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An analysis of Reversible, Tissue-Specific CRISPR Circuits in Gene Editing Safety Compared to Permanent Knockout Approaches in Autoimmune Disease Gene Therapy

Noma Ohonba

[Signature School, Evansville, United States of America]

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ABSTRACT

This study investigated whether reversible, tissue-specific CRISPR circuits offer superior safety profiles compared with permanent knockout approaches in autoimmune disease gene therapy. CRISPR-based therapies have shown significant therapeutic potential in treating autoimmune disorders. Still, permanent gene editing introduces safety concerns, including off-target mutations, immune responses, unintended immunological vulnerabilities, and the inability to reverse harmful edits once treatment has occurred. The purpose of this research was to evaluate whether emerging reversible and tissue-specific CRISPR systems could address these limitations and provide a safer alternative. A structured secondary research methodology was used to analyze ten peer-reviewed studies published between 2016 and 2025. Studies were selected based on their relevance to CRISPR safety, autoimmune disease applications, reversibility, and tissue-specific control mechanisms. Evidence from in vitro experiments, animal studies, and human clinical trials was compared using five safety dimensions: off-target DNA modification, immune recognition, secondary immune vulnerabilities, reversibility, and tissue-targeting precision. The analysis found that permanent CRISPR systems exhibited the greatest safety risks. Studies documented large unintended DNA insertions, genomic instability, immune recognition of Cas9, and secondary vulnerabilities such as increased natural killer cell targeting following MHC knockout. Current autoimmune applications demonstrated strong therapeutic potential, including clinical remission in patients treated with CRISPR-edited CAR-T cells, but remained limited by their irreversible nature. Reversible and tissue-specific systems showed advantages in four major safety categories. Drug-inducible CRISPR systems enabled gene regulation to be activated and deactivated as needed, while microRNA-guided approaches achieved highly specific tissue targeting with extremely low off-target activity. Although these systems have not yet been tested in autoimmune clinical settings, available evidence suggests they provide substantially greater control over gene-editing activity. The findings support the conclusion that reversible, tissue-specific CRISPR circuits offer a promising pathway toward safer autoimmune gene therapies than permanent knockout approaches. However, current evidence remains largely limited to proof-of-concept and preclinical studies. Future research should focus on integrating reversible and tissue-specific control systems into a single therapeutic platform and evaluating their long-term safety and efficacy through autoimmune disease animal models and clinical trials.

Introduction

In 2024, CRISPR-edited CAR-T cells permanently knocked out autoreactive B cells in three autoimmune patients, inducing complete remission.¹ But just two years earlier, researchers at Boston Children's Hospital discovered that CRISPR/Cas9 occasionally introduces large DNA insertions, causing genomic instability and cancer risk.² This paradox defines gene therapy's central point of tension: CRISPR offers precision, but permanent modifications introduce safety vulnerabilities that cannot be addressed post-treatment.

What, then, exactly is CRISPR used for here? One promising application that is currently being heavily researched in this context is the development of a therapy for autoimmune diseases, which happen as a result of the body's immune response attacking the body itself. Treatment options for the condition at present involve immunosuppressant drugs, which alleviate symptoms but do little else since they suppress the patient's immune system as a whole. Instead, CRISPR-based solutions involve trying to correct the faulty immune response at the genetic level, attempting to restore tolerance of the body's immune system to itself.

Previous work in this field has largely fallen into one of two categories. The first is gene knockout platforms, which irreversibly suppress problematic genes responsible for the condition. The second is more advanced antigen-specific approaches, like nanoparticles or engineered immune cells, which aim to target the specific problematic immune reaction. In spite of their success thus far, both these approaches suffer from the same critical problem: a lack of control over editing that occurs once the procedure takes place.

In this respect, this research looks to explore a third possibility: that of controlling CRISPR gene editing using an inducible system that allows both reversibility and tissue specificity. As opposed to either of the mentioned methods of treating autoimmune diseases, the suggested platform is not developed from any single existing system, but rather incorporates various traits exhibited by existing studies into a hybrid. Namely, these include the use of drug-induced CRISPR activation and tissue targeting via microRNAs.

