WHITE PAPER

ARCUS® Shows Promise for *In Vivo* and *Ex Vivo* Therapeutics

Over time, the cost of DNA analysis technologies has dropped drastically, making them accessible to the entire life sciences community and allowing for the identification of pathogenic genetic variants with remarkable speed and accuracy.[1-3] With each new discovery, there is increased interest and rapid investment in gene editing as a clinical intervention to potentially treat or even cure diseases.[2,4] Some early results have given hope for a new approach to diseases that have long been considered incurable.[2,5]

Unfortunately, gene editing is much more challenging to accomplish in the clinic than in the laboratory.[4] There are a number of challenges associated with making targeted edits for both *in vivo* (DNA edited within a patient) and *ex vivo* (DNA edited in cells that are then transplanted into a patient) therapeutics. Consistently achieving the appropriate edit (ie, deletion, insertion, or repair) in the desired target tissue and achieving a lasting response without introducing consequential off-target effects are among the major hurdles this field needs to overcome for gene editing to earn its place as a proven therapeutic option.[6] These challenges have continued across decades of innovation in gene-editing techniques, including zinc-finger nucleases (ZFNs), transcription activator–like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR).[7]

The ARCUS gene-editing platform was conceived by Derek Jantz, PhD, and Jeff Smith, PhD, more than 15 years ago and became the foundation for Precision BioSciences, Inc., when the company was founded in 2006. Based on a naturally occurring gene-editing enzyme from the homing endonuclease (also referred to as meganuclease) family, ARCUS nucleases have a unique set of traits optimal for clinical gene editing. The utility and efficacy of the ARCUS platform have been validated through extensive preclinical *in vivo* studies in large animal models and encouraging results observed in clinical trials evaluating *ex vivo* manufactured allogeneic chimeric antigen receptor (CAR) T-cell therapies.

Gene editing in brief

Each approach for gene editing has been geared toward finding a reliable way to target a gene at a precise location and edit the DNA sequence.[8] Most gene-editing technologies, including ZFNs, TALENs, homing endonucleases, and CRISPR, accomplish this by generating a DNA double-strand break repair at a target sequence. During repair of the break, the target DNA can be removed and/or DNA can be added.

For therapeutic purposes, gene editing can be performed *in vivo* or *ex vivo*.[9] *In vivo* editing involves introducing genome-editing components directly into a patient so that the gene-editing process occurs within that patient's cells.[10] This is a promising approach to correcting genetic or infectious diseases.[4,11] In contrast, *ex vivo* editing involves taking cells from a patient or donor, genetically altering them, and transplanting the modified cells into the patient.[10] This approach is useful for applications in fields such as immuno-oncology, where immune cells are genetically programmed to recognize and kill cancer cells and are then transplanted into the body to target the cancer.

Although vast improvements have been made to gene-editing technologies over the years, they still face a number of challenges, including imprecise editing at the target site (due to mixed modes of DNA double-strand break repair), delivery of and immunity against gene-editing components, and long-term safety.[9,12,13] One of the biggest concerns for all current

Snapshot: *in vivo* gene editing for primary hyperoxaluria type 1

One gene-editing program wholly owned by Precision BioSciences, Inc., focuses on the treatment of primary hyperoxaluria type 1 (PH1), a disease caused by mutations in the glycine biosynthesis pathway that results in the buildup of oxalate, a toxic metabolite that can cause a severe and potentially fatal accumulation of kidney and urinary tract stones.[44,45] Targeting the well-validated *HAO1* (hydroxyacid oxidase 1) gene has been shown to result in an accumulation of glycolate, a nontoxic soluble metabolite that is easily excreted, instead of oxalate.[46] Lumasiran (Alnylam Pharmaceuticals Inc.), a small interfering RNA that targets *HAO1* mRNA, was Food and Drug Administration approved in November 2020 for the treatment of PH1.[47,48] The therapy requires 3 up-front loading doses administered once monthly followed by maintenance doses administered once every 1 to 3 months depending on body weight.[47]

In preclinical studies, Precision BioSciences has administered an *HAO1*-specific ARCUS nuclease via an adeno-associated virus vector to nonhuman primates. The single treatment led to a 98% reduction in *HAO1*'s resulting protein (glycolate oxidase) as well as higher levels of glycolate in serum (80 µM with ARCUS treatment versus <20 µM with the control). This indicates that knockdown of *HAO1* with ARCUS may be a potential single-dose treatment option for patients with PH1 as opposed to siRNA therapy, which requires periodic redosing.

