Canadian Journal of Health Technologies
July 2024 Volume 4 Issue 7

CADTH Horizon Scan

CRISPR Technologies for In Vivo and Ex Vivo Gene Editing

Health Technology Update

Key Messages

What Is the Issue?

The first therapeutics based on clustered regularly interspaced short palindromic repeats (CRISPR) technologies are entering the market. These gene editing technologies have the potential to change treatment paradigms and may be used to treat conditions that cannot be treated or cured with current methods. This report aims to provide an overview of the technologies and their current and potential roles in health care.

What Are the Technologies?

CRISPR is a part of bacterial immune systems that can cut DNA strands and is used as a gene editing tool. A guide ribonucleic acid (RNA) sequence leads the CRISPR-associated nuclease to the target DNA sequence where the cut is made. These edits change the function of the gene, making genes nonfunctional or replacing the coding sequence for 1 gene with another. CRISPR can also be used to increase or decrease the expression of specific genes.

What Is the Potential Impact?

CRISPR-based technologies have a variety of potential applications in health care, including:

- treating genetic diseases
- understanding the genetic mechanisms of diseases and investigating the relevance of potential drug treatments
- managing infectious diseases through detection, treatment, and elimination.

What Else Do We Need to Know?

Ethical issues pertinent to the use of CRISPR include the ability to obtain adequately informed consent, the potential future consequences of gene editing and its potential unintended effects, and the impact gene editing could have on future generations. The long-term effects of CRISPR-based therapies are currently unknown. Further research into emerging applications is required. Long-term follow-up of the patients who have received the first CRISPR-based therapeutics will help inform understanding of the safety and effectiveness of these treatments. While the first of these therapies have been granted regulatory authorization, the next viable CRISPR-based therapies are still in the early phases of development, with the pivotal clinical trials not expected to be completed until at least 2027.

Purpose and Scope

The purpose of this report is to present health care decision-makers in Canada with an overview of gene editing technologies based on clustered regularly interspaced short palindromic repeats (CRISPR), how they are currently being used in health care settings, and how ongoing clinical developments may impact health care in the future.

To inform this report, a research information specialist conducted a tailored literature search, balancing comprehensiveness and relevance, across various sources, including databases and a focused internet search conducted on May 22, 2024. These searches were supplemented with handsearching of the grey literature by the author. Content experts were consulted for input and peer review. Appendix 1 provides the detailed literature search methods.

What Is the Technology and How Does It Work?

CRISPR was discovered by Dr. Jennifer Doudna and Dr. Emmanuelle Charpentier and described in their 2012 publication in the journal *Science*.[1] CRISPR is a part of bacterial immune systems that can cut DNA and is used as a gene editing tool. There are 2 key parts to the CRISPR system: a guide RNA sequence and a CRISPR-associated (Cas) nuclease. The customizable guide ribonucleic acid (RNA) sequence is used to direct the Cas nuclease to a target DNA sequence where the Cas nuclease binds and cuts the DNA.[2] CRISPR was discovered in bacterial immune systems where it was observed to cut the DNA and disable bacteriophages that were invading the bacteria.[2] Drs. Doudna and Charpentier determined that the CRISPR Cas9 bacterial immune system could be repurposed as a gene editing tool.[1] CRISPR Cas9 is currently the most commonly used nuclease in gene editing.[2] Others include Cas12, Cas13, and Cas14. CRISPR Cas9 is guided by 2 RNAs to the DNA target and operates by creating double-stranded breaks in the DNA and leveraging cellular DNA repair mechanisms.[1,2] Drs. Doudna and Charpentier became the first all-female team to win the Nobel Prize in Chemistry for this discovery in 2020.[2]

> **CRISPR technologies can be used to edit genes within the body (in vivo) or to edit cells that have been removed from the body (ex vivo) that are returned to the body after their editing is complete.**

Three common uses for CRISPR include gene knockouts, gene knock-ins, and regulating gene expression. Among the various pathways involved, nonhomologous end joining (NHEJ) and homology-directed repair (HDR) are pivotal for gene editing. NHEJ is used to disrupt genes and make them nonfunctional, whereas HDR is used to insert new genes or genetic material fragments.[2]

