WHITEPAPER

Advancements in CRISPR-Cas9 Gene Editing for Cardiovascular Disorders

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CONTENTS:

Recent developments surrounding CRISPR-Cas9 gene editing technology

CRISPR gaining traction

Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-Associated 9 (CRISPR-Cas9) technology has become the most widely used gene editing technology in recent years due to its simple design, low cost, high efficiency, and straightforward operation, compared to ZINC Finger Nucleases (ZFN) and transcription-activator-like effector nucleases (TALENS; Figure 1). CRISPR-Cas9 is an adaptive immune response found in bacteria and unlike other gene editing techniques, it can utilize both viral and non-viral platforms to deliver proficient genome editing in double-strand breaks (DSBs) in a wide range of organisms and cell types.[1] CRISPR-Cas9 technology is being rapidly adopted into all fields of biomedical research, including the cardiovascular (CV) field, where it has facilitated a greater understanding of cardiovascular disorders (CVDs), cardiomyopathies, electrophysiology, and lipid metabolism, as well as creating a variety of cellular and animal models for the evaluation of new therapies.[2]

Figure 1: Pros and cons of gene editing tools

CRISPR-Cas9

- Very efficient
- Inexpensive
- Moderate off target effects
- Protein-RNA-DNA interaction based
- Widespread use — animal, cell lines, plant, and protozoal parasites
- Extensive use in animal models

TALE Nuclease

- Efficient
- Expensive
- Low off target effects
- Protein-DNA interaction based
- Limited applications

ZINC finger

- Less efficient
- Very expensive
- Variable off target effects
- Protein-DNA interaction based
- Limited applications

Source: Authors' research[3]

Importantly, CRISPR has many applications both *in vitro* and *in vivo* and as a potential route for fixing faulty genes associated with certain illnesses. CRISPR allows researchers to study loss-of-function (LOF) or gain-of-function (GOF) mutations whereby mutations in a single gene that encodes for a protein product can either result in the protein structure changing so it no longer functions effectively as LOF mutation, or it generates a new protein isoform that can perform a new and important function, i.e., a GOF mutation. Thus, CRISPR technology provides researchers with a high degree of flexibility allowing the generation of gene knockouts (CRISPRn), interference (CRISPRi) or activation (CRISPRa), the regulation of endogenous gene expression, live-cell labeling of chromosomal loci, addition of single-stranded RNA, and high-throughput gene screening as well as the generation of CRISPR-based disease models.[4]

Clinical trials

In recent years, extensive studies have been conducted to improve the gene-editing specificity of CRISPR-Cas9 nucleases to reduce off-target effects such as RGEN (RNA-guided endonuclease)-induced mutations (at sites other than the intended on-target site)[5] and improve genome and tissue targeted delivery.[6] Technological advances have led to the development of a plethora of potential gene editing therapies. According to TOPRA (The Organisation for Professionals in Regulatory Affairs), there are currently 87 active clinical trials involving gene-editing[7] in a wide variety of genetic disorders, including inherited blood disorders, malignancies, and metabolic disorders.[8]

The first CRISPR-based therapy under regulatory review

The first gene editing product Exa-cel (exagamglogene autotemcel, formerly CTX001) developed by Vertex Pharma and CRISPR Therapeutics is expected to be approved by the regulators by the end of 2023 for the treatment of severe sickle cell disease (SCD) and transfusion-dependent beta-thalassemia (TDT). This CRISPR-based treatment induces the expression of fetal hemoglobin (HbF) via non-viral, *ex vivo* CRISPR/Cas9 gene editing, which restores hemoglobin levels in individuals with SCD and beta-thalassemia.[9] In phase III trials, Exa-cel met primary and key secondary endpoints in trials for both disorders.