gene-editing platforms, however, is the potential for off-target gene editing, which occurs when the site-specific nuclease binds to and alters an unintended genomic location.[9,12] This can occur when there are sites with closely related sequences that differ by only a few base pairs, making it challenging for the system to differentiate between the target site and the similar unintended site.[9,14] Different gene-editing technologies have varying abilities to discriminate between these closely related sites.[4] This has clinical implications because off-target editing may interfere with normal gene function (via insertions, deletions, and/or chromosomal rearrangements, including translocations) and/or lead to the expression of tumor-promoting genes; therefore, challenges with both nuclease specificity and the ability to detect off-target edits must be resolved before gene editing can be used as a mainstream therapeutic application.[9,15]

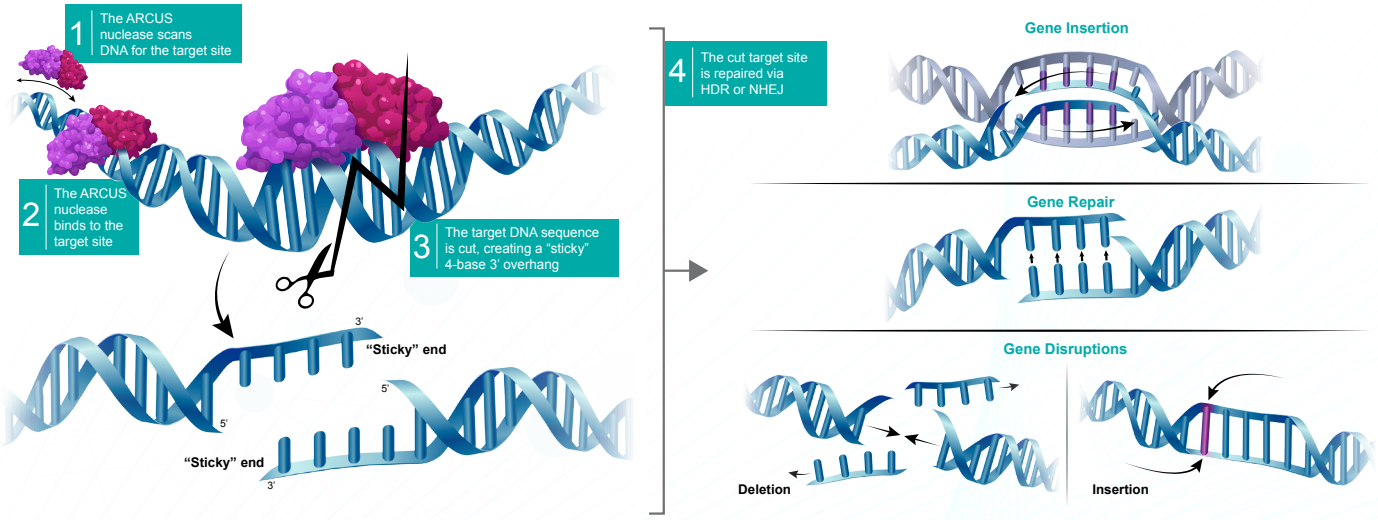
The ARCUS difference: precision, versatility, and delivery

The ARCUS gene-editing platform is based on a naturally occurring gene-editing nuclease called I-CreI (pronounced I-Crē-I), which comes from the alga *Chlamydomonas reinhard ii*.[16] Unlike bacterial CRISPR enzymes, which in their natural state cut frequently to evade viruses, I-CreI evolved in nature to perform a single, highly specific DNA edit.[17,18] Each ARCUS nuclease is generated through an iterative protein-engineering process to optimize on-target binding and cutting activity while minimizing off-target editing. The proteins' DNA-binding site is customizable to prevent the cutting of similar off-target sequences, including sequences that differ by only 1 base from the intended site. Additionally, the cutting activity can be customized to match the type of delivery, the concentration, and the amount of time that the nuclease is active in the cells.