Gene Knockouts

Cas-9 typically repairs a double-strand break in DNA through NHEJ, which often introduces indels (i.e., insertion or deletion of 1 or more nucleotides) into the repaired region.[2] When these indels cause a frameshift mutation within the gene's coding region, the gene becomes nonfunctional, resulting in a gene

knockout. Gene knockouts are often used in research fields such as functional genomics, pathway analysis, drug discovery, screening, and disease modelling.[2] Using multiple guide RNAs targeting different parts of the gene ensures highly efficient gene knockout, a method that is becoming more popular. Recent research has found that genes that have been knocked out by CRISPR Cas9 may not be completely nonfunctional.[3] Residual functional proteins could be generated to make up for the loss of function in a process called "knockout escaping."[3]

Gene Knock-Ins

When Cas9 induces a double-strand break in DNA, cells can also repair themselves through HDR. This repair process provides an opportunity for researchers to insert new DNA, such as an entire gene, known as gene knock-in.[2] To achieve a gene knock-in, a donor template containing the desired DNA sequence flanked by homologous regions matching the cut site is required. This template, along with Cas9 and single-guide RNA, is delivered into the engineered cells.[2] Gene knock-ins have been used in biotechnology, enabling the production of recombinant proteins, improving immortalized cell lines, and enhancing disease modelling precision. Additionally, CRISPR knock-ins hold promise for correcting genetic mutations in cell and gene therapies for human diseases.[2] Gene knock-ins are more challenging than knockouts because HDR is less common than NHEJ and occurs only during specific cell cycle stages. The lower frequency of HDR typically results in lower knock-in efficiencies.[2] However, scientists are developing methods to overcome these challenges, such as experimental optimization, cell cycle synchronization, and treatments that enhance HDR or inhibit NHEJ during knock-in experiments.[2]

Regulating Gene Expression

CRISPR can also be used to regulate gene expression.[2] CRISPR activation ($CRISPR_a$) increases the expression of a gene and CRISPR interference ($CRISPR_i$) decreases expression using catalytically dead Cas9 (dCas9). These gene modulators are being used for a variety of research purposes, including developmental biology, infectious disease, disease progression, functional genomics, and screening for genetic elements that mediate drug resistance.[2]

What Is CRISPR Used For?

CRISPR has been used as a treatment for medical conditions in 2 ways: ex vivo and in vivo gene editing.[2] Ex vivo gene editing, or cell therapy, involves harvesting cells from the patient, editing the cells with CRISPR while they are outside of the body, then returning them to the patient through an infusion.[2] In vivo gene editing occurs when the instructions for the CRISPR gene editor are injected directly into a patient with the intention that the CRISPR tool will cut out the problematic gene in the cells within the patient's body.[4] CRISPR can also be used to generate chimeric antigen receptor (CAR) T cells, which are used for cancer immunotherapy.

CRISPR-based gene editing technologies have a variety of potential applications in health care, such as:[5]

- treatment for human genetic diseases (e.g., sickle cell disease, beta-thalassemia, cystic fibrosis, Duchenne muscular dystrophy, retinitis pigmentosa, cardiac disease)
- understanding the genetic mechanisms underlying cancer development and investigating the functional relevance of potential drug targets in cancer cells
- the management of infectious diseases via:
  - detection (e.g., diagnostic assays for viral detection)
  - treatment (e.g., treating diseases by directly targeting viral genomes and inhibiting viral load and replication)
  - elimination (e.g., editing the genome of viruses and parasites to disrupt their life cycle and prevent their ability to infect humans).

What Is the Evidence?

Clinical Evidence

There is well-established evidence that demonstrates the CRISPR Cas9 system is capable of inducing precise gene editing in a wide range of organisms.[5] Ex vivo and in vivo gene editing are 2 ways CRISPR has been used to develop treatments for medical conditions.[2]

Ex Vivo Gene Editing (Cell Therapy)

