber and Hajjar, 2018).

For CRISPR/Cas9-based genome editing, all Cas9 and gRNAs must be administered to the target tissues, either separately or together. A repair template is also needed for HDR-mediated gene correction. To accomplish the desired genome editing, selecting the right expression system and distribution mechanism is crucial. Early research using methods such as plasmids or viruses to deliver the Cas9 and gRNA coding sequences (Mishchenko, Mishchenko and Ivanisenko, 2021). While these DNA-delivery approaches have been used in several trials, both in vitro and in vivo, there are several possible drawbacks to consider, such as strong off-target effects due to prolonged Cas9 expression, the need for active promoters in the target cells, the chance of DNA absorption into the genome, and so on. Methods for delivering Cas9 as mRNA or protein to target cells have been established to prevent these complications (Mishchenko, Mishchenko and Ivanisenko, 2021). Current experiments have favored the use of a ribonucleoprotein (RNP), which is a Cas9 protein in complex with a gRNA. RNPs will function directly after transmission because they don't need Cas9 transcription and have less off-target effects because Cas9 expression isn't prolonged. Nanoparticles, peptides, lipofection or electroporation in vitro may be used to deliver RNPs to target cells (Dowaidar, Abdelhamid, Hällbrink, Freimann, *et al.*, 2017; Dowaidar, Abdelhamid, Hällbrink, Zou, *et al.*, 2017; Abdelhamid, Dowaidar and Langel, 2020).

Many expression mechanisms and distribution methods designed for in vitro platforms can be used for ex vivo therapeutic applications, such as blood cell editing (Mishchenko, Mishchenko and Ivanisenko, 2021). RNPs with electroporation are the best tool for achieving transient expression of

genome editors currently available. RNPs may also be administered using chemical conjugation or engineered cell-penetrating peptides. However, this form of application is restricted to the cardiovascular field (Dowaidar, Abdelhamid, Hällbrink, Freimann, *et al.*, 2017; Dowaidar, Abdelhamid, Hällbrink, Zou, *et al.*, 2017; Abdelhamid, Dowaidar and Langel, 2020).
The distribution options for in vivo applications are currently limited to viral vectors (Mishchenko, Mishchenko and Ivanisenko, 2021). Because of their low immunogenic capacity, decreased genome integration, and wide range of serotype specificity, AAV vectors are applied in a number of tissues, including the eye, brain, liver, and muscle, each AAV serotype has high efficacy (Riyad and Weber, 2021). AAV9 has also been used in clinical trials of cardiac gene therapy in the cardiovascular region (Ishikawa, Weber and Hajjar, 2018).

Conclusion
Genome editing may be used to cure or prevent two forms of cardiovascular disorders. The first type is affected by genetic regions in the heart or arteries that are responsible for the disease. This category includes a wide range of hereditary and acquired genetic diseases in cardiovascular tissues. The liver is currently the organ that has been researched the most for therapeutic genome editing of cardiovascular diseases. There are three major techniques for altering genomic DNA sequences to prevent or cure disease. The most straightforward approach to using genome editing is to disrupt a gene that is unhealthy or whose suppression is beneficial. Targeting PCSK9 to lower blood cholesterol levels is a promising example of this strategy. The "exon skipping" technique for Duchenne muscular dystrophy (DMD) is a clear example. Dystrophin, a protein required for muscle membrane stabilization, is the gene responsible for DMD. Exon deletions often interrupt the dystrophin gene's reading frame and result in complete loss of functional dystrophicin expression. Ex on skipping has been suggested as a treatment choice for DMD patients. Exons can be targeted in genome editing, which can potentially target any exon. It remains to be seen if exon skipping via genome editing will prevent heart failure, the most common cause of death in DMD patients.
CRISPR/Cas9-based methods have emerged, with applications in gene control, epigenome editing, genomic imaging, and other areas. DNA knock-in is possible with HITI also in non-dividing cells. Research into base-editing therapy for cardiovascular disorders has been restricted to PCSK9 in the liver. "Prime editing" works by combining a Cas9 H840A nickase with a reverse transcriptase. Without DSBs or donor DNA templates, prime editing allows for targeted insertions, deletions, and all 12 possible base-to-base transformations. In theory, this new method could correct identified genetic variants associated with human diseases, making it a promising candidate for therapeutic genome editing.
Designing suitable gRNAs to carry Cas9 to a target site that is within repeat sequences or has no adjacent sequences that are unusual relative to the rest of the genome can be challenging. It is important to achieve high editing efficiency at a desired genomic site in a desired tissue for therapeutic genome editing. In vitro genome editing in the heart has a much lower performance than in the liver. The heart's poor editing efficiency will be a major barrier to genome-editing therapy. All forms of genome editing carry the risk of off-target mutagenesis. Off-target mutations in target cells or tissues may trigger unwanted functional phenotypes like oncogenesis. Inhibiting NHEJ, activating HDR, or modifying cell cycle status are all possible options.
Cell-based approaches can be used to test off-target editing in a genome-wide unbiased manner. Unbiased cell-free in vitro assays have been developed to detect CRISPR/Cas9 action. Well-designed gRNAs are important for achieving low off-target effects. High fidelity Cas9 variants have lower editing performance, and delivery methods must be optimized. Delivering genome editors to target tissues and cells is a big obstacle for clinical application. Therapeutic genome editing necessitates the effective and precise distribution of genome editors. In vitro delivery methods for most gene therapies currently depend on AAV vectors.

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