Precision

ARCUS nucleases have a number of inherent traits that make them promising tools for targeted, precise, and safe gene-editing therapeutic applications. Most importantly, they have a high degree of specificity that minimizes the cutting of off-target sites in the genome.[19,20] This is partly because they recognize a long, 22-base DNA sequence to home in on the correct sequence at the target site.[7,19] The high specificity of ARCUS nucleases may also be due to the structure and location of the nuclease and DNA-binding domains. Unlike many competing technologies, such as ZFNs and TALENs, which are fusions of separate DNA-binding and -cutting domains, the ARCUS nuclease cutting domain is integrated into the DNA-binding domain. ARCUS nucleases, like other technologies, are able to discriminate against many off-target sites by having tighter binding affinity for the correct site. The close integration of the cutting and binding functions provides ARCUS nucleases with the added advantage that binding the correct target site causes a conformational change that allows cutting. Other technologies may not be able to differentiate binding sites with sequences that are only different by 1 base; however, the added conformational change that is unique to ARCUS nucleases reduces the likelihood of cutting these off-target sites.

FIGURE 1: ARCUS GENE EDITING PLATFORM



1) The ARCUS nuclease scans DNA for the target site. 2) The ARCUS nuclease binds to the target site. 3) The target DNA sequence is cut, creating a "sticky" 4-base 3' overhang. 4) The cut target site is repaired via HDR or NHEJ. If a template DNA sequence is supplied, the DNA can be precisely repaired or altered (including gene insertion) via HDR. Without a template DNA sequence, the "sticky" ends are repaired via NHEJ; however, this process can lead to insertions and/or deletions, both of which can lead to gene disruption.

HDR, homology-directed repair; NHEJ, nonhomologous end joining.

Versatility

Another key differentiator between the ARCUS gene-editing platform and competing technologies is the type of cut made by ARCUS nucleases. After cleaving its target site, an ARCUS nuclease leaves behind a pair of 3' sticky ends, each consisting of 4 bases (Figure 1).[20] These sticky ends can be repaired by a high-fidelity process called homology-directed repair (HDR), which uses a DNA template to accurately repair the DNA break.[20,21] In contrast, other gene-editing technologies, such as CRISPR and ZFNs, require additional processing of the DNA ends for HDR and more often use nonhomologous end joining (NHEJ) to repair DNA breaks, which is a method that is more likely to introduce errors and potentially knock out the gene.[13,20,21] However, if the 4-base 3' sticky ends created by ARCUS are repaired via NHEJ, they are more likely to be correctly put back together; this is an important safety feature that makes it less likely that a mutation will be introduced if an off-target site is cut.[22]

Delivery

Compared with some other gene-editing technologies, an ARCUS nuclease is relatively small (~1.1 kilobases [kb] and ~360 amino acids), making it possible to deliver via lipid nanoparticles or by a single gene therapy vector, such as an adeno-associated virus (AAV) , which has a packaging capacity of ~4.4 kb.[20,23-26] Some other gene-editing tools, including many CRISPR systems, base editors, and prime editors, are too large to fit into a single AAV vector.[27-29] In some cases, elements of the tool can be split into multiple vectors; however, this can limit successful delivery of the system because each cell must receive all vectors for the edit to occur.

Snapshot: *in vivo* gene editing to lower cholesterol

In collaboration with scientists at the University of Pennsylvania, Precision BioSciences, Inc., developed an ARCUS nuclease capable of inactivating *PCSK9*, a gene that regulates the levels of low-density lipoprotein (LDL) cholesterol by preventing removal of excess LDL in the liver.[49,50] The *PCSK9*-specific ARCUS nuclease was administered to nonhuman primates and effects on PCSK9 and LDL levels were evaluated.[31] Following treatment, the animals experienced up to an 84% drop in PCSK9 levels, which was associated with up to a 60% reduction in serum LDL. Three years later, these animals still exhibited lower LDL levels, indicating that the effects of the one-time treatment may be permanent.[23] Furthermore, at this longer follow-up, stable gene editing was maintained without any obvious adverse effects.[50] This project provided the first peer-reviewed validation of clinically relevant gene editing in large animal models.

Application: *in vivo* editing to target a patient's genes

Precision BioSciences has shown early preclinical success in several large animal models for different genetic conditions, including primary hyperoxaluria type 1 (PH1),[30] chronic hepatitis B virus (HBV),[25] familial hypercholesterolemia,[23,31] familial amyloid polyneuropathy/cardiomyopathy,[32] autosomal dominant retinitis pigmentosa,[33] and lipoprotein lipase deficiency. Please see the snapshots throughout for more information on these programs.