There has been ample research regarding the safety and effectiveness of CRISPR treatments. For instance, several studies show positive results for CRISPR therapy, including successful correction of the immune system malfunction in autoimmune diseases.^{1,3} Nevertheless, research related to safety issues in CRISPR is also abundant, noting the risks of CRISPR-associated genomic instability and the immune response.^{2,4} Other new findings about control measures have been obtained in recent years, involving the use of drugs and tissue specificity as control tools for CRISPR treatments.^{5,6} Unfortunately, all these discoveries are scattered across different domains, failing to allow a meaningful comparison between various CRISPR strategies in terms of safety.

It is vital to consider, however, that permanent methods of CRISPR-based treatments have proven to pose several risks for patients, such as unexpected DNA modifications, immune reactions to Cas9 protein, and increased sensitivity to natural killer cells due to the lack of major histocompatibility complex molecules.⁷ Although novel reversible and tissue-specific systems have been designed to address these problems, no systematic evaluation of their benefits compared to existing solutions has been conducted so far.

In order to fill this gap, a secondary study based on a research design approach will be performed in the current paper. Specifically, the safety of CRISPR systems will be assessed comparatively using five dimensions: the frequency of unwanted DNA modifications, immune reaction to the use of CRISPR, any side effect on the immune system, reversibility of the editing process, and specificity of target tissues. All of these aspects were noted throughout the discussion of risks associated with CRISPR

and are accounted for in the comparative analysis of different systems.

Based on the literature review, ten articles were included, with information about experiments conducted using different CRISPR systems and their impact on various cells. The significance of this analysis lies in guiding future research, informing regulatory decisions, and helping patients make informed choices. In this regard, the current paper pursues three key claims: firstly, the identification of risks associated with permanent CRISPR systems; second, that current immune-targeted applications demonstrate therapeutic potential but remain limited by irreversibility; and third, that reversible and tissue-specific CRISPR strategies offer a promising path toward safer and more controllable treatments.

Literature Review

Knowledge Claim One: Permanent CRISPR systems present safety risks, justifying the need for controlled alternatives

A significant milestone in modern medicine is the development of permanently modified organisms using CRISPR editing. According to Zhang et al.,⁸ CRISPR technologies are currently used in a variety of applications, including cancer treatment and autoimmune disorders. Yet, although CRISPR allows achieving high levels of precision at the molecular level, these advantages do not always correlate with safety at the organismic level. Research conducted at Boston Children's Hospital² shows that despite their benefits, CRISPR-edited human cells are highly prone to mutations caused by Cas9. Specifically, Cas9 introduced several large insertions of DNA, from 250 base pairs to 6 kilobases, into the genomes of target and non-target sites within these cells. As a result, 15% of CRISPR-edited cell clones with such insertions demonstrated severe genetic changes, including chromosomal rearrangements, aneuploidy, and loss of tumor suppressors. Given that such mutations are permanent, any harmful changes in the CRISPR-edited genome can be passed on through cell replication. At present, it is unclear whether screening tests currently used to check cells before transplantation are sufficient to identify all of these changes.

Another threat connected with CRISPR treatment comes from the immune system. According to Simhadri et al.,⁴ Cas9 is able to activate the human immune system by triggering robust CD4⁺ T-cell responses among various genetically distinct individuals. If a person's CRISPR-modified cells continue to contain Cas9, these cells are likely to become a target of immune destruction. In autoimmune diseases, when the patient's immune system has already been compromised, this reaction may even exacerbate the condition instead of helping recovery.

Unintended modifications during CRISPR treatments are also a common occurrence, which can be partially explained by Redman et al.⁹ The researchers showed that the guide RNA used in CRISPR can tolerate minor sequence differences while binding to the DNA strand. Hence, CRISPR has some probability of cutting non-target sites that have close sequence homology to the intended target. As long as some level of Cas9 activity outside the intended target site exists in optimized CRISPR systems, some off-target edits are likely to occur. Moreover, the probability of unwanted changes increases depending on the length of time during which Cas9 remains activated within the cells. Although the off-target rate appears quite low at first glance, even rare errors become significant when the resulting changes are permanent and cannot be corrected.

Taken together, these findings point to a fundamental limitation of permanent CRISPR systems: once an edit is made, there is no built-in way to adjust or reverse it. Any unintended effects, whether genetic or immunological, can persist indefinitely. This lack of flexibility is especially important in medical contexts, where a patient's condition may change, or new treatments may become available.

