

Genome Editing: From Science to New Therapies

A Non-Technical Guide to Clinical Applications, Best Practices, and Future Perspectives
(Version 2.4)

Francisco J Molina-Estévez and Karim Benabdellah

GENYO, Centre for Genomics and Oncological Research: Pfizer, University of Granada,
18016 Granada, Spain

0. Graphical Abstract



1. Summary: What is this document about?

Over the past three decades, genome editing has undergone a monumental shift, evolving from an exploratory research tool in molecular biology into a transformative clinical platform. Rather than merely alleviating the external symptoms of chronic conditions, **genome editing addresses disease at its ultimate source: the human genetic code.** This clinical revolution is built upon decades of foundational research into cellular DNA repair mechanisms, which paved the way for early customizable gene-cutting tools, including zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). The subsequent discovery and adaptation of bacterial CRISPR-Cas systems catalysed an unprecedented acceleration across the field, offering researchers a modular, RNA-guided platform that is significantly simpler, more versatile, and more cost-effective to engineer than its protein-guided predecessors.

Pioneering clinical investigations—such as early ZFN-based trials modifying the CCR5 receptor to protect human immune cells from HIV infection—first established that target genomic modification could be performed safely in human patients. In recent years, this experimental foundation has yielded therapeutic breakthroughs. A historic milestone occurred with the regulatory approval of *CASGEVY*® (*exagamglogene autotemcel*), a CRISPR-Cas9-edited cell therapy developed for severe inherited blood disorders, specifically sickle cell disease and transfusion-dependent β -thalassemia. Beyond hematology, clinical genome editing is expanding into oncology to engineer allogeneic "off-the-shelf" CAR-T cell therapies that evade immune rejection, as well as into hepatology, where in vivo systemic therapies target liver cells directly to halt the production of disease-causing circulating proteins.

Translating these powerful molecular engines from the laboratory to global clinical practice requires more than effective cutting tools. Scientific discovery must be paired with rigorous multi-omic safety evaluations, scalable bioreactor manufacturing, customized clinical trial protocols, clear regulatory frameworks, and proactive strategies to ensure equitable global access. This White Paper presents a comprehensive, structured overview of the journey from basic science to approved therapies, detailing the molecular mechanisms, delivery vehicles, clinical applications, and regulatory pathways governing the field.

2. Introduction: What is Genome Editing?

Every cell in the human body contains a set of genetic instructions written in a chemical code called DNA. Genome editing refers to a group of laboratory techniques that allow scientists to add, remove, or alter specific sequences in this genetic code.

To edit DNA, scientists take advantage of the body's natural cell repair mechanisms. When a DNA strand breaks, the cell quickly uses specialized molecular pathways to stitch the broken ends back together. Modern genome editing relies on exploiting endogenous cellular DNA repair machinery. When a double-strand break (DSB) occurs in the DNA double helix, the cell activates one of two primary repair pathways: **Non-Homologous End Joining (NHEJ)**: An efficient, fast-acting pathway that directly re-joins the severed DNA ends. Because NHEJ is prone to introducing small insertions or deletions (indels) at the break site, it is routinely used by scientists to disrupt or "knock out" a disease-causing gene; and **Homology-Directed Repair (HDR)**: A precise repair pathway that uses a matching DNA template to reconstruct the broken sequence. By introducing an artificial DNA template alongside a cutting tool, scientists can harness HDR to correct mutated sequences or insert new genetic material with high fidelity.

By intentionally creating a controlled cut at a precise genomic location, genome editing tools prompt the cell to repair the break—allowing scientists to intentionally disrupt a faulty gene or insert a corrected genetic sequence.

A major actor that facilitated in speeding up genome editing development has been the **evolution of Editing Tools**: scientists moved from semi-natural nucleases, which were very hard to engineer, towards versatile and user friendly CRISPR-based approaches:

Zinc-finger nucleases (ZFNs): The first custom DNA cutters!

Developed in the late 1990s, ZFNs were among the first engineered tools capable of cutting specific DNA sequences. They combine customized DNA-binding proteins ("zinc fingers") with a molecular cutting enzyme (the FokI nuclease), see **Figure 1**. Because the cutting enzyme must pair up (dimerize) to work, two ZFN molecules are designed to bind on opposite sides of the target sequence and cut the DNA together.

While ZFNs proved targeted editing was possible, designing custom proteins for every new target was slow, complex, and expensive.

TALENs: Increasing DNA Target Accuracy

TALENs (Transcription Activator-Like Effector Nucleases) were derived from proteins found in plant-infecting bacteria. Like ZFNs, TALENs fuse a DNA-recognition protein domain with the FokI cutting enzyme. However, TALENs use a simpler "code" to recognize DNA letters, making them easier to design and more flexible than ZFNs, **Figure 2**. Nevertheless, because TALENs still rely on re-engineering complex proteins for every single DNA target, they remained labour-intensive.

Zinc-Finger Nucleases (ZFNs)