Application: Creating "off-the-shelf" CAR T-cell therapies for cancer

Unlike *in vivo* gene-editing technologies, cell therapies can be created using *ex vivo* editing, which has a number of advantages, including fewer delivery challenges, less concern with off-target editing in other cells of the body, and the ability to screen for successful editing prior to administration.[10,34]

CAR T-cell therapy is a type of cell therapy that uses T cells that have been manipulated to express cell-surface CARs, which enable the T cells to recognize and attack specific cancer cells.[35] Autologous CAR T-cell therapies, which use a patient's own cells, are programmed to produce CARs using viral vectors, and a number of therapies have been Food and Drug Administration (FDA) approved in several relapsed or refractory (r/r) hematologic malignancies.[36] While these treatments have expanded options for these patients, autologous CAR T-cell therapies have some limitations. First, because the cells are taken from the patient, the extraction, manufacturing, and shipping time can take weeks, increasing the time a patient must wait to receive treatment, which is especially problematic for patients with aggressive cancers who are at risk of clinical deterioration that could make them ineligible for CAR T-cell treatment.[37-39] Second, some patients eligible for CAR T-cell therapy have already received multiple treatments that can leave them with few functioning T cells with proliferative capacity, making it challenging or unfeasible to manufacture enough CAR T cells for treatment. While not without their own challenges, "off-the-shelf" allogeneic CAR T-cell therapies, which use healthy donor cells, have the potential to overcome these issues by providing a readily available and higher-quality product.[37,38] Currently, there are no FDA-approved allogeneic CAR T-cell treatments; however, Precision BioSciences has made significant progress developing several product candidates for *ex vivo* applications.

One of the main concerns with allogeneic cell therapies is the potential for graft-versus-host disease (GvHD), which is when the genetically modified cells recognize the patient's cells as foreign and attack them.[38,39] Precision BioSciences has successfully circumvented this issue by developing a single-step gene-editing approach in which the endogenous T-cell receptor (TCR) is knocked out while a CAR coding sequence is knocked in. The TCR knockout prevents CAR T cells from recognizing the patient's cells as foreign, while CAR expression enables targeting of the cancer cells.

Researchers at Precision BioSciences have designed several allogeneic CAR T-cell product candidates using ARCUS gene editing that are currently being investigated in clinical trials.

Snapshot: *in vivo* gene editing for chronic hepatitis B virus

Precision BioSciences, Inc., has developed and optimized a sequence-specific ARCUS nuclease that targets hepatitis B virus (HBV) covalently closed circular DNA (cccDNA), and genome-integrated HBV DNA, *in vivo*.[25] After HBV enters the nucleus, it undergoes a repair process and is converted to cccDNA that encodes HBV proteins needed for the viral life cycle. Current HBV therapies are unable to target cccDNA, thereby requiring lifelong treatment. Precision BioSciences' gene-editing approach is designed to use an ARCUS nuclease to delete persistent cccDNA and inactivate integrated hepatitis B genomes.

In an *in vitro* analysis using HBV-infected human hepatocytes, ARCUS nuclease treatment led to an 80% reduction in extracellular HBV DNA. It also resulted in a 77% reduction in secreted hepatitis B surface antigen (HBsAg), a major viral component of the envelope for infectious HBV particles that is thought to contribute to chronic immune dysfunction in patients. Sustained loss of HBsAg is considered an important clinical endpoint for HBV therapies and therefore a key parameter associated with functional cure. The ARCUS nuclease was also tested *in vivo* using an adeno-associated virus (AAV) vector containing a partial HBV sequence (surrogate for cccDNA) in mouse and nonhuman primate (NHP) animal models. In mice, treatment with the ARCUS nuclease significantly decreased AAV copy number and reduced HBsAg serum levels by 96%. In NHPs, there was no change in AAV copy number; however, there was a significant reduction in liver HBsAg.

The lead program is a phase 1/2a clinical trial evaluating PBCAR0191 (NCT03666000), Precision BioSciences' first-generation allogeneic CAR T-cell therapy, in patients with CD19+ non-Hodgkin lymphoma and B-cell acute lymphoblastic leukemia. Early results have shown that CAR T cells modified by ARCUS have the ability to expand *in vivo*, a key activity associated with the clinical outcome of CAR T-cell therapies, and induce a response in the majority of patients.[40] Please see "Snapshot: allogeneic CAR T cells for CD19+ blood cancers" for more details.