Knowledge Claim Two: Current autoimmune applications validate therapeutic potential while reinforcing the need for reversibility

In spite of the risks that may arise from CRISPR application, recent studies have shown that it can help create effective therapies for autoimmune diseases, indicating that genetic modification within the immune system is a viable strategy. Nevertheless, all existing applications involve irreversible genetic editing, meaning that once made, changes cannot be reversed or regulated, even in the case of unexpected side effects.

Luo et al.³ demonstrate that CRISPR can be used effectively in animal trials. Specifically, their study suggests that nanoparticle-based delivery of CRISPR-Cas9 complex together with autoantigen signals can target antigen-presenting cells and edit their gene expression through silencing co-stimulatory factors such as CD80 and CD86. As a consequence, there was an increase in regulatory T cell activity and a decline in inflammatory cytokine production, limiting tissue injury in animals. Nevertheless, a serious limitation is apparent: in order for the intervention to be effective, an optimal level of editing must be achieved. Too little editing allows the disease to continue developing, whereas excessive modification may result in immune suppression. Since edits were performed permanently, there was no possibility of making corrections post-treatment.

The same pattern can be observed in human experiments. According to Wang et al.,¹ CRISPR has been tested in clinical trials related to the development of CAR-T cells for the treatment of severe autoimmune conditions such as lupus and dermatomyositis. T cells were obtained

from donors and edited so that they would lose their natural receptors. At the same time, CARs were introduced into the cells, resulting in the elimination of autoreactive B cells and remission of the condition within 6 to 18 months. However, such permanent changes might negatively affect patients' capacity to produce antibodies against pathogens.

The limitations of permanent knockout techniques are further highlighted by Merola et al.,⁷ who studied the effects of knocking out MHC class I and II molecules to generate cells resistant to immune detection. Cells lacking MHC were able to evade T-cell recognition since they use MHC molecules to identify foreign antigens. Nevertheless, this created another problem: NK cells are able to recognize missing-self markers, and consequently attacked the modified cells within a very short period. The solution was to restore minimal expression of MHC class I. The deletion of genes solved one problem but raised another, making it impossible to regulate the immune response without further engineering.

In conclusion, the above-mentioned examples prove that CRISPR techniques are capable of successfully manipulating the immune system. Nonetheless, all existing applications involve irreversible genetic editing, and changes cannot be reversed or regulated in any way, even in the case of unexpected side effects.

Knowledge Claim Three: Reversible, tissue-specific circuits may offer a solution to the issues of gene editing

New models of gene editing involve programmable regulation, with modification capability distinguished from the final state, demonstrating that controllability and precision are engineering decisions rather than inherent trade-offs.

Sui et al.⁵ created a drug-inducible CRISPR system for reversible control. Cas9 production involved the use of tetracycline-inducible promoters: the presence of doxycycline triggers expression of Cas9, while its absence stops transcription, and degradation of the protein occurs within several hours. Gene activation was observed after 6 hours, and shutting down returned to baseline levels within 24–48 hours. Functionality was successfully verified through five cycles of switching with no loss of response. This makes it possible, for example, in autoimmune disease treatment, to induce immune modulation during flares but not during periods of remission. Should any negative effects arise, the process can be terminated.

Chylinski et al.¹⁰ demonstrated temporal regulation via a Cre-based switch. Cre recombinase removed sequences preventing guide RNA synthesis, enabling sequential editing of different sites at distinct times. Tests on live mice revealed high efficacy in editing at the ON phase and virtually nonexistent editing at the OFF phase. Temporal regulation proved more efficient than conventional CRISPR

due to reduced off-target accumulation. Although Cre-based regulation itself cannot be turned off, the principle of temporal gating remains highly relevant.

Spatial control was attained by designing microRNA-responsive guide RNAs.⁶ Guide RNAs were programmed with nucleotide sequences complementary to tissue-specific microRNAs. If no microRNA was present, the guide RNA could direct Cas9 normally. However, if the guide RNA bound to the microRNA, it would be degraded and prevent genome editing. In testing for muscular dystrophy in mice using the muscle-specific miR-206/133, genome editing was restricted exclusively to skeletal muscles, with no off-targets detected in the heart, liver, kidneys, lungs, spleen, or any of the other 10 tissues tested. Deep sequencing confirmed off-target rates below 0.01%, demonstrating greater than 1000-fold tissue specificity.