Exagamglogene autotemcel (exa-cel) (Casgevy) is the first CRISPR-based therapy to receive international regulatory approval.[6,7] Exa-cel is a CRISPR Cas9 gene-edited therapy intended to treat certain people with sickle cell disease or transfusion-dependent beta-thalassemia.[8] Exa-cel uses CRISPR Cas9–based gene editing to increase the amount of fetal hemoglobin in red blood cells by deleting a portion of the *BC11A* gene.[8] The treatment involves harvesting hematopoietic stem cells from the patient, which are sent to a facility where they are modified using CRISPR Cas9. While the cells are being modified, the patient receives chemotherapy to clear out the existing stem cells in their bone marrow before their edited cells are reintroduced.[9] As the red blood cells are regenerated, the newly edited cells replace the damaged ones, which significantly modifies sickle cell disease and transfusion-dependent beta-thalassemia.[8]

The pivotal clinical trials (CLIMB-111, CLIMB-121, and CLIMB-131) of exa-cel for sickle cell disease and transfusion-dependent beta-thalassemia are ongoing, with the intention to follow the enrolled patients to evaluate their longer-term outcomes after receiving treatment.[10-12] The manufacturer presented interim results of these studies at the Annual European Hematology Association Congress in June 2024.[10]
The results summarized here were not critically appraised by Canada's Drug Agency. The manufacturer presented the results for 46 patients with sickle cell disease and 56 patients with transfusion-dependent beta-thalassemia with at least 16 months of follow-up data. The longest patient follow-up was more than

5 years since receiving treatment.[10] In the CLIMB-121 trial, enrolled patients with sickle cell disease were experiencing an average of 4.1 (± 3.0) vaso-occlusive crises per year before treatment, with 59% (26 of 44) having had 3 or more vaso-occlusive crises per year for the previous 2 years.[11] The sponsor reported that, following treatment, 92.3% (36 of 39) of the patients with sickle cell disease included in the long-term follow-up data were free from vaso-occlusive crises for at least 12 months, with a mean duration of 27.9 months.[10] Vaso-occlusive crises occur when circulation is limited by the sickled red blood cells causing organ injury and pain.[13] Of patients with at least 16 months of follow-up, 97.4% (38 of 39) were free from hospitalizations related to vaso-occlusive crisis for at least 12 consecutive months.[10]

People with transfusion-dependent beta-thalassemia require monthly transfusions and iron chelation. In total, 94.2% of patients (49 of 52) were transfusion independent for at least 12 consecutive months, with a mean duration of 31.0 months.[10] Patients in both disease groups reported sustained and meaningful improvements in their quality of life, including physical, emotional, social and/or family, and functional well-being, and overall health status. The intervention is administered only once with the expectation that the effects will be lifelong; however, final long-term effectiveness data are not yet available.[14] After their participation in the pivotal studies, patients will be asked to enrol in the long-term, open-label CLIMB-131 study, which aims to follow these patients for up to 15 years after their treatment with exa-cel.[10]

In Vivo Gene Editing

In 2020, a CRISPR gene therapy was injected directly into a patient's eye as a potential treatment for hereditary blindness. They were the first person to receive the treatment with the intention that the CRISPR tool would cut out the gene causing blindness and restore the patient's vision.[4] Subsequently, initial results of a phase I and II open-label, single-ascending-dose study were published in 2024.[15] Study participants 3 years of age or older with *CEP290*-associated inherited retinal degeneration caused by a homozygous or compound heterozygous IVS26 variant received a subretinal injection in their worse eye. No serious adverse events were observed in the 14 participants and no dose-limiting toxic effects were recorded.[15] Four of the 14 participants achieved clinically meaningful improvements in best corrected visual acuity.[15]

Regulatory Status

Exa-cel is the first CRISPR-based therapeutic to gain regulatory approval anywhere internationally. It was approved for use by the US FDA and the UK Medicines and Healthcare products Regulatory Agency for patients with sickle cell disease and transfusion-dependent beta-thalassemia in late 2023 and early 2024.[6,7] The drug also received conditional authorization from the European Medicines Agency in 2024.

> As of June 2024, the drug submission for exa-cel is under priority review by Health Canada for the same patient groups.[16]

What Does It Cost?

The reported cost of treatment with exa-cel is US$2.2 million for a 1-time treatment.[17,18] The cost is expected to be the same for both sickle cell disease and transfusion-dependent beta-thalassemia. Chemotherapy is required before receiving the treatment and is included in the reported treatment cost.[18] Additionally, patients receiving treatment are also required to be admitted to the hospital for approximately 1 month, which might add to the treatment cost.