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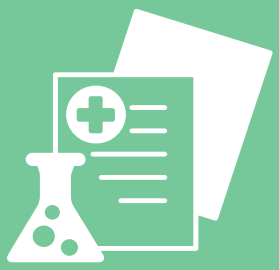
In TDT, almost 89% of patients did not require blood transfusions for at least 12 months. In sickle cell disease, 94% of patients remained free from vaso-occlusive pain crises typical of the disorder for at least one year. In January 2023, the European Medicines Agency (EMA) and the Medicines and Healthcare Regulatory Agency (MHRA) validated its Marketing Authorization Applications (MAAs) and the U.S. Food and Drug Administration (FDA) accepted a rolling biologic license application (BLA) in June and assigned a Prescription Drug User Fee Act (PDUFA) target action date of December 8, 2023 for SCD, and March 30, 2024 for TDT.[10]

Benefits and challenges of utilizing CRISPR gene editing as therapies for cardiovascular diseases

Identifying disease-causing mutations

Cardiovascular diseases (CVDs) are associated with several risk factors including lifestyle habits, environmental factors, and genetic predisposition.[11] CRISPR-Cas 9 techniques have greatly accelerated the identification of disease-causing mutations and led to the development of patient-specific induced pluripotent stem cells (iPSCs) and murine models for a variety of CVDs.[12][13,14] There are more than 100 monogenic inherited CVDs, such as hypertrophic cardiomyopathy, Marfan syndrome, and familial pulmonary hypertension whereby a single mutation can result in a lethal clinical manifestation.[15]

Single genetic mutations responsible for cardiomyopathies, such as arrhythmogenic cardiomyopathy (ACM),[16] dilated cardiomyopathy,[17] hypertrophic cardiomyopathy (HCM),[18] [19] Barth syndrome,[20] long-QT syndrome,[21] and Duchene Muscular Dystrophy (DMD),[22] have been corrected by genome editing in patient-specific iPSC-derived cardiomyocytes.[23] For instance, Atmanli and colleagues generated iPSC lines from a DMD patient and his healthy brother and then used adenoviral delivery of CRISPR Cas9/gRNA to correct dysfunctional cardiomyocytes in DMD mice models. [24]



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CRISPR-based therapies for rare CVDs

CRISPR gene editing and pluripotent stem cell technology have enabled a new class of cell replacement therapies to come to the fore. Several CRISPR-based gene-editing therapies are progressing through research and development and entering early clinical development for CVDs.

- NTLA-2001 is a CRISPR therapy candidate developed by Intellia Therapeutics in collaboration with Regeneron to treat transthyretin amyloidosis (TTA), which results in amyloid deposits accumulating in multiple tissues, leading to polyneuropathy and cardiomyopathy. NTLA-2001 deploys lipid nanoparticles (LNPs) to deliver sgRNAs targeting transthyretin (TTR) and mRNAs for Cas9 to permanently knock out TTR in the liver. Preliminary Phase I/II data showed that NTLA-2001 treatment at two different doses achieved mean reductions of 86% and 93% in serum TTR levels by day 28.[25]

- Intellia Therapeutics is also evaluating NTLA-2002 in Phase I/II for the treatment of Hereditary Angioedema (HAE) (NCT05120830). NTLA-2002 is a systemically administered LNP therapy designed to inactivate the target gene kallikrein B1 (KLKB1) to reduce plasma kallikrein activity and thus prevent HAE attacks; the estimated completion date for this study is 15 April 2024.