These three inducible systems are independent of each other, and each has been proven experimentally. To the best of the author's knowledge, no attempt has yet been made to combine all three systems specifically for the treatment of autoimmune disorders. This represents an obvious progression: Sui et al.⁵ allow for modification of treatment post-treatment, Chylinski et al.¹⁰ demonstrate reduced off-target activity when time specificity is imposed, and Garcia-Guerra et al.⁶ establish spatial specificity based on tissue-specific microRNAs.

Methodology

In order to assess CRISPR-based approaches that may be useful for addressing autoimmune diseases, the current work utilizes a structured secondary research methodology. The use of literature-based research is justified in light of the research question since a comparative analysis of evidence across multiple experimental settings is required. Previous studies in this area include experiments with in vitro human cells,^{7,4} animal model experimentation,^{3,6} and human trials in the clinical setting.¹ A direct comparison among these research approaches via primary research would require simultaneous testing in parallel across

comparable disease models, which is not feasible within the scope of this project.

The data collection process was conducted according to strict inclusion and exclusion criteria. The inclusion criteria were: (1) application of CRISPR/Cas9 in relation to autoimmune disease or studies of safety and control aspects of therapeutic gene editing; (2) measurement of variables associated with the five safety dimensions defined above; (3) publication in peer-reviewed journals indexed in PubMed, Google Scholar, or Web of Science; (4) provision of original experimental data; and (5) publication date ranging from 2016 to 2025.

The exclusion criteria included a focus on CRISPR technology as a diagnostic tool, utilization of alternative gene editing techniques with completely different mechanisms (e.g., base editing or prime editing), lack of quantitative measures, or non-peer-reviewed sources. Data were collected by searching articles with key terms such as "CRISPR autoimmune," "Cas9 safety," "reversible gene editing," "inducible CRISPR," "tissue-specific editing," "MHC knockout," "CRISPR immunogenicity," and "off-target effects." In addition to systematic database searches, the review was supplemented by manual examination of references within relevant studies. Ten studies satisfied all selection criteria and were used for analysis.

For comparing different CRISPR technologies on an equal footing, an analysis framework was developed that takes into account five critical safety factors: off-target modifications, recognition by the immune system, secondary immuno-vulnerabilities, possibility for reversal, and specificity of targeting. The choice of these criteria was determined both by the regulatory context and by the most frequently discussed safety issues in the literature. Each factor represents a separate kind of risk arising from a specific biological mechanism, enabling more thorough comparisons. Safety information obtained from each article was sorted into the five categories, with quantified results provided where possible, and qualitative results classified as "documented," "reduced," or "absent" relative to a reference value.

Data & Analysis

Table 1 presents the Comparative Safety Profile Matrix, summarizing data across five safety dimensions for three CRISPR approaches based on the ten reviewed studies.

Table 1. Comparative Safety Profile Matrix

Safety Dimension	Permanent Knockout	Antigen-Specific Editing	Reversible Tissue-Specific
Off-Target DNA Changes	High frequency; large insertions documented ^{2,9}	Moderate; targeted delivery reduces but does not eliminate ³	Low; temporal/spatial control limits exposure ^{5,6}
Immune Recognition of Cas9	CD4+ T cell responses confirmed in humans ⁴	Present; delivery method affects magnitude ¹	Reduced; brief expression limits antigen presentation ⁵

Secondary Immune Vulnerabilities	NK cell targeting post-MHC knockout documented ⁷	Tissue-dependent; less characterized	Theoretically controllable via titration
Reversibility	None; all changes are permanent	None; all changes are permanent	Yes, drug-inducible or recombinase-controlled ^{5,10}
Tissue Targeting Precision	Poor; systemic delivery affects multiple organs ⁹	Moderate; nanoparticle targeting improves specificity ³	High; miRNA sensing achieves tissue exclusivity ⁶
Clinical Efficacy (Human Data)	Proven; complete remission in autoimmune patients ¹	Emerging; mouse models demonstrate promise ³	Theoretical; no autoimmune clinical trials

Off-Target DNA Modification

The most dangerous CRISPR edits are the permanent ones, with off-target effects at their highest. According to Boston Children's Hospital,² 6–8% of edited cells exhibited major insertions of DNA between 250 bp and 6 kb due to errors in DNA repair mechanisms. These off-target effects were not always identified using regular sequencing techniques, but further analysis demonstrated that an additional 15% of such cells exhibited genomic rearrangements, deletions of critical tumor suppressor genes, or other substantial alterations. Because these edits are permanent, any mutation will be passed along to all successive cell divisions.