Issues to Consider

Ethics, Equality, and Health Inequities

The ethical issues associated with human gene editing are important because they relate to fundamental questions about editing human genetic material and what those changes mean for the individual and society. If gene editing is performed on germ cells, there will be ethical challenges related to future generations of people who may be impacted by these genetic changes. These concerns are not unique to the use of CRISPR-based technologies — they apply to all gene editing technologies. Altering the genetic make-up of any organism, human or otherwise, comes with potential risks and unknowns that should be considered before gene editing is undertaken.

CRISPR Cas9 for Disease Models

D'Souza and colleagues (2023)[19] conducted a scoping review to examine the ethical issues associated with the use of CRISPR Cas9 in the creation of human disease models. The authors found the main ethical issues discussed in the articles included discussions of safety and effectiveness and the uncertainty associated with unintended consequences, harm to the environment, off-target effects, obtaining informed consent, and potential misuse of the technology.[19] The authors recommended the development of ethical and legal guidelines and best practices to guide the use of CRISPR in creating human disease models. They identified gaps in the literature, including inconsistency in the standards for choosing target diseases and uncertainty in the safety and effectiveness of CRISPR Cas9.[19]

Gene Editing of Human Embryos

One of the more commonly understood ethical issues related to CRISPR technologies and gene editing is the concept of "designer babies," in which gene editing is used to select or modify certain traits in human embryos.[19] In 2015, 2 Chinese scientists used CRISPR for human embryo genome editing.[20] International consensus following this event was that human germ line editing should not be done until safety and effectiveness are better understood and ethical issues have been addressed.[20] However, in 2018 a Chinese biologist edited a gene in 3 human embryos in an attempt to make them resistant to infection with HIV. These embryos were implanted in females and resulted in the live birth of twin females.[20,21] The biologist was sentenced to 3 years in prison for conducting illegal medical practices.

Parents may face ethical dilemmas when making genetic choices for, and about, their unborn children. The decision will be a balance between the parental desire for their children to possess desirable genetic traits with the best interest for the child and being mindful of the potential psychological and social consequences their child may experience.[21] Genetic engineering for specific traits could impact human diversity by decreasing genetic variation, which raises ethical concerns about the consequences of biodiversity within society.[20] Additionally, there are concerns about eugenics and changes being made to create future generations that possess the characteristics that a certain group of people with access to this expensive technology choose as being desirable or correct.[20] There is a distinction between using gene editing to treat or cure disease versus using gene editing to create biological enhancement for the recipient (e.g., physical ability or intelligence). Aside from the potential health-related consequences of gene editing, there is concern that the use of gene editing technologies may worsen discrimination against people based on the perceived quality of their genome.[20]

Safety and Long-Term Effects

The uncertainties in gene editing include the unknown effects to the individual that result from the editing of their genes and the consequences these gene edits may have on them in the future.[21] There is a chance for off-target edits (when the Cas nuclease cuts at unintended genetic sites), which could result in unknown consequences to an individual's health. There is also little known about the interactions edited genes may have with unedited genes and whether those interactions may impact biological functions and pathways.[21]

The key risk associated with any gene editing is off-target effects in which genes other than the intended target become altered. CRISPR causes double-stranded breaks in DNA and results in indels that could cause large deletions and chromosome instability. This could result in a loss of individual autonomy for the person who would be living with the changes to their genetic make-up that they did not consent to.[20] As a result, informed consent has been highlighted as an important ethical consideration. Newer techniques, such as base editing and prime editing, induce only single-strand breaks and therefore lower levels of indels.[22] These newer techniques precisely correct base substitutions in genetic diseases and reduce the risk of off-target effects.

> We can look to other gene-based therapies to get an idea of what the future might hold. Chimeric antigen receptor (CAR) T-cell therapy is an example of a potentially promising treatment that had unknown long-term effects when it was introduced as part of patient care in 2017.[23]

CAR T-cell therapy alters a patient's immune cells so they begin to target their malignant cells.[23] The therapy is currently used to treat some blood cancers and is being studied in the context of other types of cancer. Since they have been introduced, there have been some reports of blood cancers originating in the T cells of people who have received CAR T-cell therapy. Other long-term effects that are being monitored include autoimmune effects and long-term effects on the immune system. Some patients have also experienced a relapse in disease or developed resistance to the therapy.[23] The safety and long-term effects of gene

modification, off-target effects, and unintended alterations in the genome associated with CRISPR-based therapies are not yet known[19] and will need to be monitored over time.