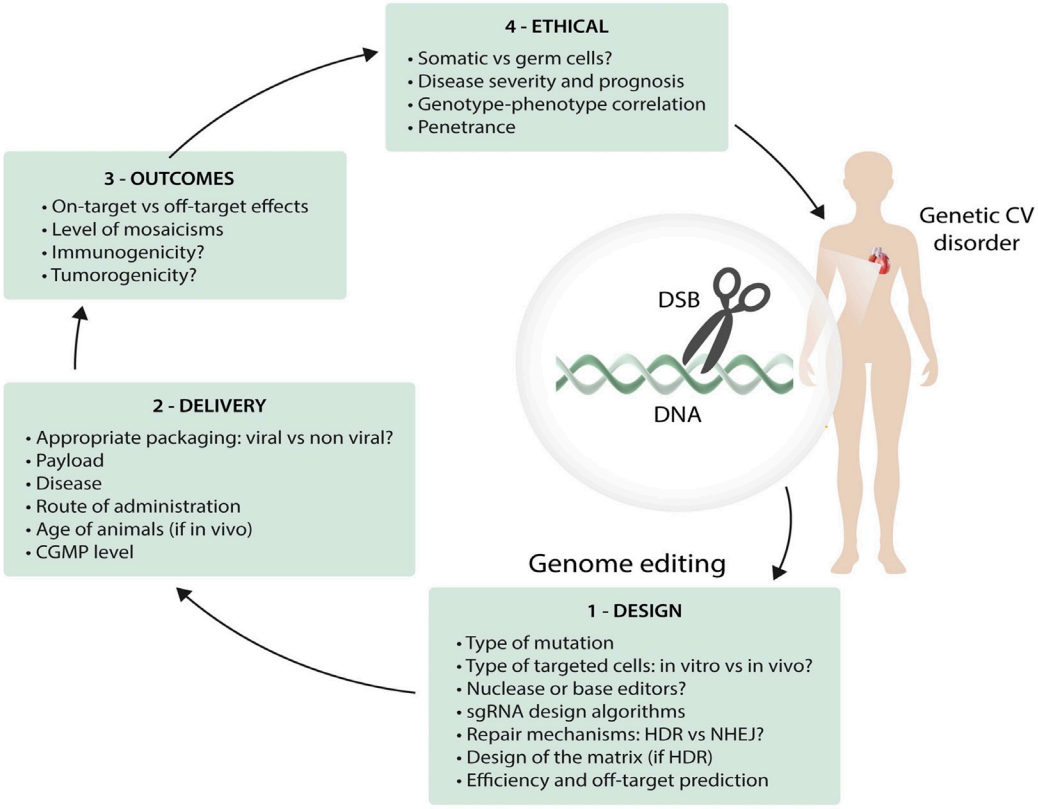
- CRISPR Therapeutics are evaluating two pre-clinical candidates, CTX310 and CTX320, targeting angiopoietin-related protein 3 (ANGPTL3) and lipoprotein(a) (Lp(a)), respectively, two validated targets for CVD.[26] [27] In preclinical studies in non-human primates (NHP) a single dose of CTX310-ANGPTL3 results in a significant and prolonged reduction in serum ANGPLT3 protein and triglycerides by 89% and >50% from baseline, respectively. Similarly, CTX320, Lp(a) 3mg/kg dose reduces Lp(a) levels by up to 92% from baseline. CST310 is scheduled to enter clinical trials during 2023 followed by CTX320.[28]

- CRISPR therapies may also play a role in cardiomyopathy associated with DMD. In August 2022, the FDA approved the first-in-human dosing of Cure Rare Diseases, CRD-TMH-001 investigational CRISPR therapy to treat DMD.[29] CRD-TMH-001 targets mutations in the promoter region and exon 1 of the DMD gene and the aim is to stabilize or reverse the progression of the disease by upregulating a dystrophin protein isoform to reduce muscle weakness and wasting as well as cardiomyopathy associated with the later stages of the disease. Unfortunately, the first patient treated with the therapy died four months after receiving treatment; however, the cause of death is unknown but may be due to an adverse reaction to the viral vector.[30]

Importance of monitoring on- and off-target effects

Despite these initial successes and promising developments, the path to CRISPR-based therapies has not been a smooth one. CRISPR-Cas9 gene editing can result in significant on-target mutagenesis, such as large deletions and more complex genomic rearrangements at the targeted sites and the Cas9 protein can evoke off-target immunogenic effects by binding to MHC binding epitopes. In addition, the DSBs induced by Cas9 may also be toxic to cells and induce apoptosis.[28] Thus, it is essential to optimize CRISPR–Cas9 design, delivery, and outcomes to ensure safety, specificity, and efficacy before it's used in clinical trials to correct genetic CVDs (Figure 2).[31]