Redman et al.⁹ also described varying off-target mutation rates, ranging from 0.1% to 5% depending on the quality of the guide RNA. These errors increased with the duration of Cas9 protein presence, indicating that continuous expression of the CRISPR system results in more extensive accumulation of unwanted mutations.

Targeting CRISPR to specific antigens will help lower the likelihood of off-target mutations, but is not foolproof. Luo et al.³ utilized nanoscale particle systems to deliver CRISPR components to cells responsible for autoimmune diseases. Although this technique significantly decreased the total number of cells affected by editing, the percentage of off-target mutations per cell remained unchanged. CRISPR systems can also be controlled by restricting the location and duration of expression. Garcia-Guerra et al.⁶ demonstrated the ability to confine CRISPR activity mostly to skeletal muscle, resulting in 12–18% of cells affected in the target tissue while only 0.01% or less in non-target tissue. Time-based CRISPR control can also significantly reduce adverse effects on the genome. Sui et al.⁵ discovered that 72 hours of CRISPR activity achieved comparable editing efficiency to 7 days of continuous activity, while reducing off-target mutations by fourfold.

Immune Recognition

All CRISPR systems face immune risk because Cas9 is a bacterial protein that the human immune system can recognize as foreign. Simhadri et al.⁴ tested Cas9 protein fragments against human donor T cells and found that multiple regions triggered immune responses across

different genetic backgrounds. The response levels were similar to known immune memory reactions, showing that many people already have T cells capable of recognizing Cas9.

From the clinical perspective, Wang et al.¹ found that some patients exhibited fever and inflammation alongside T cell activation after undergoing CRISPR-based cell therapy treatment. While the exact cause of these phenomena was not confirmed, it is reasonable to associate it with an immune response to edited cells. Sui et al.⁵ proved that Cas9 could be effectively silenced within 48 hours after discontinuing administration of the inducing drug. Therefore, the key differentiator between permanent and reversible methods is exposure time to the protein.

Secondary Vulnerabilities

A complete knockout liability is evident in Merola et al.⁷ CRISPR deletion of MHC I/II from endothelial cells prevented T cell recognition but increased NK cell cytotoxicity sixfold through missing-self recognition. While complete knockout resolved the issue with T cells, it created a problem with NK cells. Partial rescue required restoring minimal amounts of MHC I through additional engineering. Irreversible modifications lack the ability to adjust themselves when unforeseen liabilities occur. Reversible mechanisms, in theory, allow for titration—for instance, if the expression of MHC were inducible, MHC I could be regulated to ensure NK inhibition without excessive T cell epitope presentation. This idea remains speculative, with no empirical proof of reversible MHC modification published to date.

Reversibility and Controllability

Irreversibility of impact is a critical distinguishing parameter between the two paradigms. The effects of permanent systems cannot be undone: patients undergoing Wang et al.'s¹ B-cell depletion therapy require a sustained capacity for antibody production that is permanently compromised. Similarly, Luo et al.³ found a stable reduction in co-stimulatory molecules independent of the disease's activity level. In contrast, Sui et al.⁵ found that 72 hours of treatment with doxycycline reverted to baseline values after 96 hours across five consecutive experiments. Chylinski et al.¹⁰ demonstrated temporal control of guide RNA activity,

and although Cre switching is irrevocable, temporal gating can limit the overall editing burden. With respect to safety, reversible circuits offer the opportunity to stop the inducer if adverse effects occur; permanent editing provides no such recourse.

Tissue Targeting Precision

CRISPR-based therapies with permanent effects are usually introduced into the entire body via viral vectors or lipid nanoparticles, making it extremely hard to control the localization of editing events. Despite the use of tissue-specific promoters, some gene modification takes place in other tissues. Redman et al.⁹ estimate this at 1 to 10% of the intended value, sufficient to produce measurable off-target effects.

The antigen-specific delivery of CRISPR tools improves results significantly but cannot fully eliminate the risk of unwanted editing. Luo et al.³ used nanoparticles designed to interact specifically with receptors found on immune system cells, achieving delivery to the target cells at eight times the rate compared to non-target organs such as the liver and kidneys. However, researchers could not prevent gene editing entirely outside the targeted tissues.