A modular tool for targeted genome editing

Separating DNA recognition from DNA cleavage

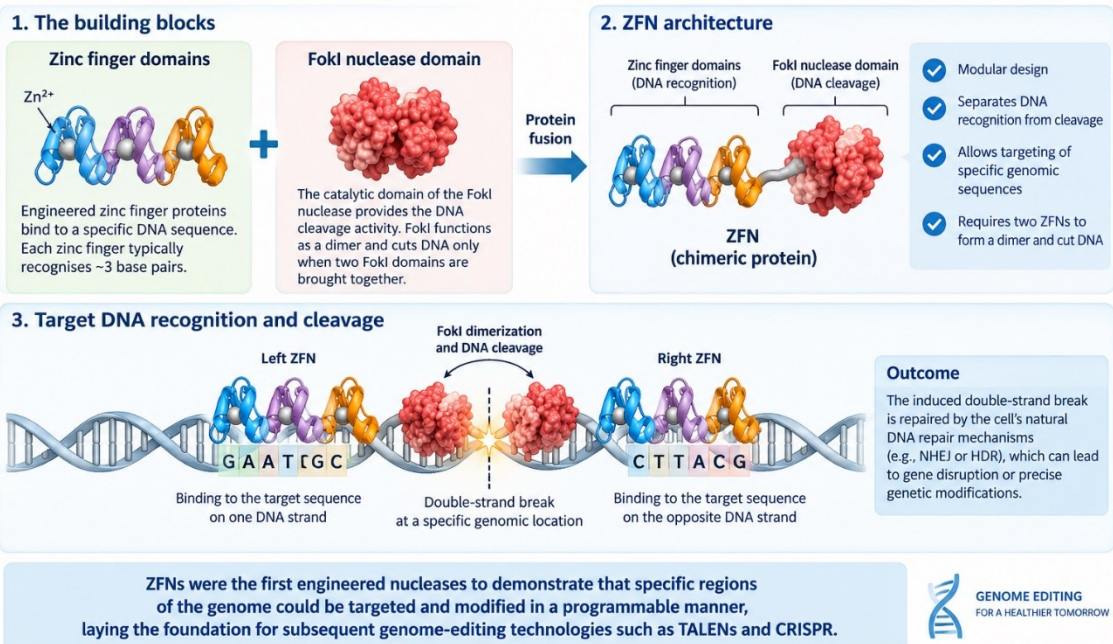


Figure 1: Modular Architecture and Cleavage Mechanism of Zinc-Finger Nucleases (ZFNs). Structural blueprint of ZFNs, highlighting engineered zinc-finger DNA-binding domains fused to the catalytic domain of the FokI restriction nuclease. Dimerization of two adjacent FokI domains introduces a site-specific double-strand break (DSB), which is subsequently resolved by endogenous cellular repair machinery (NHEJ or HDR) to achieve targeted gene disruption or modification).

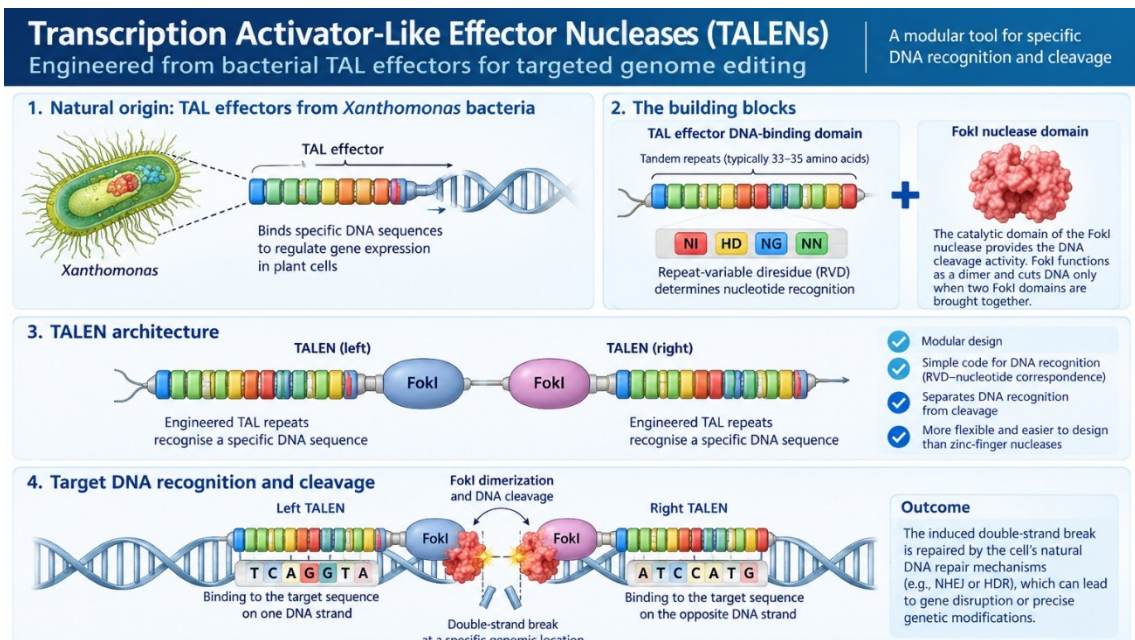


Figure 2. Transcription activator-like effector nucleases (TALENs) Overview of TALEN technology, showing repeat-variable diresidues (RVDs) derived from *Xanthomonas* bacterial proteins engineered to bind target DNA bases. Dimerization of fused FokI domains at the target site induces a double-strand break to enable targeted gene disruption or precise sequence alteration.

CRISPR-Cas: the RNA-guided revolution!

CRISPR-Cas systems originated as an adaptive immune defence used by bacteria to destroy invading viruses. When a virus attacks a bacterium, the bacterium stores small fragments of the viral DNA inside its own genome (the CRISPR locus). The bacterium uses these stored fragments to create short RNA molecules that guide a DNA-cutting protein (such as Cas9) to recognize and destroy matching viral DNA upon future infections, **Figure 3**.

In 2012, researchers adapted this bacterial system into a universal gene editor. Instead of building expensive new proteins for every DNA target, scientists simply change a short piece of "guide RNA." The guide RNA acts like a GPS navigator, directing the Cas protein directly to the exact matching spot in the human genome to execute a precise cut. This modular simplicity made genome editing accessible to research laboratories around the globe. This scientific advance was such a game changer that only 8 years after, in 2020, awarded the *Nobel Prize* to Emmanuelle Charpentier and Jennifer A. Doudna! By simply altering a 20-nucleotide sequence within this guide RNA, scientists can direct the Cas endonuclease to virtually any genomic location containing a short adjacent landmark known as a Protospacer Adjacent Motif (PAM). Decoupling DNA recognition (handled by RNA base-pairing) from DNA cleavage (handled by the Cas protein) democratized genome editing, enabling rapid, high-throughput, and cost-effective genomic targeting worldwide.