Figure 2: Application of CRISPR in cardiovascular disease



CGMP - cyclic guanosine monophosphate; CV - cardiovascular; DSB - double strand break; HDR - homology-directed repair; NHEJ - non-homologous end joining

Source: Vermersch et al., 2020[32]

Regulatory considerations for the use of gene editing in treating cardiovascular conditions

Regulatory framework evolving

Steady progress has been made by the regulators in the publication of guidelines regarding conducting human clinical trials in rare diseases and the use of cellular or gene-based therapies[33] and have established a framework to review and approve these life-changing therapies and their respective companion diagnostics (CDx) or in-vitro diagnostic device (IVD). Despite these changes, the regulators have limited experience with the clinical evaluation of medicinal products manufactured with the CRISPR-Cas9 system and current regulatory knowledge in gene editing comes from experience with previous gene editing methods. It will take time for the regulators to learn, adapt, and harmonize guidelines and it will be essential for the regulatory authorities to share information with other stakeholders to move the field forward. [34] In the meantime, the lack of formal interaction between regulators and limited harmonization across regions for clinical development poses a significant challenge for sponsors; however, programs like Project Orbis coordinated by the FDA and MHRA alongside several other regulatory agencies and the recent announcement by FDA of their plan to launch an Operation Warp Speed-style pilot program for rare disease cell and gene therapies, should help to facilitate the development of innovative cell and gene therapies.[35]

Currently, the FDA considers any use of CRISPR/Cas9 gene editing in humans to be gene therapy and is subject to regulation by the FDA's Center for Biologics Evaluation and Research (CBER). Therefore, clinical studies of gene therapy in humans require the submission of an investigational new drug application (IND) before their initiation in the U.S., and marketing of a gene therapy product requires submission and approval of a BLA. In addition, companies developing CRISPR therapies can request regenerative medicine advanced therapy (RMAT) designation concurrently with the submission of their IND or as an amendment to an existing IND, providing the therapy meets the criteria as a regenerative medicine therapy that is intended to treat, modify, reverse, or cure a serious or life-threatening disease or condition, and clinical evidence indicates it has the potential to address an unmet

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medical need. RMAT is a designation specifically for regenerative advanced therapy products and provides all the advantages of a Breakthrough Therapy designation, including early interactions with the FDA and also the potential for accelerated approval.

The EMA follows a stepwise process for the approval of cell and gene therapies in the European Union (EU). This involves preclinical and clinical studies, submission of an MAA, and evaluation by the Committee for Advanced Therapies (CAT). Like the FDA, the EMA offers mechanisms such as conditional approval and priority medicines (PRIME) designation to expedite access to therapies with high potential for public health benefit.[36] The EMA evaluates gene editing medicines including CRISPR/Cas9 products for *in vivo* use under advanced therapy medicinal products (ATMP) regulation (Council Regulation [EC] 1394/2007 of 13 November 2007 on ATMPs and amending Directive 2001/83/EC and Regulation [EC] 726/2004). In addition, for some genome-editing products in the EU, the genetically modified organisms (GMO) regulation and requirements may apply (Directive 2001/18/EC on the deliberate release into the environment of GMOs; [37] Directive 2009/41/EC on the contained use of genetically modified microorganisms[38]). In January 2022, the European Union (EU) initiated a new legal framework Clinical Trial Regulation 536/2014 (EU-CTR) to increase transparency and harmonize the submission, assessment, and supervision processes for clinical trials. This is expected to facilitate the clinical development of novel products for rare CVDs.[39]