There is a concern regarding the risk of using genetically modifying organisms for bioterrorism or biowarfare.[5,19] There are also concerns that unintended genetic modifications to humans, animals, bacteria, and viruses may have harmful effects on agriculture and ecosystems.[5,19]

Cost and Implementation

CRISPR technologies are currently expensive. The cost and limited availability of these technologies raise issues of equitable access.[5] The high cost may limit the ability of certain patient groups, communities, or countries to gain access to them, including the patient populations that will be eligible for the new treatments that will soon become available. These access issues might exacerbate existing health and social inequalities and contribute to further disparities in health care delivery and availability.[5]

Other Considerations

There are technical limitations to the widespread implementation of CRISPR technologies.[5] Editing inherited epigenetic modifications can be challenging because of its complex nature and our lack understanding of the interplay between epigenetic markers and how they influence gene expression.[5] It is also difficult to gain proficiency with using the technology while maintaining an adequate safety profile.[14]

Related Developments

Clinical Therapeutics

The concept of using gene therapies, including those created using CRISPR-based techniques, is being studied for a range of diseases, and most of the available information outlines the preclinical and discovery phases of this research. Gene editing treatments for genetic diseases caused by single gene mutations will have earlier clinical applications than those caused by multiple gene mutations because of the complexity of gene interactions for diseases caused by multiple mutations. Experience from the development of therapeutics for genetic diseases caused by single gene mutations will inform the therapy's development and extension to more complex genetic disorders. In this review we included overview articles that were identified that describe the progress of this research in cardiovascular disease and osteoarthritis.

Cardiovascular Disease

Ganipineni and colleagues (2023)[24] conducted a clinical review of the use of CRISPR in cardiovascular disease, in particular, its use for dilated cardiomyopathy. Dilated cardiomyopathy is a major cause of heart failure. Currently available therapies focus on slowing disease progression and managing symptoms.[25] Most people with dilated cardiomyopathy who survive to the late stages of the disease will require a heart transplant as their only remaining treatment option and the only available cure. The hope is that future gene therapies will be developed that will be able to reverse cardiac damage.[25]

CRISPR system applications that have been studied in dilated cardiomyopathy include:[26]

- ex vivo gene editing for disease and therapeutic modelling
- in vivo gene editing for disease modelling.

The authors determined from their literature review that gene editing has shown some promise when developing precise genotype-specific therapeutic strategies; however, limitations remain. The mechanics of delivering CRISPR Cas9 to human cardiomyocytes using viral vectors is a challenge, as the vectors can potentially cause an immune response or other adverse reactions.[25] Unintended gene targeting is another limitation. The genes involved in dilated cardiomyopathy are complex and have multiple functions, and CRISPR Cas9 can sometimes target the wrong part of the genome and result in unintended effects that could be benign or harmful, depending on which area has been targeted.[25] A phase III clinical trial is currently under way for a CRISPR-based therapy for familial amyloid cardiomyopathy that is anticipated to be completed at the end of 2027.[14]

Osteoarthritis

Jia and colleagues (2024)[24] systematically reviewed current studies on CRISPR-based technologies for the improvement of cell deterioration, inflammation, and cartilage damage associated with osteoarthritis. Researchers have estimated that between 40% and 60% of osteoarthritis is gene related and hypothesize that gene therapy might provide a viable treatment option.[24] There are currently no treatments available to cure osteoarthritis. Lifestyle modification and pain management are the main management options. Existing drug therapies that are recommended to manage symptoms usually have dose-dependent toxicity.[24] The concept of using CRISPR applications has been investigated to target the genes believed to be associated with the osteoarthritis inflammation process and the cartilage repair process.

The main areas of focus for potential future gene therapies for osteoarthritis include:[24]

- engineering undifferentiated adult stem cells to give them the ability to repair inflammation or differentiating differentiated adult stem cells into chondrocytes to repair damaged cartilage
- editing the mitochondrial DNA of damaged chondrocytes to treat osteoarthritis by inhibiting and regulating immune processes
- modulating the inflammatory pathways of osteoarthritis to improve or possibly reverse the damaged cells to maintain the cellular lifespan.