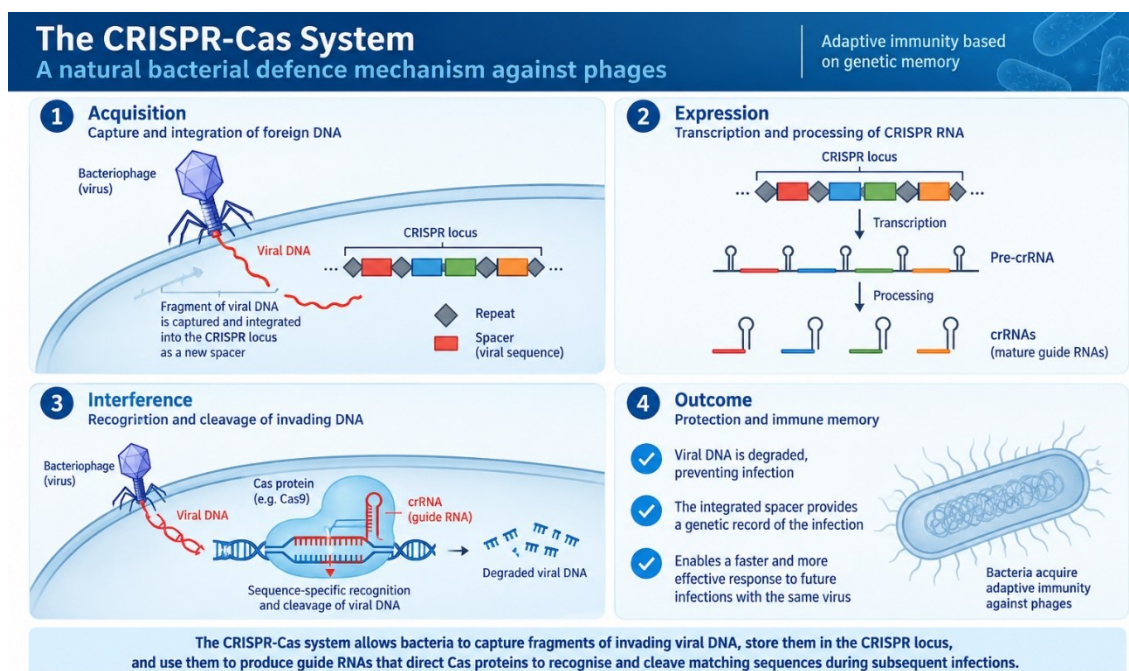


Figure 3. Biological Stages of the Bacterial CRISPR-Cas Adaptive Immune System. Four sequential steps of natural CRISPR-Cas adaptive immunity: foreign viral DNA acquisition into the bacterial genome (1), transcription into mature guide crRNAs (2), sequence-specific targeting and cleavage of invading bacteriophage DNA by Cas nucleases (3), and ultimate bacterial immune protection (4)

Next-Generation Tools: Base Editing and Prime Editing!

It is worth mentioning that tools are evolving beyond programmable DNA cutters. While traditional CRISPR tools act like "molecular scissors" by cutting both strands of the DNA ladder (causing double-strand breaks), newer tools act like precision word processors:

- **Base Editors:** Convert single DNA letters (e.g., changing a C to a T, or an A to a G) directly without severing the DNA backbone, minimizing unwanted genetic damage. Created by fusing a catalytically impaired Cas nuclease ("deaminase-Cas nickase") to a nucleobase deaminase enzyme. Base editors perform direct, targeted chemical conversion of single DNA bases (such as converting

C:G to T:A, or A:T to G:C) with high precision, bypassing double-strand breaks and dramatically reducing unwanted indels or translocations.

- **Prime Editors:** Function like a complete "search-and-replace" word processor, capable of correcting longer sequence errors or inserting new genetic code with high precision and without full double-strand breaks. Composed of an engineered Cas nickase fused to a reverse transcriptase enzyme, guided by an expanded prime editing guide RNA (pegRNA). Prime editors execute direct "search-and-replace" editing, capable of writing new genetic sequences or fixing point mutations, insertions, and deletions without double-strand breaks or donor DNA templates.

3. Delivering Genome Editing Tools to Target Cells:

Having an accurate molecular tool is only half the battle. A successful therapy must safely deliver the editing machinery into the correct cells inside the human body. Delivery represents one of the single biggest challenges in modern gene therapy.

In vivo vs. Ex vivo

We have optimised procedures to harvest, manipulate and reintroduce a reduced set of adult stem cells. These are capable of homing back to their source-tissue and contribute to regenerate the tissue as a consequence of normal tear-and-wear (Physiologic) or as response to injury. For these isolated cells we can set a controlled gene transfer protocol that falls under "*Ex vivo*" modification, **Figure 4**. For most adult tissues the better strategy is using vectors to target these tissues in the patient. The "*in vivo*" approaches resource in powerful vehicles that need to specifically deliver the cargo into the right tissue and also shield the cargo to avoid that the natural defences could be triggered and react against the therapeutic molecules, **Figure 4**.