Ethical issues will also need to be addressed, in particular for human heritable/germline editing, as any potential use for functional enhancement of people and unconventional trial designs may or may not be acceptable for different stakeholders. [40] In addition, placebo-controlled trials are often not possible due to ethical reasons; regulators and the industry are reliant on the use of natural history data, although for some (ultra) rare diseases this type of data may not be readily accessible or available. Similarly, the inclusion of pediatrics generates additional challenges as the benefit/risk ratio may not be favorable or clear during the initial stages of the clinical development where there is limited efficacy and safety data. For instance, off-target effects are of greater concern with *in vivo* editing compared to *ex vivo*

During the development of a gene-editing therapy, CDx is often used to help define the population for inclusion in the gene therapy trial, and therefore clinical research organizations (CROs) need to be able to identify the regulatory pathways available for the device component in parallel to the clinical trials.

editing. Consequently, companies will need to ensure they undertake appropriate risk-benefit assessments to ensure that they are fully aware of the potential risks associated with the different methods of gene editing and to put in place protocols/procedures to mitigate risks that could impact clinical trial development.

Therefore, the timely development of these innovative medicinal products requires regulatory oversight and changes to the regulatory framework to clarify the definition and classification of genome-editing products as well as international harmonization is one of the key recommendations of the EU-IN Horizon Scanning Report on genome editing (EMA/319248/2020). Moving forward, regulators will require a better understanding of how novel methods will affect immunogenicity, dosing, long-term clinical efficacy, and safety and whether repeat administration is beneficial.

Operational and regulatory efficiencies need careful consideration

During the development of a gene-editing therapy, CDx is often used to help define the population for inclusion in the gene therapy trial, and therefore clinical research organizations (CROs) need to be able to identify the regulatory pathways available for the device component in parallel to the clinical trials. The simultaneous development of therapy and diagnostics requires careful consideration by developers, specifically in terms of the timing of two different regulatory pathways; one for their gene editing product and another for a CDx/IVD where the goal is that these are completed around the same time to ensure the CDx is available at time of the product launch, thereby maximizing market access and reducing barriers to entry.

For example, the FDA recently approved BioMarin's Roctavian (valoctocogene roxaparvovec), an adeno-associated virus vector-based gene therapy for the treatment of adults with severe hemophilia A without pre-existing antibodies to adeno-associated virus serotype 5 detected by an FDA-approved test AAV5 DetectCDx.[41] In the U.S., a CDx can be made available through labs that are certified under the Clinical Laboratory Improvement Amendments (CLIA) whereas in the EU, developers are advised to use an EU-based lab to ensure that the device is developed in line with IVDR to minimize potential regulatory hurdles during clinical development.

In addition, there are logistical and operational considerations that a CRO and study sponsor need to consider when using *in vivo* gene editing versus *ex vivo* editing. For instance, impeccable planning is required for the transportation of patient cells to the manufacturer within a given timeframe, and site licenses are required to retrieve cells from patients and transport the product following manufacturing for *ex vivo* editing.

Non-clinical disease models are limited

Given the infancy of CRISPR technologies, relatively few non-clinical disease models are currently available to test gene editing products and there is also limited regulatory guidance on the use of these models to support product development. In addition, the data generated in available models may not be translatable to human studies in terms of mechanism of action, safety, or efficacy. Therefore, it is important to determine what the aim behind using a specific model is and whether it can address the questions that the sponsor/CRO is trying to answer. Overall, gene-editing therapies present unique challenges in terms of long-term safety and efficacy, delivery, and operational complexity; therefore, CROs must be prepared for these challenges and be ready to work with study sponsors to solve them.

Choosing the right CRO partner for cardiovascular and gene therapy clinical development

Multifunctional/multidisciplinary teams

When it comes to conducting clinical trials in cardiology, choosing the right CRO can make a huge difference in terms of accelerating the drug development process, reducing operational complexity, and ensuring regulatory compliance.[42] Biotech/pharma must partner with a CRO with a proven track record that has access to multifunctional (e.g. drug metabolism and pharmacokinetics (DMPK), toxicology, bioanalysis, translational medicine) and multidisciplinary teams (e.g. medical, regulatory, and operational leadership, as well as experienced certified cardiologists) with global experience in drug and device studies. [43]