Diagnostic Testing

In addition to being a treatment for medical conditions, CRISPR can also be used as the base for diagnostic tests. CRISPR Cas12 and Cas13 are often used in diagnostic assays.[27] In their systematic review and bibliometric analysis, Leta and colleagues (2024)[27] identified 580 different CRISPR-based diagnostic assays that target 170 pathogens, with around 72% of those assays being developed for point-of-care testing. CRISPR Cas12 and Cas13 demonstrated collateral nucleic acid cleavage activity, a characteristic that has been used to develop diagnostic tests that are specific, sensitive, fast, and portable.[27] The majority of the tests identified in the review used a lateral flow assay to display the results. Lateral flow assays detect the presence of antibodies or antigens in a liquid sample. The sample is drawn through a conjugate pad and,

if present, the target of the test will bind to the immunoreagent and a line will appear to indicate a positive result.[28] A familiar example of a lateral flow assay is a simple 2-line pregnancy test or at-home tests for COVID-19. Lateral flow assays do not require complicated equipment, which makes them suitable for point-of-care testing.[27] Multiplex assays that can detect more than 1 pathogen using a single test are of great use in detecting and differentiating between infectious diseases; however, at the time of drafting this report, there appears to be limited development of these tests using CRISPR technologies.

During the COVID-19 pandemic, emergency use authorization was granted by the US FDA to several CRISPR-based diagnostic tests. These include the Sherlock CRISPR SARS-CoV-2 lab-based test kit[2,27] and the Accula SARS-CoV-2 test.[27] The Lucira Check It COVID-19 test kit was authorized for use in both the US and Canada. There does not appear to be much publicly available information about the real-world performance or use of these.

Looking Ahead

Although the first CRISPR-based therapeutic is now available to treat patients in some countries, it is unlikely that a rush of new CRISPR-based gene therapies will follow its entry to the market. Other therapeutics are in the works, but they remain in the early phases of clinical development. Based on the information available about clinical trials that are currently under way, the next phase III trials of viable CRISPR-based therapies (for the treatment of familial amyloid cardiomyopathy) are anticipated to be completed at the end of 2027.[14] There are more than 20 phase II trials ongoing for CRISPR-based therapies, including for cancers and cardiovascular disease, with the earliest safety trial set to be complete in December 2025.[14,18]

> **CRISPR-based therapies may represent a paradigm shift in health care by providing management options or curative treatment for conditions that previously had no cure or limited options for treatment.**

Looking ahead, balancing the hope for these therapies with the uncertainty that remains about the long-term safety and effectiveness will be critical. Long-term follow-up studies of the recipients of CRISPR-based therapies will further understanding of how these treatments impact human health in the long term. Reproductive counselling following effective somatic cell treatment will be required to ensure patients are aware that they are still carriers of a genetic disease despite receiving therapy. Long-term clinical effectiveness and safety (e.g., unintended effects, off-target gene edits) in addition to several ethical challenges will need to be considered when contemplating widespread implementation.