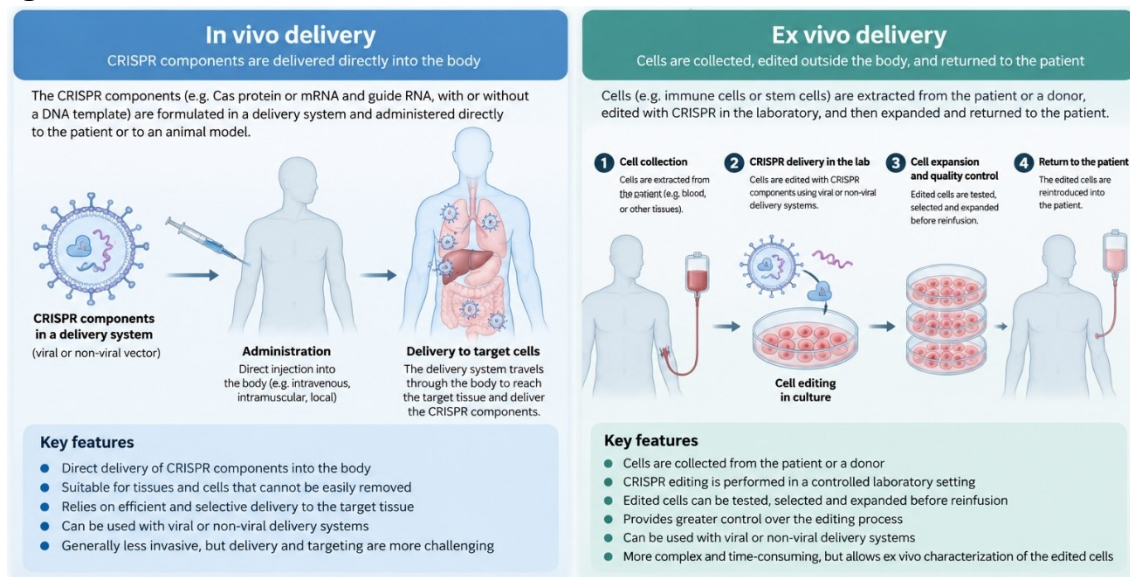


Figure 4. *In vivo* versus *ex vivo* CRISPR delivery strategies. Schematic operational workflow contrasting direct in vivo administration of CRISPR components via delivery vehicles against ex vivo harvesting, laboratory modification, quality control selection, and autologous reinfusion of host cells.

Viral vs. Non-Viral Delivery Systems

Delivery methods fall into two primary categories based on the nature of the molecular spacecraft used to bring the genome editing tools into the target genetic material that needs to

be changed. Different vehicles are exploited in gene editing due to their features, **Figure 5**, but they are normally separated in two categories:

Viral Vectors: Modified, harmless versions of natural viruses (such as Adeno-Associated Viruses/AAVs or Lentiviruses). Viruses have naturally evolved to enter human cells efficiently, but they can sometimes trigger immune responses or carry size limits for genetic payloads.

Non-Viral Vehicles: Synthetic microscopic carriers, such as Lipid Nanoparticles (LNPs)—tiny bubbles of fatty molecules similar to those used in modern mRNA vaccines. LNPs are easier to manufacture, do not permanently remain in the body, and carry less risk of long-term immune reaction.

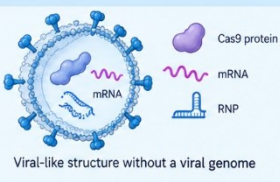
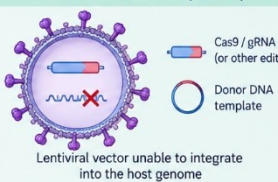
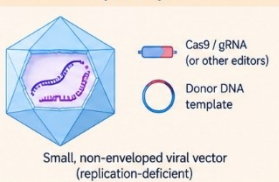
	Virus-like particles (VLPs)	Integrase-defective lentiviral vectors (IDLVs)	Adeno-associated viral vectors (rAAVs)
Schematic structure	 Viral-like structure without a viral genome	 Lentiviral vector unable to integrate into the host genome	 Small, non-enveloped viral vector (replication-deficient)
Viral genome	No viral genome	Incomplete lentiviral genome	Single-stranded DNA genome (no Rep/Cap)
Integration into host genome	✗ No integration	✗ No integration (integrase-defective)	✗ Very low integration frequency (mainly episomal)
Cargo capacity (approximate)	Flexible (depends on cargo)	Up to ~8–10 kb	~4.7–4.9 kb
Main cargo types	CRISPR proteins, mRNA, RNPs, protein-RNA complexes	Cas9 or other nucleases, gRNA, donor DNA templates	Cas9 or other editors, gRNA, donor DNA templates (size limited)
Typical use	Ex vivo and in vivo (transient delivery)	Ex vivo and in vivo (transient delivery)	Mainly in vivo (broad tissue tropism)
Key advantages	• Transient delivery • No viral genetic material • Can deliver proteins, mRNA or RNPs • Reduced risk of genomic integration	• Efficient cell entry • Relatively large cargo capacity • Transient expression • Reduced integration-related risks	• High in vivo transduction efficiency • Broad tissue tropism • Persists in non-dividing cells • Low immunogenicity
Main limitations	• Delivery efficiency can vary • Manufacturing and scalability • Stability in vivo	• Lower expression than integrating vectors • Limited cargo capacity • Manufacturing and targeting	• Small cargo capacity • Pre-existing immunity • Manufacturing and large-scale production

Figure 5. Gene Editing Delivery Vehicles. Summary of viral and non-viral delivery platforms—Virus-Like Particles (VLPs), Integrase-Defective Lentiviral Vectors (IDLVs), and Recombinant Adeno-Associated Viruses (rAAVs)—outlining their structural compositions, cargo capacities, integration risks, and primary clinical applications.

4. Therapeutic Applications: From Lab to Clinic.

Thanks to the growing molecular knowledge that we are gaining on many diseases, doctors and scientist can envision together how to tackle discrete disease causing mutations or , in some cases, how to re-route the genomic instructions to counteract the unbalance created by a set of mutations causing the same illness. We provide some of these strategies:

Blood Disorders: Reactivating Healthy Hemoglobin

Sickle Cell Disease (SCD) and Transfusion-Dependent β -Thalassemia (TDT) are severe genetic disorders caused by mutations in the HBB gene, which encodes adult β -globin—a core component of hemoglobin required for red blood cell function. In SCD, a point mutation causes hemoglobin molecules to polymerize, bending red blood cells into rigid, sickle shapes that block blood vessels, causing severe pain and organ damage. In β -thalassemia, deficient β -globin production results in severe, life-threatening anemia requiring lifelong blood transfusions.