Research networks, data analytics, and assay development

Access to a global cardiovascular research network is critical to driving patient recruitment and retention. Early engagement with regulators, innovative clinical trial design (virtual and hybrid), and adequate post-market confirmatory studies to monitor the long-term risks are all essential when performing gene therapy studies. Ideally, the CRO will have comprehensive knowledge and expertise in designing custom

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genomic assays and have access to technological platforms that can support the quantitation of on-target and off-target effects. The CRO will also need experience in developing validated GLP assays and have a track record for getting these assays CLIA-certified to support gene therapy trials should sponsors seek to develop a CDx.

CRO-pharma: a cohesive partnership

A new era of gene-editing therapies is dawning; these treatments have the potential to revolutionize the treatment of CVDs with high unmet medical needs.[44] However, the unique nature of these therapies poses many challenges to regulators and developers, and therefore stakeholders need to work together to develop a regulatory framework that will ensure the delivery of safe and effective therapies in the future. Medpace works closely with pharma partners to provide high-quality integrated services, medical and scientific expertise, operational support, and regulatory guidance to help mitigate developmental risks, overcome regulatory uncertainties, and ensure long-term data management that demonstrates the value that these life-changing CVD therapies bring to those in need.

Endnotes

1. Zhu Y (2022) Advances in CRISPR/Cas9. Biomed Res Int. :9978571. doi: 10.1155/2022/9978571. PMID: 36193328; PMCID: PMC9525763.
2. Strong A, Musunuru K (2016) Genome editing in cardiovascular diseases. Nat Rev Cardiol. 2017 Jan;14(1):11-20. doi: 10.1038/nrcardio.2016.139. Epub Sep 9. PMID: 27609628.
3. Gupta (2015) https://varuncnmicro.blogspot.com/2015/05/lab-series-6-short-introduction-to-talen.html
4. Gupta D, Bhattacharjee O, Mandal D, et al. (2019) CRISPR-Cas9 system: A new-fangled dawn in gene editing. Life Sci. 2019 Sep 1;232:116636. doi: 10.1016/j.lfs.116636. Epub 2019 Jul 8. PMID: 31295471.
5. Huang X, Yang D, Zhang J, Xu J, Chen YE (2022) Recent Advances in Improving Gene-Editing Specificity through CRISPR-Cas9 Nuclease Engineering. Cells. ;11(14):2186. doi: 10.3390/cells11142186. PMID: 35883629; PMCID: PMC9319960.
6. Sinclair F, Begum AA, Dai CC, et al. (2023) Recent advances in the delivery and applications of nonviral CRISPR/Cas9 gene editing. Drug Deliv Transl Res. ;13(5):1500-1519. doi: 10.1007/s13346-023-01320-z. Epub 2023 Mar 29. PMID: 36988873; PMCID: PMC10052255.
7. Edwards H (2023), G-E-T-ting there – how gene editing technologies could change more than gene. Retrieved from https://www.regulatoryrapporteur.org/pharmaceuticals/g-e-t-ting-there-how-gene-editing-technologies-could-change-more-than-genes/276.article
8. U.S. National Library of Medicine. Clinicaltrials.gov. 2022. https://clinicaltrials.gov/.