References

1. Jinek M, Chylinsky K, Fonfara I, Hauer M, Doudna J, Charpentier E. A programmable dual RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science.* 2012;337(6096):816-821. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6286148/pdf/nihms-995853.pdf. Accessed 2024 Jun 13. PubMed
2. Synthego. What Is CRISPR: The Ultimate Guide To CRISPR Mechanisms, Applications, Methods & More. 2024; https://www.synthego.com/learn/crispr. Accessed 2024 Jun 12.
3. Wang Y, Zhai Y, Zhang M, Song C, Zhang Y, Zhang G. Escaping from CRISPR-Cas-mediated knockout: the facts, mechanisms, and applications. *Cell Mol Biol Lett.* 2024;29(1):48. PubMed
4. Scharping N. CRISPR Treatment Is Injected Directly Into a Patient's Body — a First for Gene Editing. *Discover Magazine.* 2023: https://www.discovermagazine.com/health/crispr-treatment-is-injected-directly-into-a-patients-body-a-first-for-gene. Accessed 2024 Jun 18.
5. Kolanu ND. CRISPR-Cas9 Gene Editing: Curing Genetic Diseases by Inherited Epigenetic Modifications. *Glob Med Genet.* 2024;11(1):113-122. PubMed
6. Medicines and Healthcare products Regulatory Agency. MHRA authorises world-first gene therapy that aims to cure sickle-cell disease and transfusion-dependent β-thalassemia. London (GB): Government of the United Kingdon; 2023: https://www.gov.uk/government/news/mhra-authorises-world-first-gene-therapy-that-aims-to-cure-sickle-cell-disease-and-transfusion-dependent-thalassemia. Accessed 2024 Jun 17.
7. FDA Approves First Gene Therapies to Treat Patients with Sickle Cell Disease. Silver Sring (MD): U.S. Food and Drug Administration; 2023: https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease. Accessed 2024 Jun 21.
8. CRISPR Therapeutics Pipeline. Zug (CHE): CRISPR Therapeutics: https://crisprtx.com/pipeline. Accessed 2024 Jun 11.
9. Pagliarulo N. What if a CRISPR cure isn't such an easy choice? *BioPharmaDive*: Industry Dive; 2023: https://www.biopharmadive.com/news/sickle-cell-crispr-gene-editing-vertex-exa-cel-barriers/698121/. Accessed 2024 Jun 11.
10. Vertex Presents Positive Long-Term Data On CASGEVY™ (exagamglogene autotemcel) at the 2024 Annual European Hematology Association (EHA) Congress. Boston (MA): Vertex Pharmaceuticals, Inc.; 2024: https://news.vrtx.com/news-releases/news-release-details/vertex-presents-positive-long-term-data-casgevytm-exagamglogene. Accessed 2024 Jun 21.
11. Frangoul H, Locatelli F, Sharma A, et al. Exagamglogene Autotemcel for Severe Sickle Cell Disease. *N Engl J Med.* 2024;390(18):1649-1662. PubMed
12. Locatelli F, Lang P, Wall D, et al. Exagamglogene Autotemcel for Transfusion-Dependent β-Thalassemia. *N Engl J Med.* 2024;390(18):1663-1676. PubMed
13. Maakaron J. Sickle Cell Disease (SCD) Clinical Presentation. *MedScape*: MedMD; 2023: https://emedicine.medscape.com/article/205926-clinical?form=fpf. Accessed 09 Jul 2024.
14. Beaney A. Trials to watch: CRISPR pipeline accelerates after milestone Casgevy approval. Clinical Trials Arena; 2024: https://www.clinicaltrialsarena.com/features/trials-to-watch-crispr-pipeline-accelerates-after-milestone-casgevy-approval/. Accessed 2024 Jun 11.
15. Pierce E, Aleman T, Jayasundera K, et al. Gene Editing for CEP290-Associated Retinal Degeneration. *N Engl J Med.* 2024;390(21):1972-1984. PubMed
16. Health Canada. Drug and Health Product Submissions Under Review (SUR): New drug submissions under review. Ottawa (ON): Government of Canada; 2024: https://www.canada.ca/en/health-canada/services/drug-health-product-review-approval/submissions-under-review/new-drug-submissions-under-review.html. Accessed 2024 Jun 11.
17. Bellamy C. New sickle cell treatment sparks feelings of hope and some hesitancy among Black patients. *NBC News.* 2023: https://www.nbcnews.com/health/health-news/new-sickle-cell-treatment-sparks-feelings-hope-hesitancy-black-patient-rcna128116. Accessed 2024 Jun 17.