During fetal development, human red blood cells produce fetal hemoglobin (HbF), which possesses high oxygen affinity and lacks sickling properties. Shortly after birth, the master transcriptional repressor gene BCL11A turns off HbF production. The landmark approval of

CASGEVY® (exagamglogene autotemcel) utilizes ex vivo CRISPR-Cas9 editing of autologous CD34⁺ hematopoietic stem cells to disrupt the erythroid-specific enhancer region of BCL11A. Disabling this repressor switch reactivates robust fetal hemoglobin production in mature red blood cells, functionalizing oxygen transport, preventing sickling, and eliminating painful vaso-occlusive crises with a single, curative intervention. Advanced clinical trials are currently evaluating Cas12a nucleases, base editors, and prime editors to achieve similar reactivation profiles or directly correct the underlying HBB mutation, **Figure 6**.

Genome editing for blood disorders

From genetic insight to durable patient benefit

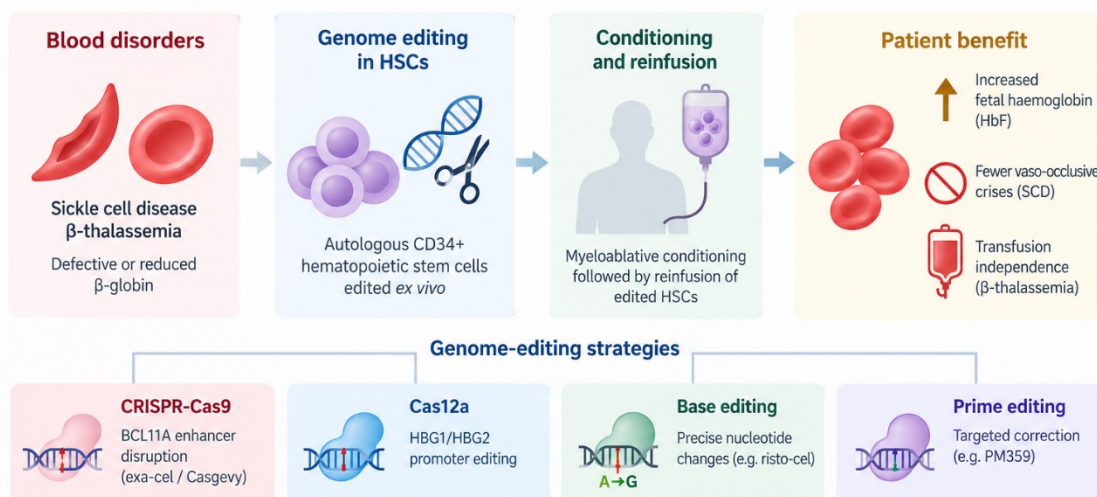


Figure 6. Ex Vivo Hematopoietic Stem Cell Editing for Inherited Blood Disorders. Clinical pathway for treating Sickle Cell Disease and Transfusion-Dependent β-Thalassemia through ex vivo editing of autologous CD34⁺ stem cells. Disruption of the BCL11A enhancer or direct mutation correction via Cas9, Cas12a, Base Editing, or Prime Editing reactivates fetal hemoglobin (HbF) to deliver long-term patient benefit.

Cancer Immunotherapy: Supercharging Immune Soldiers

In CAR-T cell therapy, doctors extract immune T-cells and re-engineer them to recognize and destroy cancer cells, **Figure 7**. Genome editing is dramatically enhancing CAR-T cell potency and accessibility through multiplex gene modifications:

Allogeneic "Off-the-Shelf" Products: Conventional CAR-T therapies require custom manufacturing using each individual patient's T-cells. By using TALENs or CRISPR to knock out the TRAC gene (preventing Graft-versus-Host Disease) and the CD52 gene (conferring resistance to lymphodepleting drugs), scientists can create universal donor T-cells suitable for dozens of patients on demand.

Preventing Mutual T-Cell Destruction ("Fratricide"): In T-cell acute lymphoblastic leukemia (T-ALL), target malignant cells express markers (such as CD7) that are also present on healthy therapeutic T-cells. Knocking out CD7 in CAR-T cells prevents fratricide during expansion.

Overcoming Immunosuppression: Disrupting immune checkpoint genes, such as PD-1 (PDCD1), prevents tumor-mediated immune exhaustion, enabling CAR-T cells to maintain cytotoxic activity inside solid tumor microenvironments.

Liver-Directed In Vivo Editing: Fixing the Body's Protein Factory

The liver produces hundreds of vital proteins that circulate throughout the human body. Because intravenously administered Lipid Nanoparticles naturally accumulate in hepatocytes, the liver has become a major target for direct in vivo genome editing, **Figure 8**:

Genome editing for cancer immunotherapy

More precise. More persistent. More patients.