The newest methods increase selectivity to a far greater extent. Garcia-Guerra et al.⁶ introduced a system for CRISPR activation in the presence of specific microRNAs, finding that gene editing occurred in approximately 12 to 18% of muscle cells but was almost absent in other tissues. The proportion of off-target editing was less than 0.01%, representing more than 1000-fold selectivity compared to earlier methods.

Clinical Validation Status

The key differentiating factor between the three approaches is validation maturity. Wang et al.¹ demonstrated successful permanent CRISPR-based CAR-T cell therapy results in human remission after an 18-month follow-up, confirming clinical efficacy. Antigen-specific efficacy was proven in mice: Luo et al.³ provided evidence of decreased tissue damage and enhanced scores, but the absence of human data leads to doubts regarding reproducibility. There are no applications of reversible and tissue-specific circuits in autoimmunity for any organism. The systems developed by Sui et al.⁵ and Garcia-Guerra et al.⁶ were tested in non-immune environments, specifically reporter genes and muscular dystrophy models.

Data Synthesis

Across five safety dimensions, reversible tissue-specific circuits demonstrate advantages in four of them (off-target frequency, immune kinetics, reversibility, and precision), with a theoretical advantage in the fifth (secondary vulnerabilities), although empirical testing is lacking. However, this safety differential exists primarily at the

proof-of-concept level, comparing clinically proven permanent systems with mature data against laboratory-validated reversible systems that remain untested in autoimmune applications or human subjects.

Conclusion

In summary, while this review did not produce any direct experimental findings, all ten sources collectively suggest that CRISPR systems based on reversibility and tissue specificity are more reliable and provide better results in terms of safety than gene knockout approaches. According to the reviewed literature, permanent CRISPR systems present several unavoidable risks: off-target mutations due to prolonged Cas9 presence, immune activation through sustained contact with CRISPR components, the potential for NK cell responses due to MHC depletion, and no mechanism to change or eliminate edits once made. At the same time, modern reversible and tissue-specific CRISPR approaches address these problems through additional mechanisms of control. Drug-inducible systems⁵ and temporal systems¹⁰ can limit exposure to editing activities and stop them at a particular point in time. In addition, systems based on microRNA signals⁶ are able to edit genes only within certain tissues, allowing side effects to be avoided without necessitating total gene deletions.

Therefore, the evidence allows concluding in favor of the proposed hypothesis. Modern CRISPR systems have addressed many risks associated with older technology in their architecture and mechanism of action. However, further clinical experiments are required to confirm this and assess long-term outcomes.

Discussion and Evaluation

Using a secondary research methodology enabled comparison and synthesis of results that would be difficult or impossible to achieve via primary research alone. The analysis of information across several scholarly works allowed the identification of regularities regarding CRISPR safety, precision, and immune system effects within various experiments. The literature review approach also facilitated the inclusion of evidence from diverse research methods, including in vitro and animal experiments, and initial clinical research. The application of a structured analytical framework provided a thorough examination of all evidence according to specific criteria regarding reversibility, targeting precision, immune impact, and long-term safety.

The analysis of multiple CRISPR approaches via primary research in a laboratory setting would involve extensive costs, complex equipment, and long-term research not feasible within the limits of this project. Secondary research allowed for a rigorous and highly efficient assessment of available knowledge on CRISPR technologies.

However, this study had four main limitations. First, the studies being conducted in different subjects creates incomparability. For example, Merola et al.'s⁷ *in vitro* findings may not translate quantitatively to *in vivo* contexts. While the underlying principles of CRISPR effectiveness are established, the clinical magnitude of comparisons remains uncertain.

Second, publication bias skews evidence toward favorable outcomes. Successful applications generate high-impact publications, while failures tend to remain unpublished. This overrepresents the advantages of newer technologies. During the research for this literary analysis, zero reports of completely failed reversible systems or negative effects were found, which would be statistically implausible given the engineering complexity involved and the many potential errors possible.

Third, temporal asymmetry limits long-term comparison. Some original permanent systems have been investigated since the earliest stages of this technology's development, while newer ideas, such as reversible circuits, were first reported in 2019. Long-term adverse effects from newer approaches may not yet have appeared.

Lastly, theoretical extrapolation introduces unverified assumptions. Integrating drug-inducible with microRNA-gated control assumes cooperative function without interference, but inducible promoters and microRNA elements might compete for cellular machinery, or drug administration could alter microRNA levels. Since no published work has tested this integration, potential incompatibilities remain unexplored.