18. Goodwin K. What's Next for CRISPR?: BiosSpace.com; 2024: https://www.biospace.com/article/what-s-next-for-crispr-/. Accessed 2024 Jun 11.
19. D'Souza R, Mathew M, Surapaneni K. A Scoping Review on the Ethical Issues in the Use of CRISPR-Cas9 in the Creation of Human Disease Models. *JCDR.* 2023;17(12):JE01-JE08. https://www.jcdr.net/articles/PDF/18809/68275_CE%5bRa1%5d_F(SHU)_QC(AK_RDW_SHU)_PF1(RI_OM)_PFA(RI_OM)_PN(KM).pdf. Accessed 2024 Jun 11.
20. Astarastoae V, Ioan BG, Rogozea LM, Hanganu B. Advances in Genetic Editing of the Human Embryo. *Am J Ther.* 2023;30(2):e126-e133. PubMed
21. Gumer JM. The Wisdom of Germline Editing: An Ethical Analysis of the Use of CRISPR-Cas9 to Edit Human Embryos. *New Bioeth.* 2019;25(2):137-152. PubMed
22. Kaya H. Base Editing and Prime Editing. In: Ricroch A, Eriksson D, Miladinović D, Sweet J, Van Laere K, Woźniak-Gientka E, eds. *A Roadmap for Plant Genome Editing*: Springer, Cham; 2023: https://link.springer.com/chapter/10.1007/978-3-031-46150-7_2#:~:text=While%20base%20editing%20can%20introduce,%2Dand%2Dreplace%E2%80%9D%20method. Accessed 09 Jul 2024.
23. Couzin-Frankel J. Explainer: What's behind FDA's concern that a cancer-fighting cell therapy can also cause the disease? *ScienceInsider.* 2023: https://www.science.org/content/article/explainer-what-s-behind-fda-s-concern-cancer-fighting-cell-therapy-can-also-cause. Accessed 2024 Jun 20.
24. Jia S, Liang R, Chen J, Liao S, Lin J, Li W. Emerging technology has a brilliant future: the CRISPR-Cas system for senescence, inflammation, and cartilage repair in osteoarthritis. *Cell Mol Biol Lett.* 2024;29(1):64. PubMed
25. Ganipineni VDP, Gutlapalli SD, Danda S, et al. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in Cardiovascular Disease: A Comprehensive Clinical Review on Dilated Cardiomyopathy. *Cureus.* 2023;15(3):e35774. PubMed
26. Geslewitz WE, Cardenas A, Zhou X, Zhang Y, Criss AK, Seifert HS. Development and implementation of a Type I-C CRISPR-based programmable repression system for Neisseria gonorrhoeae. *mBio.* 2024;15(2):e0302523. PubMed
27. Leta S, Chibssa TR, Paeshuyse J. CRISPR-Cas12/Cas13: Bibliometric analysis and systematic review of its application in infectious disease detection. *J Infect Public Health.* 2024;17(5):741-747. PubMed
28. Steward K. An Introduction to the Lateral Flow Test: Strengths, Limitations and Applications. *Technology Networks Diagnostics*: LabX Media Group; 2023: https://www.technologynetworks.com/diagnostics/articles/an-introduction-to-the-lateral-flow-test-strengths-limitations-and-applications-370382. Accessed 10 Jul 2024.

Appendix 1: Methods

Note that this appendix has not been copy-edited.

Literature Search Strategy

An information specialist conducted a literature search on key resources including MEDLINE, the Cochrane Database of Systematic Reviews, the International HTA Database, the websites of Canadian and major international health technology agencies, as well as a focused internet search. The search approach was customized to retrieve a limited set of results, balancing comprehensiveness with relevancy. The search strategy comprised both controlled vocabulary, such as the National Library of Medicine's MeSH (Medical Subject Headings), and keywords. Search concepts were developed based on the elements of the research questions and selection criteria. The main search concept was CRISPR. Search filters were applied to limit retrieval to health technology assessments, systematic reviews, and meta-analyses. The search was completed on May 22, 2024, and limited to English-language documents published since January 1, 2019. Internet links were provided, where available.

Selection Criteria

One author screened the literature search results and reviewed the full text of all potentially relevant publications. Publications were considered for inclusion if the intervention was related to CRISPR-based technologies. Grey literature was included when it provided additional information to that available in the published studies.

ISSN: 2563-6596

About CADTH: CADTH is an independent, not-for-profit organization responsible for providing Canada's health care decision-makers with objective evidence to help make informed decisions about the optimal use of drugs, medical devices, diagnostics, and procedures in our health care system.

Funding: CADTH receives funding from Canada's federal, provincial, and territorial governments, with the exception of Quebec.

Questions or requests for information about this report can be directed to **Requests@CADTH.ca**.

cadth

Canada's Drug and
Health Technology Agency

cadth.ca