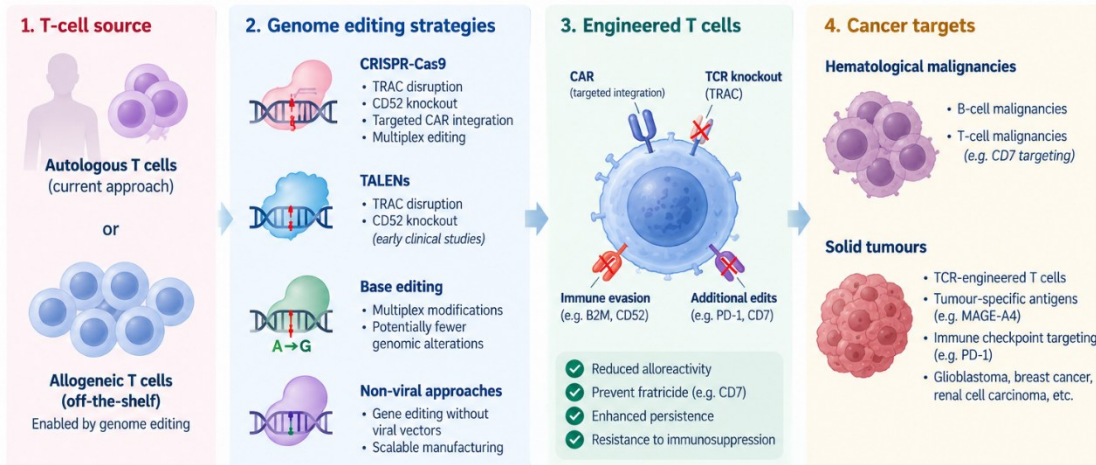


Figure 7. Re-engineering T-Cells for Advanced Cancer Immunotherapy. Strategies for generating autologous and allogeneic "off-the-shelf" CAR-T therapies using multiplex genome editing. Targeted knockouts (TRAC, CD52, B2M, PD-1, CD7) prevent alloreactivity, avoid T-cell fratricide, counter immune exhaustion, and enhance persistence against hematological and solid tumors.

Hereditary Transthyretin Amyloidosis (ATTR): Mutations in the TTR gene cause misfolded transthyretin proteins to form amyloid deposits in heart and nerve tissue. In vivo delivery of Cas9 mRNA and TTR-targeting guide RNA via LNPs leads to a permanent reduction in circulating toxic protein levels following a single infusion.

Hereditary Angioedema (HAE): In vivo knockout of the KLKB1 gene in hepatocytes blocks kallikrein protein production, preventing life-threatening inflammatory swelling attacks.

Hypercholesterolemia: Knocking out or base-editing the PCSK9 gene in hepatocytes permanently upregulates low-density lipoprotein (LDL) receptors, dramatically lowering circulating cholesterol levels to protect against cardiovascular disease

In vivo genome editing of the liver

A single intervention with systemic impact

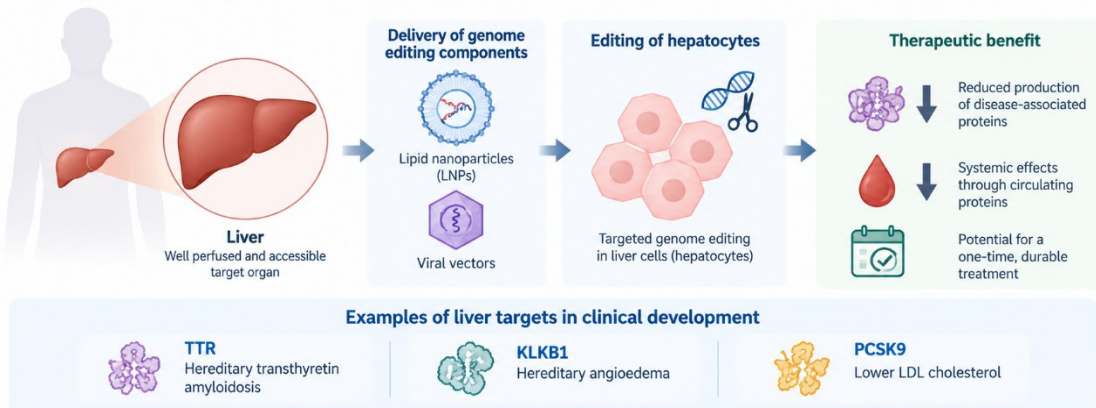


Figure 8. Liver-directed in vivo genome editing. This figure illustrates how genome-editing components can be delivered to hepatocytes using lipid nanoparticles (LNPs) or viral vectors to modify genes involved in the production of circulating proteins. Examples include targeting *TTR* for hereditary transthyretin amyloidosis, *KLKB1* for hereditary angioedema and *PCSK9* to reduce LDL cholesterol, highlighting the potential of a single intervention to provide durable systemic benefits.

5. Safety and Risk Assessment.

Because genetic modifications are permanent, proving safety before human administration is critical, **Figure 9**. Safety evaluations focus on three main pillars:

Off-Target Effects (Accidental Cuts Elsewhere)

Although guide RNAs target specific genomic addresses, similar DNA sequences elsewhere in the genome can occasionally mislead the Cas enzyme into making unwanted cuts. To protect patients, researchers use advanced computer predictions and Next-Generation Sequencing (NGS) to scan the entire genome for accidental cuts. High-fidelity Cas proteins engineered for near-perfect accuracy help prevent these off-target events, **Figure 9**.

On-Target Unintended Damage

Even when a cut happens at the exact intended spot, the cell's natural repair process can occasionally produce unpredictable outcomes, such as large DNA deletions or rearrangement of chromosome segments (translocations), **Figure 9**. Making multiple cuts at the same time increases this risk. Transitioning to non-cleaving tools like Base Editors and Prime Editors significantly reduces these structural hazards.

Immunogenicity (Immune System Reactions)

Because Cas proteins originate from common bacteria, many humans already possess pre-existing antibodies or immune memory against them, **Figure 9**. If injected directly into the body, the immune system might destroy the edited cells or neutralize the delivery vehicle. Doctors overcome this by performing editing outside the body (ex vivo), using "humanized" or hidden protein variants, or administering brief immune-soothing medications during treatment.

6. Manufacturing, Quality Control, and Clinical Trials.

Once the scientific tools turn into medicines, all governments, through Specialised agencies, secure that any product labeled as medicine passes a minimum number of quality controls to ensure that the product is beneficial and also that is manufactured in a way that is safe for the patients, **Figure 10**.

High-Purity Mass Production

Moving from small lab experiments to commercial production requires scaling up to industrial bioreactors while maintaining strict purity. Manufacturers must ensure every batch is identical, active stem cells remain healthy, and no lingering cutting enzymes remain to cause unwanted delayed edits inside the patient.

SAFETY & RISK ASSESSMENT OF GENOME EDITING

A Balance of Potential and Precision for a Cure.