9. Locatelli F, Lang P, Li A,er al. (2022); Efficacy and Safety of a Single Dose of Exagamglogene Autotemcel for Transfusion-Dependent-Thalassemia. Blood ; 140 (S1): 4899–4901. doi: https://doi.org/10.1182/blood-2022-166881
10. CRISPR Therapeutics (June 2023) FDA Accepts Biologics License Applications for exagamglogene autotemcel (exa-cel) for Severe Sickle Cell Disease and Transfusion-Dependent Beta Thalassemia. Retrieved from http://ir.crisprtx.com/news-releases/news-release-details/fda-accepts-biologics-license-applications-exagamglogene
11. Roshanravan N, Tutunchi H, Najafipour F, et al., (2022) A glance at the application of CRISPR/Cas9 gene-editing technology in cardiovascular diseases. J Cardiovasc Thorac Res. ;14(2):77-83. doi: 10.34172/jcvtr.2022.14. Epub 2022 Jun 14. PMID: 35935390; PMCID: PMC9339732.
12. Khera AV, Kathiresan S (2017) Genetics of coronary artery disease: discovery, biology and clinical translation. Nat Rev Genet. 18(6):331–344. doi: 10.1038/nrg.2016.160.
13. Liu N, Olson EN (2022). CRISPR Modeling and Correction of Cardiovascular Disease. Circ Res.;130(12):1827-1850. doi: 10.1161/CIRCRESAHA.122.320496. Epub 2022 Jun 9. PMID: 35679361; PMCID: PMC9202442.
14. Ledford, H. (2015) CRISPR, the disruptor. Nature 522, 20–24. https://doi.org/10.1038/522020a
15. Cao G, Xuan X, Zhang R, Hu J, Dong H. (2021) Gene Therapy for Cardiovascular Disease: Basic Research and Clinical Prospects. Front Cardiovasc Med. Nov 5;8:760140. doi: 10.3389/fcvm.2021.760140. PMID: 34805315; PMCID: PMC8602679.
16. Janz A, Zink M, Cirnu A, et al. (2021)CRISPR/Cas9-edited PKP2 knock-out (JMUi001-A-2) and DSG2 knock-out (JMUi001-A-3) iPSC lines as an isogenic human model system for arrhythmogenic cardiomyopathy (ACM). Stem Cell Res. 2021 May;53:102256. doi: 10.1016/j.scr..102256. Epub 2021 Feb 18. PMID: 33640690.
17. Karakikes I, Stillitano F, Nonnenmacher M, et al. (2015). Correction of human phospholamban R14del mutation associated with cardiomyopathy using targeted nucleases and combination therapy. Nat Commun;6:1–10.
18. Hinson JT, Chopra A, Nafissi N, et al. (2015) Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. Science;349:982–986.
19. Strong, A. (2023) CRISPR gene-editing therapies for hypertrophic cardiomyopathy. Nat Med 29, 305–306. https://doi.org/10.1038/s41591-022-02184-5
20. Wang G, McCain ML, Yang L, et al. (2014). Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. Nat Med ;20:616–623.
21. Limpitikul WB, Dick IE, Tester DJ, et al (2017) A precision medicine approach to the rescue of function on malignant calmodulinopathic long-QT syndrome. Circ Res;120:39–48.
22. Choi IY, Lim HT, Estrellas K, et al. (2016). Concordant but varied phenotypes among Duchenne muscular dystrophy patient-specific myoblasts derived using a human iPSC-based model. Cell Rep;15:2301–2312.
23. Vermersch E, Jouve C, Hulot JS. (2020) CRISPR/Cas9 gene-editing strategies in cardiovascular cells Cardiovasc Res, Volume 116, Issue 5,, Pages 894–907, https://doi.org/10.1093/cvr/cvz250
24. Atmanli A, Chai AC, Cui M, et al. (2021) Cardiac myoediting attenuates cardiac abnormalities in human and mouse models of Duchenne muscular dystrophy. Circ Res.. doi:10.1161/CIRCRESAHA.121.319579