In addition to GvHD, another concern with allogeneic CAR T cells is rapid elimination of the cells by the patient's immune system due to recognition of the cells as foreign.[38] PBCAR19B is Precision BioSciences' next-generation allogeneic CAR T-cell therapy that aims to extend CAR T-cell persistence by evading the patient's T cells and natural killer (NK) cells. This stealth-cell strategy builds on the company's base CAR T-cell platform and incorporates a short hairpin RNA that reduces, but does not completely eliminate, expression of β_2-microglobulin (B2M), a key component of major histocompatibility complex (MHC) class I molecules on the cell surface that are recognized as foreign by a patient's T cells.[38] Precision BioSciences uses this B2M knockdown approach because a complete knockout of MHC class I molecules would make allogeneic CAR T cells more susceptible to a host NK cell attack,[41] whereas a reduction can reduce host T-cell responses without promoting an NK cell attack. The PBCAR19B stealth vector also carries an *HLA-E* gene, which further inhibits NK cell activation.[38] A phase 1 clinical trial of PBCAR19B (NCT04649112) is now enrolling patients with CD19+ B-cell malignancies treated with at least 2 prior standard regimens.

Looking ahead

As ARCUS-based preclinical and clinical studies move forward, the path toward incorporating gene editing for reliable medical use seems more promising than ever. Preclinical results from ARCUS gene editing offer the potential of cures through single gene-editing treatments and strong cancer-fighting responses facilitated by powerful cell therapies. Furthermore, data generated so far indicate that the ARCUS gene-editing platform, developed through over 15 years of dedicated research, could be more effective for clinical applications than other gene therapy techniques. In the future, this approach has the potential to help clinicians and their patients confront a wide range of medical challenges ranging from various genetic diseases or conditions to cancer.

Snapshot: allogeneic CAR T for CD19+ blood cancers

Precision BioSciences, Inc., is evaluating PBCAR0191, an allogeneic chimeric antigen receptor (CAR) T-cell therapy that targets CD19+ cells, in a nonrandomized, open-label, phase 1/2a dose-escalation and dose-expansion trial of patients with relapsed or refractory CD19+ non-Hodgkin lymphoma (NHL) or B-cell acute lymphoblastic leukemia (ALL) who received at least 2 prior therapies (NCT03666000).[42] Early results demonstrated antitumor activity and a well-tolerated safety profile, including no evidence of graft-versus-host disease, in both cohorts of patients administered an enhanced lymphodepletion (eLD) regimen (high dosages of fludarabine and cyclophosphamide).[40] A single dose of PBCAR0191 cells following eLD resulted in a clinical response in the majority of patients at day 28, with overall response rates of 85% in patients with NHL and 80% with B-cell ALL; complete response (CR) rates were 62% and 80%, respectively. Additionally, patients saw improved peak cell expansion with eLD vs standard lymphodepletion (LD). An earlier analysis of the study also found that the median interval from confirmation of eligibility to the start of LD was 1 day, reinforcing the potential feasibility of rapid delivery of an off-the-shelf allogeneic cell therapy in high-risk patients.[43]

A subgroup analysis of this study found a notable benefit among 11 patients who received prior autologous CAR T-cell therapy. All patients had a response, 8 (73%) of whom had a CR. Furthermore, half of patients had a durable response rate >6 months. As a result of these data, additional patients who relapse on autologous CAR T-cell therapy are being actively enrolled in the study. Given that there are currently no Food and Drug Administration–approved treatments for patients with lymphoma who have relapsed following autologous CAR T-cell therapy, PBCAR0191 has the potential to become a salvage therapy for this patient population.