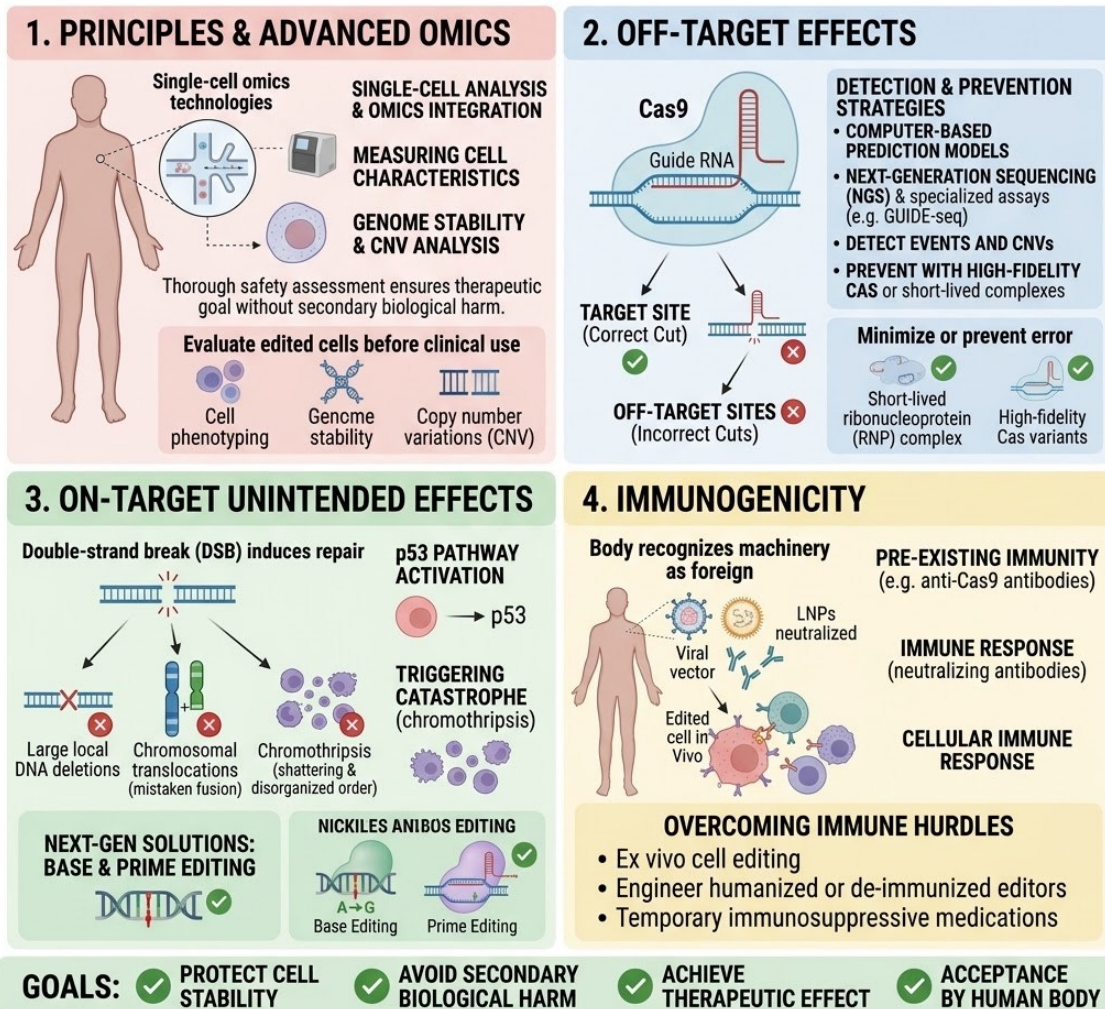


Figure 9. The Four Pillars of Comprehensive Safety and Risk Assessment. Multi-faceted safety evaluation framework for clinical genome editing, encompassing multi-omic single-cell profiling (1), off-target cleavage detection and prevention (2), on-target structural damage mitigation via nickase platforms (3), and clinical strategies to overcome immunogenicity (4)

Cautious Clinical Trial Design

Because gene edits are designed as permanent, single-dose treatments that cannot simply be stopped if side effects occur, clinical testing follows extra precautions:

Staggered Patient Enrollment: Researchers treat a single patient first and observe them extensively over months before enrolling additional participants.

Decade-Long Surveillance: Patients are monitored for 15 years or more to ensure cellular stability and track potential delayed complications.

Clear Biological Endpoints: Success is measured by concrete biological improvements—such as sustained protein production or reduced hospitalizations—rather than temporary symptom relief.

MANUFACTURING, CLINICAL & REGULATORY PATHWAY IN GENOME EDITING

Scaling Precision Cures from the Laboratory to the Patient Safely and Responsibly