25. Tongtong C, Li B, li W (2022), NTLA-2001: opening a new era for gene therapy, Life Medicine, 1, 2, 49–51 https://doi.org/10.1093/lifemedi/lnac036
26. Jiang S, Qiu GH, et al. (2019) ANGPTL3: a novel biomarker and promising therapeutic target. J Drug Target. Sep;27(8):876-884. doi: 10.1080/1061186X.2019.1566342. Epub 2019 Jan 25. PMID: 30615486.
27. Tromp TR, Stroes ESG, Hovingh GK (2020). Gene-based therapy in lipid management: the winding road from promise to practice. Expert Opin Investig Drugs.;29(5):483-493. doi: 10.1080/13543784.2020.1757070. Epub 2020 Apr 29. PMID: 32349563.
28. CRISPR Therapeutics (2022) Corporate Slide Deck Q4 2022. Retrieved from https://s3.us-east-1.amazonaws.com/crisprtx-assets.investeddigital.com/CRISPR-Tx-Corporate-Deck-Q4-2022.pdf
29. Cure Rare Disease (2021) FDA Approval CRISPR Therapeutic. Retreived from https://www.cureraredisease.org/fda-approval
30. STAT (2023) Early look at gene therapy death suggests CRISPR wasn't cause. Retreived from https://www.statnews.com/2023/04/26/muscular-dystrophy-crispr-gene-therapy-death-early-findings/
31. Roshanravan N, Tutunchi H, Najafipour F, et al. (2022). A glance at the application of CRISPR/Cas9 gene-editing technology in cardiovascular diseases. J Cardiovasc Thorac Res. 14(2):77-83. doi: 10.34172/jcvtr.2022.14. Epub 2022 Jun 14. PMID: 35935390; PMCID: PMC9339732.
32. Vermersch E, Jouve C, Hulot JS. (2020) CRISPR/Cas9 gene-editing strategies in cardiovascular cells Cardiovasc Res, Volume 116, Issue 5,, Pages 894–907, https://doi.org/10.1093/cvr/cvz250
33. FDA (Accessed July 2023) Cellular & Gene Therapy Guidance. Retrieved from https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/cellular-gene-therapy-guidances
34. Anliker B, Childs L, Rau J, et al. (2022) Regulatory Considerations for Clinical Trial Applications with CRISPR-Based Medicinal Products. CRISPR J. 5(3):364-376. doi: 10.1089/crispr.2021.0148. Epub 2022 Apr 22. PMID: 35452274.
35. Gov.UK (2023) Project Orbis. Retrieved from https://www.gov.uk/guidance/guidance-on-project-orbis
36. Bluecord (2023) Understanding the Differences Between FDA and EMA in Cell and Gene Therapy Regulation. Retrieved from https://www.bluecord.com/post/understanding-the-differences-between-fda-and-ema-in-cell-and-gene-therapy-regulation
37. EMA, (2001) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms. Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32001L0018
38. EMA, (2009) Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. Retrieved from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:125:0075:0097:EN:PDF
39. EC (accessed June 2023) Clinical trials—Directive 2001/20/EC, Public Health Directive, ec.europa.eu
40. Europa.eu (accessed June 2023) Genome Editing EU-IN Horizon Scanning Report. Retrieved from https://www.ema.europa.eu/en/documents/report/genome-editing-eu-horizon-scanning-report_en.pdf
41. FDA (2023) FDA Approves First Gene Therapy for Adults with Severe Hemophilia A. Retrieved from https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapy-adults-severe-hemophilia
42. Vial (2023) CROs in Cardiology: Advancing Treatments for Heart Disease ★ https://vial.com/blog/articles/cros-in-cardiology-advancing-treatments-for-heart-disease/&https:/vial.com/blog/articles/cros-in-cardiology-advancing-treatments-for-heart-disease/?utm_source=organic
43. Cell And Gene (2019) 3 Strategies To Use When Selecting A CRO For A Gene Therapy Study. Retrieved from https://www.cellandgene.com/doc/strategies-to-use-when-selecting-a-cro-for-a-gene-therapy-study-0001#:~:text=Here%20are%20three%20helpful%20strategies%20to%20follow%20when,Anticipate%20the%20challenges%20associated%20with%20gene%20therapy%20studies.
44. Drago D, Foss-Campbell B, Wonnacott K, et al. (2021). Global regulatory progress in delivering on the promise of gene therapies for unmet medical needs. Mol Ther Methods Clin Dev. 2021 Apr 5;21:524-529. doi: 10.1016/j.omtm.2021.04.001. PMID: 33997101; PMCID: PMC8099595.