References

1. Khan SH. Genome-Editing Technologies: Concept, Pros, and Cons of Various Genome-Editing Techniques and Bioethical Concerns for Clinical Application. *Mol Ther Nucleic Acids*. 2019;16:326-334.
2. Cox DBT, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. *Nat Med. 2015*;21(2):121-131.
3. Wetterstrand KA. National Human Genome Research Institute. DNA Sequencing Costs: Data. Accessed April 27, 2022. https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data.
4. Li H, et al. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal Transduct Target Ther*. 2020;5(1):1.
5. Hirakawa MP, et al. Gene editing and CRISPR in the clinic: current and future perspectives. *Biosci Rep*. 2020;40(4):BSR20200127.
6. Ernst MPT, et al. Ready for Repair? Gene Editing Enters the Clinic for the Treatment of Human Disease. *Mol Ther Methods Clin Dev*. 2020;18:532-557.
7. Khalil AM. The genome editing revolution: review. *J Genet Eng Biotechnol*. 2020;18(1):68.
8. Gaj T, et al. Genome-Editing Technologies: Principles and Applications. *Cold Spring Harb Perspect Biol*. 2016;8(12):a023754.
9. Doudna JA. The promise and challenge of therapeutic genome editing. *Nature*. 2020;578(7794):229-236.
10. Li Y, et al. Ex vivo cell-based CRISPR/Cas9 genome editing for therapeutic applications. *Biomaterials*. 2020;234:119711.
11. Ho BX, et al. In Vivo Genome Editing as a Therapeutic Approach. *Int J Mol Sci*. 2018;19(9):2721.
12. Taha EA, et al. Delivery of CRISPR-Cas tools for in vivo genome editing therapy: Trends and challenges. *J Control Release*. 2022;342:345-361.
13. Lino CA, et al. Delivering CRISPR: a review of the challenges and approaches. *Drug Deliv*. 2018;25(1):1234-1257.
14. Zhang XH, et al. Off-target Effects in CRISPR/Cas9-mediated Genome Engineering. *Mol Ther Nucleic Acids*. 2015;4(11):e264.
15. Naeem M, et al. Latest Developed Strategies to Minimize the Off-Target Effects in CRISPR-Cas-Mediated Genome Editing. *Cells*. 2020;9(7):1608.
16. Seligman LM, et al. Genetic analysis of the Chlamydomonas reinhardtii I-CreI mobile intron homing system in Escherichia coli. *Genetics*. 1997;147(4):1653-1664.
17. Shabbir MAB, et al. Survival and Evolution of CRISPR-Cas System in Prokaryotes and Its Applications. *Front Immunol*. 2016;7:375.
18. Arnould S, et al. The I-CreI meganuclease and its engineered derivatives: applications from cell modification to gene therapy. *Protein Eng Des Sel*. 2011;24(1-2):27-31.
19. Zheng N, et al. Molecular mechanisms, off-target activities, and clinical potentials of genome editing systems. *Clin Transl Med*. 2020;10(1):412-426.
20. Guha TK, Edgell DR. Applications of Alternative Nucleases in the Age of CRISPR/Cas9. *Int J Mol Sci*. 2017;18(12):2565.
21. Liu M, et al. Methodologies for Improving HDR Efficiency. *Front Genet*. 2019;9:691.
22. Lin WY, et al. Repair of chromosomal double-strand breaks by precise ligation in human cells. *DNA Repair (Amst)*. 2013;12(7):480-487.
23. Wang L, et al. Long-term stable reduction of low-density lipoprotein in nonhuman primates following in vivo genome editing of PCSK9. *Mol Ther*. 2021;29(6):2019-2029.
24. Certo MT, Morgan RA. Salient Features of Endonuclease Platforms for Therapeutic Genome Editing. *Mol Ther*. 2016;24(3):422-429.
25. Gorsuch CL, et al. Targeting the hepatitis B cccDNA with a sequence-specific ARCUS nuclease to eliminate hepatitis B virus in vivo. *Mol Ther*. 2022;S1525-0016(22):00311-2.
26. Zekonyte U, et al. Mitochondrial targeted meganuclease as a platform to eliminate mutant mtDNA in vivo. *Nat Commun*. 2021;12(1):3210.
27. Rasul MF, et al. Strategies to overcome the main challenges of the use of CRISPR/Cas9 as a replacement for cancer therapy. *Mol Cancer*. 2022;21(1):64.
28. Zhou L, et al. A universal strategy for AAV delivery of base editors to correct genetic point mutations in neonatal PKU mice. *Mol Ther Methods Clin Dev*. 2022;24:230-240.
29. Wang Q, et al. Broadening the reach and investigating the potential of prime editors through fully viral gene-deleted adenoviral vector delivery. *Nucleic Acids Res*. 2021;49(20):11986-12001.