There was also an unexpected experimental finding that strengthened the study's conclusions. The NK vulnerability discovery⁷ challenges assumptions that solving immune recognition through knockout would have unidirectional effects, demonstrating that permanent knockout actually creates new problems while solving intended ones, and thereby supporting the necessity for newer, more controlled technology.

Future Implications

This literary analysis shows that reversible, tissue-specific CRISPR circuits offer significant safety advantages across multiple dimensions compared to permanent knockout systems. These findings carry strong implications for therapeutic development, clinical practice, and regulatory frameworks in autoimmune gene therapy.

The documented safety differential implies it would be of greater benefit to the field to prioritize developing integrated reversible tissue-specific platforms over continuing with traditional permanent editing approaches. The evidence presented demonstrates that controllability and precision are not just theoretical ideals but achievable

engineering features with demonstrated proof-of-concept validation. This should position reversible CRISPR methods as a new standard for autoimmune CRISPR therapy, indicating that research investments should shift towards creating systems that offer therapeutic benefit without permanent genomic consequences.

For patients with autoimmune disease, these findings imply that safer treatment options may become available that address the limitations of current permanent editing approaches. Patients who might decline gene therapy due to concerns about irreversibility could access CRISPR-based treatments if reversible options were available, thereby expanding the eligible treatment population.

The increased safety of controlled systems may indicate the necessity to rethink regulatory frameworks and risk-benefit calculations. Currently used FDA frameworks designed for permanent changes might prove either too conservative or insufficiently cautious when dealing with reversible systems. The standards for approving gene editing architecture must clearly specify the nature of editing—whether permanent or reversible—as the basis for assessment.

Moreover, the absence in the existing literature of any work coupling temporally reversible techniques with tissue specificity, despite both having been demonstrated independently, indicates a crucial translational gap. The building blocks are present, but the assembly is not. Translation efforts should focus on the integration aspect as the natural follow-up to safe autoimmune gene therapy development.

Declaration of Competing Interests

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If applicable: During the preparation of this work the author(s) used ChatGPT in order to aid in the process of finding suitable articles that would fit the premise of this research. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.]

References

1. Wang X, Hu C, Qiao S, et al. Treatment of autoimmune diseases with CD19 CAR T cells generated by

- CRISPR/Cas9-mediated gene editing. *Nat Med.* 2024;30(1):178–187.
2. Boston Children's Hospital. Safety risks of permanent CRISPR edits in human cells: research findings on genomic instability and cancer risk from CRISPR/Cas9 editing. 2022. [Online research report]
 3. Luo YL, Xu CF, Li HJ, et al. Macrophage-specific in vivo gene editing using cationic lipid-assisted polymeric nanoparticles. *ACS Nano.* 2020;14(7):7759–7771.
 4. Simhadri VL, McGill J, McMahon S, Wang J, Jiang H, Sauna ZE. Prevalence of pre-existing antibodies to CRISPR-associated nuclease Cas9 in the USA population. *Mol Ther Methods Clin Dev.* 2021;10:105–112.
 5. Sui Y, Chen Q, Sun X, et al. Drug-inducible CRISPR/Cas9 system for reversible gene regulation in mammalian cells. *Nucleic Acids Res.* 2025;53(2):847–862.
 6. Garcia-Guerra A, Dunbar CE, Persons DA. CRISPR MiRAGE: MicroRNA-activated genome editing for tissue-specific therapeutic applications. *Cell.* 2025;188(4):823–839.
 7. Merola J, Reschke M, Pierce RW, et al. Progenitor-derived human endothelial cells evade alloimmunity by CRISPR/Cas9-mediated complete ablation of MHC expression. *JCI Insight.* 2019;4(20):e129739.
 8. Zhang ML, Li Y, Rao PJ, Huang K, Luo D, Cai X. Application and perspective of CRISPR/Cas9 genome editing in regulatory sequences of autoimmune risk loci. *Front Genet.* 2024;15:1364742.
 9. Redman M, King A, Watson C, King D. What is CRISPR/Cas9? *Arch Dis Child Educ Pract Ed.* 2016;101(4):213–215.
 10. Chylinski K, Hubmann M, Hanna R, et al. CRISPR-Switch: Temporal control of CRISPR/Cas9 gene editing. *Nat Biotechnol.* 2019;37(6):657–666.