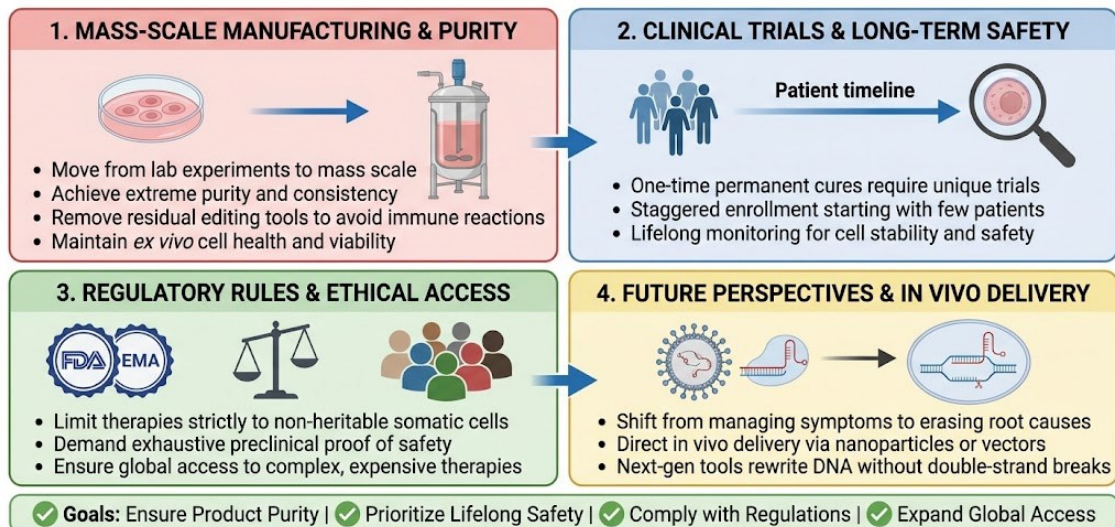


Figure 10. Manufacturing, Clinical Translation, and Regulatory Pathway. Four key phases in scaling genetic therapies from bench to bedside: bioreactor mass production and purity control (1), staggered clinical enrolment and lifelong monitoring (2), strict regulatory boundaries restricting edits to somatic cells alongside global equity initiatives (3), and the transition toward next-generation non-cleaving in vivo delivery tools (4).

7. Regulatory and Ethical Considerations

While the ultimate goal of therapeutic genome editing is to cure disease, healthcare professionals must carefully consider potential risks and future handicaps due to the use of these powerful technologies, **Figure 11**. Hence, we need to set beforehand the redlines needed to maintain the medical intervention within reasonable grounds considering the individual and the society potential gains and loss.

REGULATORY & ETHICAL PATHWAY FOR GENOME EDITING

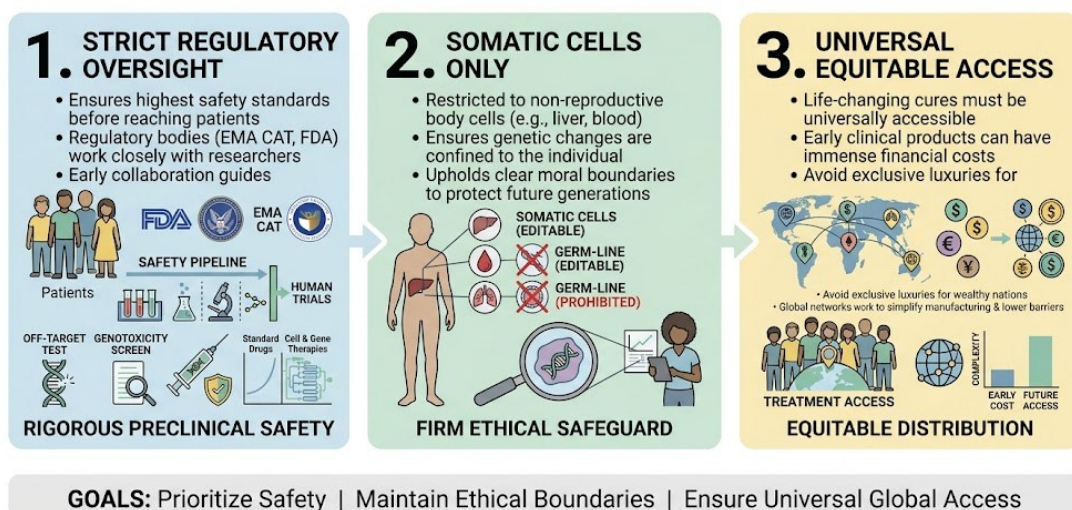


Figure 11. Regulatory and Ethical Pathway for Genome Editing. Overview of regulatory oversight frameworks (FDA/EMA), somatic cell boundaries, and global equity initiatives to ensure universal access.

Somatic vs. Germline Editing

Global regulatory agencies, such as the U.S. FDA and European Medicines Agency (EMA), enforce strict boundary lines regarding which cells can be edited:

Somatic Cell Editing (Permitted): Modifies non-reproductive body cells (e.g., blood, liver, or eye cells). Changes affect only the individual patient treated and cannot be inherited by future generations.

Germline Editing (Prohibited): Modifies reproductive cells (sperm, eggs, or early embryos). Any genetic changes would be passed down permanently to future children and descendants. Clinical germline editing remains strictly banned worldwide.

Universal Access and Global Equity

The most urgent ethical challenge in modern gene therapy is affordability and access. Current single-dose genome treatments can cost millions of dollars per patient due to complex individual manufacturing. International health organizations are advocating for streamlined manufacturing and non-viral in vivo therapies to bring costs down, ensuring these transformative cures reach patient populations in developing regions where conditions like sickle cell disease are most prevalent.

8. Conclusions and Future Perspectives

Genome editing represents a fundamental shift in human medicine—moving from lifelong symptom management to single-dose genetic cures. The approval of therapies for blood disorders has proven the clinical validity of this approach, while advances in cancer immunotherapies and liver-directed treatments continue to expand its medical horizons.

As next-generation tools like Base Editing and Prime Editing mature, therapies will become increasingly precise, avoiding double-strand DNA breaks and reducing safety risks. By uniting scientific innovation with rigorous safety standards, clear ethical boundaries, public funding, and global health equity, genome editing will serve as a cornerstone of 21st-century medicine for patients around the world.

Acknowledgments and Disclaimers

This document is part of the divulgation efforts funded by GenE-HumDi (COST Action CA21113; www.genehumdi.eu)

Cooperation in Science and Technology (COST) is an EU-Funded Programme (www.cost.eu).

Authors want to acknowledge all COST Action CA21113, GenE-HumDi members who engage in fruitful discussion and providing fuel for the network to move forward for the past 4 years. We also are in debt to former and current Grant Holder Managers who inspired the initiative to make a more accessible review of genome editing to reach a wider audience.

KB and FJME have no financial interest in any of the companies and medicines mentioned. Figures were rendered using different AI models using authors instructions to illustrate the contents and were reviewed after for compliance.

Last revision and publication: Granada, October 2026