30. Owens G, et al. Optimization of Hydroxyacidoxidase 1 (HAO1) Targeting ARCUS Nucleases for the Treatment of Primary Hyperoxaluria Type 1 (PH1). *Mol Ther*. 2022;30(4S1):116.
31. Wang L, et al. Meganuclease targeting of PCSK9 in macaque liver leads to stable reduction in serum cholesterol. *Nat Biotechnol*. 2018;36(8):717-725.
32. Greig J, et al. Translation of an AAV-Delivered Gene Editing Approach for Transthyretin Amyloidosis from Mice to Nonhuman Primates. *Mol Ther*. 2021;29(4; suppl 1):24.
33. Jalligampala A, et al. Rho 1-2 meganuclease, an allele-specific gene-editing therapy, rejuvenates rod photoreceptor structure and function in a pig model of autosomal dominant Retinitis Pigmentosa (adRP). *Invest Ophthalmol Vis Sci*. 2021;62:1478.
34. Bachtarzi H. Ex vivo and in vivo genome editing: a regulatory scientific framework from early development to clinical implementation. *Regen Med*. 2017 Dec 15.doi:10.2217/rme-2017-0095.
35. Kankeu Fonkoua LA, et al. CAR T cell therapy and the tumor microenvironment: Current challenges and opportunities. *Mol Ther Oncolytics*. 2022;25:69-77.
36. Crees ZD, Ghobadi A. Cellular Therapy Updates in B-Cell Lymphoma: The State of the CAR-T. *Cancers (Basel)*. 2021;13(20):5181.
37. Caldwell KJ, et al. Allogeneic CAR Cell Therapy-More Than a Pipe Dream. *Front Immunol*. 2021;11:618427.
38. Depil S, et al. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. *Nat Rev Drug Discov*. 2020;19(3):185-199.
39. Tang TCY, et al. Donor T cells for CAR T cell therapy. *Biomark Res*. 2022;10(1):14.
40. Shah BD, et al. Allogeneic CAR-T PBCAR0191 with Intensified Lymphodepletion Is Highly Active in Patients with Relapsed/Refractory B-Cell Malignancies. *Blood*. 2021;138(suppl 1):302.
41. Kumar V, McNerney ME. A new self: MHC-class-I-independent natural-killer-cell self-tolerance. *Nat Rev Immunol*. 2005;5(5):363-374.
42. Dose-escalation, dose-expansion study of safety of PBCAR0191 in patients with r/r NHL and r/r B-cell ALL. ClinicalTrials.gov identifier: NCT03666000. Updated September 16, 2021. Accessed April 15, 2022.
43. Precision BioSciences reports progress on two strategies designed to optimize durability of allogeneic CAR T therapy in r/r non-Hodgkin lymphoma. News release. Precision BioSciences; June 4, 2021. Accessed April 18, 2022. https://investor.precisionbiosciences.com/news-releases/news-release-details/precision-biosciences-reports-progress-two-strategies-designed.
44. Dindo M, et al. Molecular basis of primary hyperoxaluria: clues to innovative treatments. *Urolithiasis*. 2019;47(1):67-78.
45. Milliner DS, et al. Primary hyperoxaluria type 1. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, eds. *GeneReviews®*. University of Washington; 1993-2022. Updated February 10, 2022. Accessed April 21, 2022. https://www.ncbi.nlm.nih.gov/books/NBK1283/.
46. Liebow A, et al. An Investigational RNAi Therapeutic Targeting Glycolate Oxidase Reduces Oxalate Production in Models of Primary Hyperoxaluria. *J Am Soc Nephrol*. 2017;28(2):494-503.
47. Oxlumo. Package insert. Alnylam Pharmaceuticals Inc; November 2020.
48. FDA approves first drug to treat rare metabolic disorder. News release. US Food and Drug Administration; November 23, 2020. Accessed July 11, 2022. https://www.fda.gov/news-events/press-announcements/fda-approves-first-drug-treat-rare-metabolic-disorder.
49. Peterson AS, et al. PCSK9 function and physiology. *J Lipid Res*. 2008;49(6):1152-1156.
50. Precision Announces 3-Year Pre-clinical Study Results Showing Long-term Durability and Safety of ARCUS In Vivo Gene Editing to Cut LDL Cholesterol Levels in Nonhuman Primates. News release. Precision BioSciences; February 19, 2021. Accessed September 9, 2022. https://investor.precisionbiosciences.com/news-releases/news-release-details/precision-announces-3-year-pre-clinical-study-results-showing.

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